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# Biochemistry in Decapod Crustaceans

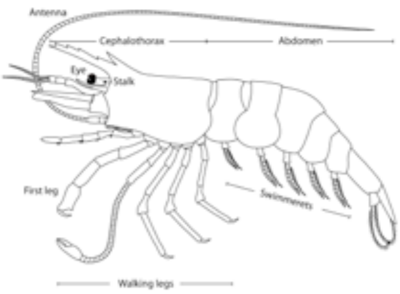

*Figure 1. Conventionalised shrimp, illustrating terms used in the description and classification of decapod crustaceans. (Adapted from* ***Crustaceans*** *by Waldo L. Schmitt, Ann Arbor: University of Michigan Press, 1965).*

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People (like anglers and seafood lovers) who are familiar with aquatic animals may consider crayfish, lobsters, shrimp and crabs to be as close as kith and kin. Although these animals are not as closely related as brothers and sisters, they nevertheless belong in the same taxonomic group (Order Decapoda, Class Crustacea, Phylum Arthropoda) [1]. They have segmented bodies which are divided into two major parts (the cephalothorax and the abdomen), two pairs of antennae and five pairs of legs, the front two of which are often equipped with claws (Fig 1). A jointed hard shell or skeleton on the outside of their bodies, the exoskeleton, provides protection, support, flexibility and increased surface area for muscle attachment.

Their five pairs of legs, used as multipurpose appendages, extend from the cephalothorax region that consists of a fused head and a thorax, and give rise to the name “decapod crustaceans”. The first pair usually ends in claws (big in crabs, lobsters and crayfish or small in shrimp) while the other pairs are generally used for crawling or walking, may also be important in the mating process, and may assist other mouthparts in the capture and manipulation of food. Swimmerets (appendages attached in pairs to the abdominal segments) help with swimming, balancing or the holding of developing eggs in species that brood their zygotes. Decapods breathe via gills protected under the unjointed portion of the exoskeleton called a carapace that covers the cephalothorax.

Each gill is attached at the outside of the innermost joints of the legs. Water is propelled over the gills of shrimp, lobsters and crayfish by the action of appendages attached to the bases of these legs. In crabs, the enclosed gill chamber permits them to retain water or humidity so that some species even can wander onto land. The compound eyes of decapods are on movable stalks to allow sight in different directions. Like our nose, ears, fingers and tongue, their two pairs of antennes are used to sense things.
A wide range of body designs and colours of decapod crustaceans may be seen in any seafood restaurants.

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## Shrimp, lobsters and crayfish come in many colours

Different species of decapod crustaceans exhibit a wide range of blue, green, brown and yellow body colours that are derived from the presence of the xanthophyll carotenoid astaxanthin (3,3′-dihydroxy-β,β′-carotene-4,4′-dione) pigments encapsulated in a protein carrier that is found embedded in the exoskeleton or in pigmented cells (chromophores) in tissues under the cuticle.

The best known carotenoprotein is the blue ɑ-crustacyanin from the lobster *Homarus gammarus*. It consists of 16 protein subunits with 16 bound astaxanthin molecules. Astaxanthin (Box 1) is a red pigment, which when complexed with various carotenoproteins, undergoes bathochromic shifts of its spectrum (Box 2): when astaxanthin is complexed with crustacyanin, the peak of the absorption spectrum is shifted to a longer wavelength by some 160 nm (λmax = 632 nm) as compared to the wavelength maximum of free astaxanthin (λmax = 472 nm).

This shift is responsible for the blue colouration of crustacean shells [2]. Molecular designers have taken advantage of this colouration mechanism to produce a variety of soluble dyestuffs and food colourants. Astaxanthin is itself a powerful antioxidant. It has the potential to be used in the prevention and treatment of some human diseases (e.g., arteriosclerosis, age-related macular degeneration, neurodegenerative diseases, cancer, *Helicobacter pylori* infections, and rheumatoid arthritis) [3]. Because of its solubility in water, crustacyanin is of pharmaceutical interest as a carrier of astaxanthin molecule.

