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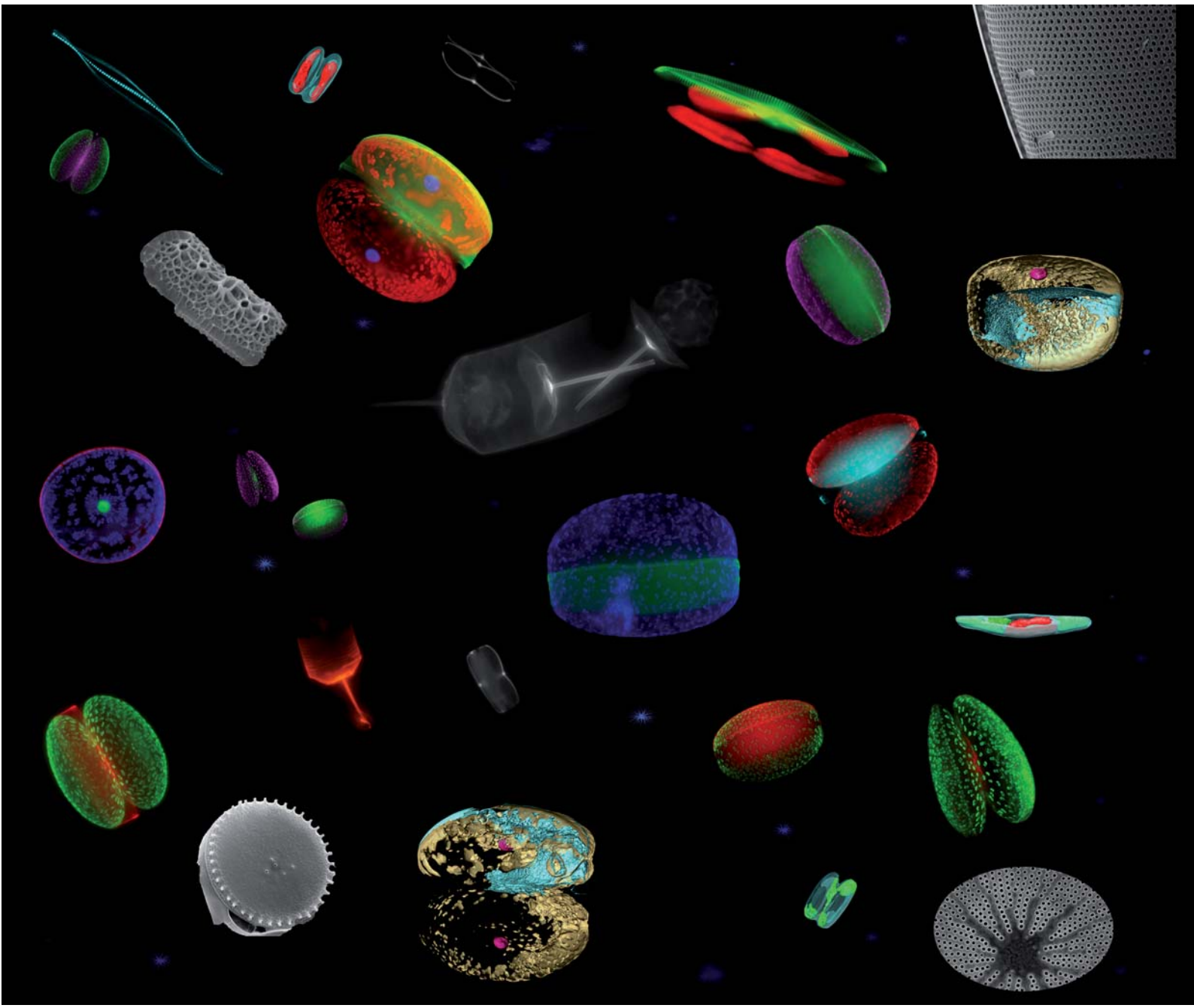

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CRITICAL REVIEW

Diatoms: Self assembled silica nanostructures, and templates for bio/chemical sensors and biomimetic membranes

Wenrong Yang,*[ab] Pascal J. Lopez[c] and Gary Rosengarten*[a]

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In this review we highlight recent advances in the understanding of biosilica production, biomodification of diatom frustules and their subsequent applications in bio/chemical sensors, and as a model membrane for filtration and separation.

Introduction

Diatoms are unicellular marine organisms that have an amazing self-assembled micro- and nanoporous silica outer thecae (called a frustule). They use photosynthesis as an energy source and convert dissolved carbon dioxide into sugars. They are essential to all life on earth, producing in the order of 20% of the oxygen we breathe by capturing atmospheric carbon.[1] When diatoms die, because their silica shell ensures they are denser than water, they sink to the bottom of the ocean, thus, in effect, producing a massive carbon dioxide sink. As diatoms are photosynthetic they can live within the top ~200 m of water where sunlight can penetrate (known as the Euphotic zone), but generally reside much closer to the surface. They are the predominant photosynthetic organism in both fresh and sea water and have even been attributed to cause the largest global cooling event in the last 100 million years.[2]

Diatoms also have a rich research history due to their unique silica shell which acts as an indicator for water quality,[3–5] and because of their abundance and critical roles in the earth's carbon cycle,[2,6] because they are a model for self assembled nanotechnology and biomimetics[7–10] and because very recently they have been proposed as a sustainable source of biofuel.[11–14] The genome of three simple diatoms has even been sequenced indicating, amongst other things, the genes that help in biosilica production and those that allow diatoms to thrive in aquatic environments.[15,31]

In the last ten years or so, since the explosion of nanotechnology as a separate science, there have been various reviews

[a]*School of Mechanical Engineering, University of New South Wales, Sydney, NSW, 2052, Australia. E-mail: wenrong.yang@sydney.edu.au; g.rosengarten@unsw.edu.au*
[b]*Australia centre for microscopy & microanalysis, The University of Sydney, Sydney, NSW, 2006, Australia*
[c]*CNRS UMR-8197, Ecole Normale Supérieure, 46 rue d'Ulm, 75005 Paris, France*

Wenrong Yang

Wenrong Yang received his PhD degree in chemistry in 2002 from the University of New South Wales under the mentorship of Prof. Justin Gooding and Prof. Bryn Hibbert. He worked then at Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO) as a CSIRO post-doctoral fellow before returning to UNSW in 2005. He was awarded a University of Sydney Research Fellowship and joined the University of Sydney in 2007. His research interest involves nanostructuring surfaces to provide them with unique functionality, nanomaterial-based biosensors and molecular electronics.

