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INVITED REVIEW

Diatom Frustule Morphogenesis and Function: a Multidisciplinary Survey

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Abstract

Diatoms represent the major component of phytoplankton and are responsible for about 20-25% of global primary production. Hundreds of millions of years of evolution led to tens of thousands of species differing in dimensions and morphologies. In particular, diatom porous silica cell walls, the frustules, are characterized by an extraordinary, species-specific diversity.

It is of great interest, among the marine biologists and geneticists community, to shed light on the origin and evolutionary advantage of this variability of dimensions, geometries and pore distributions.

In the present article all the main results related to frustule morphogenesis and functionalities with contributions from fundamental biology, genetics, mathematics, geometry and physics are reviewed.

Keywords

Diatoms; frustule; genomics; Gielis superformula; biophysics.

1. INTRODUCTION

"Few objects are more beautiful than the minute siliceous cases of diatoms: were they only created to be admired under the microscope?" [Darwin, Charles. On the Origin of Species by Means of Natural Selection. J. Murray, London, 1859]

In the latter half of the nineteenth century, German naturalist Ernst Haeckel proposed a kingdom, called Protista, composed of eukaryotic organisms that are not animals, plants nor fungi and proposed a link between ontogeny (development of form) and phylogeny (evolutionary descent). Furthermore, he developed the theory of non-random form, which culminated in the beautifully illustrated Kunstformen der Natur (Art forms of nature) (Niklas and Kutschera, 2016).

Among the classes belonging to Protista, he described also diatoms, phytoplanktonic unicellular organisms included in the Stramenopila group, which are part of the supergroup Chromalveolates, a completely separate evolutionary lineage from land plants (Katz, 2012). In the Cretaceous, around 100 million years ago, diatoms began to become widespread and developed great diversity (Gross, 2012). They are indeed one of the most diverse groups of eukaryotes (Kooistra and Medlin, 1996) and estimates suggest that there are well over 250 genera and about 10^5 marine and freshwater species (Mann and Vanormelingen 2013). These microalgae have the ability to generate a highly ornamented, fanciful and elegant porous silica cell wall, known as the frustule. These cell walls exhibit an amazing diversity of species-specific shapes and pore patterns, which made diatoms very popular organisms for microscopist community in the Nineteenth century. The intricate structure of frustules was used, indeed, to assess the quality of microscope optics (Round et al., 1990). Even nowadays, high magnification images of diatom walls continue to amaze with their huge variety of micro and nano-structures (Volcani et al. 1981) (Fig. 1). In addition, the silica cell walls produced by diatoms give these single-celled algae a distinct and influential role in the ecology and biogeochemistry of the oceans. Diatom silicification links the marine carbon and silicon cycles: they are among the most productive organisms on earth, responsible for an estimated 20% of global primary production, and a corresponding 240 Tmol of annual biogenic silica precipitation (Falkowski and Raven, 2007).

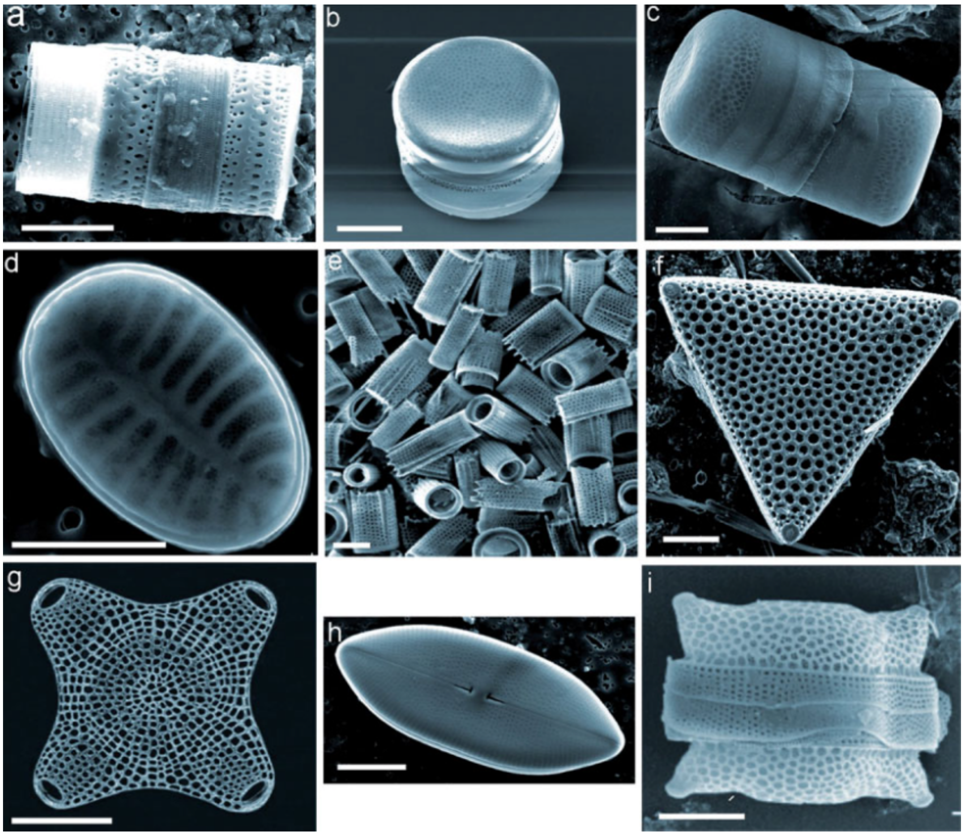

Fig. 1. Diatom shape diversity. Scanning Electron Microscopy (SEM) images of diatom frustules (a-d; f-i) and fossilized diatom biosilica (e). Scale bar: 10 μm. Reproduced with permission from Losic et al., 2007.

A first distinction among diatoms traditionally starts from the symmetry of their frustules: *Centric* or *Centrales* diatoms are characterized by a frustule with radial symmetry, and are typically planktonic. *Pennates* diatoms have bilaterally symmetric frustules and are mainly found in benthic and epipelic communities (Round et al., 1990). In both cases, diatom frustules resemble a petri-dish-like silica box, with a *hypotheca* inserted in a slightly bigger *epitheca*, enclosing the living cell (Fig.2). The size of the frustule is species-dependent, and varies from a few microns to millimeters. Both the *hypotheca* and the *epitheca* can be viewed as a valve surrounded by a lateral girdle. Valves are made of several layers (*foramen*, *cribrum* and *cribellum*), each provided with more or less regular patterns of pores, whose dimension (from micron down to nanometer scale) and spatial distribution is species- and layer-specific.

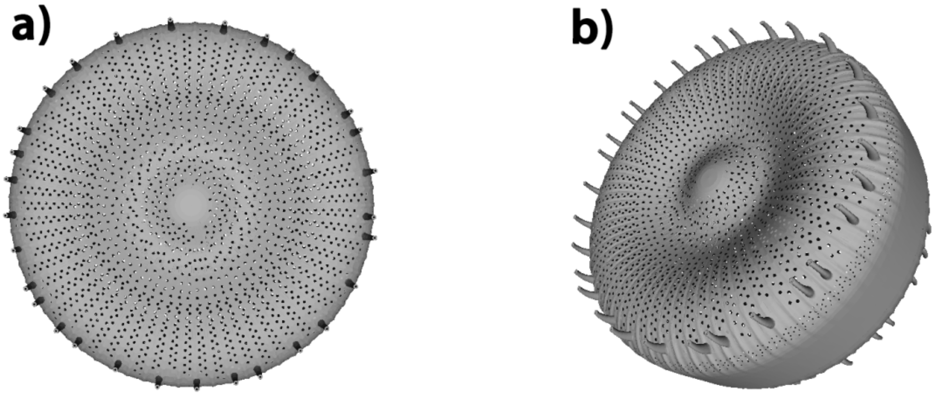

Fig. 2. Schematic front **(a)** and angled **(b)** view of a CAD replica representing a generic *Stephanodiscus* diatom frustule, provided with characteristic spines surrounding the valve.

Diatom frustules are basically composed by hydrated, porous, amorphous silica provided with several surface defects such as Si-OH (silanol) and Si-H groups (Qin et al., 2008). An accurate analysis based on Raman and FTIR spectroscopies on frustules after removal of the organic content allowed to detect also signals coming from organic residuals incorporated in the porous matrix (mainly C-H bonds) and sporadic, localized signals from sulfur composites (C-S and S-H bonds) (Kammer et al, 2010; De Tommasi, 2016). The presence of sulfur residuals may be related to the global sulfur cycle, in which the role played by phytoplankton is of fundamental importance, mainly through the release of dimethylsulfide (DMS) in the atmosphere (Simó, 2001). Furthermore, frustules are surrounded by extracellular polymers, mainly polysaccharides, whose functions comprise, among others, sessile adhesion, gliding, protection against drying, and formation of biofilms and colonies (Svetličić et al., 2013).

Through the years, applications of diatom frustules have gone far beyond microscope quality testing. Beside the use of fossilized diatom biosilica (the so called *diatomite* or *diatomaceous earth*) in toothpaste, facial scrubs and water filtration, recently the physical properties of frustules found applications in several fields of micro-, nano- and bio-technology. For example, frustules of centric diatoms have been successfully used as microlenses (De Stefano et al., 2007; De Tommasi et al., 2010), being able also to squeeze light under diffraction-limit (De Tommasi et al., 2014); their ability to collect light with high efficiency can lead to the development of new generations of bio-based and bio-inspired solar cells (Toster et al., 2013; Wang et al., 2013); metalized frustules have been successfully used as nano-structured substrates in plasmonics (Payne et al., 2005; Ren et al., 2014; Kwon et al., 2014); photoluminescence of frustules has been exploited in the realization of optical sensors and biosensors (De Stefano et al., 2005; Gale et al., 2009); functionalized diatomite nanoparticles can be used as vectors in drug-delivery (Terracciano et al., 2015; Delalat et al., 2015); diatomite and frustules have been used as scatterers in random lasers (Lamastra et al., 2014). Other interesting applications may come from a proper modification of the frustule: metabolic insertion of germanium (Jeffryes et al., 2008) or titania (Jeffryes et al., 2008 bis) allow to obtain efficient nanostructured semiconducting devices for optoelectronics, enhanced light trappers in dye-sensitized solar cells and structured photocatalysts of toxic chemicals; polymer (Losic et al., 2007 bis), silicon (Bao et al., 2007), or metallic (Fang et al., 2012) frustule replicas can be used as masters for nanofabrications, in sensing and electronic devices and as Enhanced Optical Transmission (EOT) plasmonic elements, respectively. However, the most fascinating potentiality of diatom biosilica, which would lead to the complete control of all the above mentioned applications and even more, relies on the ability to manipulate the genes which rule frustule morphogenesis. Indeed we can imagine to modify frustule morphology, geometry, shape and pore distribution in order to optimize a specific application by mutating the proper genes (Kröger and Poulsen, 2008). Unfortunately the genes involved in silica precipitation, aggregation, spatial rearrangement and relative proteins are still not well characterized.

