



Exploiting algal mineralization for nanotechnology: bringing coccoliths to the fore

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Complex mineral structures are produced by many microalgal species. Pioneering work on diatom silica has demonstrated the potential of such structures in nanotechnology. The calcified scales of coccolithophores (coccoliths) have received less attention, but the large diversity of architectures make coccoliths attractive as parts for nano-devices. Currently coccolith calcite can be modified by the incorporation of metal ions or adsorption of enzymes to the surface, but genetic modification of coccolithophores may permit the production of coccoliths with customized architectures and surface properties. Further work on the laboratory cultivation of diverse species, the physicochemical properties of coccoliths and on genetic tools for coccolithophores will be necessary to realize the full potential of coccoliths in nanotechnology.

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Introduction

Unicellular algae have been much lauded as environmentally friendly production systems for organic molecules including lipids for use in biofuels, therapeutic proteins and bioactive small molecules [1]. In contrast, much less attention has been paid to the biotechnological potential of inorganic minerals produced by algae. This is surprising as intricate mineralized structures with complex 3D architectures and nanoscale patterns are prominent features of many groups within the Ochrophyta (Figure 1). For instance, members of the Synurophyceae, Chrysophyceae, and the Palmales produce silica scales, while the Dictyochophyceae (Silicoflagellates) and Bacillariophyceae (diatoms) produce complex silica (SiO₂) skeletons [2]. Within the Haptophyta, coccolithophorid algae produce intricate scales made of calcite (CaCO₃) called coccoliths [3**].

Diatom silica and coccolithophore calcite are available in abundance in diatomite and chalk deposits respectively and so have found a range of uses including silica supports for catalysis and calcite filler in paint formulations. The use of algal biominerals in nanotechnology however is a much more recent development. Work in this field has focused on diatom silica, which has a large surface area and is readily functionalized, leading to potential applications as an enzyme immobilisation substrate, as optoelectronic components and drug delivery vehicles. Some excellent reviews have been published summarising the work on diatom silica, to which we refer the reader for further information [4–8].

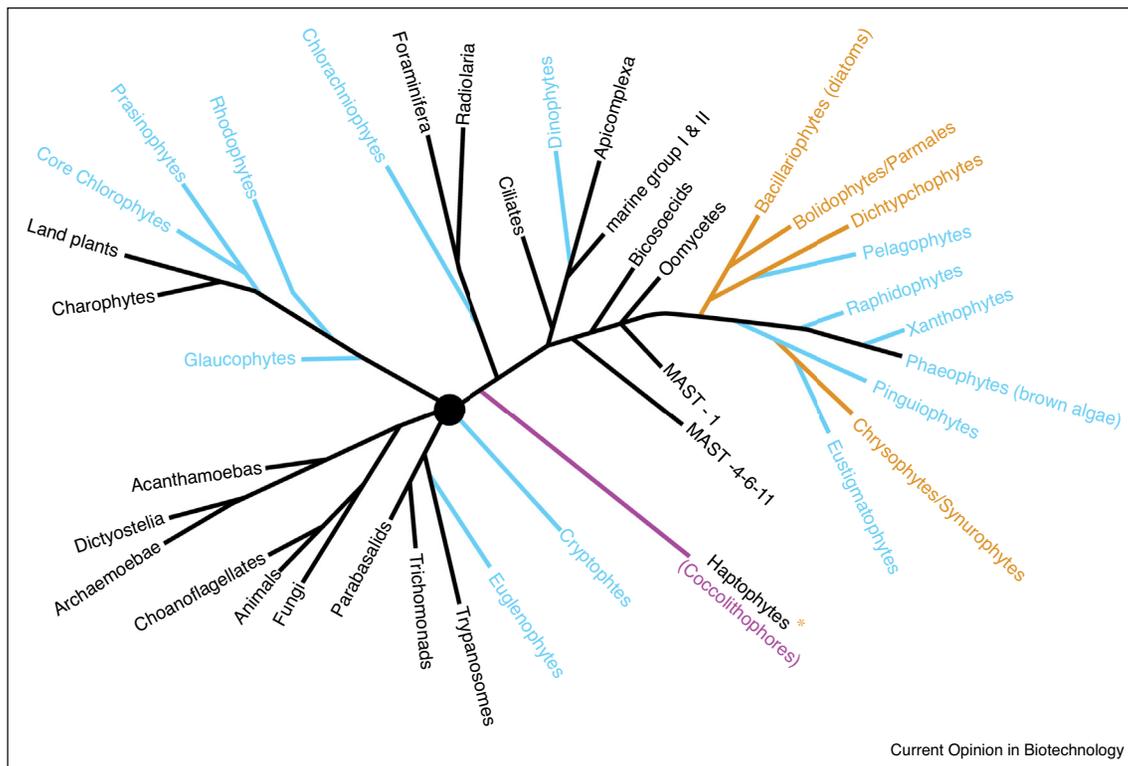
The large diversity of coccolith architectures found in nature (Figure 2), and the fact that synthetic minerals of similar complexity cannot yet be reproducibly fabricated at this scale, makes them attractive for various applications in nanotechnology. A key advantage of at least some coccolithophore species is that coccoliths are not essential for cell growth and survival in culture, as is evidenced by the many strains that have lost the ability to calcify [3**,9]. Thus, the bioengineer wishing to manipulate coccolith form will be less constrained than when working with diatom silica which appears to be essential for cell survival. The flexibility of the calcification machinery is well illustrated by members of the genus *Scyphosphaera* which produce two distinct coccolith morphologies on the same cell (Figure 2d) [10,11].