Pascal J. Lopez

Pascal Jean Lopez heads the Diatom Biomineralization and Morphogenesis group at the Ecole Normale Supérieure, Paris (France). He obtained is Ph.D. in Microbiology from the University of Paris IX-Orsay (France) and completed a post-doctoral position in the Gene Expression Unit at the European Molecular Biology Laboratory, Heidelberg (Germany). His research interests include genomic and cell biology studies of the impact of environmental factors on silica morphogenesis, and the development of biophysical approaches to characterize biomaterials.

focusing on the unique diatom biosilica frustule (for example references 11 and 16–22) and the possible insights we can gain from their nano-structure. In this review we focus specifically on recent advances in the understanding of the biosilica production, biomodification of diatom frustules and their subsequent applications in biosensors, and on the interaction of fluids with the frustule and its role as a membrane.

Diatom structure

There are, purportedly, over 100 000 species of diatoms[23] but their classification and taxonomy is still under debate.[24,25] Their shapes and sizes vary from circular to triangular and from approximately five micrometres to a few hundred micrometres. They are broadly categorised into those having radial symmetry of the nanostructured pore pattern (centric) or bilateral symmetry (pennate). Examples of some nanostructures found in centric diatoms are shown in Fig. 1.

Diatoms possess the typical characteristics of standard plant cells: a nucleus that contains the DNA, mitochondria, chloroplasts for photosynthesis and a cell membrane. What they have that is unique is the extracellular porous rigid silica outer-shell or frustule. A schematic representation of the three dimensional structure of a centric diatom, such as a *Coscinodiscus*, is shown in Fig. 2. The frustule is made up of two caps called valves joined by a cylindrical section called the girdle bands. Diatoms reproduce primarily by mitotic divisions which are constrained by the rigid cell wall. The mother cell divides with one valve contributing to each of the two daughter cells. Thus a new valve is produced for each reproductive cycle and the new generation gets smaller until a critical size is reached and sexual reproduction (generally oogamy) is used to increase their size.

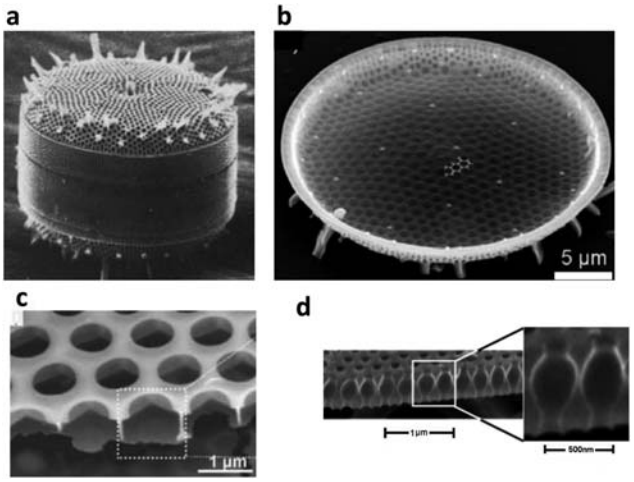

Fig. 1 Example of diatom frustule a) full *Thalassiosira* frustule,[26] b) *Thalassiosira* valve and c) close up of *Thalassiosira* valve pores,[27] d) pore structure of girdle band from *Coscinodiscus*.[28,29]

Surrounding the frustule there is an organic layer coating all the silica which is most often too thin even to be seen in material prepared for TEM sectioning, but it does sometimes obscure detail in SEM images of uncleaned diatoms.[26] The organic layer over the silica is thought to help in preventing silica dissolving.[26] Most diatoms also have a distinct, continuous organic layer of acidic polysaccharides between the siliceous frustule and the plasma membrane called the diatotepum (see Fig. 3). It is postulated that the diatotepum helps keep the silica frustule together and possibly reduce the pore size to be more selective during filtration.[30] Some diatoms, particularly those that attach to surfaces, have a considerable extracellular membrane material that forms the adhesive for attachment.[31] In most centric diatoms the cell wall does not extend into the silica frustule leaving pores open for transport of nutrients.

The intricate nanostructure of a diatom's silica frustule has been linked to a variety of functions including acting as a photonic crystal that guides light to help photosynthesis,[32] being exceedingly strong in order to stave off predators[18] and to counterbalance turgor pressure.[33] The structure is species dependent and is thus genetically controlled. At this stage, other than knowing that the silica structure depends on the chemical environment they are grown in (*e.g.* the salinity), and that energy minimization through self assembly is involved, there is little consensus to the true function of the intricate nano-patterned silica cell wall. Some diatoms have larger pores on the outside such as *Thalassiosira* and some on the inside[27] such as *Coscinodiscus*. While it is not clear why this is the case it may be linked to the environment they reside in, with those living in more turbulent water requiring quicker uptake into a larger 'storage' space where the nutrients can then diffuse into the plasma membrane (bigger pores outside). Further keys in understanding the role of the silica frustules are linked to understanding the silica formation process.

Diatom biomineralization

Over the last decade several complementary approaches have allowed important progress in our understanding of diatom biomineralization. The development of molecular tools,[34] (for a review see[35]), and then the sequencing of the complete genome of currently three diatoms: *Phaeodactylum tricornutum*,[36] *Thalassiosira pseudonana*[37] and *Fragilariopsis cylindrus* (available at http://genome.jgi-psf.org/Fracy1/Fracy1.home.html), have been essential steps. Among the other important approaches was the development of fluorescent probes used to label the silica shells. The first reported dye was the rhodamine-123 which is permeant to cell membranes, and typically concentrates in acidic

Gary Rosengarten

Gary Rosengarten heads the micro and nanotransport phenomena group in the School of Mechanical and Manufacturing Engineering at the University of New South Wales, Australia. He completed honours degrees in Physics and Mechanical Engineering at Monash University, and a Ph.D. at the University of NSW in Mechanical Engineering. His research interests include fluid flow and heat transfer for micro- and nano-systems specifically related to energy systems and surface effects in micro-fluidics.

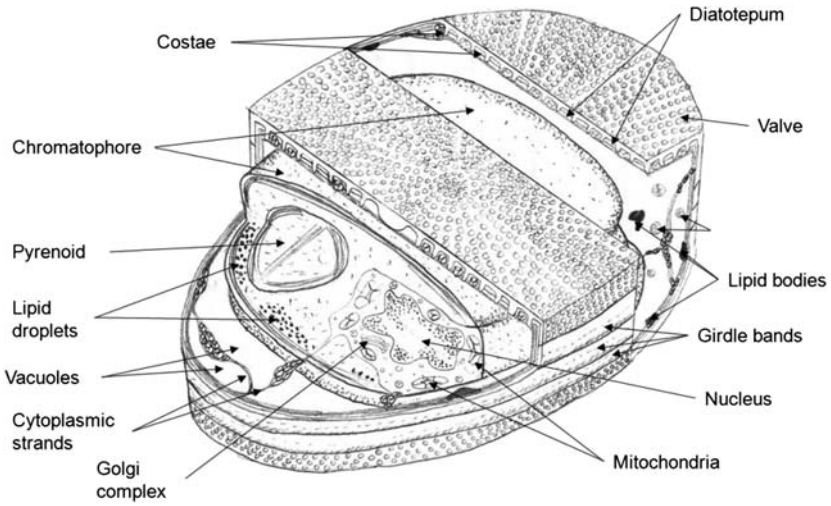

Fig. 2 Schematic representation of a typical centric diatom, indicating the major components.

