From diatoms to silica-based biohybrids

Nadine Nassif, Jacques Livage

Chem Soc Rev

This article was published as part of the

Hybrid materials themed issue

Guest editors Clément Sanchez, Kenneth J. Shea and Susumu Kitagawa

Please take a look at the issue 2 2011 table of contents to access other reviews in this themed issue

Chimie de la matière condensée de Paris, CNRS, UPMC, Collège de France, 11 place Marcelin Berthelot, 75231 Paris Cedex 05, France.
E-mail: jacques.livage@upmc.fr; Fax: +33 14427 1504;
Tel: +33 14427 1500
† Part of the themed issue on hybrid materials.

CRITICAL REVIEW

From diatoms to silica-based biohybrids†

Nadine Nassif and Jacques Livage*

Received 24th September 2010
DOI: 10.1039/c0cs00122h

This *critical review* shows that diatoms can be a source of inspiration for the synthesis of advanced nanostructured biohybrids. These single cell microalgae are living inside a porous silica shell called 'frustule'. Mimicking this model, silica-based biohybrids have been produced *via* the so-called sol–gel process. Biomolecules such as proteins, enzymes or antibodies can be trapped within a silica matrix leading to hybrid biosensors and bioreactors. Whole cells remain viable and retain their metabolic activity leading to the formation of living biohybrids that offer new possibilities in the field of biotechnology and nanomedicine. Diatom frustules exhibit an incredible variety of sophisticated shapes; they can be used as 3D hierarchically structured materials for the realization of sensors, photonic devices or microfluidics. They can also be a model for the bio-templated synthesis of nanostructured materials. Diatom nanotechnology is becoming a new field of research where biologists and materials scientists are working together! (125 references)

1. Introduction

The development of modern nanotechnologies requires the synthesis of advanced materials with controlled structures. Therefore scientists are looking for new synthetic techniques in order to be able to make such materials. The 'top-down' route is typically developed by physicists while chemists usually follow the 'bottom-up' approach in which nanostructured materials are obtained *via* the self-assembly of basic building blocks. Organic templates have received increasing attention over the last decade for the generation of inorganic structures and materials.[1] Sophisticated techniques are being developed but today's nanotechnology still has limited 3D capabilities. The realization of novel nanostructured materials will be based on our ability to synthesize and organize matter into controlled geometries from the nano to the macroscale.

Biomineralization could be described as the process by which living organisms assemble solid nanostructures from naturally occurring inorganic compounds. The resulting biomaterials could actually be described as 'biohybrids' in which biomolecules and inorganic components are intimately associated. They display an incredible variety of sophisticated nanostructures and so much better properties that are difficult to obtain even with the most advanced synthetic methods. The potential of biological scaffolds for the fabrication of novel nanostructures is then being actively explored.[2] Most biomaterials are based on calcium carbonate (shells, crustacea cuticle) and phosphates (bones, teeth). Rather few living species (diatoms, radiolaria, sponges) are using silica.

However, silica is a major component of the lithosphere and silica-based materials find widespread industrial applications in the field of glasses and ceramics. For centuries, these materials have been made at very high temperatures while biogenic silica is made by diatoms under ambient conditions. These unicellular algae are encased within a cell wall made of amorphous hydrated silica called 'frustule'.[3,4] They are well known for the beauty of their silica shells (Fig. 1), but beauty is not the only function of this biomaterial. Silica shells have to be strong in order to protect diatoms against predators. They should be porous to allow nutrients and metabolites to diffuse in and out. They must also be transparent for the photosynthetic activity of the cell. However, why do they display such an incredible variety of sophisticated shapes? we don't really know but the answer to this question could lead to new progress in the field of nanostructured materials![5]

Diatomist and material scientists have long been working separately without interaction. This is no longer the case, and 'diatom nanotechnology', a new interdisciplinary area involving biology, chemistry, physics, materials science and engineering, is growing rapidly.[6] Diatoms could then become a source of inspiration for the synthesis of novel biohybrid materials.[7,8]

2. From biogenic silica to sol–gel biohybrids

2.1 Sol–gel synthesis of silica

Biogenic silica is made by diatoms in water at room temperature from the silicic acid $Si(OH)_4$ resulting from the dissolution of silicate minerals. The silica network is formed *via* a poly-condensation process: $Si(OH)_4 \Rightarrow SiO_2 + 2H_2O$

The reaction occurs inside the cell, in a Silica Deposition Vesicle (SDV) around pH 5. Silica nanoparticles are then deposited outside the cell to form an exoskeleton.[9,10]

