CHAPTER 1

Whence Is the Diversity of Diatom Frustules Derived?

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1.1 Introduction

The silica frustules of diatoms have fascinated scientists since van Leeuwenhoek's first reports of their structure in the early 18th century.[1] As microscopy and microbiology have advanced, other protists, along with sponges, have been found to produce silica-based structures, but none with as minute and intricate detail as diatoms. The outer silica diatom frustule became, and largely remains today, the basis for the group's taxonomy, although this is beginning to be supplemented and supplanted by molecular studies.[2–5] During the last 300 years, little attention has been paid to the function of the frustule detail. Work shows that the frustule has protective and ballasting functions,[6,7] but these do not address the function of the intricate detail. There is recent work showing that frustules have good mechanical strength,[8,9] photonic properties,[10] and hypothetical buffering capacity,[11] but again these are gross properties that would exist without resorting to the need for specific detail. There are specialized or rare structures, such as spines and excretion sites, but these are a tiny fraction of the frustule structure. Some work shows that the most surficial frustule details modify particle movement over the frustule surface and diffusion through the frustule.[12–17] However, none of

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this work explains why diatoms have a variety of minute and intricate structures found among their 100 000+ species.

The purpose of this review is to examine work directly and indirectly related to the frustule structure to show what we know about the function of the frustule to date. As such, this is meant to be a jumping-off place for those readers interested in understanding how the frustule interacts with the surrounding chemical environment, and the way that this interaction is influenced by fluid flow and by the cellular processes within the diatom.

1.2 The Frustule in Context

Diatoms have rigid, silicon-based exteriors that are similar to many micro/nanofluidic devices. The surface of the former always possesses distinct surface patterns. Figure 1.1 shows the rigid exterior (frustule) for a diatom, specifically the species *Thalassiosira eccentrica*. This basic structure appears repeatedly in diatoms and may explain their success in a variety of environments. Diatoms form the base of the marine food web and are among the most abundant phototrophs on Earth.[18,19] Their physiology and nutrient uptake capacities are moderately well studied,[20–22] but it is uniformly overlooked that the membranes are recessed below the frustule, essentially layers of what are effectively rigid, but porous patterned grids. The behaviour of particles near or in this grid system is virtually unknown, as is the role of the elaborate geometry. Although these groups are the dominant ocean and freshwater phytoplankton, photosynthetic single cells that drift in the ocean, we still lack fundamental information on how they identify and take up nutrients. Many studies have shown that diatoms are essential to phytoplankton ecology, and their role in the microbial loop and colloid dynamics is unparalleled.[23–26] However, here it will be shown that multiple disciplines

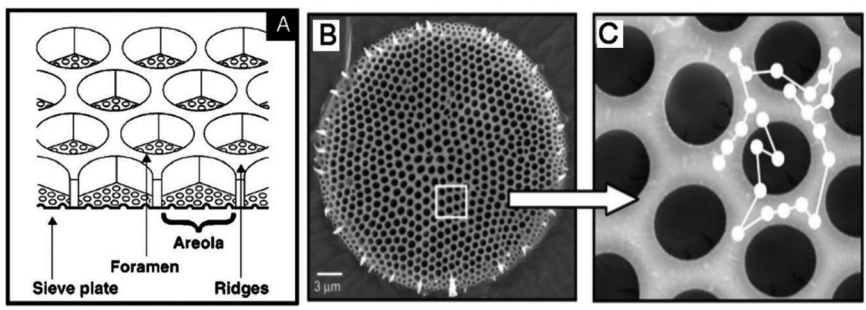

Figure 1.1 Diagram (A) and SEM (B and C) of a silica diatom frustule from *Thalassiosira eccentric* with a path of 0.25 µm particles diffusing over the surface. The opening (foramen) of the areolae is about 1 µm across. Preliminary results indicate that spines (white) along the frustule edge are particle ejection points. Adapted with permission from ref. 12. Copyright 2001 American Chemical Society.

are on the verge of providing insight into fundamental principles of particle surface interactions and that indeed progress has already been made in a variety of areas.