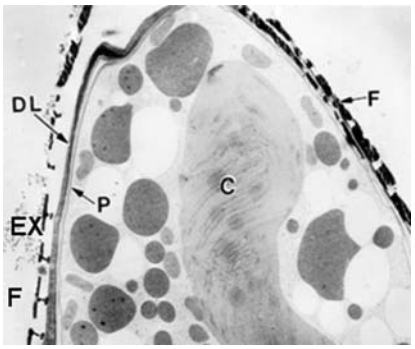

Fig. 3 TEM image of a cryopreserved adhesive diatom (Bacillariophyceae) showing the diatotepum, plasma membrane P, chromatophore C, frustule F, and extra cellular material (EX) that acts as a surface adhesive.[31] The organic casing is not visible.

organelles. Hence, because the compartment within which frustule morphogenesis occurs, namely the Silica Deposition Vesicle (*i.e.*, SDV), is acidic the rhodamine-123 accumulates inside the SDV and becomes entrapped within the newly synthesized silica structures.[38,39] Other weakly basic amines that selectively accumulate in cellular compartments with low internal, pH were also tested. Among, them, two particular dyes named DND-160[40–42] and HCK-123[43] (see Fig. 4) were shown to be particularly useful, giving for example, new information on the process of silica morphogenesis.[44,45]

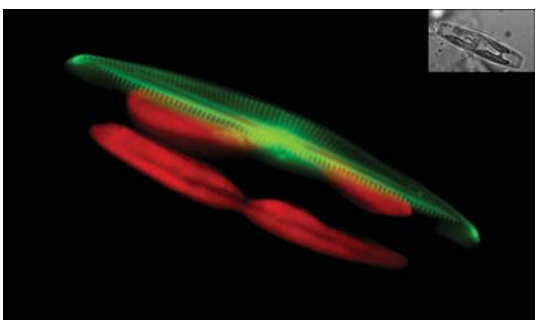

Fig. 4 Fluorescent labelling of newly synthesized silica structure in a pennate diatom. *Seminavis robusta* cells were grown in the presence of the fluorescent dye (HCK-123). After *ca.* 12 h, one valve of a dividing cell is labelled. Some details of the frustule (the central nodule, the raphe structure and the striae) can be clearly observed. In green the silica reporter, red the chloroplast auto-fluorescence, and the insert corresponds to the Nomarsky image.

One of the most successful approaches relied on the purification of the organic components associated to the frustule. This procedure involves the development of protocols to first dissociate the cell-wall components and then to dissolve the silica phase. Experiments of characterization of the organic components entrapped inside the biomaterials have identified proteins, polysaccharides and long chain polyamines (LCPAs).[46,47]

It was shown that each diatom contains a specific mixture of LCPAs that have been characterized as poly(propyleneimine) chains with up to 20 repeat units, attached to putrescine, 1,3-diaminopropane or spermine.[48] Interestingly, depending on the LCPAs fraction used it was possible to control the formation of nanostructured silica *in vitro*.[49,50] In fact, both the methylation pattern and the polyamine chain length are likely to influence their ability to promote silica condensation rate, and therefore to play a role in creating species-specific silica nanostructures.[51] The first cloned silaffins (silica affinity) gene came from the pennate diatom *Cylindrotheca fusiformis*.[52] It was shown that peptides derived from Cf-Sil1 can enhance the precipitation of silica particles *in vitro*.[52–56] A second Silaffins (Cf-Sil2) was also purified from this species but the gene has not yet been cloned. Three different silaffins have also been characterized from the model specie *T. pseudonana*.[57] Even if silaffins do not show sequence similarity, they present domains enriched in lysines and serines residues. Another common feature, likely to be essential in silica polycondensation and assembly, is the presence of different kind of post-transcriptional modification including phosphorylation, methylation, hydroxylation, glycosylation and sulfation.[58] A highly acidic phosphoprotein, named silacidins, was isolated from *T. pseudonana*.[59] From *in vitro* experiments obtained with different combinations of polyamine/silcacidin to precipitate silica from a silicic acid solution, it was proposed that silacidins may serve as the polyanion required for silica formation directed by polyamines (and/or silaffins) *in vivo*.

Recently, Brunner and colleagues[60] were able to purify chitin fibers after dissolution of *T. pseudonana* silica frustules. Remarkably they show that the network-like chitin-based scaffold resembles the size and shape of the *T. pseudonana* biosilica. Knowledge on the organic material present in the silica biomaterial of diatoms has been used to develop a number of applications. In particular one specific peptide inspired from Cf-Sil1 and named R5, was used to control of silica nano-patterning[61] or to perform enzymes immobilization.[62–67] Silica encapsulation can often stabilize an enzyme's activity,[68] making it an interesting and cost-effective route to develop new biosensors. Another attractive possibility to directly incorporate proteins within the silica matrix was to use the capability of diatom cells to target specific proteins to the frustule. One study has been published on the genetic manipulation of diatom silica wall biogenesis *per se*. Fusion between the C-terminal region of a silaffin (Tp-Sil3) and either the GFP reporter or an enzyme involved in Hydroxylamino aromatic compounds degradation (HabB) were constructed and used to transform the diatom *T. pseudonana*. It was shown that in some transformants the fusion protein was targeted to the frustule and that such incorporation into the silica biomaterial decreases the GFP photobleaching or stabilizes the hydroxylaminobenzene mutase activity.[57] Genetic engineering combined with further information on the network of genes involved in the silicon metabolism and frustule formation should find some potential uses for biotechnology and materials science.

Molecular-level bio-modification of diatom frustules

Recent developments in chemical modification and bio-functionalization of silica have made it possible to generate a new class of bioactive silica nanostructures that can be conjugated with biomolecules such as proteins. The availability of these biomodified nano-constructs has opened up entirely new and exciting research directions in the field of biosensors. The rich chemistry of the silica structure of the diatom frustule provides great potential for bio-interfacing with biomolecules. Free hydroxyl groups on the surface can be used for chemical modification of the surface and subsequent tethering of biological or chemical moieties. Popular methods use long-chain alkane thiols to create self-assembled monolayers on the metal surface such as gold. The attractiveness of thiol chemistry is that well ordered monolayers can be formed relatively easily, with a reasonably strong bond formed between the organic molecule and the surface, and that a diverse range of molecules can be synthesized with which to modify a surface. However, these have compromised long-term stability of surface coatings. The advantages of thiol chemistry are somewhat offset by a number of disadvantages, including alkanethiols being oxidatively or reductively desorbed. Other disadvantages include: alkanethiols being desorbed at temperatures over 100 °C. In contrast, silanes are tightly bound through covalent bonds with the surface, and silane molecules can link to each other as well as the surface to form a polymer network within the coating. This means that functionalized silanes provide a more robust layer, which is essential for maintaining adhesion and strength for the grafting of relatively large biological moieties. In addition, silanes are good chelating ligands because of their reactivity with hydroxyls, and they can couple organic groups to virtually any oxide surface. The frustule is hydrated glass: $SiO_2 \cdot nH_2O$, which has free hydroxyl groups on the surface. These reactive groups allow the chemical modification of the surface and subsequent functionalization.

