Review

Expanding the solar spectrum used by photosynthesis

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A limiting factor for photosynthetic organisms is their light-harvesting efficiency, that is the efficiency of their conversion of light energy to chemical energy. Small modifications or variations of chlorophylls allow photosynthetic organisms to harvest sunlight at different wavelengths. Oxygenic photosynthetic organisms usually utilize only the visible portion of the solar spectrum. The cyanobacterium *Acaryochloris marina* carries out oxygenic photosynthesis but contains mostly chlorophyll *d* and only traces of chlorophyll *a*. Chlorophyll *d* provides a potential selective advantage because it enables *Acaryochloris* to use infrared light (700–750 nm) that is not absorbed by chlorophyll *a*. Recently, an even more red-shifted chlorophyll termed chlorophyll *f* has been reported. Here, we discuss using modified chlorophylls to extend the spectral region of light that drives photosynthetic organisms.

Solar output spectra and flux

The spectrum of the output of the sun that falls on the Earth is essentially that of a black body emitter with a temperature of 5800 K (Figure 1), modified by scattering and absorption in the atmosphere [1]. All solar energy storage processes, both living and nonliving, rely on this energy source. The solar spectrum is often plotted as energy per area per time or, alternatively, as photon flux per area per time. Energy representation is more appropriate for a device such as a solar thermal system in which light is absorbed and all the energy is converted to heat, which is then used to power a device such as a steam turbine that in turn drives a generator that produces electricity. By contrast, the photon flux curve is more appropriate for a device that utilizes the quantum nature of light to generate energy in the form of an electric current in a photovoltaic cell, a dye-sensitized solar cell or chemical energy in a living photosynthetic organism. Both curves are shown in Figure 1. There is an inverse relationship between photon energy and wavelength as represented in Planck's law ($E = hc/\lambda$) where E is photon energy in Joules, h is Planck's constant (6.63×10^{-34} J s), c is the speed of light (3.0×10^{8} m s^{-1}) and λ is the photon wavelength in meters. It takes a larger number of longer wavelength photons to supply a given quantity of energy than it does shorter wavelength photons. This relationship has the effect of changing the shape of the curve represented in two different ways. The photon flux curve peaks at approximately 700 nm, whereas the peak of the energy curve is at approximately 500 nm. The importance of the spectral region at longer wavelengths than 700 nm is made more apparent when the solar spectrum is represented as photon flux. Even small increases in the ability to utilize these photons can be significant because they occur at the place where the solar spectrum is at its maximum.

Most oxygenic photosynthetic organisms are able to utilize essentially the same region of the solar spectrum that our eyes are sensitive to, namely the visible range extending from 400 nm to 700 nm [2]. This region is called photosynthetically active radiation (PAR). Using the ASTM 1.5 reference solar spectrum [1], the integrated 400–700 nm PAR photon flux is 1.05×10^{21} photons m^{-2} s^{-1}. Dividing by Avogadro's number gives the more familiar number of 1740 μE m^{-2} s^{-1}, where an Einstein (E) is a mole of photons. If the useful region of the spectrum is expanded to include the 400–750 nm region, the photon flux is 1.25×10^{21} photons m^{-2} s^{-1}, or 2070 μE m^{-2} s^{-1}. This relatively modest increment in the portion of the solar spectrum utilized (700–750 nm) increases the number of available photons by 19%. Because the amount of stored energy is proportional to photon absorption under nonsaturating conditions, this represents a substantial potential increase.

Long wavelength limit for oxygenic photosynthesis

Because photosynthesis is a quantum storage process (as opposed to a thermal process that uses heat), the energy to drive the photochemistry must be supplied in discrete packets in the form of photons. Any quantum process will have a threshold characterized by the longest wavelength photon that can drive the process. In other words, photons with wavelengths longer than the threshold do not have sufficient energy to drive the photochemistry and are usually not absorbed by the system. Similar principles apply for any quantum storage system, including photosynthetic organisms, dye-sensitized solar cells and photovoltaic cells, although the molecular details of these other systems are different.