This chemical synthesis of biogenic silica from molecular silicic acid precursors appears to be very simple. However silica is poorly soluble in water (few mg l^{-1} at pH 7) and highly basic sodium silicate solutions (water glass, pH 12) are currently used as solute precursors in industry. Silica is then obtained *via* the acidification of these alkaline solutions, but the reaction is not easy to control and precipitation occurs as soon as some acid is added to the silicate solution. Therefore, chemists currently use organic solutions of alkoxide precursors $Si(OR)_4$ ($R = CH_3$, C_2H_5,...). Two chemical reactions are involved in the formation of silica:

$$\text{Hydrolysis} \quad \text{–Si–OR} + \text{HOH} \Rightarrow \text{–Si–OH} + \text{ROH}$$

$$\text{Condensation} \quad \text{–Si–OR} + \text{HO–Si–} \Rightarrow \text{–Si–O–Si–} + \text{ROH}$$

The overall reaction can be written as:

$$Si(OR)_4 + 2H_2O \Rightarrow SiO_2 + 4ROH$$

Colloidal solutions (sols) and gels are progressively formed upon condensation and this soft solution synthesis of silica is commonly called the 'sol–gel process' (Fig. 2). These reactions can be chemically controlled *via* acid or base catalysis. Chain polymers are obtained at low pH leading to microporous silica gels whereas mesoporous gels made of spherical nanoparticles are formed above the Point of Zero Charge (pH $\approx$ 3).

One of the main advantages of the sol–gel route for industrial applications is to allow the powderless processing of glasses and ceramics. Shaped materials such as films, fibers or nanoparticles can be directly obtained from the solution. The first patent was taken in 1939 in order to make glass coatings and many new products have since then been developed on an industrial basis.[11]

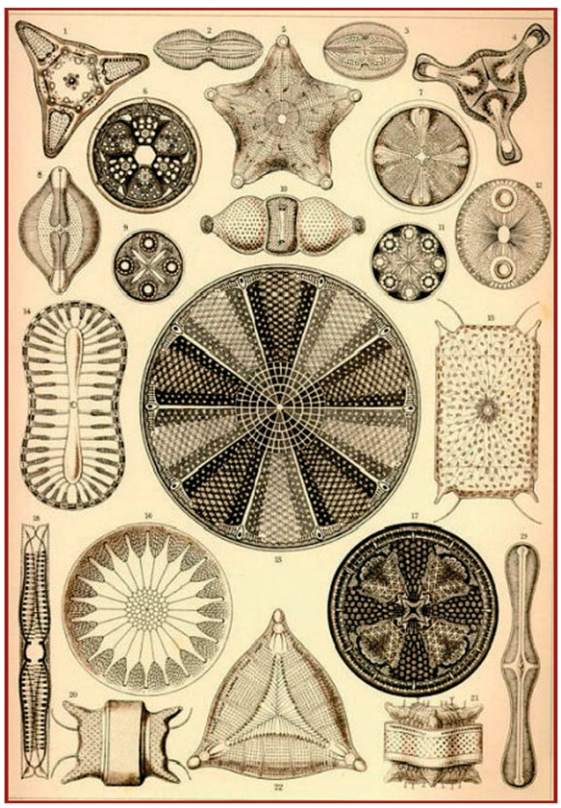


Fig. 1 Diatoms by Ernst Haeckel in 'Kunstformen der Natur' 1904.

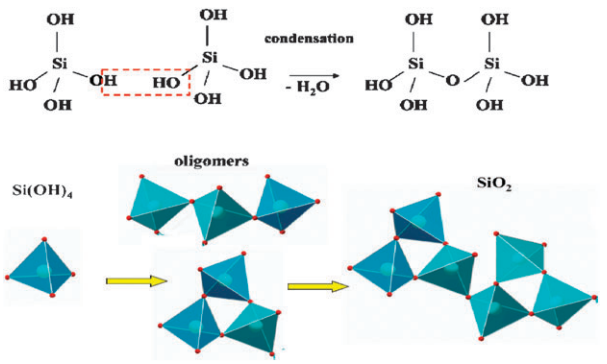


Fig. 2 Formation of silica *via* the condensation of silicic acid.

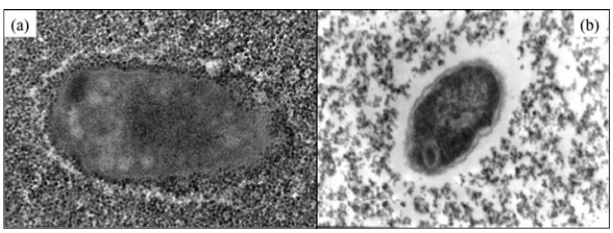


Fig. 3 Bacteria encapsulated within a silica matrix aged for (a) 1 month without glycerol and (b) 1 day with a layer of glycerol.