The immobilisation of a biological sensing element (such as an enzyme, DNA, antibody or cell) with a transducer, either electrochemical, optical or piezoelectric, is the basis of a biosensor. There are two basic approaches to immobilising biomolecules onto a surface using the characteristic of silane to self-assemble on silica surfaces: (1). The most common non-covalent approach to immobilise biomolecules to the surface has been *via* electrostatic binding. This simple and gentle method provides the potential for control over the orientation of the immobilised biomolecules depending on the charge distribution of the biomolecules. The major drawback of electrostatic binding is that the strength of the bond is dependent on the solution conditions. Changes in ionic strength and pH can cause the protein to be lost from the surface. (2). Direct covalent attachment has the greatest potential for the development of biosensors due to the stability of the resultant covalent bond. A popular and highly versatile method for covalently attaching biomolecules to the surfaces is the use of carbodiimide coupling chemistry which couples amines to carboxylic acids. In the reaction N-ethyl-*N*-[dimethylaminopropyl] carbodiimide (EDC) converts the carboxylic acid into a reactive intermediate which is susceptible to attack by amines. In some cases EDC and N-hydroxysuccinimide (NHS) or N-hydroxysulfosuccinimide (NHS) are used as they produce a more stable reactive intermediate which has been shown to give a greater reaction yield[69,70] (see Fig. 5).

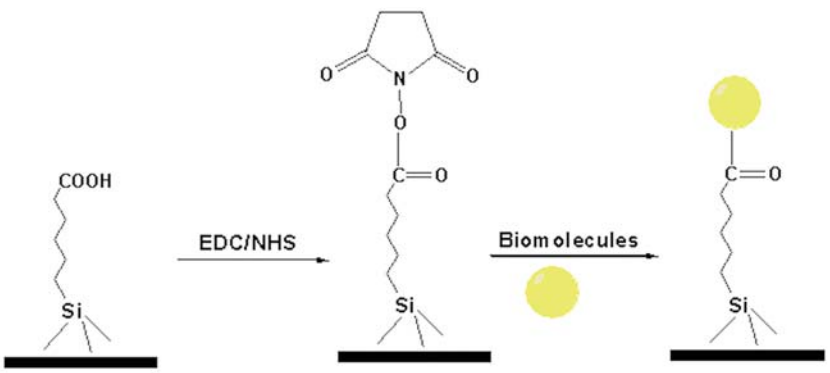

Fig. 5 Schematic diagram showing the covalent attachment of a biomolecule to a SAM using EDC and NHS.

Silanes were, for example, shown to be useful to label diatom frustules either starting with purified biomaterials or live cells.[39] The surface control provided over the molecular architecture of the sensing interface fabricated by using self-assembled monolayers of silane has been used for fabrication of elegant complex sensors. More exciting developments with nanostructured materials such as diatoms are expected in the near future with regard to biosensors.

Townley *et al.* used 3-aminopropyl trimethoxy silane (APS) to silanize diatom surfaces followed by reaction with the heterobifunctional crosslinker N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOS).[71] The crosslinker provides an amine-reactive N-hydroxysuccinimide (NHS) ester with a photoactivatable nitrophenyl azide, enabling antibodies to be tethered *via* their amine groups when exposed to UV light. They demonstrated surface modification using *anti*-IgY. The direct coupling of *anti*-IgY to the diatom surface is also of particular interest since IgY does not bind to protein A or protein G. This coupling can be performed through primary amine groups. Whilst very effective, binding can also occur near the antigen-binding domain of the antibody, which reduces the number of antibodies that retain biological activity and are able to bind antigen. A similar direct-coupling method enables orientation-dependent coupling by binding *via* the carbohydrate side chains of the antibody.

The silica surface of a diatom is amenable to simple chemical functionalization. An interesting example of this uses a DNA-modified diatom template for the control of nanoparticle assembly.[72] The amino-functionalized diatoms were coupled to fluorophore-labelled thiolated DNA by using a hetero-bifunctional crosslinking agent. Then gold particles were coated with DNA complementary to that bound to the surface of the diatom. Subsequently, the gold particles were bound to the diatom surface *via* the sequence specific DNA interaction (see Fig. 6).

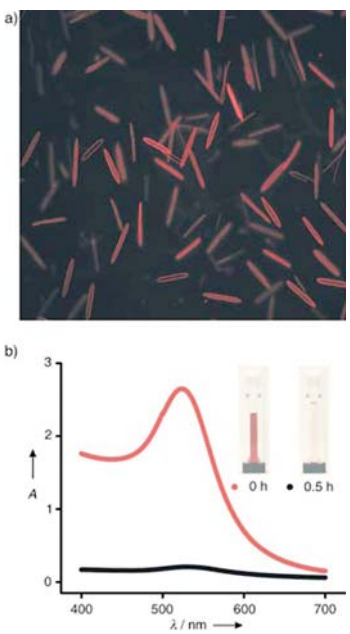

Fig. 6 (a). Fluorescence microscopy images of diatoms modified by fluorophore-labeled thiolated DNA. (b). The reaction of DNA-functionalized diatoms with complementary DNA-functionalized nanoparticles can be monitored by the naked-eye or by UV/Vis spectroscopy.