What is the threshold energy for photosynthesis, or put another way, what is the longest wavelength photon that can drive the process? Here, it is necessary to draw a distinction between anoxygenic and oxygenic photosynthesis. Anoxygenic (nonoxygen-evolving) photosynthesis, which depends on bacteriochlorophyll pigments, has a significantly longer wavelength limit than does oxygenic photosynthesis. Many anoxygenic phototrophic bacteria

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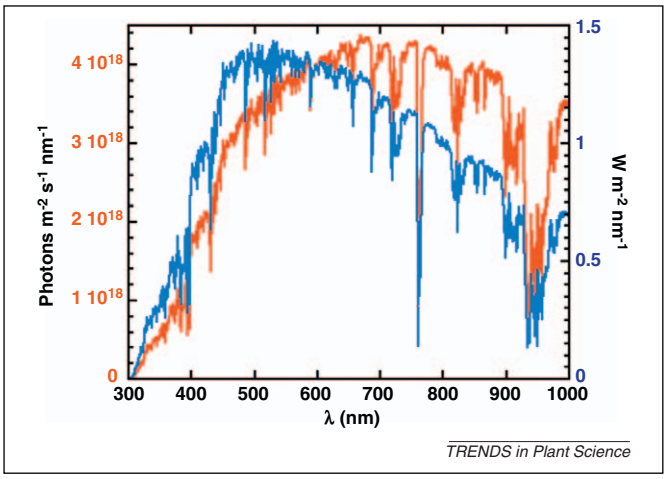

Figure 1. ASTM 1.5 solar output spectra. This is the standard reference solar spectrum used to evaluate solar cells and photosynthetic efficiency, which can be downloaded at http://rredc.nrel.gov/solar/spectra/am1.5/. The blue curve is the energy output spectrum and the red curve is the photon flux spectrum. The photon flux spectrum is more relevant for a quantum energy storage process such as photosynthesis.

are known with complexes that absorb at wavelengths longer than 900 nm and some of the Bchl *b*-containing purple bacteria such as *Blastochloris viridis* have long wavelength limits beyond 1000 nm [3]. By contrast, oxygenic phototrophs, which depend on chlorophyll pigments, have until recently been thought to have long wavelength limits of approximately 700 nm. The reason for this much higher threshold energy for oxygenic photosynthesis is thought to be the formidable energy requirements of water oxidation, which requires a redox potential for the oxidized primary electron donor pigment that is sufficiently strong to oxidize water, while at the same time having an excited state redox potential that is sufficiently negative to drive the reduction of the primary electron acceptor [4]. The redox potentials of the donor and excited state depend on the nature of the pigments and the photon energy available. A low-energy photon will fail to generate either a strong enough oxidant to oxidize water or a strong enough reductant to reduce the primary acceptor.

The discovery of the Chl *d*-containing *Acaryochloris* [5] and the newly discovered Chl *f*-containing organism [6] has forced a reevaluation of what is the minimum threshold energy for oxygenic photosynthesis. Current indications are that the photochemistry in *Acaryochloris* is as efficient as that found in Chl *a*-containing cyanobacteria; thus, the threshold seems to have been at least extended to 740 nm [7]. This issue is not yet clear and represents an important area for additional research.

Acaryochloris and chlorophyll *d*

Chlorophyll *a* is the most widespread photopigment in nature, and Chl *d* is the only known chlorophyll that can replace all or nearly all the functions of Chl *a* in oxygenic photosynthesis. The chemical structure of Chl *d* differs from Chl *a* only in the C3 position at ring A, where a formyl group in Chl *d* replaces the vinyl group in Chl *a* (Figure 2). Chl *d* was first reported from a red algal pigment extract nearly 70 years ago [8], although later it was considered likely to be an artificial byproduct of pigment extraction and not present in any organism [9]. However, this view changed dramatically with the discovery in 1996 by Miyashita and colleagues of *Acaryochloris marina*, an organism that contains over 95% Chl *d* as its major photopigment, with only small amounts of Chl *a* [5].