1.2.1 The Chemical Milieu

Oceans and lakes are among the most complex of chemical environments on Earth, particularly when variation is considered over days, years and millennia. This includes the complex chemical composition of colloids and particles. Diatoms have had to cope with particles of all sizes at their surfaces for hundreds of millions of years. The basic tenets of natural selection in a particle-laden ocean suggest that the detailed, rigid patterns of their surfaces may help to control submicrometre particle behaviour near their surface. Cellular biologists do not consider this region and, because it is too small to fall within the realm of oceanography, it falls between discipline boundaries; thus, there are relatively few papers to review. However, this region may be crucial for understanding diatom ecology and physiology, since this is the region from which nutrients are drawn for uptake and in which pathogens and fouling bacteria and chemicals attach. It is also a region that is crucial to understanding micro/nanofluidics for microchip analysis methods[27] and nanostructure assemblage.[28,29] In fact, diatom frustules can be regarded as a prototype of a natural silica-based 3D microfluidic system.

The appreciation of diatoms as key components of the biosphere continues to increase. They fix 25% of global organic carbon and oxygen, host nitrogen-fixing symbionts, and migrate vertically more than a kilometre to transfer inorganic nitrogen to the ocean surface.[30,31] For this perspective, they fix more carbon than all of the rainforests combined.[31] They reproduce and are consumed rapidly. The result is that the carbon they fix is rapidly passed through the food web compared to trees, grasses and seaweed, making them the primary biomass source of many marine, river, lake and some soil ecosystems.[31,32] Their importance far exceeds that of other microalgae. An important reason for their critical role in the biosphere appears to be their ability to precisely use silica to form complex frustules, which over evolutionary time have adapted to changing environments and chemical milieu.

Understanding how diatoms use their nanostructures to compete in ecosystems has become increasingly important as the importance of diatoms in the global food web and in biogeochemical processes has been realized. As stated above, they account for 25% of all primary production on Earth.[33,34] They are the primary cyclers of silica in the ocean.[33,34] Physiologically, they are the only group in which cadmium metalloenzymes have been found, and these are now known to be widespread as an apparent substitute for zinc in beta-class carbonic anhydrase.[35,36] This opens an unexplored area of how any cell can handle cadmium and avoid toxicity. On the opposite side of toxicity, diatoms produce toxins that can cause permanent memory loss in humans.[37,38] They are also models for nanotechnology and nanofluidics.[17] In short, diatoms play key roles in global productivity, climate change,

enzymology and human health, yet the functions of the intricate and unique designs of the frustules of this important group have been little investigated or understood. However, work on external carbonic anhydrase shows increasingly that a chemical advantage of silica is that it is an appropriately buffered chemical surface where reactions can take place more efficiently than they do inside the cell.

1.2.2 Why Are Diatom Frustules Only Now Being Appreciated?

Traditionally, research on diatoms and other environmental microbes has focused on constant laboratory conditions or monitored a narrow range of factors for environmental microbes without controlling the stimuli. For phototrophs, the emphasis is most often on light and nutrients. Because these two parameters are important to all phototrophs, they do not always provide the information resolution to discriminate why particular species dominate or how the cells that do dominate function. For example, there are relatively few papers on the influence of turbulence on microbial phototroph function, yet the few that there are consistently show a dramatic impact on growth and competition, particularly with regards to vastly different frustule structures.[39] Diatoms, for example, out-compete other microalgae in the presence of shear. Mitchell *et al.* proposed a mechanism (Figure 1.2) to explain this difference, but there has been no experimental testing or confirmation of how flow moves nutrients to cells, thus changing the surface dynamics.[40] Confer and Logan[41] did show that molecule size is important, and this was confirmed by others, but these studies were either on pure bacterial systems or focused on the bacterial nutrient flux and not on the diatoms. Furthermore, experimental microbes are almost invariably laboratory strains, whose constant growth conditions specifically select against the metabolic flexibility necessary to survive in the environment, which at best makes them the least likely to provide insights and at worst can lead researchers in the wrong direction for years or decades.

1.2.3 Paradigm of Porosity: Why Frustule Detail Matters

We know the factors that are fundamentally important for microbial growth, particularly phytoplankton, but we are unable to culture most species. For the diatoms that we can culture, controlling their growth, health and sexual reproduction is difficult to impossible. There are then subtleties to the concentration, timing or order of the fundamental factors that we do not appreciate. The need for a more nuanced appreciation of marine phototrophs and microbes in general was recently pointed out[42] for improving understanding, modelling and planning with regards to environmental change and prediction. One conclusion to be drawn from the work of Worden *et al.* is that our paradigms for diatom frustules are complete only at the coarsest levels and that if progress