Using this method, up to seven layers were added showing how a hierarchical structure could be built onto the template. So one can easily modify the diatom surfaces with many different functional groups designed specifically to interact with nanostructures of interest or to perform desired chemistry. Unlike the surfaces of some biological templates, the surface of diatoms exhibit remarkable species-specific nanoscopic details that cannot be rendered or synthesized by using conventional material fabrication techniques. These nanostructures may prove useful for various applications in sensors, catalysis and optics by using the unique properties of surface modification of diatoms.[73]

Diatoms frustules possess intricate nanoscale features imbedded within naturally micro/nano-fabricated periodic two-dimensional pore arrays, displaying unparalleled diversity in structure and morphology. The deposition of gold and silver on a diatom frustule surface has been achieved by thermal evaporation.[74,75] Furthermore, Losic *et al.*[74] developed a procedure for pore size modifications of centric diatom species, using the atomic layer deposition of ultrathin films of TiO_2. TiO_2 was deposited by sequential exposures to $TiCl_4$ and water. They also showed the controlled reduction of pore sizes while preserving the shape of the diatom membrane pores. Pore diameters of diatom membranes can be further tailored for specific applications by varying the number of cycles and by changing their surface functionality. Toster *et al.* demonstrated that the ordered pores of diatom frustule can be effectively used as templates to mediate gold nanoparticle growth by a facile method.[76] Therefore, diatom frustules offer a advantage for synthesis of nanomaterials with well-defined and precisely controlled three dimensional morphologies and micro-to-nano scale features. Recently Losic *et al.* demonstrated the surface modification of diatom frustules with dopamine terminated Fe_3O_4 nanoparticles.[77] This approach is based on a simple one-step electrostatically driven self-assembly of dopamine modified Fe_3O_4 nanoparticles onto the diatom surface. The labelling of modified diatoms using fluoro probes showed that amino groups of dopamine on diatom surface are functional and available for the further attachment of targeting molecules. It was suggested this type of three dimensional hybrid nanomaterials could be potentially used as magnetically guided micro-carriers for drug delivery applications. Further, Rorrer's research group demonstrated the biological fabrication of Ge-doped biosilica frustules by two-stage cell culture of the diatom *Pinnularia*, and they fabricated an electroluminescent device by the incorporation of these diatom frustules.[78] This study represented a first step towards the realization of optoelectronic devices that utilize components fabricated through cell culture. Furthermore, the same team used the living diatom itself to metabolically insert nanostructured TiO_2 into the periodic structure of its frustule biosilica,[79] and they found addition of titanium to the diatom cells had no detrimental effects on the growth of the organism and preserved the nano- and microstructure of the frustule biosilica. The fabrication of these unique nano- and micro-structured semiconductor materials would offer possible applications including dye-sensitized solar cells for enhanced light trapping efficiency and photocatalysts for enhanced breakdown of toxic chemicals.

Biosensors

The changes in photoluminescent (PL) properties of porous silicon and nanoscale semiconductor materials upon their interaction with biomolecules have been well studied. The research groups of Chan,[80,81] Sailor[82,83] and Gooding[84,85] have investigated the application of porous silicon as optical biosensors where biomolecules including enzymes, DNA fragments and antibodies have been immobilized. Diatoms as photonic crystals consist of a periodically arranged set of dielectric materials that affect the propagation of light in a manner analogous to the way crystalline solids influence the flow of electrons. Using diatoms both as a large surface area matrix as well as an optical transducer of biomolecular interactions is relatively new. A number of research groups have explored diatom based photonic nanostructures as large surface area matrices for the immobilization of particular biomolecular agents and through spectral measurements study biomolecular interactions with high sensitivity. Apart from the sensitivity, there are other advantages of diatoms: first of all, they can monitor the binding reactions without the need to label one of the molecules involved in the binding reaction. Label-free monitoring of binding reactions not only simplifies any analysis by reducing errors in quantification, but also allows the biomolecules to be investigated directly in their natural environment. Secondly, the porosity of diatoms enables an intimate mix between the analytical sample and the detector, allowing monitoring of the biomolecular interactions in an effective way.

Rorrer's research group[86] investigated how antibody-functionalized diatom biosilica frustules serve as a microscale biosensor platform for selective and label-free PL-based detection of immunocomplex formation. They attached antibody rabbit immunoglobulin G (IgG) covalently to the frustule biosilica of the disk-shaped, 10 μm diameter diatom *Cyclotella* sp. by using silanol amination and a crosslinking step to a surface site density. It was demonstrated that functionalization of the diatom biosilica with the nucleophilic IgG antibody amplifies the intrinsinc blue PL of diatom biosilica by a factor of six. When the rabbit-IgG-functionalized diatom biosilica was bound to its complimentary antigen (goat anti-rabbit IgG), which is also nucleophilic, the peak PL intensity increased again by at least a factor of three. In fact, the increase of PL emission with nanoscale topology has been investigated with nucleophilic moieties or biomolecules that are attached to nanoscale semiconductors or other photoluminescent surfaces.[87,88] These studies suggested that nucleophilic chemical groups increase the PL intensity by donating electrons to non-radiative defect sites on the photoluminescent surface, thereby decreasing non-radiative electron decay and increasing the radiative emission, resulting in a higher quantum efficiency of the functionalized photoluminescent surface.

Parker *et al.*[89] demonstrate how the photonic properties of a diatom can be altered by growth with a metal pollutant. Both the optical and physical properties of the silica frustule of the diatom *Coscinodiscus wailesii* were affected by the presence of nickel sulfate in sea water. It was found that a sublethal concentration of the metal both significantly modified the size of the pores of the valves and quenched the intrinsic PL of the amorphous silica. De Stafano *et al.*[90,91] recently chemically modified the frustules of the marine diatom *Coscinodiscus* to properly bind a highly selective bioprobe such as an antibody. By measuring the changes in the photoluminescence emission of diatoms frustules, they monitored the molecular recognition event between the antibody and its binding ligands.

Gas sensors

The photoluminescence (PL) emission from the silica frustule of diatoms has been explored by De Stafano[92] for an optical gas sensor. They showed that the PL of *Thalassiosira rotula* is strongly dependent on the surrounding environment. Both the optical intensity and peaks are affected by gases and organic vapours. In the presence of NO_2, acetone and ethanol, the photoluminescence was quenched because these substances attract electrons from the silica frustule of the diatoms and hence quench the PL. On the other hand, substances that donate electrons, such as xylene and pyridine, had the opposite effect, and increased PL intensity almost ten times. Both quenching and enhancements were reversible as soon as the atmosphere was replaced by air. Subsequently, the same research group investigated the modification of the PL properties of different light-emitting diatom samples induced by the presence of NO_2. They demonstrated high-sensitivity gas detection at a low concentration, with a detection limit as low as 50 ppb obtained in the case of diatom frustules of highest specific surface. This study strongly implied that the luminescence activity of diatom frustules is related to surface-oxygen stoichiometric defects.[93]

Further, Sandhage *et al.*[94] conducted an inorganic molecular conversion reaction that preserves the size, shape and morphology of the diatom whilst changing its composition. They used a displacement reaction to convert biologically derived silica structures such as frustules into new compositions. Magnesium was shown to convert SiO_2 diatoms by a vapour phase reaction at 900 °C to MgO of identical shape and structure, with a liquid Mg_2Si by-product. Similarly when diatoms were exposed to titanium fluoride gas, the titanium displaced the silicon, yielding a diatom structure made up entirely of titanium dioxide; a material used in some commercial solar cells. Recently, this group extended its work on silica diatoms to work at lower temperatures.[95] Two-dimensional microporous silicon was used as an attractive sensing interface for rapid gas detection with the high specific surface areas and structures of the 3D silicon frustule replicas allowing rapid gas detection. To test such gas detection, a simple device based on a silicon frustule replica was fabricated by using platinum electrodes connected to the ends of this replica on a silicon nitride substrate, which exhibited rapid changes in impedance upon exposure to gaseous nitric oxide (see Fig. 7). This type of sensor could be used in in microscale gas sensing. Subsequently the same group reported how the intricate nanostructured silica valves of diatom frustules may be coated with a thin (50 nm), conformal, and continuous layer of a functional oxide (SnO_2) through dendritic amplification of hydroxy groups on the silica surfaces and then use of an automated surface sol–gel process.[96] They fabricated a device from such SnO_2-coated diatom frustule valves acts as a sensitive detector for NO gas by using a general process for depositing compact, continuous, and conformal coatings of synthetic inorganic oxides onto 3D nanostructured biosilica templates. To maximise