Acaryochloris is an oxygenic photosynthetic cyanobacterium that harvests light and performs photochemistry with Chl *d*, a red-shifted Chl, with the *in vivo* absorbance maximum of ~710 nm (Figure 3). *Acaryochloris* was discovered in association with a colonial ascidian [5]. The upper layer of the ascidian harbors the Chl *a*/*b*-containing prochlorophyte *Prochloron didemni,* whereas *Acaryochloris* resides beneath the lower layer of the ascidian [10]. The light intercepted by *Acaryochloris* in this environment is deficient in the visible light region absorbed by Chl *a* and Chl *b*, but relatively enriched in the infrared light region (700–750 nm) absorbed by Chl *d* (Figure 3). *Acaryochloris* ecotypes have since been discovered in other habitats that are enriched in infrared light, such as on the undersides of red algae [11], free-living in a microbial mat community in a turbid, saltwater lake [12] and underneath crustose coralline red algae [13]. The far-red light-enriched habitat of *Acaryochloris* provides strong selection pressure to use the photons in the far-red light region because only those photons penetrate to where it lives.

Not only is *Acaryochloris* distinctive for utilizing Chl *d* in the reaction center of both photosystems [14,15], but *Acaryochloris* also captures light with two complementary light-harvesting systems: a simple, external phycobiliprotein antenna and an integral Chl *d*-binding antenna [16–18]. These systems enhance the light-harvesting ability of *Acaryochloris* to absorb not only infrared light (700–750 nm), but also visible light of 500–600 nm that penetrates through the body of the ascidian-containing symbiotic *Prochloron* [10]. *Acaryochloris* dynamically regulates these antennae in changing light conditions, and recently it was found that the upregulation of one antenna provides negative feedback to the other [18,19]. Further biogeographic studies using light-harvesting genes have revealed that nutrients and light are important elements for their light-harvesting strategies [20].

The C3 formyl substitution in Chl *d* causes the maximum absorption Q_y band to shift to a longer wavelength, from 665 nm for Chl *a* to 696 nm for Chl *d* in methanol (Figure 3). In Chl *b*, a major accessory light-absorbing pigment of eukaryotic photosynthetic organisms, the C7 position contains a formyl group, replacing the methyl group of Chl *a* at that position (Figure 2). This difference results in the blue-shifted shift of the maximum absorption Q_y band to a shorter wavelength (Figure 3). Thus, the presence of the formyl group shifts the absorption spectrum to the red when it is positioned near the Q_y molecular axis and toward the blue when it is positioned near the Q_x molecular axis. The introduction of the electronegative formyl group along different molecular axes changes the electron density distribution of the tetrapyrrole macrocycle [21–23]. The C7 formyl group in Chl *b* is an electron withdrawing substituent, and this shifts electron density along the x-axis toward the periphery of the macrocycle, causing a blue shift of the Q_y absorption band compared with Chl *a*. Because of this absorbance blue shift and its metal ligation properties, Chl *b* can only function as an