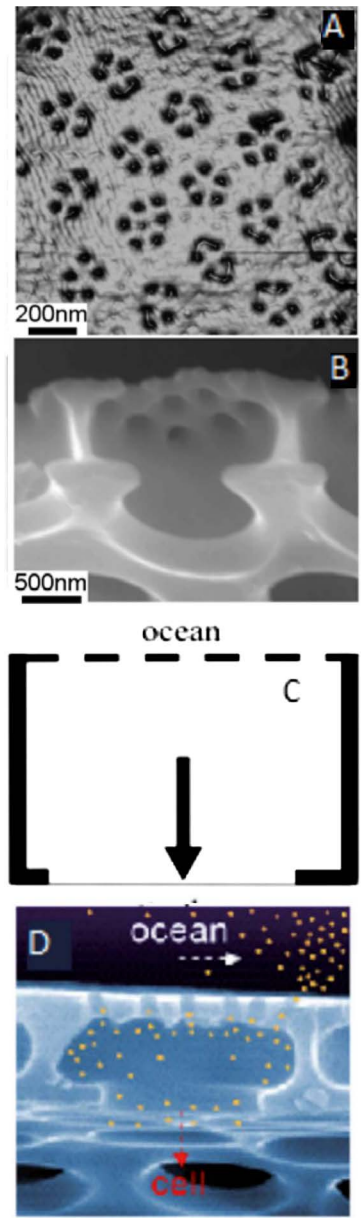

Figure 1.2 *Coscinodiscus* sp. (A) A close-up of the outer surface, (B) a cut away of the frustule showing the intrafrustule chamber, and (C) a schematic where the arrow indicates the net flux of a nutrient filled chamber. The directionality is provided by the greater diffusive resistance of the outer surface. (D) A graphical representation of the nutrient uptake process. The light blue is a side-view perspective of the frustule. The white arrow indicates flow. The yellow dots represent nitrate. (Adapted from ref. 40 with permission from PLOS ONE.)

is to be made, the diversity of signals and mechanisms needs to be taken into account in conjunction with subtle changes in the variety of shapes.[42]

1.2.4 Resolving the Porosity

Resolution of current paradigm failings, such as the inability to culture most microbes, appears to depend not so much on new paradigms, but rather on expanding and filling-in existing paradigms to account for hierarchical signals and responses in a complex environment. For too long have the nutrient environments been simplified to maximize growth over a short period. This rapid growth spurt, a bloom in the case of diatoms and other phytoplankton, is only one, often rare, part of what we increasingly understand are complex life cycles. The diatom frustule is present all of the time and must be useful across a wide range of conditions and not just in the supra-abundant nutrient conditions that most cultures imitate.

1.2.5 Chemical *versus* Physical Balance

How do cells prioritize vastly different signals? For multiple chemical signals, only the biochemical pathways and kinetics can provide insight into the preferred pathways. However, cells do not solely experience chemical inputs. Eppley *et al.*[43] in a classic experiment showed that diatoms at a tenth of the concentration of dinoflagellates would outgrow the dinoflagellates in the presence of turbulence and eventually exclude them from the culture. Gibson and Thomas[44] have shown that dinoflagellates are inhibited by turbulence, and Peters *et al.*[39] have shown that some diatom species grow best at low turbulence and some grow best at high turbulence. The mechanisms through which turbulence alters growth and makes cells more or less competitive are unknown. However, two factors about turbulence are clear. First, the turbulence overrides population abundance disadvantages, as well as favourable light and nutrient conditions. Second, unlike chemicals, which can have a variety of effects inside a cell, turbulence, or more accurately the shear produced from turbulence, can only act at the diatom surface, which is to say on the frustule. Given that the turbulence advantage of diatoms over dinoflagellates has been repeatedly established during the last 50 years, it is reasonable to investigate the cause of this, rather than continuing to leave it at the observational level.

1.2.6 Shrinking Diatoms

The silica frustule around diatoms with new matching frustules created inside the cell means that as they divide the cells become small. The size decrease continues until limits are reached, *e.g.* one chloroplast and one mitochondrion. The cell cannot be smaller than the nucleus and some ribosomes are necessary. However, the diameter decrease can be more than a factor of 10. As this decrease occurs, the relative ratios of different cell components change in number, volume and surface-to-volume ratio.

These changes can be followed by a variety of methods. Diatoms restore their largest size through sexual reproduction. The signals that trigger this are unclear for almost all diatom species.