This review will focus on the potential of coccolithophores as a source of mineralized materials for nanotechnology. After a brief summary of the process of coccolithogenesis we will discuss the properties of coccoliths which make them suitable as components in nano-devices and micro-devices. We will then outline methods by which coccoliths could be modified and functionalized and discuss the practicalities of coccolith production before highlighting the challenges and opportunities ahead.

Coccolithogenesis

There are two main types of coccolith. Heterococcoliths are produced by diploid cells, and are made of calcite crystals with complex shapes (Figure 2h, inset). The calcite crystals nucleate intracellularly on an organic baseplate within a specialised vesicle, in which they develop to their final morphologies before being secreted to the cell surface and incorporated in the shell of coccoliths surrounding the cells, called the coccosphere [10]. The coccoliths are loosely connected to the cell surface

Figure 1



Schematic showing the phylogenetic relationships of phytoplanktonic (colored) and other eukaryotic taxa (black). Phytoplankton groups producing silica based biominerals are colored in orange and those producing calcium carbonate in violet. The Haptophytes include calcifying species (coccolithophores), a silicifying species (*) [12], and non-mineralizing species. The phylogenetic tree was adapted from [13] with permission.

and are probably held together by sticky macromolecules and, in some species, through interlocking of the coccoliths [14,15]. In many species it has been found that coccoliths are coated with an acidic polysaccharide which may be involved in calcium delivery [16,17] or crystal growth and morphogenesis [18] and which partially protects the coccoliths from dissolution [19].

The second type of coccolith are produced by the haploid phase of some, but not all, coccolithophore species and are known as holococcoliths. The crystals comprising holococcoliths are typically smaller than those in heterococcoliths and have simple shapes (Figure 2c). They appear to be formed extracellularly, but within an outer organic layer [20]. For both holo- and heterococcoliths, the molecular detail of how crystal nucleation and growth is so tightly controlled remains elusive.