*Figure. 2 Shrimp turn bright orange when cooked.*

## The decapods turn orange-red when cooked

When alive, decapod shells look blue-green to brown. This provides valuable camouflage protection in their natural habitats. When cooked, they turn orange-red because of the hypsochromic shift of the absorption spectrum of the astaxanthin that is released from the denatured carotenoproteins due to high temperature (Fig 2). This appealing colour depends on the amount of astaxanthin deposited in the hypodermal chromophores and in the shell. In addition to the above astaxanthin plays many vital physiological roles, particularly in the overall health of decapod crustaceans. For them, it serves as an antioxidant, hormone precursor, immune enhancer and provitamin A, and plays a role in reproduction, growth, maturation and photoprotection. In human nutrition, astaxanthin has been used as a dietary supplement called super vitamin E due to its powerful antioxidant properties. To obtain the required amount of astaxanthin, there is no need to consume a big quantity of decapods since several astaxanthin products; both synthetic and naturally occurring (e.g., those derived from the green microalgae, *Haematococcus pluvialis*, and the red yeast, *Phaffia rhodozyma*), are available in the marketplace. However, the peeling waste discarded from edible decapod meat at seafood processing and frozen storage plants.
and frozen storage plants is an inexpensive source of this valuable orange-red pigment for use in animal feed.

## The party is filled with the fragrance of the decapods

The orange-red colour that tinges their whitish flesh and the pleasant aroma that arises during cooking make shrimp, lobsters and crabs popular seafood in many countries (Fig 3). The volatile components of crustaceans include nitrogen- and sulfur-containing compounds, ketones, aldehydes, alcohols, esters, hydrocarbons, pyrazines, pyridines, amides, amines and other compounds.

*Figure 3. Seafood cocktail with spicy lime dipping sauce.*

Some of these are not present or are present at low concentration in raw meat, but are present at higher concentration in cooked meat. More than 100 unsaturated methylketones, including 40 sulfur and/ or nitrogen-containing compounds, have been found in cooked shrimp volatiles. The key fragrant compounds isolated from shrimp and lobsters are simple bromophenols such as 2- and 4-bromophenol, 2,4- and 2,6-dibromophenol and 2,4,6-tribromophenol derived from plankton and other food sources like marine worms (polychaetes). These bromophenols contribute recognisable marine- or ocean-like flavours to seafood and occur at higher concentrations in the head than in tail meat. Thus, the taste and volatile components of shrimp heads are valued for their potential application as food flavouring agents.

*Figure 4. Photograph of the blue swimming crab Portunus pelagicus: (Top) live, (Bottom) after cooking. In contrast to shrimp, lobsters, and crayfish, the crab abdomen is minimised and most of their edible meat is in their cephalothorax region.*

## Mouth-watering meat is made up of strong muscles from decapod tails

Shrimp, lobsters and crayfish have an elongated abdomen that is often called the tail and makes for a tasty treat when properly cooked. By contrast, the crab abdomen is minimised and most of their edible meat is in their cephalothorax region and claws (Fig 4). All muscles that make up the tasty decapod tail are striated, with well-defined sarcomeres. Thus, the fascinating mechanical arrangement of a complex helical trajectory characteristic of muscles in other animals is present within these creatures and the molecular mechanism for transforming chemicals to mechanical energy is also similar, although the details of the overall process may vary. The muscles controlling movement in tails of shrimp, lobsters and crayfish are divided into four sets: the slow and fast flexors and extensors. The ventral fast flexors and dorsal fast extensors account for most of the abdominal volume being designed to create an explosive backward acceleration.