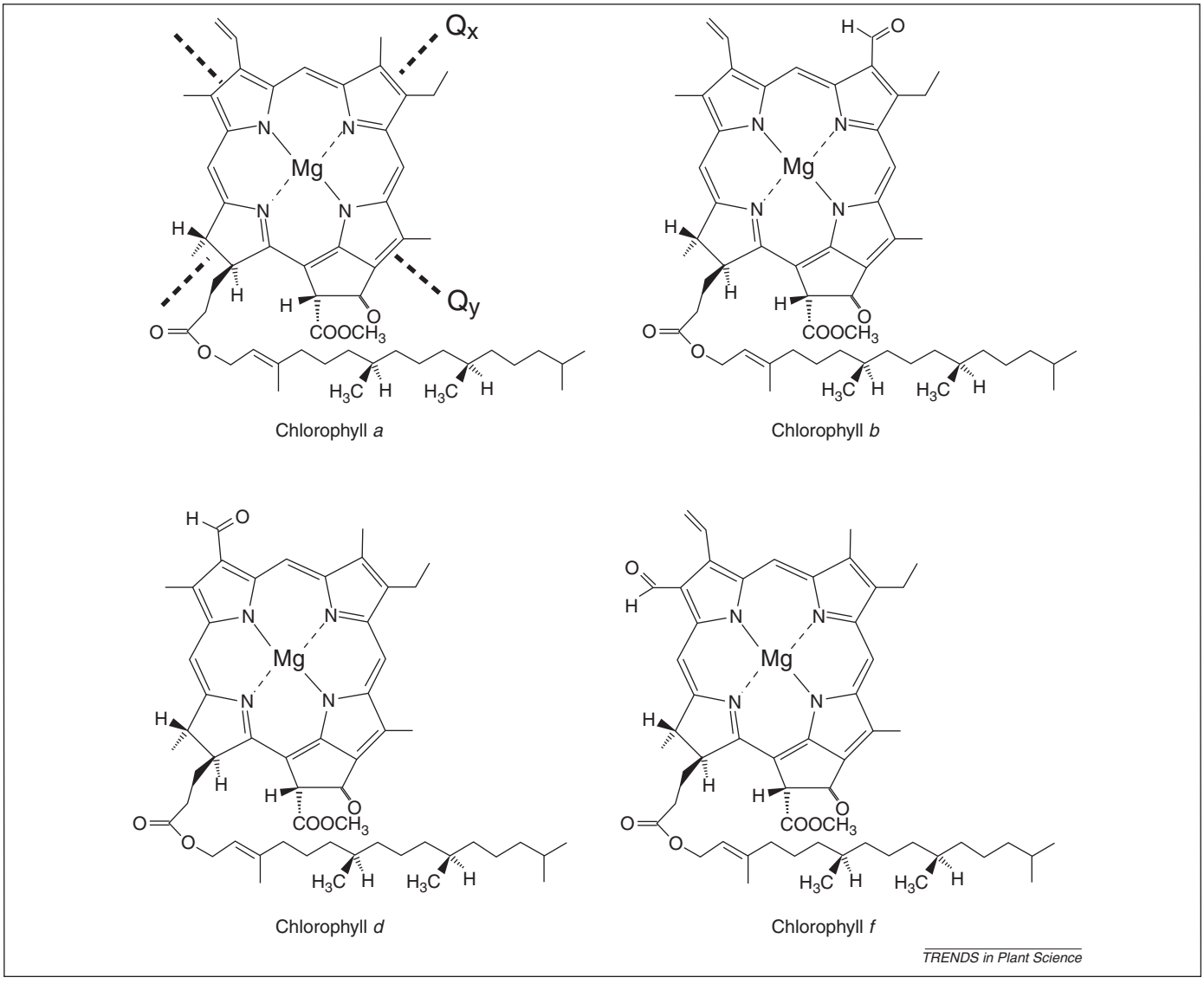

Figure 2. Chemical structures of Chl *a, b d* and *f*. The x and y molecular axes are shown for Chl *a* but are the same for the other pigments.