The diatom genome is a *mélange* of different genes, some resembling plants, others animals or prokaryotes, which arose through successive endosymbioses and horizontal gene transfers from bacteria (Bowler et al., 2008). The genetic and molecular details of frustule morphogenesis have been partially elucidated only in recent years. Accurate knowledge of diatom genome and genetics will help us to understand the processes that drive the construction of the precise structure of frustules.

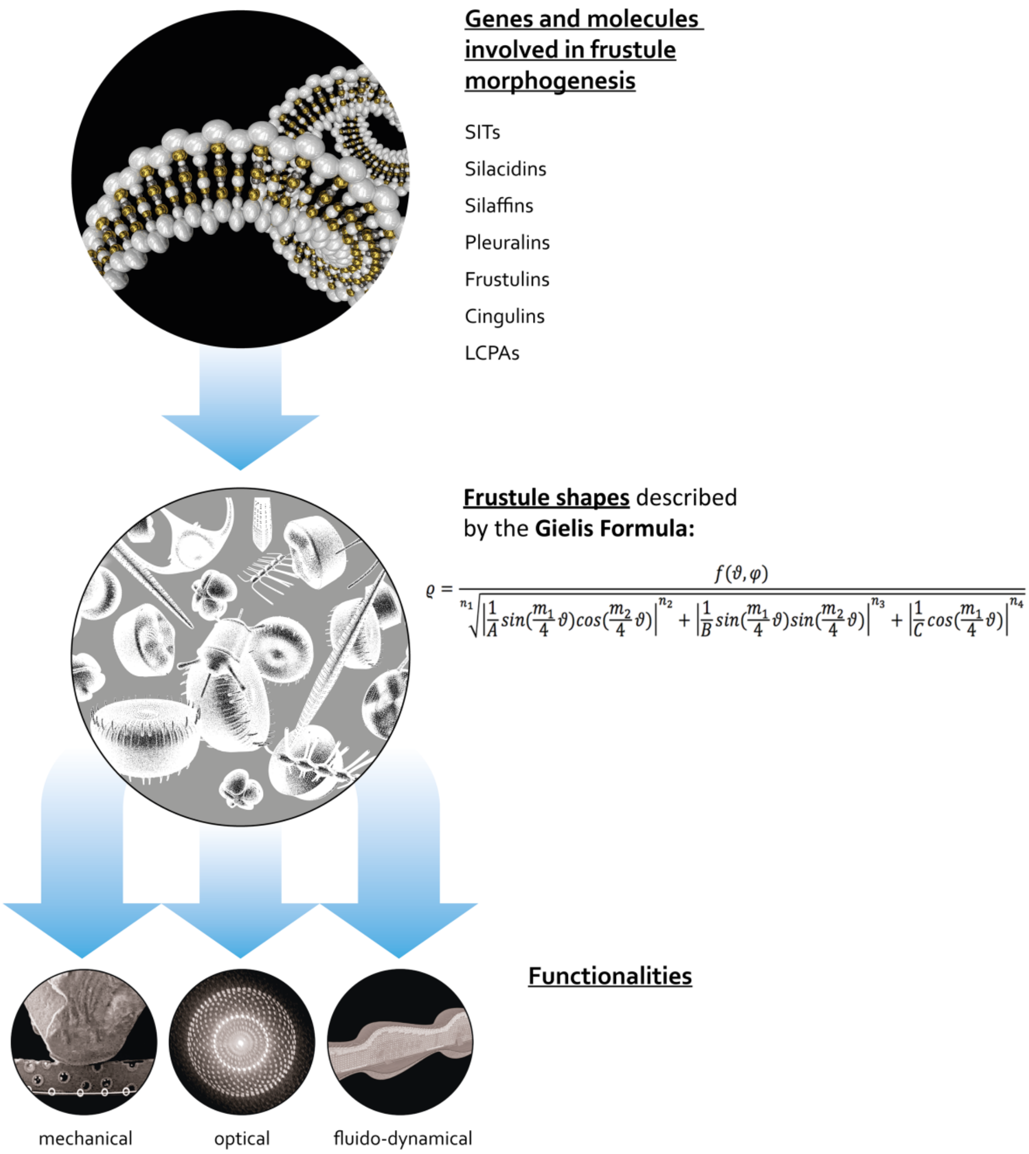

Fig. 3. Schematic representation of the interplay between genes and molecules, geometry and physics, pivotal to understand form, function and development.

In this review we propose a link between morphology and functionalities of diatom frustules through a multidisciplinary approach which is schematized in Fig. 3. First, we will report the available informations of the genes putatively implicated in the machinery aimed at silica precipitation and frustule genesis, with a close examination of the known proteins, polyamines and other macromolecules involved in the process. Secondly, the most recent mathematical techniques for the description of natural shapes when subjected to given constraints will give us the proper instrument to shed light on frustule diversity in shapes and geometries, looking at it both as a whole and at its ultrastructure, down to pore dimensions and arrangement. Finally, the main physical properties of frustules (mechanical, fluid dynamical and optical), which represent the actual link between shape and functionality, will be described.

2. GENES AND MACROMOLECULES INVOLVED IN FRUSTULE MORPHOGENESIS

Silicification in diatoms is a complex process, under strict genetic control. However, although current techniques for genetic manipulation (Apt et al., 1996, Falciatore et al., 1999; Poulsen et al., 2005 ; Kroth et al., 2007; De Riso et al., 2009; Niu et al., 2012; Daboussi et al., 2014; Weyman et al., 2014; Sabatino et al., 2015; Karas et al., 2015 ; Hopes et al., 2016; Rastogi et al., 2016) potentially allow to modulate the expression of the genes involved in biomineralization in order to understand their impact on frustule structure, architecture and functions, to date only few studies have been published on the genetic manipulation of diatom silica forming machinery and none of them allows a functional characterization of proteins regulating cell wall assembly (Knight a et al., 2016; Poulsen and Kröger, 2005; Fischer et al., 1999).

In recent decades, a variety of organic and biological molecules have been successfully separated and identified from cell-wall extracts. Most of the comprehensive information available on genes and molecules involved in frustule formation and morphogenesis comes from studies on few species, in particular *Thalassiosira pseudonana* and *Cylindrotheca fusiformis* (Kröger et al., 1996; Kröger et al., 1999; Kröger and Poulsen 2007; Hildebrand 2008; Kröger and Poulsen 2008; Sumper and Brunner 2008, Matsukizono and Jin, 2012; Lechner and Becker 2015). To date, many genomes from relevant pennate and centric diatom species (e.g.: marine, freshwater, toxic, cold-water, and oleaginous) have been sequenced and several others are in pipeline (Armbrust et al., 2004; Bowler et al., 2008; Lommer et al., 2012; Trainer et al., 2012; Galachyants et al., 2015; Tanaka, et al., 2015; Traller et al., 2016; Basu et al., 2017; Mock et al., 2017). The increased accessibility to whole genome information from other diatom representative species have contributed to further understand the mineralization process (Kröger and Poulsen 2007; Mock et al., 2008; Kröger and Poulsen, 2008; Scheffel et al., 2011; Shrestha et al., 2015; Durkin et al., 2016). Several research papers and reviews have been published presenting the advances in the characterization of the structure and function of diatom cell wall proteins and molecules. The following sections aim to review and discuss the most recent knowledge on these molecules, the state of genetic manipulation to modify frustule biosynthesis, the possible impacts of these manipulations on cell wall and the molecular information generated by other –omics data (transcriptomics, proteomics, metagenomics and metatranscriptomics) (Mock et al., 2008; Allen et al., 2008; Keeling et al., 2014; Muhseen et al., 2015) in order to reveal the role and specificity of these genes and molecules responsible for species shape.

2.1 Silicon transporters (SITs)

The most well characterized genes involved in biomineralization, encode the silicic acid transporters (SITs), which transport silicic acid from seawater into the cell (Durkin et al., 2016; Durkin et al., 2012; Shrestha et al., 2015; Thamatrakoln et al., 2006). Si (primarily in the form of silicic acid, $Si(OH)_4$) is actively taken from outside into the cell by the SITs. In marine species, SITs are sodium-coupled active transporters with specific silicic acid uptake activity, whereas in freshwater species there seems to be sodium and potassium coupled transporters. These proteins were first identified and described by Hildebrand and coworkers (Hildebrand et al., 1997) in the pennate *C. fusiformis,* and subsequently in all species studied so far. Si transporter genes are constituted by several members, with differences in SITs family size and functionality, that play different roles in the uptake of silicic acid with different cellular localizations, Si binding affinities, and transport rates. Each SIT is predicted to contain 10 transmembrane domains (TMDs) and a coiled-coil motif at C-terminus. The mechanism by which SITs recognize and bind silicic acid is still not completely known. Actually, a model has been proposed in which a transport centered around the highly conserved GXQ amino-acid sequence motif is hypothesized, directly involved in $Si(OH)_4$ binding (Hildebrand et al.,1998; Thamatrakoln et al., 2006; Kröger and Poulsen, 2008; Marron et al., 2013; Lechner and Becker., 2015; Knight et al., 2016). From an evolutionary point of view, the SITs genes encoded by diatoms display sequence homology with proteins present in bacteria, most likely acquired through horizontal gene transfer (Schroder et al., 2004; Ma et al., 2006 and 2007).