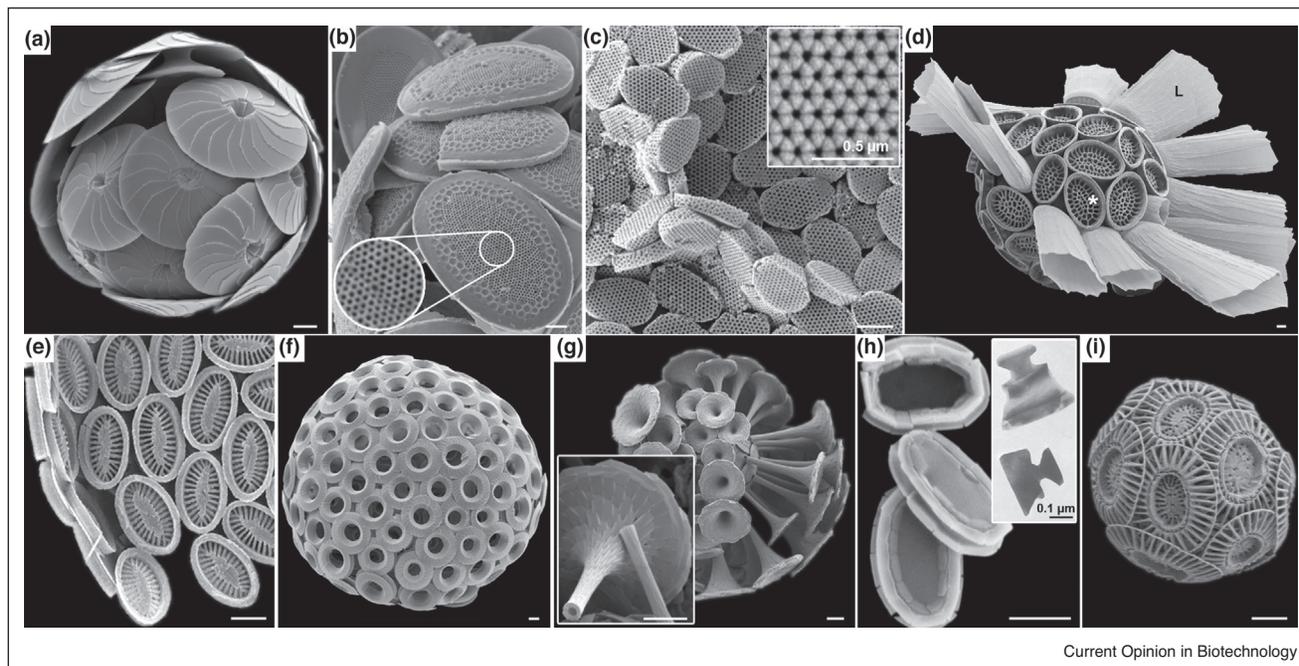
Coccoliths as parts for micro-devices and nano-devices

Devices constructed on sub-micron scales are expected to be a transforming technology in a wide range of fields, from environmental sensing to advanced electronics, medical diagnostics and drug delivery. In the last decade substantial progress has been made in the development of

nanoactuators, which can power the movement of objects at this scale [21–23]. However, inexpensive and flexible methods to generate parts of micro-devices and nano-devices in a high throughput manner are still lacking. For example, the fabrication of nanopores is often carried out by expensive and time-consuming focused ion beam milling [24].

One field in which coccoliths have clear potential is nanofluidics. When nanoscale pores and channels interact with a fluid, physical phenomena which do not apply at the microscale become relevant. For example, the surface charge of a pore can govern the transport of selected ions and asymmetric pore geometries and surface charges can lead to a net flow of certain ions in one direction (ion current rectification) [24]. Coccoliths with hollow funnel and tubular shapes such as those of *Discosphaera tubifera* (Figure 2g) and *Rhabdosphaera clavifera* [25] seem to be particularly relevant to this area, as do coccoliths that have nanoscale pores or slits such as those of *Pontosphaera japonica*, *Michaelsarsia elegans* or the holococcoliths of *Calyptrolithophora papilifera* (for pore sizes and coccolith dimensions see [3•, 11, 26, 27]) (Figure 2d, c, e). The high pore density of the latter may also allow efficient encapsulation of selected molecules by suitable control of pore

Figure 2



Electron micrographs showing the coccoliths of the extant coccolithophores (a) *Calcidiscus leptoporus* subsp. *leptoporus*, (b) *Pontosphaera japonica*, (c) *Calyptrolithophora papillifera*, (d) *Scyphosphaera porosa*, (e) *Michaelsarsia elegans*, (f) *Umbilicosphaera sibogae*, (g) *Discosphaera tubifera*, (h) *Pleurochrysis carterae*, and (i) *Emiliana huxleyi*. Panel c shows holococcoliths whereas the other panels show heterococcoliths. Inset in c shows the hexagonal array packing of the simple-shaped crystallites in these holococcoliths. *S. porosa* (d) produces dimorphic coccospheres with vase-like 'lapodoliths' (L) and oval casserole-like body coccoliths (*). Inset in g shows the narrow end of the trumpet-like spine. The spine is hollow. Inset in h shows high-magnification image of the complex-shaped calcite crystals these coccoliths are composed of (reused from [37] with permission). Images a–g are a courtesy of Jeremy Young, University College London, London, UK, and adapted from <http://www.mikrotax.org/Nannotax3/>. Scale bars: 1 μm .

chemistry. The fact that coccolith calcite can be stable, even in solutions highly undersaturated with respect to calcite [19], combined with the inducible dissolution of the calcite by modulation of pH, could be utilised in drug delivery strategies.