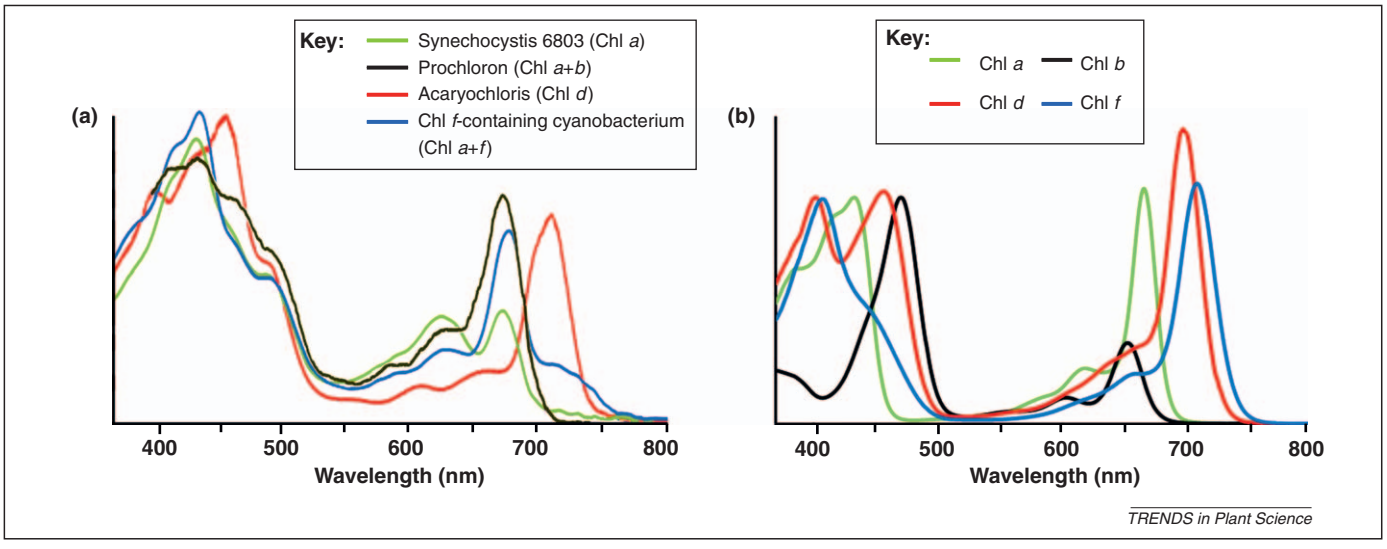

Figure 3. **(a)** *In vivo* absorption spectra of *Synechocystis* PCC6803, *Acaryochloris marina* and the newly discovered Chl *f*-containing cyanobacterium. **(b)** *In vitro* absorption spectra of Chl *a, b d* and *f* in methanol. Spectra have been normalized at their Soret bands.

antenna pigment [23,24]. By contrast, the C3 formyl group in Chl *d* is electron withdrawing along the y-axis of the macrocycle and this causes a red shift of the absorbance spectrum and similar metal ligation properties as Chl *a* [21,24]. Consequently, Chl *d* can effectively substitute for Chl *a* in most applications [21].

Discovery and properties of chlorophyll *f*

Chlorophyll *f* is the most red-shifted chlorophyll thus far isolated from an oxygenic photosynthetic organism [6]. Stromatolite samples collected from Western Australia were incubated under infrared light for the initial purpose of the isolation of new Chl *d*-containing phototrophs, but analysis revealed an even more red-shifted pigment that was named Chl *f* (a pigment called Chl *e* was reported in the 1940 s but this has not been structurally characterized and its status remains uncertain) [6]. A formyl group substitution at the C2 position in Chl *f* (Figure 2), near the Q_y molecular axis, is responsible for the most red-shifted Q_y absorbing maximum, namely 706 nm in methanol (Figure 3). The newly discovered Chl *f* was isolated from an uncharacterized cyanobacterium, which contains mainly Chl *a*. The available evidence thus suggests that Chl *f* is not the major photopigment (only approximately 10–15% total chlorophylls), but functions as an accessory chlorophyll. This is different from the role of Chl *d* in *Acaryochloris*, where it is the predominant Chl and has replaced all (or nearly all) the functions of Chl *a* in photosynthetic reactions, including PSI, PSII and light-harvesting protein complexes [14,15,25,26]. The *in vivo* absorbance spectrum of the Chl *f*-containing organism demonstrates that Chl *f* extends the photosynthetic absorbance region up to 750 nm (Figure 3).

Some oxygenic phototrophs that contain 'red chlorophylls' with Q_y absorption maxima up to 760 nm have been known for many years [27–29]. The difference between these pigments and the red-shifted Chls discussed here is that the former is accomplished only using Chl *a*, whose energetic properties are modulated by protein environmental effects. In the pigments emphasized here, the shift is accomplished by the chemical structural modification of Chl *d* and Chl *f* [6,8]. Interestingly, most red chlorophylls are located in light-harvesting complexes, and the function of those red chlorophylls in energy storage is under debate. Uphill energy transfer is needed to deliver the excitation to the reaction centers, which have the normal absorbance spectra that are well to the blue of these pigments. This might significantly reduce the efficiency of energy storage. In the case of Chl *d*, the reaction center absorption is also shifted to the red so that significant uphill energy transfer is not required. Whether the same is true for Chl *f* is not yet known.