To better understand the origin and functional divergence of SITs proteins, Durkin and colleagues in 2012 provided a comprehensive sequence-based analysis and more recently, in 2016, reconstructed a more exhaustive evolutionary scenario where SIT genes form five separate clades, called A, B, C, D and E (Durkin et al., 2016), with Clade B at the basal position within the clades . The idea is that SIT B proteins probably arose when the concentration of silicic acid was around 1000 μM, orders of magnitude greater than today, suggesting that SIT B proteins are involved in the passive transport to a specialized vesicle, called Silica Deposition Vesicle (SDV), in high silicic acid environments. Moreover, the clade contains proteins with amino acid residues that undergo post-translational modifications which can affect the function of channel gating. On the contrary, proteins of SITs A, C, D and E clades, are directly involved in the transport of silicic acid from the seawater to the SDV. SITs C and D proteins have been found in all major diatom lineages whereas clades A and E are lineage-specific with Clade A forming a monophyletic cluster, and including only sequences from pennate diatoms. The functional diversificaton and specialization of SIT members might be justified because diatoms, although unicellular organisms, may experience large differences in silicon concentration between surface and deep water, between different oceanic regions and between intracellular compartments (depending on the species). Diatoms developed such efficient, fine and ultrafine structured uptake systems to provide a quick response to the constant and rapid changes that take place in the ocean environment and that enable these organisms to adapt to their environment and thrive (Maldonado et al., 1999; Frings et al., 2016; Bhattacharyya and Volcani 1980; Shrestha et al., 2015; Frings et al., 2016; Durkin et al., 2016; Martin-Jezequel et al., 2000). In order to clarify the specific role played by each gene, fold change of SIT genes transcript studies involving different diatom species have been conducted in different conditions. Recent research indicates that SITs protein expression is closely related to the cell cycle, with a down regulation during cell division and consequent cell wall synthesis. Analysis characterizing the knockdown mutants of the two major SITs in *T. pseudonana* showed that the magnitude of this down regulation is inversely related to the concentration of the silicic acid, suggesting for these transporters also a role in the sensing of silicic acid levels, independently of the transport, that controls cell wall formation and division process (Shrestha et al., 2015). Moreover, what remains still little known, is the intracellular transport of silicic acid. Only recently, thanks to novel advances in molecular tools, Knight and coworkers made a series of targeted mutations in *T. pseudonana* and *P.*

tricornutum SITs to further determine the location and function of the different proposed conserved residues upon protein structure and function. In addition, they developed a new method based on a fluorescent probe for silicic acid that will contribute to understand the mechanisms of silicon transport *in vitro* (Knight a et al., 2016).

With the same purpose, Javeheri and colleagues introduced a mathematical model to describe silicon dynamics in the diatom *T. pseudonana*, obtaining good agreement with experimental data relative to silicon transport dynamics in four compartments: external environment, cytoplasm, SDV and deposited silica (Javaheri et al., 2014). The hypothesis is that the coordinated Si transporter actions underlie a flexible network that mobilizes Si in response to many environmental changes and that these proteins are differentially regulated at various levels. However an additional possibility is also a functional redundancy of these transporters.

A more focused analysis on the reported profiles of expression of the different silicon transporters in different diatom species will give new insights on the existence of a Si transduction pathway involved in diatom biomineralization, cell cycle and growth mediated by these proteins. A full comprehensive description of all SITs functions and evolution is beyond the scope of this review, but it has to be highlighted that only through a functional characterization it will be possible to identify the independent and specific and/or synergistic and/or reduntant activity of the individual carriers.

2.2 Frustulins, Silaffins, Silacidins, Pleuralins and Cingulins

Beside transport, diatom morphology is controlled by additional protein families required for silica precipitation and molecules working as cell wall scaffold (Durkin et al., 2012; Scheffel et al., 2011; Wenzl et al., 2008; Kröger and Poulsen, 2008; Kröger et al., 1999;

Kröger et al., 1996). Silica precipitation and polymerization occurs in a very ordered way; after uptake, silicon is transported to the SDV, that contains silica forming organic components, where silica formation takes place (Poulsen et al., 2013).

Over the last 20 years, significant progress has been made in the identification and study of many of these proteins. Herein we review the most advances in the knowledge of their functions.

The first protein was isolated from the cell wall of the diatom *C. fusiformis* (Kröger et al., 1996) and called frustulin. Later on, these glycoproteins have been identified in different diatoms species, both centric and pennate and all are characterized by the presence of a conserved large cysteine-rich domains domain (acidic and cysteine-rich domains ACR) and a species specific C-terminus. Frustulines are, amongst the protein families involved in silica formation, the only that are present across the cell wall. This suggests that they have a role in protection of the frustule rather than in its biogenesis (Kröger and Poulsen, 2008; van de Poll et al., 1999).

Whereas frustulines do not play an active role in silica formation, silaffins, silacidins, pleuralins and cingulins, are strictly confined to the silicalemma and are known as silica-forming proteins (Wenzl et al., 2008; Scheffel et al., 2011). These proteins have enzymatic activity and promote polymerization and deposition of silica from inorganic precursors. They contain common amino acid motifs (e.g., lysine-rich), and are derived from precursor polypeptides containing N-terminal signal peptides for import into the endoplasmic reticulum (ER) (Kröger et al., 1999; Kröger et al., 2002; Sumper and Kröger, 2004; Pamirsky et al., 2013;).

Silaffins, whose name derives from their high affinity to silica, have the capacity to initiate and modulate silicon dioxide precipitation. Silaffins are able to precipitate silica *in vitro*, forming spheres or plates containing regularly distributed nano and micro pores

(Kröger et al., 2002; Pamirsky et al., 2013). The primary structure of a precursor of silaffins was isolated in *C. fusiformis* (Kröger et al., 2002), and later other genes have been found in different diatom species. All the silaffins identified so far lack any sequence conservation but all are rich in serine and lysine, and are subjected to different post-translational modifications during intracellular maturation. These are essential for SDV and silica targeting and necessary for the formation of silicon dioxide (Kröger and Poulsen, 2008; Kröger et al., 1999).

Pleuralins are protein components associated with the pleural bands, the region of overlap between the two valves of the frustule. Pleuralines are not encoded in all the genomes sequenced thus far. However, and only recently, it has been reported in *C. fusiformis* that the role of pleuralins could be to bind simultaneously silaffins and frustulins , in order to connect epi and hypotecha at the girdle bands (Kröger and Poulsen, 2008, De Sanctis et al., 2016).

The silacidins play a structural role in diatom frustules and, like silaffins, are phosphorylated and the high degree of phosphorylation of the serine residues is strictly related to their ability to precipitate silica nanospheres (Richthammer et al., 2011).

Recently, Kröger and coworkers reported the discovery of a new class of proteins in several diatom species that contrarily to sillaffins and silacidins appear to be crucial in the molecular mechanisms of silica assembly (Scheffel et al., 2011). In particular, these proteins seem to be involved in the morphogenesis of the girdle band, also termed *cingulum*, and for this reason are called cingulins.

For none of the aforementioned genes functional characterization is reported in literature. A joint analysis of the available transcriptomic and proteomic data can provide useful insights in their funcions and regulation in order to build dynamic models connecting gene function with biomineralization processes.

2.3 Other frustule-related molecules

Long-chain polyamines (LCPAs) are linear polyamines constituting the main organic and soluble fraction of the biosilica matrix in all diatoms studied to date. As for the majority of the frustule related molecules, LCPAs have been identified and isolated in the diatom *C. fusiformis* (Kröger et al., 1997 and 2001) and, like silaffins and silacines, are able to precipitate silica *in vitro*. Before their identification in diatom frustules, LCPAs have been identified almost exclusively in bacteria and archeae, predominantly in extremophiles. Interestingly, in diatoms they display species-specific differences in degree of methylation, chain length and position of secondary and tertiary amines (Sumper and Brunner, 2008; Bridoux and Ingalls, 2010; Gräb et al., 2016). This suggests that they may be directly responsible for the differences in the nanopatterned biosilica frustule across different diatom species (Poulsen and Kröger, 2004; Sumper and Lehmann, 2006; Scheffel et al., 2011; Bridoux and Ingalls, 2010). Recently, Gräb and coworkers propesed a model in which LCPAs interact with the lipid membrane of the SDV and play a role in the control of expansion process of the SDV during cingulum and epi – and hypo-theca formation (Gräb et al., 2016).

Another compound embedded within the frustule is chitin, a structural polysaccharide that contributes to the rigidity of the cells. Chitin represents the most abundant polymer in the ocean widely distrubuted among eukaryotes, archaeae and bacteria and its biosynthesis requires a set of multiple chitin synthase (CHS) genes encoded by multi-copy gene family. CHSs genes have been found in different diatom species, and molecular phylogenies reveal five separate clades (Brunner et al., 2009; Durkin et al., 2009). Chitin does not seem involved in silica deposition directly, but rather it seems to direct the correct layout of the proteins involved in biomineralization (Richthammer et al., 2011). The exact mechanism by which chitin is assembled and the way it interacts with the components of the frustule are still not known. However, transcriptome analysis of the Si and Fe response in *T. pseudonana* reported an increased expression of two genes encoding chitin-binding proteins, p150 and p150-like, an overproduction of chitin and a consequent elongated cell phenotype with a tendency to aggregate and sink. This suggests that chitin may also play a role in signalling and response to changes in environmental conditions, influencing cell wall morphology and/or assembly and inducing a survival strategy for cells in hostile environments (Durkin et al., 2009 and 2012).

2.4 Frustule composition influenced by nutrient availability

The different frustule related genes and molecules involved in silica formation and composition, are influenced by different environmental constraints such as nutrient supply and light intensity. Likewise, frustule structure and architecture influence nutrient uptake and light perception (Mock et al., 2008; Allen et al., 2008; Soler et al 2010; Knight et al., 2016).

Some examples are shown of how Si concentration and availability, together with other macronutrients such as carbon (C), nitrogen (N), phosphorus (P), and iron (Fe), could limit diatom growth and division, affect diatom physiology and the mechanical properties of frustules influencing the genes and genes products involved in diatom silicification (Brzezinski et al., 2008).

In 2008, Mock and coworkers (Mock et al., 2008) and later, in 2012, Shrestha and coworkers (Shrestha et al., 2012), reported a genome-wide transcriptome analysis of the marine diatom *T. pseudonana*, experimentally identifying a direct molecular interaction between Si and Fe metabolism and frustule related genes expression. To examine which of these gene products may be involved in silicification pathways, the authors constructed a comprehensive gene expression array. Findings from the two studies revealed that nutrient availability either up- or down-regulate the expression of genes involved in silicification, in particular the SITs, in agreement with previous reported results, showing increased silicon cellular content in response to Fe, N and P limited growth conditions. Consequently, the increased Si content, due to the altered stoichiometry of Si to C- and N- ratios, results in a thicker and mechanicaly stronger frustule that makes cells less susceptible to grazers (Wilken et al., 2011; Bucciarelli et al., 2010; Mock et al., 2008; Claquin et al., 2002). Moreover, in N and P starved cells an accumulation of lipids in the frustule fraction has been reported, which could impact the frustule architecture (Soler et al., 2010; Smith et al., 2016). In addition, a silica-dependent checkpoint in diatom cell cycle progression has been demonstrated. During their vegetative reproduction, diatoms need to build up a new silica cell wall, but if the amount of silicon in the environment is depleted, cell division arrests in the G1-phase or G2-M phase (Okita and Volcani 1980; Thamatrakoln et al., 2007; Bowler et al., 2010; Huysmann et al., 2014; Tanaka et al., 2014). Finally, Durkin and collaborators in 2012 extended the transcriptional analysis of genes encoding SITs, chitin synthases, and proteins involved in silica formation in response to nutrient starvation in natural populations. Their results highlight the important role of nutrient availability in frustule-related gene transcription and consequently in shaping diatom community composition (Durkin et al., 2012).