1.3 Applying Diatom Frustule Information

To understand the utility of diatoms in micro/nanofluidics it is important to realise that their surfaces are exposed to a huge variety of particle sizes, concentrations and types. The largest and least abundant are bacteria at up to 1 μm and 10^7 cells per ml, while the smallest and most abundant are nutrient molecules, such as nitrate, at up to 10^{16} molecules per ml.[19,45] Intermediate in concentration and size are viruses, colloids and macromolecules.[18,23–26,46,47] Here, we refer to all of these groups as particles. Diatoms must deal with this entire size range as food, signals, fouling components or pathogens. Mitchell *et al.*,[40] based on earlier work by Confer and Logan,[41] proposed a general mechanism for the mechanism by which diatoms are able to passively (no moving components) sort and process this diverse range of particles, but much theoretical and experimental expansion is needed to provide a thorough understanding of this system.

1.3.1 Linking Diatoms to Lab-on-a-chip Systems

A success of the past decade has been the concerted efforts from a wide variety of international researchers to put laboratory analyses, particularly those relating to DNA processing, onto microelectronic silicon chips.[48] The electronics are well understood, but the handling and behaviour of submicrolitre volumes of particle-laden fluids needs extensive development if this technology is to achieve the easy and ubiquitous use of computer chips. The current state-of-the-art approach to achieving particle control in microfluidics primarily relies on the interaction between particles in a purified sample passing through, not over, patterned obstructions[48,49] or by dielectrophoresis.[50,51] Use of these fluid-particle systems is limited and presents considerable technical challenges, particularly for biological samples where mixtures of complex molecules are common.[52–54] In particular, particles in microfluidic systems continually encounter channel walls because of high surface area to volume ratios and the effectiveness of molecular diffusion over micrometre distances. Unfortunately, particle–surface interactions have produced unpredictable or inexplicable results.[55] Experimental systems in this area have used flat surfaces and focused on how van der Waals and electrostatic forces control particle–surface interactions.[56–59]

1.3.2 Particle Movement at the Nanoscale

In fluid systems, ranging from blood to the ocean, however, high salt concentrations reduce the Debye-Huckel length for electrostatic forces to the same distance as for van der Waals forces, less than a few nanometres.[60,61] This leaves surface-induced drag on Brownian particles as the dominant process

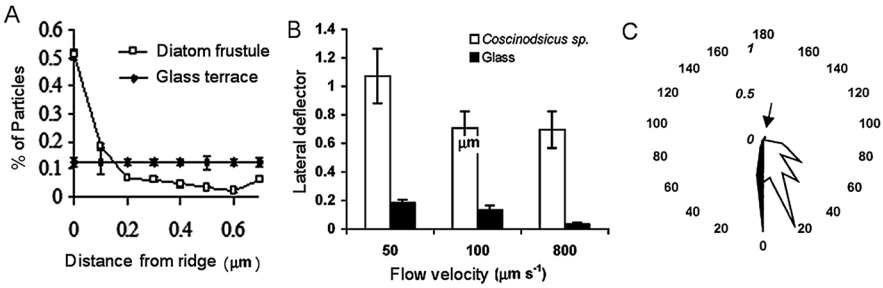

Figure 1.3 (A) Position of 0.46 μm diameter particles relative to the areolar ridge. Similar results have been obtained for 0.05 to 2 μm particles. (B) Lateral deflection of 0.46 μm beads as a fraction of forward flow in a diatom. Adapted with permission from ref. 12. Copyright 2001 American Chemical Society. (C) Map view of a diatom surface in 50 μm s^{-1} flow. The degrees of deflection are around the edge and the proportion of particles for the diatom (white) and glass slide (black) were normalised to the maximum particle bin for each. Adapted with permission from ref. 14. Copyright 2002 American Chemical Society. Bin size was 10 degrees of deflection. Minimum of 30 particles per point in (A), (B) and (C). Error bars are 95% confidence intervals.

at distances greater than a few nanometres from surfaces.[62,63] We have shown that the microtopography of diatom surfaces controls particle movement in very specific ways that appear consistent with surface-induced drag (Figure 1.3).[12–14] Figure 1.3A shows how microtopography localizes particles above the diatom frustule ridges compared to flat glass, where there is no microtopography and no localization. In contrast to the still environment of Figure 1.3A–C show an added effect of microtopography when the overlying fluid is moving. The microtopography laterally deflects particles across streamlines. The strength of the deflection is size dependent, so there is the potential for sorting of submicrometre particles based on channel microtopography. We find the same particle behaviour on live diatoms, diatom frustules and patterned mimics made of silicon, indicating that topography is a key factor in particle control.[12,13,64] The resultant particle sorting was observed whether movement was by Brownian motion or from fluid flow, but it is easier to detect and study in fluid flow. Modifying the topography and adding polymers that interfere with particle movement alters the strength and direction of the sorting effect.