The micro-scale features of coccoliths may also have applications. For example the one to two micron diameter eyelets of *Umbilicosphaera sibogae* [3**] could be used for filtration if arrayed in a suitable support, while the cable-reel type coccoliths of species such as *Emiliana huxleyi* could conceivably be used to house low-nanoscale fibers, such as the chitin fibrils produced by diatoms [28], allowing them to be reeled out when required. Entire coccospheres may also have applications as support structures within larger devices, since the coccospheres of *E. huxleyi* have been shown to have an excellent strength to weight ratio and damage tolerance [29].

Coccoliths have potential in applications relying on manipulation of the properties of light. Calcite itself is birefringent and can be used as a polarizer. Other optical properties of coccoliths may emerge from their nano and micro-scale structures. For example it is conceivable that

the somewhat lens shaped coccoliths of *Calcidiscus leptoporus* (Figure 2a) may focus light. The tight control that coccolithophores display over of the crystallographic axes of calcite [30**] is an advantage in that the optical properties of coccoliths can be expected to be reproducible between individual coccoliths. Since arrays of nanopores in diatom silica have been previously shown to effectively focus a laser beam and to have photon trapping properties [31,32], it is possible that coccoliths with nanopore and slit patterns, such as those of *M. elegans*, *Scyphosphaera porosa* and *P. japonica* (Figure 2e, d, b), may have similar properties. However, the best studied optical property of coccoliths is light-scattering. In one study, *E. huxleyi* coccolith orientations were controlled in a strong magnetic field [33] and thereby demonstrated to have a high degree of light scattering anisotropy. Coccolith morphology and calcification state have also been shown to modulate their scattering and birefringent properties, in both experimental [34,35*] and theoretical [36] studies.

Modifying and functionalizing coccoliths

The range of potential applications of coccoliths could be vastly expanded by looking beyond the natural diversity

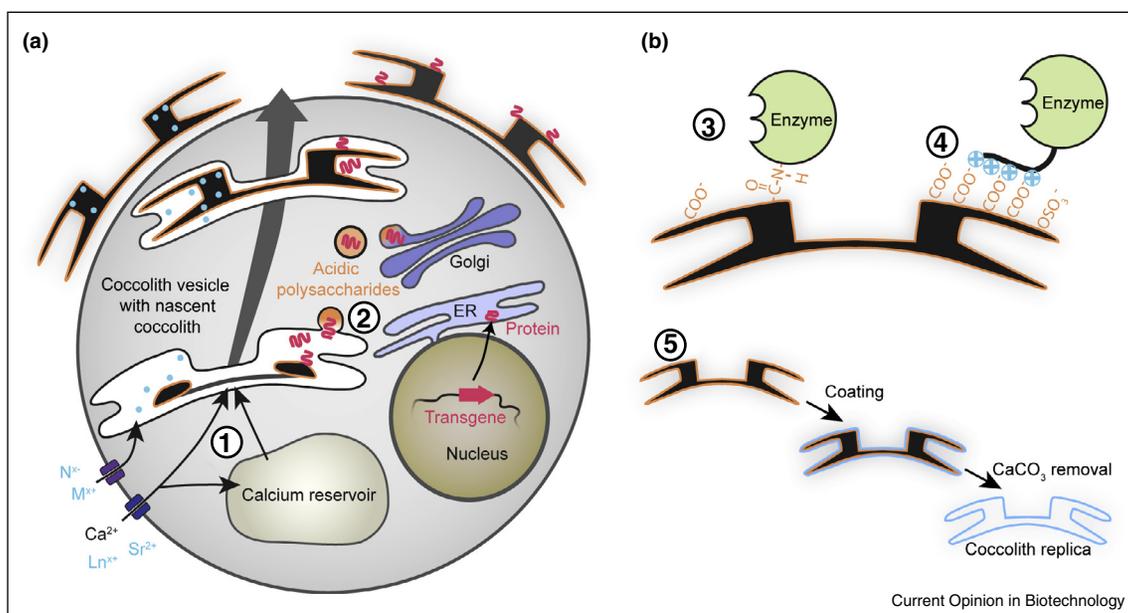
of coccoliths. Our understanding of the pathways and mechanisms of coccolith synthesis is becoming increasingly sophisticated [16,38*,39*], and genetic tools [40*] along with genomic and transcriptomic sequences [41,42] are beginning to become available for some coccolithophores, so we are approaching the stage where the manipulation of coccolith morphology and the design of custom architectures is imaginable.