All together these data clearly have a profound ecological significance and could help to explain the large-scale ecological success of diatoms, suggesting the existence of an intimate crosstalk between different processes interacting with each other and working in collaboration to respond and adapt to multiple environmental factors and conditions.

3. DIATOMS AS MODEL SYSTEMS FOR GEOMETRY

3.1. A generalized Pythagorean Theorem for the uniform description of algae

Ultimately the shape of the frustules, the structure of valves and girdles, and the distribution of pores need to be studied via mathematics, with emphasis on geometry, optimization and energy minimization. In particular, there is a need for a dedicated geometrical structure. There are many applications of mathematics in biological sciences, especially in computational genomics. Genes and genetics are important factors in development and evolution, but it can be argued that first and foremost organisms have to abide to physical and mathematical laws, globally and locally. A problem is that biology, in general, does not have laws like physics has, apart from allometric laws (Dhar and Guiliani, 2010; Niklas, 2004). To achieve the well known unreasonable effectiveness of mathematics in the natural sciences, which is in that case in physics, seems a formidable task. The mathematician I. M. Gelfand wrote: *"There exists yet another phenomenon which is comparable in its inconceivability with the inconceivable effectiveness of mathematics in physics noted by Wigner - this is the equally inconceivable ineffectiveness of mathematics in biology"* (Arnold, 1999). Currently mathematical or geometrical models or laws to describe trajectories of biological organisms through their lifetime or spacetime are simply not available (Berger, 2003).

A geometrization of biology, or more generally of nature, based on forms and formation of natural shapes (a geometrical theory of morphogenesis) is both an enormous challenge and a prerequisite for progress in science and the life sciences. What needs to be achieved is the combination of a uniform description of shapes, coupled to differential equations within a coherent geometrical framework. This is exactly how physics evolved, with the laws of gravitation based on a uniform description using circles, based on the Pythagorean Theorem, or using conic sections, based on the Pythagorean application of areas. Later the geometrical setting became Riemannian geometry and increasingly Finsler geometry with applications in marine biology (Antonelli and Miron, 2013). Only with a uniform description for biological shapes, the laws that govern shape and morphogenesis can be found, and only then biology can deal with form and function of biological organisms, in the same way physics has achieved. What is needed then is first, a uniform description of natural shapes (the Kepler-like step, May), and second, the study of the biological organisms in their ever-changing environment and the way they deal with stresses and curvatures.

Gielis Transformations are generic geometric transformations of planar functions $f(\vartheta)$, which unify a wide range of natural and abstract shapes (Gielis 2003 and 2017; Gielis et al., 2005). Equation 1 is a generalization of the circle (as a constant function) but it retains the compact structure of the Pythagorean Theorem: indeed, selecting $n_1 = n_2 = n_3 = 2$ and $f(\vartheta) = R = 1$ gives the unit circle and the Pythagorean theorem. Instead of only the classic Euclidean circle, Eq.1 defines natural shapes at all levels, such as diatoms, starfish, flowers and molluscs (Gielis 2003 and 2017; Gielis et al., 2005), as well as regular polygons (Matsuura, 2015), and even spacetime models (Gielis et al., 2005). The exponents $n_{1,2,3}$ change the basic polygons defined by the symmetry parameter *m*. Parameters *A* and *B* are scaling parameters. If *A = B*, the basic shape is a circle, but when they differ, the basic shape is an ellipse. Since shape and size parameters are real numbers, a huge diversity and variability can be described in a very compact way:

$$\varrho(\vartheta, f(\vartheta), A, B, m, n_1, n_2, n_3) = \frac{1}{\sqrt[n_1]{\left|\left(\frac{1}{A}\cos\left(\frac{m}{4}\vartheta\right)\right|^{n_2} + \left|\frac{1}{B}\sin\left(\frac{m}{4}\vartheta\right)\right|^{n_3}}} . f(\vartheta) \quad \text{Equation 1}$$

In particular, diatoms and their substructures can be described in one coherent framework (Figure 1). With *m=0* (circle) radially symmetrical cylindrical frustules can be described, and with increasing m various diatom shapes are modelled (Fig. 1). Square shapes have *m*=4 and benthic diatoms with pennate shapes have bilateral symmetry, defined by *m*=2. *Stictodiscus* diatoms can be circular but also triangular (m=3) or square (Figs 1 and 4). Higher symmetries can be observed in sectorized *Arachnodiscus* or *Cosnicodiscus*. Actually, the parameter *m* divides the plane into m sectors. For *m*=1 the sector is 360° but unlike the circle, this shape can be asymmetrical, with one axis of symmetry. In algae, such asymmetry or polarity is well known in establishment of polarity in *Fucus* or in zygotes of *Dictyota* (Bogaert et al., 2017), in oogonia (Fig. 6), or in the diatom *Podocystis*.

Equation 1 concerns planar curves, but the 3D version, Equation 2, a generalization of the sphere for $f(\vartheta,\varphi) = R$(= the radius of the sphere), can describe the shape of the complete frustule:

$$\varrho(\vartheta,\varphi, f(\vartheta,\varphi),A,B,C,m_1,m_2, n_1,n_2,n_3,n_4)0 = \frac{1}{\sqrt[n_1]{\left|\left(\frac{1}{A}sin\left(\frac{m_1}{4}\vartheta\right).cos\left(\frac{m_2}{4}\varphi\right)\right|^{n_2} + \left|\frac{1}{B}sin\left(\frac{m_1}{4}\vartheta\right).\, sin\left(\frac{m_2}{4}\varphi\right)\right|^{n_3} + \left|\frac{1}{C}cos\left(\frac{m_1}{4}\vartheta\right)\right|^{n_4}}} . f(\vartheta,\varphi)$$

Equation 2

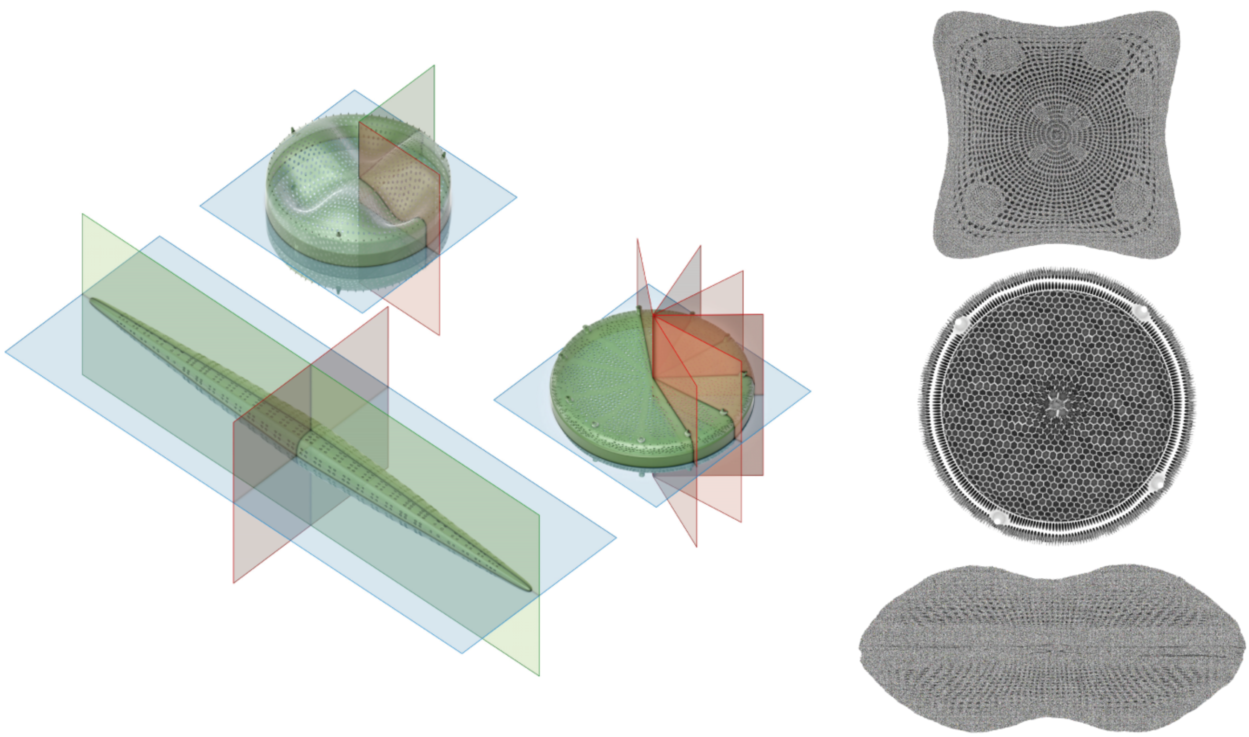

Fig. 4 Coordinate planes on CAD models (left) and Digital Diatoms (right; Courtesy of M. Kay, 2010)

The 3D coordinate planes then correspond to the valvar, radial/transapical and the (per)apical planes in diatoms (Fig. 4). The arrangement of the pores in fact provides a very specific coordinate system adapted to the shape, as a natural generalization of classical coordinate systems such as spherical and elliptical coordinates. The regularity in the distribution of pores is remarkable, both in centric and pennate diatoms, with local deviations based on the actual processes in actual environmental conditions. Using the pores as a natural coordinate system should then allow to understand the mathematical physics underlying species specific and environmental-developmental specific distribution of pores.

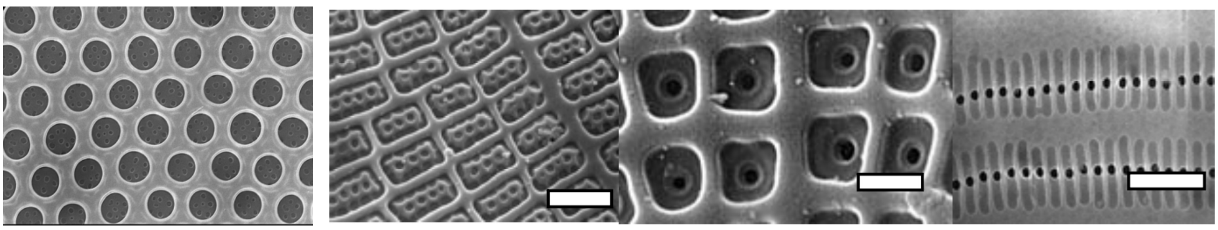

Fig. 5. Left: Pore distribution on *Coscinodiscus wailesii* valves. Right: Areolae array, microchambers and microchannels of *Cocconeis scutellum* vars. Scale bars 1.5μm (Courtesy of De Stefano et al., 2008, 2011).