1.3.3 Ongoing Development

Colloidal flow is especially important for biomedical point-of-care lab-on-a-chip systems, which rely on the movement and filtration of whole blood samples that are laden with a range of particle sizes. These systems still need accurate and quick diagnosis from small blood samples and will thus benefit greatly from efficient particle handling systems. Similarly, portable pollution

or bio-terrorism detection systems rely on handling, sorting, and concentrating particles of interest using similar methods to those used by diatoms. Thus, detailed understanding of particle–diatom interactions may reveal principles and patterns that will be extremely useful in designing micro/nanofluidic components and "lab-on-a-chip" systems. Apart from particle control, diatom microtopography could be applied to the design of microchannels to enhance fluid mixing, which remains difficult to achieve without the help of expensive active mixers or long mixing lengths ($\gg$1 cm). Chaotic mixing has been demonstrated in microchannels using bas-relief structures similar to diatom frustules on channel floors.[27]

1.3.4 Imaging Diatom Structures

The continued increase in the sensitivity of CCD cameras and improved tracking software have continued to improve our ability to follow submicrometre particles over distances of tens of micrometres with light microscopy.[65–69] Light microscopy provides information on particle dynamics, including over diatom surfaces. 3D control is achieved through the use of confocal laser optics. However, for investigating the structures underlying the mechanisms of the dynamics, atomic force microscopy (AFM), which generates surface images by "feeling" with a sharp probe rather than "looking" at the sample surfaces, is superior. AFM is able to image biological systems in real time, with nanometre scale resolution, under natural conditions.[70,71] It has the added advantages of being able to measure friction between the tip or a microparticle attached to the tip. The probe tip can further be functionalized to achieve control over surface chemistry and to study its dependence on adhesion forces.[72–74]

AFM has been applied by Losic *et al.* to examine the frustule surfaces of marine diatoms, including *Coscinodiscus species* and *Thalassiosira eccentrica* (Figure 1.4).[17,75] Images of the frustule surfaces showed that the frustule silica not only exhibited microstructures, but also complex nanoscale patterning of the frustule topography, suggesting that particle localization and control of molecules also occurs (Figure 1.4A–C). The nanotopography on the outer frustule surface (cribellum and cribrum) was composed of hexagonally grouped silica nodules and perforations. Images of the inner surface revealed radial channels, which could facilitate flow between the plasma membrane and the frustule surface (Figure 1.4D). A subtlety of imaging is mimicking the interactions between the frustule surface and particles of different sizes when comparing images acquired with 5–20 nm radius tips and images captured using a 300 nm radius colloid probe. Different sized probes encountered different aspects of frustule structure (Figure 1.4E–F) and shed light on the differential treatment of submicron particles by diatoms.[17,75] Comparison between images of the surface topography and the corresponding subtracted friction images revealed that lateral adhesion did vary at different structural positions on the frustule (Figure 1.4G–H). These differences did not result from tripping of the tip or surface contamination.