A good understanding of the calcification process is a prerequisite for the manipulation of coccolith shapes via targeted genetic modification, but untargeted mutagenesis approaches may also allow us to alter coccolith morphologies with substantially less knowledge of the process. Marsh and co-workers have successfully isolated chemically mutagenized clones of *Pleurochrysis carterae* which produce coccoliths which are less mineralized than those of wildtype cells [18]. One challenge for mutagenesis approaches is that screening for strains with altered morphologies using electron microscopy is very laborious. A further challenge is that heterococcolith-producing

cells are diploid and the likelihood that both copies of a gene are simultaneously mutagenized is very small [43]. The absence of reliable methods to induce meiosis and syngamy in coccolithophores precludes the use of classical genetic techniques to generate homozygous strains. Holococcolith morphology may be more susceptible to mutagenesis approaches as holococcolith-producing cells are haploid.

The modification of coccoliths, with the aim of modulating their properties, could be carried out *in vivo* or *in vitro* (Figure 3). The most straightforward method of *in vivo* modification is the incorporation of metal ions supplied via the growth medium into coccoliths. The calcium transport pathway from the external media to coccolith vesicles is to some extent leaky to other ions, meaning that Sr, Ba, B, and Zn present in the media can also be incorporated into coccolith calcite [44,45*,46], which may then affect the properties of the coccoliths. The incorporation of lanthanides, some of which have a similar ionic radius to Ca^{2+} , into the lattice of inorganic calcite has been

Figure 3



Strategies for the functionalization of coccoliths. **(a)** *In vivo* functionalization of coccoliths. (1) Through manipulation of the elemental composition of the growth medium, cations (M^{x+}) and anions (N^{x-}) of interest may become incorporated into coccolith calcite. This approach was successfully used for producing Zn-doped and Sr-doped coccolith calcite [38*,45*]. Traces of barium and borate can be also incorporated into coccolith calcite [44,46]. A similar approach is likely to work with cations transported through membranes by the same transport proteins as Ca^{2+} , such as rare earth metals (Ln^{3+}), if non-toxic concentrations are used. After uptake into the cell, the ions may either be transported directly into the coccolith vesicle or first into a storage compartment, such as the recently discovered compartment that serves as calcium reservoir in coccolith calcite formation [39*], and from there into the coccolith vesicle [38*]. (2) Genetic manipulation of coccolithophores is expected to allow the incorporation of proteins, e.g. enzymes, into coccoliths. Macromolecules have been found occluded within coccolith calcite [55,56]. The proteins destined for coccolith incorporation may be delivered into the coccolith vesicle together with the acidic polysaccharides known to be tightly associated with coccolith calcite. **(b)** *In vitro* functionalization of coccoliths. Taking advantage of the acidic polysaccharide coat of coccoliths, enzymes or antibodies could be either (3) covalently attached using established crosslinking approaches, or (4) electrostatically adsorbed to the coccolith surface. The latter approach has already been used for the immobilisation of uricase and glucose oxidase on coccoliths [49]. (5) CaCO_3 free replicas of coccoliths may be produced by depositing other materials onto their surface by vapor or liquid phase techniques. The deposited material may either react with the calcite to a new mineral phase or form a non-reactive layer that retains the shape of the coccolith after dissolution of the CaCO_3 [52–54].

demonstrated [47] so it may be possible to endow coccoliths with useful luminescent properties if lanthanides are provided to coccolithophores and toxicity is not severe.