In particular, every shape can be assigned its own set of parameters, allowing to deal with phenoplastic variability, within single diatoms or among species and individuals, to any desired degree of accuracy. The ‘round’ or ‘square’ shapes in Fig. 5, can be quantified precisely and individually, using methods described for tree rings (Shi et al., 2015), bamboo leaves (Lin et al., 2016) and seeds. It is noted that this geometrical strategy is far more general than the geometrical ones used for automatic identification of diatoms, e.g. based on contour shape analysis (Hicks et al., 2006). The main difference is that earlier approaches are based on Fourier descriptors or curvature profiling, but now we have a uniform description in a Pythagorean-compact way (i.e. the structure of Equation 1 is the same as the Pythagorean Theorem defining the circle; the Pytagorean Theorem results when, in Equation 1, *A*=*B*=1 and the exponents $n_{1,2,3}$=2). In this way we can also encompass all different symmetries encountered in diatoms, and provide for a quantitative measure for qualitative terms such as elliptical or oval, (sub)circular and (sub-)spherical, crescent shapes, lanceolate, clavate, naviculoid, sigmoid and more.

It should be noted that defining symmetry is still a major open problem in shape description, and consequently in image analysis and automated detection. With Equations 1 and 2 however, no prior information is necessary since the system can itself determine the symmetry of the shape (Gielis et al., 2011; Fougerolle et al., 2013). With the compact description, associated characteristics such as surface area, volume, perimeter, curvature etc. can be quantified immediately, and ratios can be used to understand optimal shapes and distribution of pore. This leads to understanding of minimizing material use and optimization of material distribution, mechanical strength and light focusing properties as discussed in section 4. The very same principles apply in connected diatoms or in optimal packing of cells in multicellular algae (Fig. 6). Given this uniform description, these characteristics are *a priori*, whereas in existing systems where shapes are digitized, the characteristics need to be computed *a posteriori*. Moreover, once geometrized, studies on mechanical stress can be based on mesh free modeling, solving boundary value problems directly on the surfaces and shells and complete frustrule. This allows for a global strategy for the geometric study of diatoms, rather than based only on local properties.

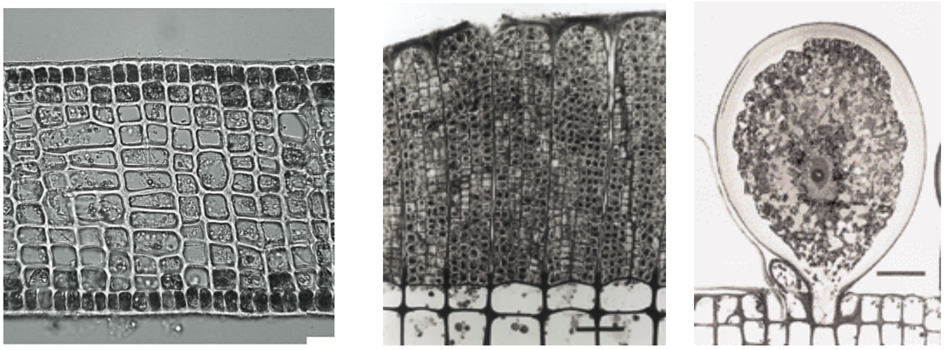

Fig. 6. Left: Cell arrangement in *Zonaria turneriana*, Center: Antheridia of *Homoeostrichus sinclairii*, Right: Oogonium in *Homoeostrichus sinclairii*

3.2. Diatoms dealing with anisotropic stress

The proposed uniform description in diatoms can be very effective in the study of global properties, rather than local. Beyond shape description, we have to understand the forces that generate the shapes. The nearly universal principle in the natural sciences is that the equilibrium configuration of a system can be found by minimizing its total energy among all admissible configurations. When considering the surface as interface between two (or more) immiscible materials, the surface geometry is determined by minimizing the surface tension subject to whatever additional constraints are imposed by the environment. There is a canonical equilibrium surface, called the "Wulff shape", that can be characterized as the absolute minimizer of the free energy F among all surfaces enclosing the same three-dimensional volume (Koiso and Palmer, 2008). Diatoms can be considered as Wulff shapes, on which surface stresses act, and they are the "unit spheres" for an anisotropic energy.

Surface stresses are determined geometrically by the curvatures of the surface (Gielis, 2017). In surface theory it is well known that in each point of a surface one can find one maximum $\kappa_{max} = \kappa_1$ and one minimum curvature $\kappa_{min} = \kappa_2$ and the directions of these principal curvatures are always perpendicular. There are inevitable mathematical relations between these principal curvatures that fundamentally relate to the intrinsic nature of these shapes (the Gaussian curvature $K = \kappa_1 \cdot \kappa_2$ or equivalently, is the square of the geometric mean $GM = \sqrt{\kappa_1 \cdot \kappa_2}$ of the principal curvatures), or curvatures that fundamentally relate to the shape, which these shapes assume in their ambient world, i.e. the extrinsic nature (given by the mean curvature $H = \frac{\kappa_1 + \kappa_2}{2}$). Soap bubbles have constant mean curvature everywhere (H is positive, not zero), but soap films (e.g. catenoids) are minimal surfaces with both K and H equal zero, attaining equality $K = H^2 = 0$, in the inequality $K \leq H^2$.

Finding extremals of H^2 is another natural choice, embodied in the Willmore functional $\int H^2 dA$ (with dA area element) on closed surfaces in 3 dimensional space (Ferrandez, 2017). The Willmore Theorem states that this functional is $\int H^2 dA \geq 4\pi$ with equality (and thus a minimum) for the round sphere. The natural problem is to determine the minima when the surfaces have additional constraints, topologically or metrically. This finds applications in biology as the Helfrich energy for membranes (Ferrandez, 2017), relating to the cell membrane of diatoms, which is the boundary between a living cell and its environment with the frustule as the boundary shell, whose formation is guided by the silicalemma. With Equations 1 and 2, we can study diatoms as Wulff shapes, minimizing certain anisotropic energy functionals (Koiso and Palmer, 2008). The morphology of diatoms then can be studied in the same way in which crystals, snowflakes, or soap films are studied. Many structures in marine organisms are known to be periodic minimal surfaces, and the frustule structure of diatoms is no exception. However, with the natural coordinate systems we can study deviations from periodicity in real natural shapes.

3.3. Diatoms as biological model for mathematical physics and biomimicry

Diatoms are a most natural domain for global and local studies of shape and morphogenesis with geometry. Such shapes are striving for the status of ideal submanifold, adapted to the surrounding space in a best possible way, in a dynamic equilibrium. The surrounding space in this case contains large quantities of salt (silicic acid, NaOH etc.), typical of generating specific shape variation. In very brine waters, archaebacteria have been found with triangular, square or box shapes (Bolhuis et al., 2006; Oren, 1999). The use of advanced imaging techniques allows to determine both the global shape as well as the finest details of the frustule, but from a mathematical point of view it is assumed that the frustule results as the solution to boundary value problems by the organisms, within their environment. To determine the associated boundary value problems and their solutions is the goal.

Fortunately, when the Gielis transformations were discovered, it was understood soon that this could open the way for solving boundary value problems on any domain, using the classical Fourier analysis. So far analytic Fourier-like solutions were restricted only to a few domains, but can not be extended to a wide range of domains in 2D and 3D, without the need for meshing and finite elements. Analytic Fourier-like solutions to Laplace, Helmholtz and wave equations have been found for various 2D and 3D domains, including shells (Natalini et al., 2008 and 2009; Caratelli et al., 2009; Caratelli et al., 2017). Fourier-like refers to analytic solutions combining Fourier series, with special functions of Hankel and Bessel type. The domains or shells are normal with respect to a suitable spherical coordinate system so that the relevant boundary may be regarded as an anisotropically stretched unit circles or spheres. The Laplacian is then defined for this anisotropically stretched coordinate system. In this way, accurate solutions can be obtained with very low orders of expansions of spherical harmonics. Fig. 7 shows one solution for the Robin problem for the Laplace equation on closed shells with fourfold symmetry in the XY plane (Caratelli et al., 2017). This is generally applicable for all 3D shells. These results show that, dependent on the boundary value problem and the boundary conditions chosen, the solutions reflect the way shapes deal with tensions on surfaces. In turn, these tensions are described by the mean curvature H, which is directly related to the Laplacian Δ, due to a Theorem of Beltrami, namely $\Delta = 2H$ for surfaces.

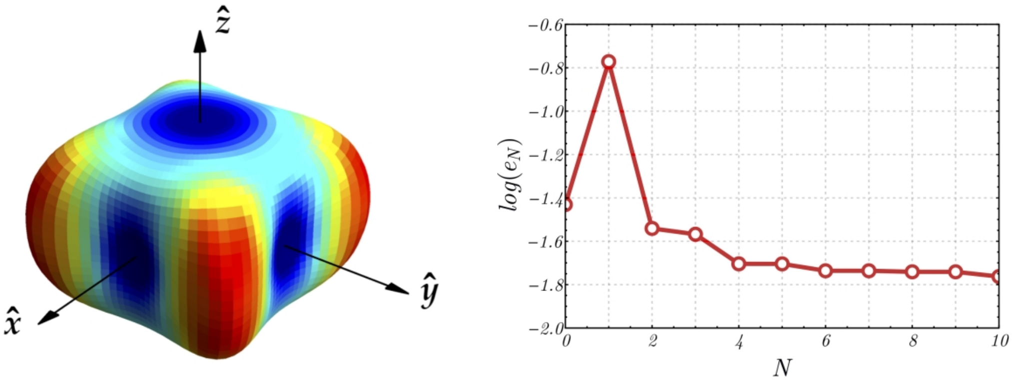

Fig. 7. The solution of the Robin problem for the Laplace equation in a shell S with fourfold symmetry in XY direction. Left: Boundary behaviour along the inner shell surface of the partial sum U_N of order $N = 11$. Right: relative boundary error e_N as function of the order N of the truncated spherical harmonic expansion for the super-shaped shell on the left.