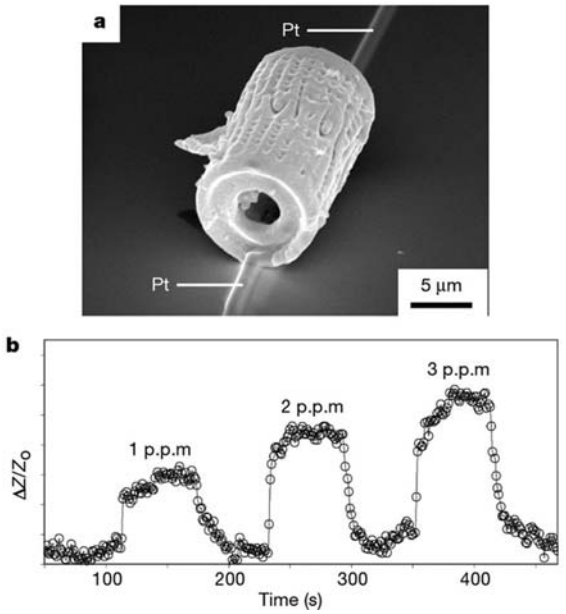

Fig. 7 (a) Secondary electron image of an electroded microporous silicon frustule replica. (b) Electrical response of this single silicon frustule sensor to $NO_{(g)}$. ΔZ is the impedance change upon exposure to $NO_{(g)}$, and Z_o is the sensor impedance in pure flowing argon.

the sensitivity and response of sensors an understanding of how fluids interacts with the diatom surfaces is imperative. In the next section we discuss recent progress in this field.

How diatoms interact with fluids and their use as biomimetic membranes

In their natural aquatic environment diatoms are exposed to a large variety of molecules/particles that range in size from less than one nanometre (nitrates for example) to viruses and bacteria that are in the micrometre range. Their silica membrane surface is exposed to not only a large size range of particles but also particles in varying concentrations- anything from concentrations of 10^7 ml^{-1} for large bacteria (~1 μm), to concentrations of 10^{16} ml^{-1} for nitrates.[97,98] Diatoms manage with minimal energy consumption to dominate by somehow filtering deleterious particles from useful ones, without the use of complex high-energy-using moving components to control their microenvironment. Their surface does not foul (but they manage to foul water purification membranes[99] themselves), they sort useful particles from harmful ones, and dominate environments with little energy consumption which leads the inevitable question: how and why? And what role does their silica frustule have in their success? Can we utilize the diatom's structure to design more efficient membranes?

Diatoms have a long history of being used by mankind for filtration purposes. Diatomaceous earth, or diatomite, which is made from the rock consisting of sedimented dead diatoms has long been known as an excellent filter due to its relatively high porosity but very fine pores, and is used as a filter in a range of applications, such as in swimming pool filters and for water purification. Recently chemically modified diatomite has been shown to be effective in removing dissolved uranium ions from water (see Fig. 8).[100] The authors used pure diatomite and hexadecyltrimethylammonium (HDTMA) modified diatomite and found the adsorption of uranium(VI) on the pure and the HDTMA diatomite varied with initial uranium concentration, sorbent/solution contact time and pH values of solution. The maximum adsorption capacity of the HDTMA-diatomite was 25.63 mol g^{-1} (158.8 mg g^{-1} or 15.9 wt/%), and for a pH of 8 and above 100% purification was achieved illustrating the usefulness of their high surface area to volume ratio.

In order to help understand how diatoms filter we must examine their natural habitat. Free living diatoms are at the mercy of the ocean or lake flow, currents and turbulence. They are subject to the bulk water motion and, due to their size, are sensitive to very small scale motion. Here we look at what flow they are subject to (depicted in Fig. 9) and how it may affect their transport and thus filtration properties.

Macroscale flow

We define macroscale as sizes greater than approximately 1 mm where the flow is affected by turbulence, currents and buoyancy changes. At this scale, and the microscale (~1 μm to 1 mm), fluid properties can be considered as a continuum and the standard advection diffusion equation can be used to determine the transport phenomena. On the macroscale the diffusion coefficient, D, may vary in space due to local changes in temperature and salinity, but in the local microenvironment around a diatom D can be considered homogenous thus simplifying the problem. Numerical simulations to solve the advection/diffusion equation for specific geometries coupled with a nutrient uptake kinetic equations can be used determine impact of shape and external

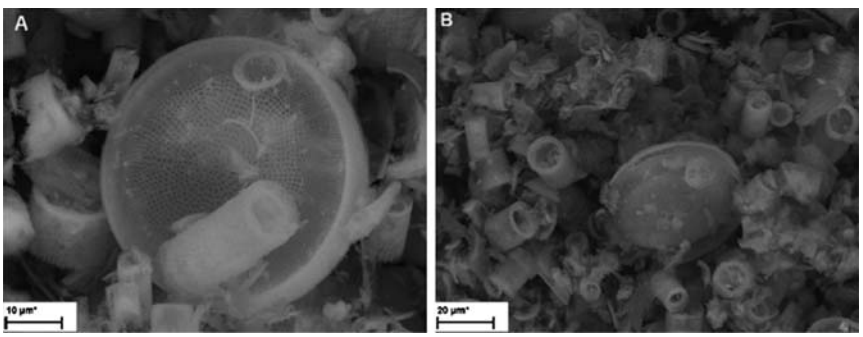

Fig. 8 SEM of diatomite[100] showing a range of different diatom frustules used in removing dissolved uranium irons.

flow for diatoms nutrient acquisition.[101–104] The information obtained using these methods may, for example, also be used for finding the best diatom for rapid drug absorption/release or biosensor response.