## The decapods spring backwards when frightened

Normally, decapods walk or swim about slowly. However, when threatened, they are capable of using their tails to propel themselves upwards and backwards in a series of rapid jerks to get away from threat or touch. At the onset of sudden muscular activity, the rate of ATP used increases many fold. Rephosphorylation of ADP at first makes use of the high-energy phosphate group in phosphagen. When this is exhausted, the muscles degrade glycogen to meet their energy requirements [4]. Phosphagens are phosphorylated guanidinium compounds (i.e., phosphorylated creatine and arginine that are reacting with ATP by a reversible reaction catalysed by phosphagen kinases). Our muscle cells contain a creatine phosphate/ creatine kinase system that supplies energy needs for muscles to work at a high rate for only 8-10 seconds. This is the major energy system used by the muscles of Olympic sprinters or weightlifters when rapid acceleration, short-duration exertion is needed. Decapod tail muscles contain an arginine phosphate/ arginine kinase system that allows for explosive escape responses that are very short in duration. The fast flexor and extensor muscles are responsible for this escape tail flip behaviour and swimming.

## The decapods can make some people ill

Many people dream of dining on cocktail dressed shrimp, crayfish, lobster on the half shell and king crab. But for people with seafood allergies, this would be a nightmare. The main allergens are specific sequences of amino acids that bind with immunoglobulin E (IgE) and can lead to responses such as itchiness around the eyes, throat, mouth, and skin. Among decapod crustaceans, shrimp are frequently identified as the cause of IgE-mediated adverse reactions. Up to 13 IgE-binding proteins have been detected in shrimp meat. The 34-39 kDa muscle protein tropomyosin is considered to be the major shrimp allergen. It is cross-reactive with tropomyosins found in other decapods. However, IgE molecules from patients allergic to crustaceans do not bind to tropomyosins from poultry and mammals. Other naturally-occurring proteins, the arginine kinase and the sarcoplasmic calcium-binding protein from the shrimp *Penaeus monodon*, and the myosin light chain from the shrimp *Litopenaeus vannamei*, can also cause allergic symptoms in some individuals [5]. These allergens are resistant to food processing and human digestion. If you are thinking of the possibility of getting rid of them by whatever means, you need to pause a moment. These allergens happen to be essential for crustacean survival. For example, tropomyosin, sarcoplasmic protein, and myosin light chain are involved in muscle contraction while arginine kinase catalyses the reversible transfer of the high-energy phosphoryl group in the reaction ATP + arginine «» ADP + phosphoarginine (Box 3). Their removal from the decapod genome would be counterproductive. Instead, one group of scientists is modifying the tropomyosin amino acid sequence so that it cannot bind IgE. Mutated tropomyosin would then be expressed from one shrimp generation to the next. This may reduce allergenicity provided that no new allergenic reactivity arises from the alterations.

## Decapods are blue bloods

Animal blood comes in several colours besides red. The characteristic colours arise from oxygen-binding proteins in their oxygen binding state. Arthropods including the decapods have copper metal in the oxygen-binding protein named haemocyanin that gives them blue blood when oxygenated (Box 4). Arthropod haemocyanins consist of multiples of hexamers of 75 kDa subunits. The oxygen molecule is bound to two copper ions, each of which is coordinated by three histidines. Cu(I) is present in the deoxygenated state and as Cu(II) when bound to O22-. X-ray diffraction studies of haemocyanin of the spider *Eurypelma californicum*, the horseshoe crab *Limulus polyphemus*, the abalone *Haliotis tuberculata* and the giant octopus *Octopus dofleini* have shown that the dioxygen-binding sites of arthropod and molluscan heamocyanins are very similar both in the coordination of copper via histidine ligands and in the way the oxygen is bound. Arthropod haemocyanins share sequence similarities with arthropod haemolymph phenoloxidases which are involved in cuticle sclerotisation and in the innate immune response. The arthropod haemocyanins are multifunctional molecules acting as oxygen carriers, as buffers and osmolytes, as carriers of moulting hormones and probably as constituents of the cuticle [6].