In Fig. 7, tensions are diverted to the corners in the shells, while top and bottom zones are essentially stress free. This solution is in line with the girdle of the frustule along one direction, while in the perpendicular direction (the lower and upper surface) the pores can form with their various functions. This is in line with the anisotropic mean curvature $\Lambda = \frac{T_1}{R_1} + \frac{T_2}{R_2}$ (Koiso and Palmer, 2008; Thompson, 1917), the modification of the Laplace-Young formula, wherein for given curvatures R_1 and R_2, the tensions T_1 and T_2 in other directions determine a weighted arithmetic mean. This is observed in elongating plant cells, keeping the same width. In dealing with stress, organisms can either avoid stress by aligning with the stress (e.g. wind is converted into harmonics by trees) or by incorporating the stresses into form and function, for example the floral shape on the test of sand dollars (Gielis, 2017). It is to be expected that the shape of the upper and lower side of the frustrule is the result of similar diverting of stresses. In diverting and incorporating stresses, the shape of these upper and lower parts of the frustule in centric diatoms for example may be considered as analogous to first modes of vibration in drums. Likewise the precise shape and distribution of valves is the result of an optimization problems.

These geometrical methods provide a more global view, which can be aligned in the future with existing CAD and Finite Element studies in diatom frustules, but such global geometric view is indispensable if we wish to understand form and function in diatoms. From a geometrical point of view, diatoms are extremely interesting because the overall stable geometrical structure of frustules, *costae* and other structures, is combined with local optimization of the precise distribution of the pores. This distribution and the diversity make diatoms individually unique; they are the snowflakes of (the liquid state of) water and they are evolutionary very stable solutions.

The concept of stress imposed by the environment onto the boundary surface or shells, has resulted in diatoms in structures capable of efficiently capturing light and radiation and of dealing with specialized fluid and particle dynamics. In this sense diatoms have evolved as excellent solutions, since the structure of the frustule gives the ability to focus light. This is a concept also known in antenna, in particular in antenna arrays (Bia et al., 2017). Understanding the structure of frustules can lead to new applications in technology (biomimicry), but alternatively, concepts known only in technology, may be discovered in living organisms as well. As one example, metamaterials are man-made materials with negative index of refraction, not known in nature. However, metamaterials can easily be obtained in various parts of the EM spectrum, by an array of supershapes (defined by equation 1), to optimize or fine-tune spectral efficiency in antennas (Zhargooni et al., 2015) or in light harvesting (Zhou et al., 2014).

It seems likely that the structure of the frustule provides the diatom with capabilities, not only to focus light, but also to tune the reception of light in an optimal way. It aligns with novel developments in antenna technology where electromagnetic bandgap structures are used to create low loss dielectric structures based on periodicity of supershapes to prevent propagation of certain frequencies, and increase isolation between antennas, and this can be tailored on angle of incidence and polarization.

Also in fluid dynamics (see section 4) shapes defined by Equations 1 and 2 have been used (Wang 2008; Legay and Zillian, 2008). Equations 1 and 2 have also been used in many studies in nanotechnology (e.g. Tassadit et al., 2011; Macías et al., 2012; Forestiere et al., 2015), in optimizing electro-osmotic pumps based on asymmetric silicon microchannel membranes (Parashchenko et al., 2014) and in the study of dielectric properties of the skin (Huclova et al., 2010).

This geometrical treatment of diatoms from a global viewpoint, should be integral part of a systems biological approach, coupling insights on form and function to understand genomic and genetic diversity in diatoms, during evolution and development. To solve the boundary value problems they encounter, organisms can rely on a broad genetic toolbox and set up to address problems through micro-RNAs, neofunctionalization, mobilisation of transposable elements, epigenetic imprinting and so on. In diatoms this genetic toolbox is known to be very complex with a rich history, and it is thus important to understand form and function, since mathematical and physical laws are prevalent in our universe.

4. PHYSICAL PROPERTIES OF THE FRUSTULE

4.1 Unveiling the frustule secrets

While, in the first studies devoted to diatom biology, the main functionalities ascribed to the frustule were related to protection of the cytoplasm from external, noxious agents and to nutrient diffusion and uptake through its porous matrix, in the last decade new possible evolutionary advantages have been hypothesized (Townley et al., 2011), including light harvesting and focusing, carbon assimilation and sinking (Finkel et al., 2010).

Since the ultra-structured "anatomy" of the frustule, strictly related to its physical properties and functionalities, is not visible in all its complexity by means of light microscopy, it started to be revealed with the introduction, among other techniques, of transmission electron microscopy (TEM), scanning electron microscopy (SEM), SEM stereo-imaging (Chen et al., 2010) and atomic force microscopy (AFM) (Losic et al., 2007). In some cases two different kinds of microscopy have been merged (photogrammetric surfaces derived from SEM pictures plus confocal microscopy in Friedrichs et al., 2012 ; SEM plus digital holography and SEM plus AFM in Ferrara et al., 2016), thus guaranteeing high levels of resolution and accuracy in all three spatial dimensions. An accurate, precise and highly detailed representation of frustule morphology is fundamental not only *per se*, but also in the retrieval of CAD models for the numerical simulation and systematic study of its physical properties, mainly mechanical, fluid-dynamical and optical.

A recent technique introduced by Pan et al. (Pan et al., 2014), allowed the detailed investigation of the internal structure of diatom frustules without sectioning the silica walls of the shell. Indeed they obtained graphene replicas of *Aulacoseirea* genus frustules by means of chemical vapor deposition of methane. Since graphene is highly transparent to electron beams, this allows the visualization of the internal morphology and structure of valves and girdles, unveiling the intricate, interconnected nanotubes linking their different layers (see Fig. 8). This is of fundamental importance in the understanding of the interactions of the living cell with the external environment and relative exchanges of matter.

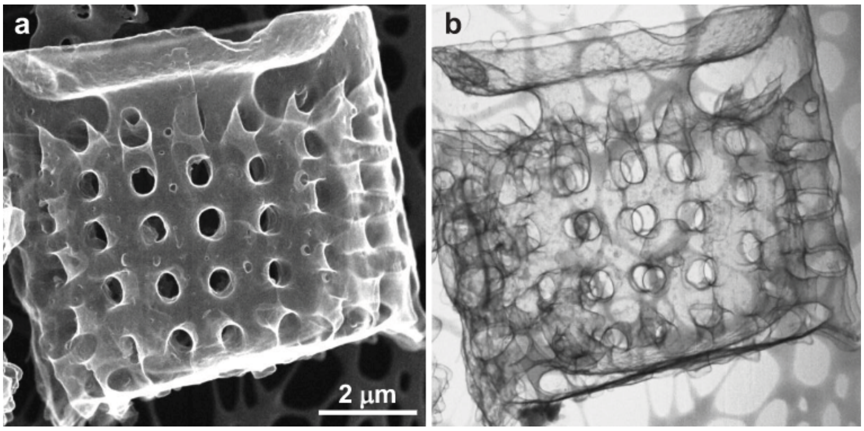

Fig. 8. SEM mode (a) and TEM mode (b) STEM images of a graphene replica of a single *Alaucoseira* frustule. Courtesy of Pan et al., 2014.

4.2 Mechanical properties of the frustule

The study of the mechanical properties of frustules is of great interest not only in the understanding of their functionalities, but also in the framework of biomimetics applied to nanotechnology (Gordon et al., 2009), architecture (Bach et al., 1985), and lightweight constructions (Hamm et al., 2005).

In 2003, Hamm and coworkers (Hamm et al., 2003) experimentally measured and numerically simulated the forces necessary to break the frustules of three species of diatoms: *Thalassiosira punctigera* (centric), *Coscinodiscus granii* (centric and much larger than *T. punctigera*) and *Fragilariopsis kerguelensis* (pennate). Using calibrated glass microneedles to load and break the frustules with defined forces, they found that they were able to resist to pressures ranging from 1 to 7 N/mm^2, equivalent to 100-700 $tonnes/m^2$. Measurements on an isolated pleura (i.e. an open, hoop-shaped segment of the girdle) of *T. punctigera* allowed to estimate its Young's modulus E in about 22.4 GPa, comparable to those of cortical bone or medical dental composites.

One way to understand how stresses are distributed through the ultrastructure of a frustule after mechanical solicitation is to make use of the Finite Element Method (FEM). In this kind of analysis, the object under study is discretized into a finite number of parts (the elements, indeed), which is equivalent to say that the domain is reduced into a limited number of degrees of freedom. Once a complete set of elements (the so called *mesh*) is obtained, the response of the system to applied forces is described by a system of partial differential equations acting at the interconnected joints between the elements (the so called *nodes*) which cannot be solved analytically. We can bypass this difficulty assuming that, in each element, the response to the solicitation is predefined (e.g. linear in case of mechanical forces). Even though FEM can readily handle complex geometries and a wide variety of physical problems (mechanical, thermodynamical, fluid-dynamical and electrostatic problems), we have to keep in mind that it is able to produce only approximate, not closed-form solutions (see section 3). In the next generation studies of diatoms, these methods can be combined with the geometrical model of section 3, which allow for analytic solutions and global geometry.

Looking at finite element model (FEM) numerical simulations performed on a rendered, complete frustule of *F. kerguelensis*, Hamm et al. concluded that the presence of ribs (the so called *costae*) allowed to deflect stress concentration, smoothly absorbing it from the fragile areas in between.

Moreno et al. (Moreno et al., 2015) tried to quantify even more in detail the different response to mechanical stress of centric (*Coscinodiscus* sp.) and pennate (*Synedra* sp.) diatom frustules. By means of nanoindentation measurements they derived, for *Coscinodiscus* sp. frustules, values of E ranging in the interval 1.1-10.6 GPa and a hardness module H of 0.10-1.03 GPa, respectively. The analogous values for *Synedra* sp. varied between 13.7 and 18.6 GPa and 0.85 and 1.41 GPa, respectively. Successive FEM simulations on accurate CAD models allowed to shed light on this different response to mechanical stimuli. The higher robustness of *Synedra* sp. frustules is mostly due to their high aspect ratio and rib-slit structure. On the other hand, stress levels in *Coscinodiscus* sp. are strongly dependent on pore dimension:

increasing pore size leads to an improved and more flexible structure.

Recently, the combination of nanoindentation and three-point bending tests allowed to retrieve even more accurately the elastic modulus of valves and girdles of *Coscinodiscus* sp. frustules, obtaining an average value of 36±8 GPa (Aitken et al., 2016): it follows that the diatom frustule is characterized by a specific strength much larger than any other reported natural biomaterial, mainly due to the honeycomb multi-layer architecture of the valves.