2.2 Sol–gel encapsulation in silica

The mild conditions associated with the sol–gel synthesis of silica are compatible with fragile biospecies allowing the formation of biohybrids in which biomolecules are entrapped within a silica matrix. Many biomolecules such as peptides, proteins, enzymes, nucleic acids or antibodies have already been trapped in sol–gel matrices.[12,13] The encapsulation procedure is quite straightforward. Hydrolyzed alkoxides are mixed with biomolecules in a buffered solution around pH 7. Condensation is then quite fast and biomolecules remain trapped within the growing porous oxide network.[13]

Trapped enzymes have been shown to retain their bio-catalytic properties and could even exhibit improved performances. The porous oxide matrix prevents leaching, protects enzymes from external aggression and provides the possibility to set locally non-conventional thermodynamic conditions. Alkaline phosphatases, for instance, that naturally exhibit their best activity around pH 9.5 remain active down to pH 1.[14]

Upon encapsulation, enzymes are trapped within a silica cage tailored to their size and shape. Their mobility is restricted in such a confined medium preventing denaturation due to conformational changes. Creatine kinases can therefore be protected against thermal degradation. They retain half of their activity at 47 °C, ten times more than for the free enzyme.[15]

Moreover, the chemical nature of the silica matrix can be chemically modified in order to improve the bioactivity of trapped enzymes. A nice example is provided by the encapsulation of lipases. These enzymes are involved in hydrolysis and esterification reactions:

$$R\text{–}COO\text{–}R' + H_2O \Leftrightarrow R\text{–}COOH + R'OH$$

They are widely used in food, oil or organic industries. However, R–COO–R′ esters are often non-soluble in water, leading to the formation of emulsions. The catalytic reaction then occurs at the interface between both hydrophilic and lipophilic phases. A special 'interfacial activation process' occurs in which the catalytic site of the lipase remains active only within a specific hydrophilic–hydrophobic range. The activity of lipases in hydrophilic silica gels is very poor, but it becomes two orders of magnitude larger in hybrid organic–inorganic matrices.[16] Such sol–gel lipases are now commercially available.

Another advantage of sol–gel biohybrids is to provide a versatile interface between biological species and electronic devices, allowing the realization of bio-sensors in which enzymes are trapped within shaped silica matrices (films, fibers, micro-arrays,...). Amperometric glucose sensors have been made *via* the deposition of bio-doped sol–gel films on electrodes. Microdot arrays can be deposited by ink jet printing for optical detection.[17,18]

Nanomedicine has emerged as a novel field which involves the application of nanotechnology to human health. Among all the materials that have been investigated, silica nanoparticles with well defined structures and surface properties appear to be promising candidates as carriers for *in situ* cell delivery. They are becoming increasingly important for oral, transdermal or implantable therapeutic systems.[19,20]

The sol–gel process offers new possibilities for embedding organic compounds within silica particles and for controlling their release from the host matrix into the surrounding medium. Bioactive molecules can be easily encapsulated within silica particles that offer a biocompatible, stable and stealthy vector.[21,22]

Recent advancements in controlling the surface properties of mesoporous silica microparticles have significantly enhanced their biocompatibility. Both the internal pore and exterior particle surfaces can be functionalized with a tunable pore diameter ranging from 2 to 30 nm and a narrow pore size distribution.[23,24]

Targeting and drug delivery to cancer cells is a key aim in biomedical science today. The drug carrier together with the therapeutic agent should then be delivered inside cancer cells. Targeting is especially important for cancer therapy, as most of the commonly used anticancer drugs also have serious side-effects on healthy cells. Moreover, nanoparticles provide a novel class of contrast agents that has great potential for medical imaging and diagnosis. Mesoporous silica nanoparticles have been synthesized combining three different moieties for targeting, imaging, and therapeutic. Folic acid was used as a targeting ligand for selective delivery, poly(ethylene imine) as a gene transfection agent and a fluorescent dye for imaging.[25,26]

A critical obstacle and challenge for cancer therapy is the fact that most anticancer drugs are hydrophobic. It is particularly important to improve the aqueous solubility of these drugs, as they have to be administered through the intravenous route. It has been shown that mesoporous silica nanoparticles might be used as a vehicle to overcome this insolubility problem.