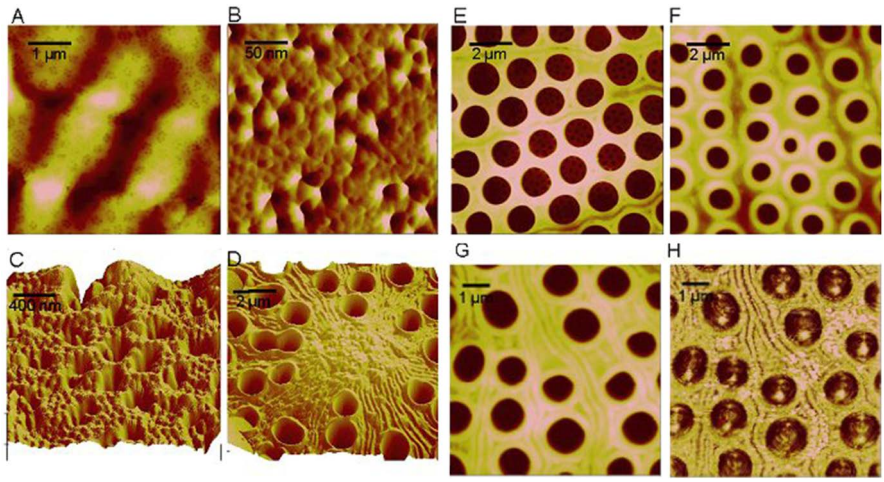

Figure 1.4 (A) Height image of the cribellum surface in tapping mode (TM) atomic force microscopy. (B) Deflection image of the cribellum surface in contact mode (CM) (zoom). (C) Height image of the cribrum layer in TM. (D) Height image of the center of the inner frustule surface in CM. (E) Height image of the inner frustule surface in TM. (F) Inner frustule surface (CM with colloid probe of 300 nm radius, foramen surrounded by lip artefact). (G) Height image of inner frustule surface in CM. (H) Friction map (trace-retrace) of the same spot as in G. (Used with permission from J. G. Mitchell, D. Losic, and N. H. Volecker.)

These results showed that the nanostructure present on the frustule is indeed of crucial importance for differential treatment of particles >100 nm (bacteria, viruses) and nutrients by diatoms. The observation of this nanostructure in the AFM images was consistent with research into diatom silica biomineralisation, as it confirmed the hexagonal arrangement of self-identical patterning in the frustule and the presence of silica nanoparticles.[76–78] The continued tracking of particles across these surfaces will require comprehensive understanding of flow around the structures and the ability to perform 3D tracking of particles within the flow with increasingly refined analysis.[79–81]

1.3.5 Exploring Diatom Diversity

Microbes have been cultured for over a century and growing diatoms in bulk culture is well established[82] to the point that the recipes and cells grown are standardized.[31] This has led to hundreds of studies that look at bulk features, such as culture growth rate, chlorophyll concentration, photosynthetic rates, silification rates and many other processes.[83] However, the extensive work by many investigators over the last half century has been primarily on cells long in culture or infrequently reisolated. The 'isolation' process and artificial nature of the experiments makes extrapolation to natural or industrial microbial communities difficult. The transition began with ref. 84–86, where it was found that salinity alters the pore structure and

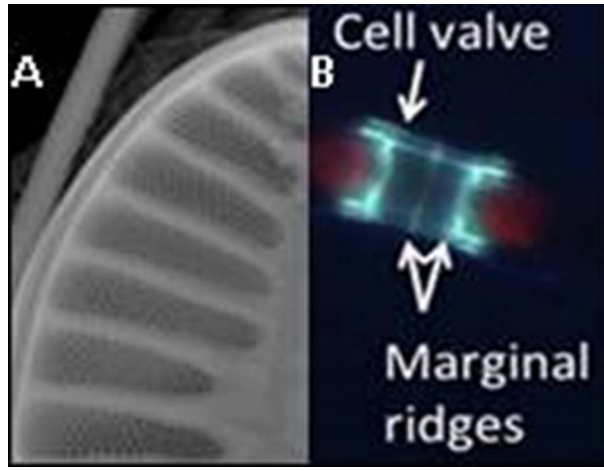

Figure 1.5 (A) Diatom analysis by the proposal team (Leterme *et al.*[84,85]) for the measurement of areolar circularity as a physiological indicator of salinity stress in *Cocconeis placentula*, with samples from the Coorong, South Australia Ramsar site, and (B) image showing the fluorescent tracking of frustule formation in a live diatom *Lithodesmium undulatum*. ((A) with permission from S. C. Leterme *et al.* and (B) with permission from ref. 86, PLOS ONE).

by inference the particle handling properties, and that these change during cell growth as well. As an added complication, the frustule is made elastic and hardens over time, allowing for changing particle and chemical interactions.[86] Figure 1.5 shows the natural populations studied and the increased diversity under study.

1.4 Conclusions

As a specific conclusion, increasingly the nanoscale detail of the frustule is associated with essential physical and chemical functions of the cell. It is a complex structure that has been found to contain nutrient traps and particle channels for nutrient processing. Although the time scale is slow, it changes in stiffness, and subtly in dimensions over time. The variation is likely to reflect a variety of functions that include nutrient uptake, nutrient sorting, viral protection, bacterial protection and chemical protection.

As a general conclusion, the frustule defines a cell and controls the passage of molecules. It is the key biological barrier in diatom function. The frustule as a barrier is made rigid and precise with silica. The precision allows a hierarchy of choice and exclusion. Diatoms are ideal to study micro- and nanoscale manipulations because their geometric regularity makes changes apparent, their production of 20% of global organic carbon and oxygen makes them key in the biosphere, and their occurrence from sunlit soils to the open ocean makes the principles learned general across most of the biosphere, as well as in chemistry and engineering applications.

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