## Decapods use multiple defense systems

As prey decapods constitute energy and nutrient sources for human and non-human consumers. Thus they must avoid, tolerate or defend themselves against their enemies such as predators, parasites and competitors. The external structures that protect their vulnerable soft tissues include the chitinous exoskeleton and the claws that may be so powerful in some species as to crush a finger with a single squeeze! In species with smaller claws, like shrimp and some crayfish, explosive backward jerks by the powerful tail are the main method of protection. Decapods also use their hard shell as a first line of defense against pathogens. Once this is breached, a complex interaction of innate humoral and cellular immune reactions is induced that can result in elimination of microorganisms. For example, melanisation of parasites is well known in decapod crustaceans. Melanin synthesis is initiated by phenoloxidase that can be activated via an enzymatic cascade and microbial cell wall components. Haemocyanin, a copper containing oxygen-transport protein, is also involved in defending against invaders since it too behaves like a phenoloxidase [7]. In addition, decapod crustaceans produce at least three families of antimicrobial peptides named penaeidins, crustins, and antilipopolysaccharide factors that display a broad spectrum of activity against fungi, viruses, bacteria, and parasites.

## Decapods occasionally cast off their exoskeletons

The exoskeleton of decapod crustaceans consists of multilayered, noncellular, chitinised material generated by the underlying epidermal tissue. Their bodies, appendages, eye balls and stomach surfaces are covered by this exoskeleton. A calcified and hard exoskeleton limits size and growth. Thus, decapods must moult periodically or shed their exoskeleton in order to grow especially when young. For example, larval black tiger shrimp moult everyday while juveniles in shrimp ponds moult once every 7-14 days. At the appropriate time a new, soft exoskeleton is first generated under the old one from which calcium is then removed and the shrimp swell up and squeeze out through a tear along the dorsal surface between the carapace and the abdomen, with rapid shedding of the old exoskeleton. They are then vulnerable to attack up to several days until calcification and hardening (sclerosis) of the new exoskeleton occurs. The major crustacean shell component is chitin, a polymer of N-acetyl-β-D-glucosamine, associated with sclerotised proteins and impregnated with calcium carbonate. Moulting (or ecdysis) is initiated by the formation of a new cuticle under the old one that involves secretion of moulting fluid that contains proteases and chitinases that digest some main constituents of the old shell that separates from the epidermis. Synthesis of new exoskeleton begins with secretion of cuticle proteins and chitin fibers through the apical membrane of the epidermal cells. All the while, moulting fluid remains between the old and new cuticles. The epidermis is protected against the digestive enzymes of the moulting fluid by the formation of the epicuticle (outermost part of the cuticle) [8].

## Moulting is controlled by hormones

The onset of periodic shedding off the exoskeleton is triggered by moulting hormones called ecdysteroids, a group of polyhydroxy steroids. In crustaceans, the Y-organ located at the base of the primary antennae, produces and secretes ecdysone and 3-dehydroecdysone which are then converted to 20-hydroxyecdysone, the biologically active ecdysteroid. By regulation of this hormone, shrimp periodically loosen the connection between their epidermis and the exoskeleton and take up water to expand the new shell and result in discontinuous size increases. After each moult, the exoskeleton hardens and water is gradually replaced by tissue. Ecdysteroid is negatively controlled by moult-inhibiting hormone (MIH) secreted from the X-organ located in the medulla terminalis of the eyestalk. MIH is a member of the crustacean hyperglycemic hormone (CHH) family. A cDNA for MIH of shrimp *Penaeus monodon* (MIH 1) encodes 77 amino acid residues including six cysteines that are also conserved in other CHH family members, and a glycine residue at position 12 that is characteristic of type II peptides of the CHH family [9].