Formation of red-shifted chlorophylls and their evolutionary significance

Chl *d* is synthesized by the oxidation of the C3 vinyl group Chl *a* to a C3 formyl group in Chl *d* [30]. Based on the similarity of the position of the C3 formyl group in Chl *d* to the C3 acetyl group in BChl *a* and the red-shifted absorption properties, it was suggested that Chl *d* could be an evolutionary intermediate between BChl *a* and Chl *a* [31,32]. An ^{18}O labeling experiment revealed that the oxygen in the C3 formyl group comes from molecular oxygen (O_2) [30]. Chl *d* is synthesized via an oxygenase-type reaction mechanism. The enzyme responsible for the conversion of Chl *a* to Chl *d* has been identified as a P450 oxygenase [33], although the details of the mechanism have not yet been worked out.

The evolutionary origin of Chl *d* is still debated. Proposals are that Chl *d* is an intermediate between Bchl *a* and Chl *a* during the transition from anoxygenic to oxygenic photosynthesis [31,32] or that it has evolved more recently during the adaptation of *Acaryochloris* to its specific niche. The former theory is supported by its intermediate absorption spectrum and redox potential as well as the primitive nature of the phycobiliproteins of *Acaryochloris*. However, the latter idea seems more probable given the strong selection pressures evident in the environment where Chl *d*-containing organisms have been found and also from the position of *Acaryochloris* in phylogenetic trees constructed using either individual genes or the complete genome [34,35].

Five types of Chls have been identified to date. Chl *c* shows a different structure from the other four types of Chls because it lacks a phytol chain and a single bond between C17 and C18, which makes it a porphyrin rather than a chlorin, as are all other chlorophylls [36]. The common feature of Chl *b*, Chl *d* and Chl *f* can be seen to be their formyl group substitution (they are probably all derived from Chl *a*), although they are substituted at different positions (Figure 2). The formation of the formyl group in Chls might reflect the evolutionary pathway of Chl development [37]. Molecular oxygen is the essential substrate for the substitution of the formyl group in Chl *b* and Chl *d*, although they are formed by different reaction mechanisms and enzymes. Chlorophyll *a* oxygenase (CAO) contains a Rieske center for Chl *b* synthesis [38], and P450 oxygenase is a key enzyme for Chl *d* synthesis [30,33]. Both of these enzymes require O_2 as a substrate so it is unlikely that they appeared before the Earth became aerobic. The aerobic pathways for the formation of Chl *b* and Chl *d* suggest that they evolved after the atmosphere of the Earth became oxygenated approximately 2.4 billion years ago. Chl *f* also possesses a formyl group at the C2 position, where a methyl group is found in Chl *a*. If it is synthesized from Chl *a* as seems probable, it might use a similar mechanism to CAO, which also catalyzes the conversion of a methyl group to a formyl group at the C7 position. The possibility of a chemical reaction catalyzed by a CAO-type enzyme might be hindered by strong influences from its neighbor substituent, the vinyl group at C3 in Chl *a*, which remains in Chl *f*. Further work on the mechanism of Chl *f* synthesis will be facilitated by the genome sequencing of this organism, which is currently underway.

Concluding remarks

The discovery of Chl *d* and Chl *f* in oxygenic photosynthetic organisms has suggested that they might be able to be introduced into algae or higher plants and permit them to utilize the 700–750 nm spectral region that no known eukaryotic photosynthetic organism can use. As discussed above, this would give access to 19% additional photon flux compared with standard PAR. Although preliminary evidence suggests that the efficiency of photosynthetic energy

storage in *Acaryochloris* is as high as it is in Chl *a*-containing cyanobacteria [7], it is not yet clear whether the same will be true for transgenic organisms, in which the ability to make Chl *d* has been inserted via genetic engineering. This is an important area for further research.

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