In general, numerical simulations are unavoidable if the complex relationship between mechanical properties of the frustule and its detailed features (porosity, shape, thickness, pore size etc.) has to be analyzed. Lu et al. (Lu et al., 2015) modeled with high accuracy the valve of a *Coscinodiscus* sp*.* diatom starting from a unit cell which comprised all the three layers of the valve (*foramen, cribrum* and *cribellum*) and even taking into account the curvature of the walls of foramen pores (*areolae* chambers). The actual lattice which repeats periodically throughout the valve is constituted by seven of these unit cells arranged with hexagonal symmetry (see Fig. 9). This model has been compared with a reference, solid structure with no pores at all but with the same amount of material. FEM simulations allowed to conclude that, in presence of pores, the seven-unit-cell (and the corresponding larger scale structure obtained by expanding it in two dimensions) is able to lower the stress concentration at the edges (in elastic regime) with respect to the solid reference; the average stress level and edge stress concentration are lower than those in the reference structure; the stress is lower between the pores. Hence, in a diatom frustule, material is saved while a certain level of strength is maintained (this allows to explain the elevated silica circle around the pores of the foramen, which allows to strengthen the structure locally). Analogous simulations performed on a simplified model of the girdle led to the conclusion that a larger reaction force is observed compared to the reference structure, which indicates that the girdle band can withstand larger compression forces (see again Hamm et al., 2003).

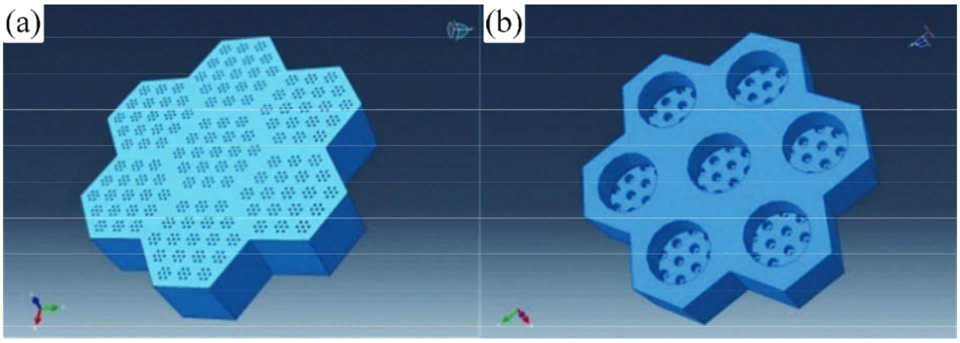

Fig. 9. Outer (a) and inner (b) view of the seven-unit cell used in Lu et al. (2015) to simulate the response of *Coscinodiscus* sp. valves to mechanical stimuli. Reproduced with permission.

Even though many organisms, from protists to crustacean zooplankton, feed on diatoms, the hypothesis that one of the main functionalities of diatom frustules is protection and defense from predators is not to be excluded. Indeed, any mechanism able to reduce population mortality in an environment strongly dominated by grazing pressure is advantageous. Furthermore, diatoms can survive gut passage if frustules are not crushed and it is likely that silica-edged mandibles of copepods and euphausiids co-evolved with their diatom preys (Hamm et al., 2003). The intimate relationship between the silification process and grazing pressure has been deeply studied by Pondaven and coworkers (Pondaven et al., 2007). As we mentioned in section 2, it was already known that, under iron, nitrogen, phosphorous or light limitations a decrease in diatom growth rate and an increase in frustule silica content take place (Brzezinski, 1985). What has been observed further is that grazing pressure induces variations in cell wall silification of the same order of magnitude as those determined by changes in growth environment, with consequent, significative variations in Si:N and Si:C ratios. This behavior could, to some extent, allow to understand the variability in the grade of silification that can be found from species to species according to their specific environment.

4.3 Diatom frustule fluid dynamics

One of the main functionalities that has been ascribed to diatom frustules is the capability to sort and filter nutrients (such as NH_4^+, HCO_3^-, NO_3^- etc.) from harmful agents (e.g. bacteria and viruses). The involved concentrations range from ~10^6 ml^{-1} for bacteria (0.2-1 μm in dimension), ~10^7 ml^{-1} for viruses (20-200 nm) up to ~10^9 ml^{-1} for colloids (5 nm-2 μm) and ~10^{14} ml^{-1} for nutrient molecules.

The first interaction of these living and non-living brownian particles with diatoms is, of course, with the external surface of frustules. Hale and coworkers (Hale et al., 2001; Hale et al., 2002) showed experimentally, for *Coscinodiscus* sp. and *Thalassiosira eccentrica* diatoms, that both diffusion and advection of sub-micrometric beads are altered by the micro-topographies of the frustule. In particular, beads following streamlines in a fluid in proximity of the frustule are directed around the edges of the areolae localizing themselves in proximity of areolae ridges, thus deflecting from the direction of bulk flow. Since the magnitude of this effect is strictly dependent on the ratio a/R_0 of the bead and areola radii, it could constitute an elegant way to sort particles according to their size. Furthermore, looking at the transverse section of a *Coscinodiscus* sp girdle, it can be noticed how the profile of the pore channels presents impressive similarity with that of a typical silicon drift ratchet (Losic et al., 2009; see Fig. 9). A drift ratchet membrane is provided with microchannels made of a periodic series of ratchet-shaped pores (i.e. with a periodic but asymmetric variation of the diameter along the pore axis), which, in zero-mean fluid flow conditions, generates a rectified motion in finite-sized brownian particles. This means that, even though the mean value of the periodic pressure profile is zero, a net motion of particles suspended in the fluid from one side of the membrane to the other can be observed, with no motion of the liquid itself (Matthias et al., 2003). In a drift ratchet, the direction of the particle current depends very sensitively on the size of the particles, even though this effect is difficult to predict intuitively (Kettner et al., 2000). This could represent a further contribute to particle sorting according to size. Nevertheless, the main differences between an artificial drift ratchet and the section of a *Coscinodiscus* sp girdle are quite relevant. The girdle band pores have only two repeating units in series respect to the tens of units of an artificial drift ratchet (Fig. 10a), so we are definitively far from the assumption of an idealized infinitely long and exactly periodic channel, on which the stochastic models relating the direction of motion of the particles and particle size are based; the girdle pores are subjected to different and more variable fluid oscillations in the oceanic environment; finally, the dimensions of the pores are of the order of the fractions of micrometer versus the several micrometers of a typical artificial drift ratchet silicon membrane. However, the fabrication of biomimetic, diatom-inspired silica ratchets would shed light on the possible ability of frustule girdles to induce asymmetric diffusion on particles dispersed in an aquatic environment.

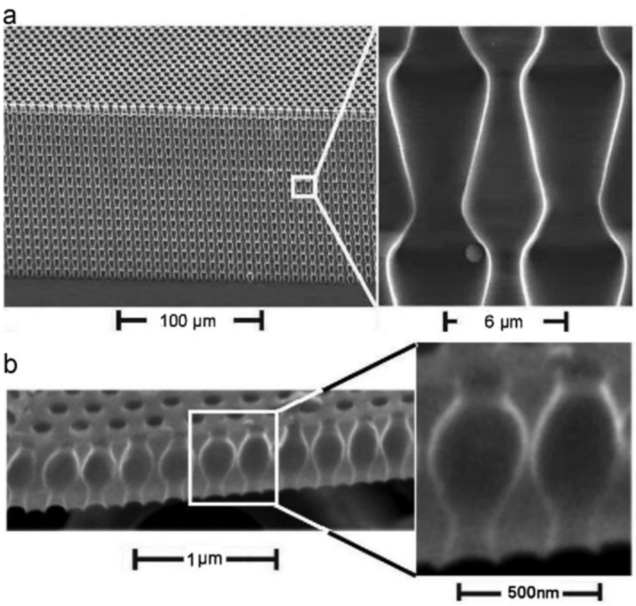

Fig. 10. Artificial silicon ratchet membrane (a) and cross-section of a *Coscinodiscus* sp. diatom girdle (b). Courtesy of Losic et al., 2009.

Even the formation of colonies in the shape of chains, which characterizes several species of diatoms (Round et al., 1990), and their fluid dynamic interaction with the environment, seems to have some influence on the efficiency of nutrients uptake. Srajer et al. (Srajer et al., 2009) applied simulations based on Computational Fluid Dynamics (CFD) on a simplified model describing a colony comprised of single units of 10 cells repeated endlessly. It turns out that the interaction of the chain with unsteady flow conditions triggers oscillatory variations in the distance between adjacent cells, increasing advective diffusion through the surface of the diatoms and therefore nutrient supply. However, this simplified model does not take into account the presence of linking spines or periplekton between adjacent cells. Since, actually, the spaces between single diatoms of a chain contain linking elements, viscosity will tend to suppress inter-cell flows. Nevertheless, the gaps still contribute to reduction of the biomass per unit chain length, thus increasing the nutrient supply for each cell (Pahlow et al., 1997).

Another fundamental phenomenon involving frustule fluid dynamics is certainly sinking rate (Miklasz et al., 2010)]. Being the main photosynthesizers of the ocean and generating half of its fixed carbon every year (Field et al., 1998), the maximum speed at which diatoms sink strongly influences global carbon cycle besides silicon fluxes. The accurate prediction of maximum sinking speed could thus allow to calculate carbon and silicon oceanic fluxes starting from local measurement of production (Nelson et al., 1995).

Usually, the sinking rate U of a spherical particle immersed in a fluid is dependent by the radius r and density ρ of the particle, the density ρ_f of the fluid and its dynamic viscosity μ, according to the well known Stokes' law:

$$U = \frac{2(\rho - \rho_f)gr^2}{9\mu}$$

where g is the gravitational acceleration.

Diatoms are able to reduce their density through ion exchange (Anderson et al., 1978;

Waite et al., 1997), but in the absence of physiological density reduction, they sink at their maximum speed. According to Stokes' law, the sinking velocity of a spherical particle scales with the radius squared, which is not true for a diatom (both live or dead), since its approximation to a sphere is quite inaccurate. Miklasz et al. (Miklasz et al., 2010) corrected Stokes' law taking into account the relative contribution of frustule and cytoplasm densities, the geometry of the frustule and its thickness, thus obtaining predictions that fit more accurately with experimental data. Indeed, typically diatom sinking rate depends on diatom radius with a scaling exponent between 1.2 and 1.6 (Smayda et al., 1970; Waite et al., 1997). Thus it is straightforward to deduce that, lowering the sinking speed, the peculiar shape and morphology of the frustule allows for a longer stay of diatoms near ocean surface and, consequently, a longer exposition to sunlight than if they were provided with a simple, spherical porous silica armor.

4.4 Optical properties of the frustule

The impressive similarity of diatom frustules with artificial photonic crystals (Fuhrmann et al., 2004; De Stefano et al., 2009) (Fig. 11) induced to hypothesize that, beyond mechanical defense and filtering of nutrients from harmful agents, valves and girdles could play a fundamental role in light manipulation and harvesting, thus enhancing photosynthetic efficiency in environments where light is not so easily accessible.