## Female brooder shrimp may have to sacrifice an eye for fertility’s sake

Ovarian maturation, spawning and production of high quality seed are among the main goals of aquaculturists. Unilateral eyestalk ablation of various species of female shrimp during maturation and reproduction is a common practice in hatcheries worldwide. This is a key discovery in making the cultivation of shrimp an economically viable, large-scale enterprise. Following ablation the female brooders become “egg laying machines” for approximately one month, when they become exhausted and are discarded. It is commonly thought that a gonad inhibitory hormone (GIH) is produced in the neurosecretory complexes of the eyestalk and that its inhibiting effect is reduced following ablation of one eyestalk and its attached eye. GIH apparently occurs in the non-breeding season and is absent or present in low levels during the breeding season. Ablation of the eyestalk also controls MIH secretion from the X-organ. In many shrimp, moulting occurs during oogenesis being closely synchronised with reproduction. Therefore, hormonal manipulation of shrimp reproduction is limited to eyestalk ablation although its effects may extend to other physiological processes. Shrimp may not have to undergo eyestalk ablation when the full diversity of hormones involved in their reproduction is sufficiently understood and can be controlled more gently. This could be particularly advantageous when genetically improved, domesticated shrimp stocks become available to the shrimp industry. After stimulation to spawn, shrimp could be left to rest and recover so that they could be used again in several spawning cycles.

## Soft moulting female tiger shrimp seek their hardened soulmates

Mating in black tiger shrimp, *Penaeus monodon*, usually takes place immediately after the female moults, while her exoskeleton is still soft and mature, and hard-shelled male shrimp can perform their romantic act. Female shrimp receive sperm packets from the males and store them in a special ventral chamber covered by a thick cuticular cover or thylecum. The sperm mature in the chamber during the inter-moult period and are released with the eggs during spawning. Thus, males need not be present during spawning. If females develop ripe ovaries but have not previously mated, sperm packets obtained from mature males can be inserted into the thylecal chamber (artificial insemination). By contrast, the white shrimp *Penaeus vannamei* has no closed thylecal chamber and males must be present during spawning. Clearly, mating and fertilisation in decapod crustaceans involves species-specific behaviour. In the shrimp *Penaeus monodon* sperm attachment to and penetration of the egg must occur within one minute after spawning. After the acrosomal reaction, a thick hatching envelope prevents penetration of other sperm. However, more than one sperm has sometimes been observed to penetrate the egg surface. If polyspermy occurs, it does not appear to cause fertilisation problems; it is thought to be a physiological adaptation for many crustaceans.

## Penaeid shrimp spermatophores have a predetermined expiry date

In male decapods the endocrine and gamatogenic functions are separated into two distinct organs, i.e., the androgenic gland (AG) and the testis. Androgenic hormone secreted by AG appears to be a glycosylated protein and is found to masculinize primary (e.g., spermatogenesis) and secondary (e.g., external morphology) sexual characteristics including agonistic behaviour. In freshwater shrimp *Macrobrachium rosenbergii* complete sex reversal from males to neo-females and from females to neo-males is achieved at an early immature stage by bilateral androgenic gland ablation and by transplanting it. *M. rosenbergii* that have undergone sex reversal are capable of mating with normal shrimp and producing progeny [10]. Penaeid males exhibit successive pairs of spermatophores (seminal fluid) inside the reproductive tract for mating within the same moult cycle. Spermatophores in prolonged disuse will become melanised and subsequently hardened rendering the male impotent. Manually or electrically induced ejaculation can remove melanised spermatophores. These methods are used widely to keep males in good condition in the absence of receptive females. Males, however, go through a periodic sperm replacement process. This involves acellular matrix degradation and phagocytosis of the spermatozoa induced by the premoult surge of ecdysteroid. This regular happening associated with the moult cycle may possibly contribute to male shrimp prowess.