Turbulence

Turbulence is a natural phenomenon that occurs in fluids when the inertial motion of the fluid overcomes the viscous forces tending to damp-out the motion. The ratio of these forces is called the Reynolds number. Turbulence thus occurs above a critical Reynolds number that depends on the nature and geometry of the flow. Turbulence is characterised by highly irregular, isotropic and fluctuating three dimensional flow with most of the energy contained in eddies (rotating packets of fluid). Diatoms are unique in water micro-organisms in that they have large vacuole that can store inorganic nutrients (*e.g.* nitrates and phosphates) meaning that they tend to thrive in areas with pulsating nutrients supplies such as highly turbulent areas of the ocean.[6]

Sources of turbulent energy in the ocean, for example, are wave motion, winds, currents and moving marine creatures. At small scales, viscous forces dominate and turbulent energy is dissipated due to fluid friction and thus as heat. The Kolmogorov scale, L_k, defines the region where viscous forces start to dominate the dissipation and thus describes the size of the smallest eddies in the flow[105] and is given by:

$$L_k \approx \left(\frac{\nu^3}{\varepsilon}\right)^{1/4}$$

where ν is the kinematic viscosity and ε is the turbulent kinetic energy dissipation. Measured values for the kinetic energy in the ocean ranges from 10^{-7} to 47 cm^2 s^{-3},[106] with the magnitude depending on the depth and the weather conditions. The energy of turbulence associated with velocity fluctuations is spread over a large frequency (or wavenumber) range. Generally ocean water in the depth range 1–4 m has most of its turbulent energy at around 1 Hz.[107] There are, however, velocity fluctuations in the order of 10 Hz right up to 100 Hz albeit with considerably lower energy. Turbulence is known to have an effect on the bio-silicon production of diatom frustules,[108] the nutrient uptake of diatoms,[109] and the collision of marine colloids to produce marine snow.[110] While diatoms reside in the inherently macroscale turbulent flow, because they are smaller than the smallest eddies, they only experience an oscillating linear shear field as depicted in Fig. 9.

Microscale flow—the relationship between frustules shape and nutrient uptake

The typical Reynolds number of a 100 μm diameter diatom sinking at 350 μm s^{-1} is 0.35, which is well in the laminar regime. This value will vary depending on the relative speed and the size of the diatom but not enough to induce turbulent flow. In addition, as the ocean's turbulence manifests itself as linear laminar shear at the scale of a diatom, only laminar flow needs to be considered. The impact of cell shape on diffusion and advective nutrient transport to individual diatoms and diatom chains was investigated by Pahlow *et al.*[102] For individual diatoms the nutrient uptake was shown to be higher than for elongated diatom shapes due to their higher surface area to volume ratio, particularly relative to that for spheres. This indicated that elongated diatoms may form a better sensor substrate.

It has been shown[111] using the analysis of[101] that simply due to the shear forces associated with diatoms such as *Coscinodiscus wailesii* sinking and floating they will rotate with a period ranging from approximately 0.5 to 2 s, which is similar to the frequency of maximum turbulent energy dissipation in the ocean. This rotation will produce periodic pressure fluctuations in the pores that are in the order of the stagnation pressure, 0.5 ρv^2, associated with the sinking velocity. That gives pressure fluctuations ranging from approximately 3.2 μPa to 60 μPa. These fluctuations may be superimposed onto the changing hydrostatic pressure associated with sinking and floating and help the diatom with filtration.

For an excellent review on the microscale flow around the surface of diatoms see Karp-Boss *et al.*[103] who assume throughout their analysis that diatoms are perfect sinks so that nutrient concentration at the surface is zero. While admitting this is not physically correct it makes the problem a lot easier to solve.

When considering the uptake of nutrients into diatoms Paskiak and Gravis (1974) defined a new parameter being the ratio of diffusive transport to nutrient uptake kinetics using the Michaelis–Menten equation. This ratio defines the rate limiting process as either being diffusion limited or uptake limited. It was shown by them, and since by other authors, that various processes are diffusion limited (*e.g.* CO_2 uptake[112]). However in

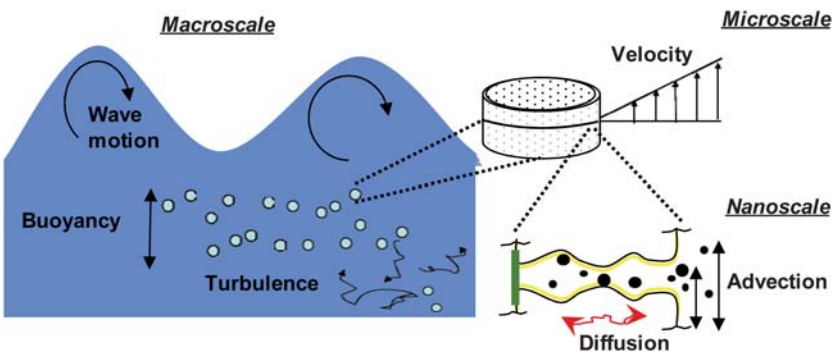

Fig. 9 Flow regimes for diatoms ranging from macroscale motion of the ocean to nanoscale transport through the frustules pores.

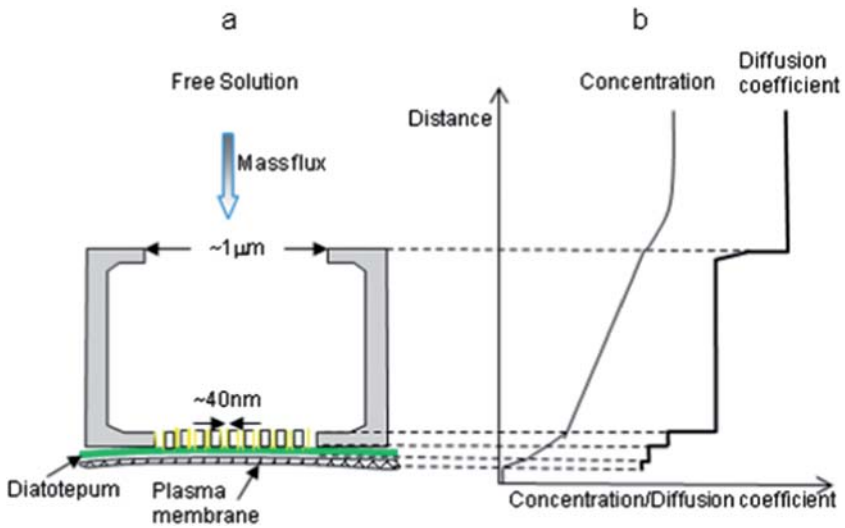

Fig. 10 Schematic of a diatom valve pore structure (not to scale) and associated diffusion coefficients and concentration though the pore assuming pure diffusion and no advection.

all these analyses a single diffusion coefficient has been used based on the free solution value.

Nanoscale effects and methods of filtering

Currently all the studies of nutrient uptake in diatoms have only considered microscale flow around diatoms, or in other words have considered only a bulk free diffusion coefficient, and a diatom plasma membrane that has a nutrient uptake limit. In reality, however, the nanostructured frustule between the bulk solution and the cell plasma membrane will have a significant effect on the transport properties. This is depicted in Fig. 9 for a girdle band pore and in Fig. 10 for a valve pore showing the pore structure and the associated diffusion coefficients and concentration gradient taking into account the nanoscale confining effects.