Coccoliths have high specific surface areas ($19 \text{ m}^2 \text{ g}^{-1}$ in *E. huxleyi* [48]) for adsorption or attachment of other molecules. For example, enzyme immobilisation to coccoliths or specific sites on coccoliths may also broaden the range of their nanotechnological application. The charged nature of calcite surfaces and the coccolith associated polysaccharides mean that some enzymes will adsorb electrostatically to coccoliths. This could be achieved *in vivo*, via expression of a transgene whose product is targeted to the coccolith vesicle, or *in vitro* by incubation of the coccolith with the protein of interest. The former approach may result in concomitant modulation of crystal morphology, whereas the latter should not. One study found that uricase and glucose oxidase could be adsorbed to coccolith calcite to a greater degree than onto synthetic calcite of the same mass [49], a fact that the authors attributed the higher specific surface area of the coccolith calcite. The use of peptide tags that have high affinity to the coccolith associated polysaccharide or to particular calcite facets may allow targeted and high affinity attachment of enzymes to coccolith calcite.

Calcite has properties that may be undesirable for many applications, such as dissolution at low pH, instability at high temperatures and brittleness. Thus it would be useful to be able to ‘convert’ coccolith calcite to other minerals with different properties. This type of process has been achieved with diatom silica, which has been chemically converted to silicon/MgO composites that maintain the structure of the diatom cell wall [50**]. Analogous approaches may be possible with coccoliths since the chemical conversion of calcium carbonate to CaTiO_3 , a promising material in microwave communication technologies, has been accomplished [51]. However, it remains to be seen if coccolith morphologies could be preserved through such processes. The potential of solid state reactions in which a metal oxide is deposited on the calcite surface and reacts with the calcite [52] should be further explored. Another approach would be to use coccoliths as sacrificial templates, whereby a new material is deposited on the calcite before the calcite is dissolved, leaving a new material with a morphology dependent on the template. This approach has already been used with calcium carbonate rods to produce hollow tubes of titania [53] or silica [54].

Coccolith production for nanotechnology

Rates of coccolith production vary between species, but can be as fast as one coccolith $\text{cell}^{-1} \text{ hour}^{-1}$ [57]. Cell densities in laboratory cultures can easily reach more than $1 \times 10^6 \text{ cells ml}^{-1}$, so a relatively small culture volume can be used to produce millions of individual coccolith-based parts for nanotechnology per day.

Methods that allow coccoliths to be removed without disrupting the cells will much simplify coccolith harvesting and purification. Indeed, a continuous coccolith harvesting system has been developed for *P. carterae*, in which optimised bubbling of the culture causes coccoliths to detach from the cell surface which then sediment through a nylon mesh too fine for the cells to pass through [15]. This system has the added advantage that it stimulates *P. carterae* cells to make more coccoliths, since this species otherwise stops making coccoliths when its coccosphere is complete [58]. Alternatives to bubbling for coccolith removal include osmotic shock, which induces *Pleurochrysis* species to shed their coccospheres [9,16]. If an application were to require significant quantities of coccoliths, then cheap, large scale culture systems would be required. *P. carterae* has been successfully grown in open outdoor raceway ponds, with calcium carbonate yields of $5.5 \text{ tonnes ha}^{-1} \text{ year}^{-1}$ [59].

Challenges and perspectives

The potential of coccoliths in advanced applications is clear, but the challenges remain substantial. To make use of the natural diversity in coccolith morphology, cultivation systems must be developed for more species, as presently only a limited number of species can be grown to good densities in the laboratory [60]. In some cases this may require more knowledge of biotic interactions in the oceans and the development of co-culture systems with other organisms. Further characterisation of the properties of coccoliths of different species will also be necessary in order to present a ‘tool box’ of well understood coccolith parts to nanotechnologists. In particular, detailed studies on the mechanical and physiochemical properties of coccoliths are currently lacking for almost all species, with *E. huxleyi* being the only exception [29,48].

The development of methods for the orientation-specific assembly of coccoliths into arrays may also lead to broader range of applications. An example for the extension of the application range is given by microlenses which in arrays are used in directional displays and signal-routing connectors [61]. Finally, to realise the full potential of coccoliths in nanotechnology robust methods for the genetic manipulation of coccolithophores must be developed. This will allow the *in vivo* exploration of hypotheses relating to the pathways and mechanisms of calcification and enable the synthesis of the first ‘custom coccoliths’.

Conflicts of interest

None.

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