## Decapods are what they eat

Decapod crustaceans are not only important predators but also prey in aquatic food chains. They comprise carnivores, omnivores and scavengers. In the wild, they eat a wide variety of small invertebrates and plants and their high quality diets are key to overall health and successful production of new generations. As mentioned earlier, astaxanthin, a high-value carotenoid produced from microalgae or phytoplankton, is an important antioxidant and is responsible for the desirable orange-red body colour that appears upon cooking. Polychaetes [11] , a kind of segmented marine worms, are a major dietary component for wild shrimp providing highly unsaturated fatty acids important for stimulating ovarian maturation. They also produce high concentrations of bromophenols which impart a seafood-like odour. Minerals absorbed from water are required for physiological activities of decapods, but calcium for hardening their shells is also acquired from zooplankton and shellfish that they eat. Lastly, amino acids provided by protein-rich foods such as squid are important for building muscle strength and for contributing the meaty taste of their tails. However, an unbalanced diet (nutritional deficiency or excess) can cause decapod crustaceans to develop illnesses similar to those in humans.

*Figure 5A. An illustration of 4 months old Penaeus monodon. (Left) Normal size shrimp with common greenish brown colour (Right) Stunted shrimp from a pond with a previous history of Hepatopancreatic Parvo-like Virus (HPV) outbreak. The lack of planktons in this cultured shrimp caused the Blue Colour Syndrome.*

## Shrimp succumb to microbial infections, an aquaculturist's nightmare

Decapod crustaceans encounter many microorganisms that may be beneficial and benign or opportunistic and virulent pathogens. Potential pathogens include viruses, bacteria, fungi, rickettsiae and parasites from protozoans to metazoans. The onset of disease occurs when the animals come into contact with a critical mass of pathogens under conditions suitable for its development (Fig 5A + 5B). Serious economic losses in aquaculture in recent years have been caused by viral pathogens. Surprisingly, infection by single or mixed viruses with no gross signs of disease is common in shrimp, other crustaceans and arthropods in general. However, the interaction between shrimp and viruses at the cellular and molecular level is poorly understood.

This contrasts with the knowledge available on the response of shrimp to bacterial and fungal pathogens [12]. When infected with the pathogens some shrimp manage to stay healthy while others in the same pond become sick. Knowledge of shrimp genetics would provide the mechanism(s) of how shrimp maintain tolerance to persistent viral infections. Shrimp genome sequencing should discover the genes with the ability to boost the animal's immune system and genetic markers for disease-resistance genes. Stronger varieties of shrimp could then be developed through selective breeding of domesticated stocks. Shrimp farmers with access to domesticated shrimp fry selected for disease resistance and high growth rate, would face less risk in shrimp farming and derive a more stable and reliable income from a higher quality product. The realisation of such robust shrimp will benefit scientists, aquaculturists and consumers in more ways than one.

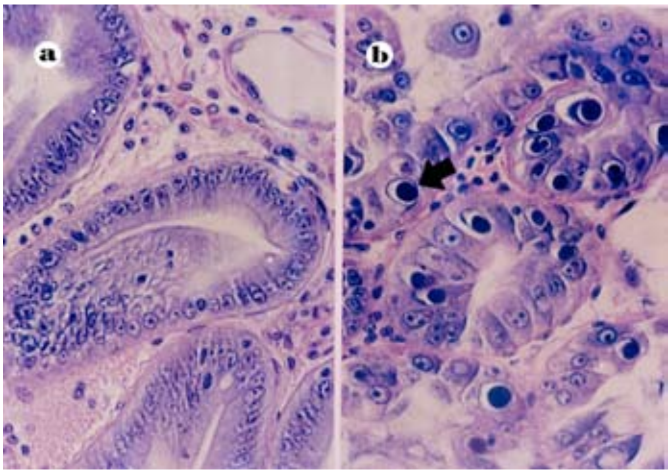
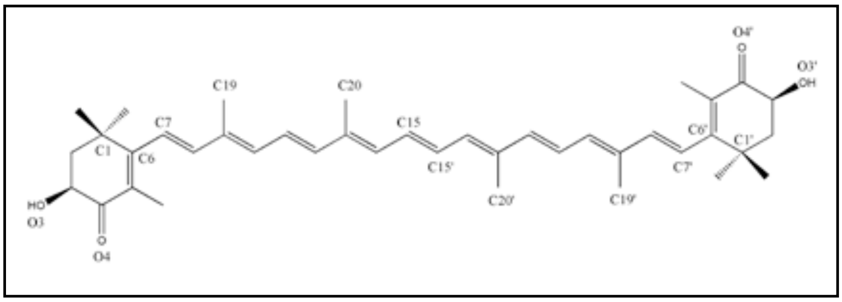