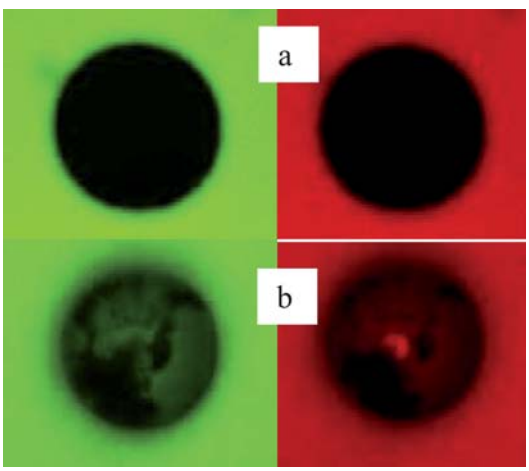

Fig. 11 Confocal images in the midplane of a *Coscinodiscus wailesii* in fluorescein (left) and propidium iodide (right). a) In a live diatom, b) Same diatom after organic membrane ruptured with methanol.

While allowing nutrients through the frustule, diatoms reject suspended bacteria and viruses. The method they use to do this is not understood, especially with regard to the role of the frustule. It is clear that the organic components play a role similar to regular cells without a hard outer silica layer. This is illustrated in Fig. 11 that shows small molecules like fluorescein (equivalent diameter ~1 nm) and propidium iodide (<1 nm) cannot penetrate a live diatom, but when the organic membrane was ruptured with methanol, the dyes readily entered the cell.

There have been a handful of studies investigating the role of the nano-structure on the transport through and along diatoms. The first in the area were by Hale and Mitchell[113,114] who looked at the lateral motion of Brownian fluorescent particles over cleaned diatom frustules and over mimics etched in silicon. They showed how the surface topography deflected the path of Brownian particles (~0.3–0.5 μm) away from the mean flow direction even when the flow was not dominated by diffusion. They proposed that the diatom surface topography may help diatoms sort and filter.

There are a few roles that the nanostructures of the frustule can play in terms of facilitating filtration and sorting based on the type of flow the diatoms experience. The methods that the solvent (water) and the solutes (molecules and nanoparticles, virus, bacteria *etc.*) interact with the diatom are dictated by advection and diffusion. In order to determine the dominate transport mechanism around diatoms, the Peclet number, is often used which is defined as the ratio of advective to diffusive transport and is given by

$$Pe = \frac{Ur}{D}$$

where U is the average velocity and r is the characteristic length scale. For flow around diatoms where the characteristic length is the diatom radius Pe for nutrient transport is often in the order of 1 to 10 meaning that both diffusion and advection are important. Once particles enter the pores the characteristic

length changes from the diatom radius to the pore radius, the *Pe* is much smaller; thus diffusion typically dominates. Thus the nanostructured pores can affect the transport both outside the frustule and though the frustule pores.

The first attempt at measuring the diffusion coefficient through clean diatom valves was by Losic *et al.*[115] Their basic set-up used a valve glued to the end of a capillary tube and they measured the fluorescent emission spectra, showing selectivity of the frustule to particle size. Bhatta *et al.*[116] then did more detailed experiments showing the diffusion coefficient of small molecules like fluoresceine was reduced through a *Coscinodiscus* valve. In order to get a more accurate value of the diffusion coefficient Bhatta *et al.*[117] then used fluorescent correlation spectroscopy FCS, which has a measurement volume very close to that of the large pores in a *Coscinodiscus* valve. The diffusion coefficient of a fluorescent dye in a pore was shown to be reduced significantly relative to the free solution value.[117] The decrease of the diffusion coefficient is well documented for confined diffusion. Experiments show that even with molecules that are only 5% of the pore diameter the diffusion coefficient is reduced by about 20% relative to free solution.[118] This decrease due to confinement is shown schematically in Fig. 10. For a good review on all aspects of hindered diffusion see Deen.[119] Additionally as diatoms live in a coilloidal mixture, the rapid changes in pore area in the valve could help in entropic trapping.[120]

As well as pore shape effecting diffusive transport, it is also known to effect advection of both solvent and solute, the frictional pressure drop and fouling.[121] For example Bowen and Sharif[122] showed a rounded pore shape is the best for maximising flux when electrostatic interactions are involved. The unique diatom pore may be optimised as an ideal antifouling membrane.

Depending on the macroscale conditions diatoms may be subject to a constant shear field, fluctuating shear, or stagnant water. The fluctuating pressure field mentioned previously associated with turbulence and rotation may provide the periodic force required to drive Brownian ratchet filtering mechanism.[123,124] The girdle band pores shown in Fig. 1d offer the change in shear forces required for operation of a ratchet, with the diatom pore shape being similar to that made artificially in silicon for a Brownian ratchet.[28]

Additionally filtering may also utilize electrostatic interactions *via* the electric double layer or even Van de Waals forces. If the negatively charged silica frustule was exposed to diatoms in sea water with an ionic strength of 0.7 M the Debye length would be very small relative to the pore size so that it would have little effect on the transport properties. However, when a very thin layer of polysaccharides are attached to the silica this may change with interaction lengths possibly approaching around 30 nm[125] which is close the size of the smallest diatom pores without the organic layer. This is area ripe for further investigation. Thus there is still much to learn about transport through diatoms frustules and we expect soon that they may form a model for efficient and selective nanofluidic transport for both solvents and solutes.[126]

While we believe the main application for the study of diatom frustules will be to inspire new architectures for synthetic membranes, diatoms may also be used directly, not only as thick random porous membrane filters such as diatomite as shown in Fig. 8, but more interestingly, as a single layer array of diatom valves to form a macroscale membrane. Cleaned valves could be self-assembled on a porous silicon support substrate and then permanently attached by forming an oxide layer on the silicon. As the biosilica is very thin for the smallest pore layer (~50–100 nm), such a membrane could offer excellent selectivity with very low hydrodynamic resistance.

Conclusion and future perspectives

Diatoms possess intricate nanoscale features imbedded within naturally micro/nano-fabricated periodic two-dimensional pore arrays, displaying unparalleled diversity in structure and morphology. Undoubtedly, diatoms will play an important role in biosensors and separation science because of their many unique properties. Diatoms generate silica structures without the need for complex chemical processes, providing an exceptionally cheap material for analytical science. Recent developments in chemical modification and biofunctionalization of these materials have made it possible to generate a new class of bioactive silica nanostructures that are conjugated with biomolecules such as proteins. The nanoporus structures of the frustules may shed light on how to best structure a membrane for low energy separation and to avoid fouling as they contain many features know for low energy separation, and even form the active structure of a manmade membrane themselves. The availability of these biomodified nano-constructs has opened up entirely new and exciting research directions in the field of biosensors and filtration.

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