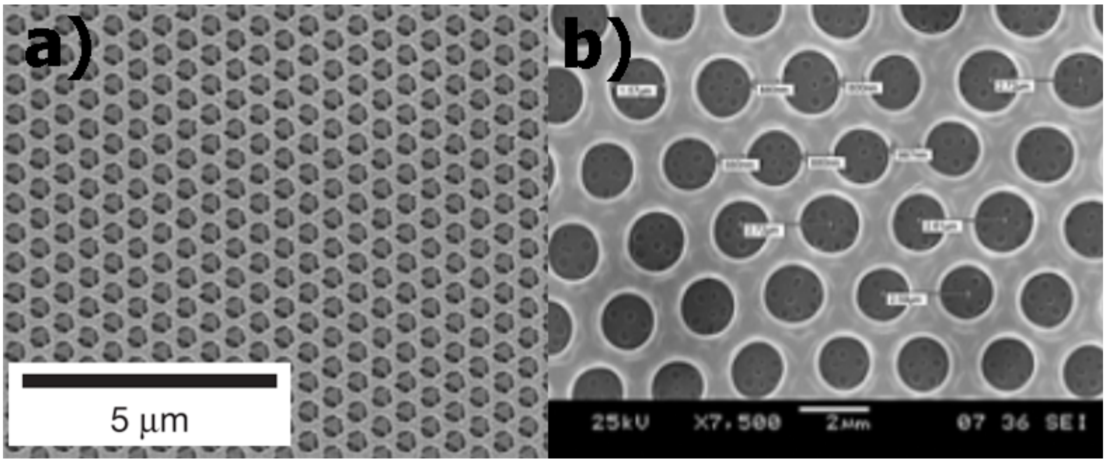

Fig. 11. Artificial photonic crystal (a) and detail of a single valve of *Coscinodiscus wailesii* diatom (b). (a) is reproduced with permission from Qi et al., 2004; (b) is reproduced with permission from De Stefano et al., 2007.

A photonic crystal is constituted by a spatial, periodic distribution of refractive index which, properly dimensioned, can block the propagation of light in specific wavelength ranges (the so-called *photonic band-gaps*) (Joannopoulos et al., 2008). Several animals and plants, mainly insects, birds and flowers, developed, through billions of years of evolution and as constitutive part of their organisms, sub-micron, periodic or quasi-periodic architectures which substantially act as photonic crystals, giving rise, by means of selective transmittance and reflectance at different wavelengths, to so-called structural colors (Vukusic et al., 2003; Parker et al., 2007; Kolle, 2011; Greanya, 2016). Structural colors are not due to any pigment but only to the geometrical characteristics and refractive index of micro- and nanostructured scales, cuticles, plumage and similar features.

The interaction of diatom frustules with light is even more articulate, and consists of three main phenomena: light confinement, selective transmission and photoluminescence.

In 2007 De Luca et al. (De Stefano et al., 2007) first observed light confinement by a single valve of *C. wailesii*, about 150 μm in diameter. The experiment was conducted with red coherent radiation, and the incoming beam was focused by the valve in a tiny spot < 10 μm wide at a distance of about 100 μm from it (Fig. 12). Even though, from this point of view, *C.*

wailesii valves can be assimilated to a sort of microlenses, the phenomenon is to be ascribed to diffraction rather than refraction: numerical simulations (De Tommasi et al., 2010; Ferrara et al., 2014) confirmed indeed that light confinement is due to the coherent superposition of the diffraction contributions coming from the single pores. The same effect can be observed making use of digital holography (DH) (Di Caprio et al, 2014; Ferrara et al., 2014), which allows to retrieve both intensity and phase of the radiation transmitted by the valve. After the acquisition of the hologram of a single diatom valve in a typical Mach-Zender interferometer and the application of a proper algorithm (described in detail in Di Caprio et al., 2014), the intensity and phase of the optical field interacting with it can be retrieved at every point of the direction of propagation of the laser beam. Among the advantages of this method, there is the possibility to analyze light propagation in a medium different than air, just substituting the proper refractive index in the reconstruction algorithm. Both DH measurements and previous numerical simulations based on Wide Angle Beam Propagation Method (De Tommasi et al., 2010; Ferrara et al., 2014) demonstrated that, in water and in cytoplasm, the confinement of incoming light takes place closer to the valve if compared to air, thus increasing light collection inside the cell.

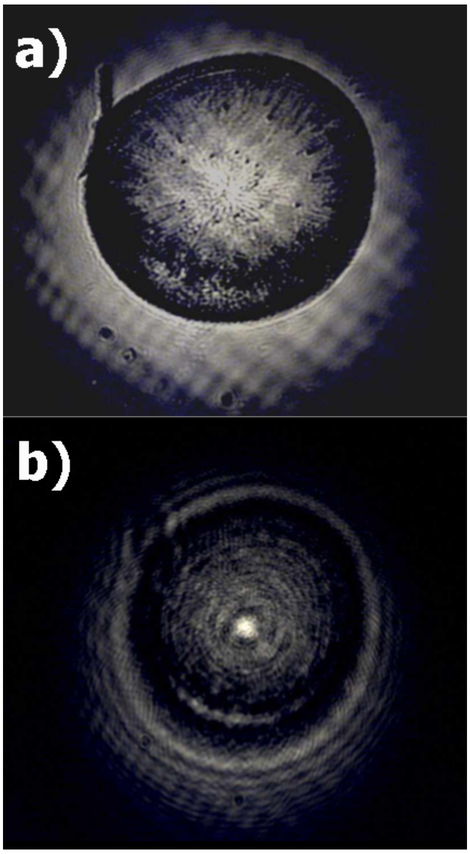

Fig. 12. "Focusing effect" by a single valve of *Coscinodiscus wailesii* diatom. Image acquired at 4 (a) and 104 (b) microns from the valve Courtesy of De Stefano et al., 2007.

The diffractive character of the effect was scrutinized by Noyes et al. (Noyes et al., 2008). An Euler cradle (traditionally applied in X-ray diffraction characterization of crystals), allowed to analyze intensity and orientation of light transmitted by single valves of *C. wailesii* diatoms, after irradiation with blue (472 nm), green (543 nm) and red (633 nm) laser light. Red light transmission was more efficient than blue and green ones, by a factor of 3.5 and 2.5 respectively. Light which is not transmitted, reflected or scattered by the valve, is supposed to couple to girdle and valves (acting as sort of waveguides) as already experimentally demonstrated by Fuhrmann et al. (Fuhrmann et al., 2004). It is noteworthy that, in the spectral range between 630 and 675 nm, we can find a peak in the absorption spectrum of chlorophyll *a*, one of the main molecules involved in photosynthesis (Kirk, 2011). Since diatoms interact with sunlight and not with laser radiation, a lot of work has been performed in recent years and by several authors (De Tommasi et al., 2010; Hsu et al., 2012; Ferrara et al., 2014; Romann et al., 2015) making use of incoherent sources of illumination. The obtained results confirm the presence of the same focusing effect, which is characterized by a precise and reproducible behavior as a function of the incoming wavelength, also confirmed by numerical simulations. The longer the wavelength, indeed, the closer to the valve the "focal spot" takes place, which is easily understandable if we look at the dependence of diffraction angle from a circular aperture (in this case the single pore of the valve) by the wavelength of the incoming radiation (Born and Wolf, 1993). This implies that, for sufficiently short wavelengths, the superposition of diffractive contributions takes place far beyond the frustule or it does not takes place at all. In case of *Arachnoidiscus* sp diatoms, for example, UVB transmitted radiation reaches, in air, the first intensity peak at about 500 μm from the valve, while, on the opposite, for red light we have a first maximum at about 90 μm from the valve and an intensity enhancement of more than three times respect to incident radiation (Ferrara et al., 2014). This behavior is one of the mechanisms by which diatom frustules are able to screen the cell from harmful UV radiation, the other being absorption by the amorphous, hydrated silica of the frustule itself. Actually still another photonic property of diatom frustules results in efficient conversion of harmful UV radiation into photosynthetically active radiation (PAR). Indeed, since diatom frustules are made by nanostructured silica full of surface defects and incorporated organic compounds, they are characterized by intense photoluminescence if irradiated at the proper wavelength (Qin et al., 2008). In particular, they emit blue radiation if irradiated with UVB light, which means that noxious radiation able to promote the formation of pyrimidine dimers in DNA strands is converted into radiation spectrally located in correspondence of one of the maxima of photosynthesis action spectrum (De Tommasi, 2016). Indeed, around 450 nm fucoxanthine (which is one of the main pigments found in diatom chloroplasts) has a significant absorption band.

The ability of centric diatom frustules to couple efficiently to visible radiation and confine it in local "hot spots" may be strictly related to chloroplast mobility as a function of illumination conditions. It is known indeed that, under dim light conditions, chloroplasts of centric diatoms tend to locate close to frustule walls (Furukawa et al., 1998), where light is guided by valves and girdles (as observed in Fuhrmann et al., 2004). On the other side, under intense illumination, chloroplasts migrate towards the centre of the cell, where light confinement has its maximum, this migration being pronounced for blue irradiation (Shihira-Ishikawa et al., 2007). This could represent a double advantage, since it would allow both to protect the nucleus from photodamage and simultaneously collect with high efficiency radiation which fits the absorption spectra of chlorophylls.

5. CONCLUSIONS

Many questions concerning diatom frustule morphogenesis remain unresolved, and only an integrated, multidisciplinary approach, spanning from genetics to geometry and physics, would represent an effective instrument in the understanding of the complex relationships which link frustule formation, shape diversity, and functionalities. Species-specific shapes, dimensions and pore patterns indicate a strict genetic control on frustule morphogenesis and functional analyses of the related proteins are necessary. Such analyses require the ability to generate overexpressing strains and knockout cell wall mutants. The potential applications of such tools in basic diatoms biology research, as well as in applied research are huge. However, all the contributions are complementary: the characterization of biosilica synthesis and the possibility to modulate the expression of the genes encoding for the frustule-associated proteins will provide unique samples for optical, photonic, microfluidic and mechanical analysis in order to understand which shape optimize better light collection, nutrient uptake and mechanical resistance. In this context, shape is the fundamental link between frustule morphogenesis and its functionalities. Finding the more suitable mathematical tool to generate, describe and understand shapes under specific environmental and functional constraints will give an extraordinary contribution in unveiling diatom diversity and dominance as major photosynthetic group in oceans.

COMPETING INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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