*Figure 5B. Histological section of Penaeus monodon hepatopancreas (a) Hepatopancreatic Parvo-like virus (HPV) negative specimen (b) HPV positive specimen showing hypertrophied nuclei in the infected cells (arrow). Both pictures were taken under the same magnification. HPV may cause slow growth in shrimp by damaging the cells of hepatopancreas, whose main function is the production of enzymes for digestion and food storage.*

## Boxes

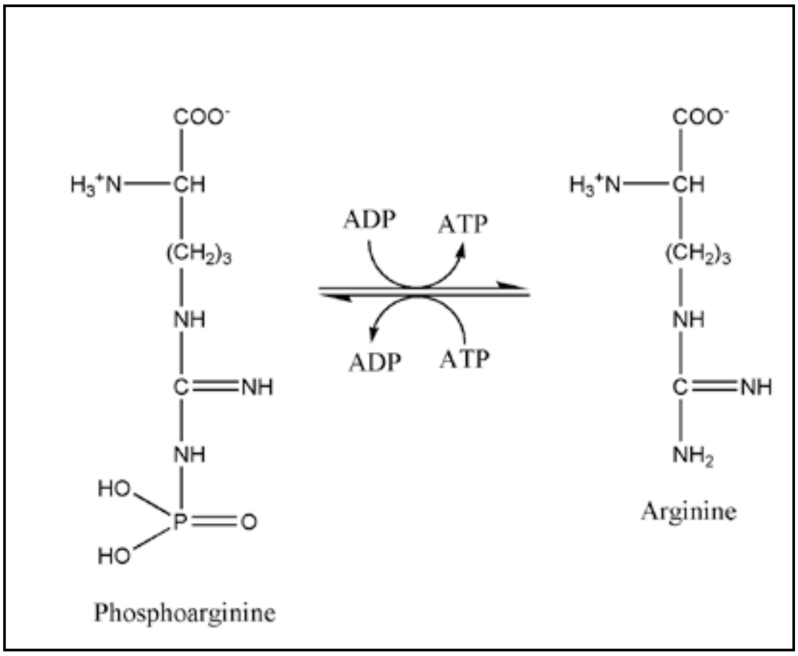


*Box 1. The chemical scheme of astaxanthin.*

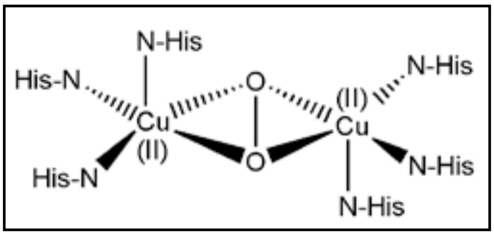
Bathochromic shift is a change of the maximum position of the absorption band of a molecule to a longer wavelength. This can occur because of a change in environmental conditions: for example, a change in solvent polarity. In this case the changed environments are the protein bound to the chromophores.

Hypsochromic shift is a change in band position to a shorter wavelength.

*Box 2.*



*Box 3. Forward and reverse phosphate-transfer reactions catalysed by the enzyme arginine phosphokinase.*



*Box 4. The chemical scheme of arthropod haemocyanin.*

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