Camptothecin is an hydrophobic anticancer drug that was incorporated into the pores of fluorescent mesoporous silica nanoparticles and successfully delivered into a variety of human cancer cells to induce cell death. This example shows that mesoporous silica particles possess several attractive features for use in the delivery of water-insoluble drugs. They have large surface areas and porous interiors that can be used as reservoirs for storing hydrophobic drugs. The pore size can be tailored to selectively store different molecules while the size and shape of the particles can be tuned to maximize cellular uptake.[27]

Silica nanoparticles have also been used for photodynamic therapy applications. Photosensitizers are injected into cancer cells. Upon light irradiation, they transfer energy to neighboring O_2 molecules giving cytotoxic $^1O_2$ singlet oxygen. One of the main drawbacks of this process is that dyes are progressively released in the blood so that tissues remain light sensitive for several weeks. This is no longer the case when photosensitizers are trapped inside nanoparticles. They cannot escape from the silica matrix.[28]

3. Living biohybrids

3.1 Whole cell encapsulation

Diatoms are one of the major components of the phytoplankton. They are living inside a 'glasshouse' and could be described as 'living biohybrids'.[29] The food chain in the

marine ecosystem is mainly based on the photosynthetic activity of these microalgae. They are responsible for 40% of the primary production of organic compounds from carbon dioxide in the ocean.[30]

The example of diatoms emphasizes that single cells could live and exhibit a metabolic activity when encased within a silica box. It suggests that sol–gel encapsulation could be extended to whole cells as reported by Carturan *et al*. about 20 years ago, who showed that *Saccharomyces cerevisiae* yeast cells retained their bio-activity in silica gels.[31]

Many papers have been published since then and a large variety of cells such as bacteria, fungi, microalgae, lichens, plant cells and even animal cells have now been trapped within sol–gel silica. These living biohybrids offer new possibilities in the field of biotechnology.

Biosensors based on immobilized microorganisms are used for the detection of chemicals, drugs and toxins.[12,32–34] Porous thin films containing biosensing species have been described during the past few years.[35] Biochemical oxygen demand (BOD) optical sensors based on *Bacillus subtilis* cells trapped in hybrid organic–inorganic sol–gel films have been developed for the analysis of pollutants in waste water[36] while microalgae fiber optic biosensors have been used for herbicide monitoring.[37]

Lichens, that are able to accumulate heavy metals, are commonly used as environmental pollution biomonitors. Immobilized in a sol–gel matrix, they have been spin-coated onto graphite electrodes in order to make electrochemical sensors. These devices exhibit long-term stability and can operate in organic solvents as well as in aqueous solutions.[38]

Moraxella spp. cells engineered to express recombinant organophosphorus hydrolase have been used for the bio-detection of organophosphates, a class of toxic compounds widely used as pesticides and insecticides. Entrapped cells appear to be protected by the silica matrix. Only 5% of their activity is lost after 2 months compared to 30% for free cells in a buffer solution.[39]

Microbial cells are also widely used for the production of valuable compounds such as chemicals, food, cosmetics or drugs. Whole cells can preserve their metabolic activity within silica matrices. Sol–gel bioreactors could then be made in which encapsulated cells would be used for the decontamination of soils or waste waters and the production of chemicals.

As mentioned above, bacteria are known to be able to bind selectively large amounts of metals, a property that has been used for the bioremediation of uranium from mining waste pile waters by entrapped *Bacillus sphaericus* cells.[40]

Filamentous fungi *Stereum hirsutum* have been encapsulated within alginate–silica hybrids. They are known to degrade lignin in wood *via* the action of extracellular oxidases. These hybrid biodevices appear to be effective for the degradation and removal of malachite green, even in the presence of a high concentration of the dye.[41]

Astaxanthin, a carotenoid dye used as fish food and dyestuff, is produced by the *Haematococcus pluvialis* algae. Large photobioreactors are currently used for commercial production but algae are destroyed when the dye is extracted either under high-pressure or in the presence of organic solvents. After encapsulation in a silica film, astaxanthin can be efficiently extracted using diethylenepropyleneglycol as a solvent. After extraction about 30% of the cells remain viable. They can be cultivated again, opening the route to the design of continuous bioreactors.[42]

Mimicking the photosynthetic behavior of diatoms within their silica shell, photobioreactors based on photosynthetic microorganisms encapsulated within transparent silica matrices have been recently developed.[43] Trapped cyano-bacteria and plant cells behave like artificial leaves. Under light irradiation, they split water into oxygen and CO_2 into biofuels, sugars or drugs.[34,44] Photobiological H_2 has also been produced with *Synechocystis* sp. These cells contain a hydrogenase enzyme that is able to reduce protons into molecular hydrogen.[45]

Whole cell encapsulation also finds applications for medicine. Blood tests for the detection of antibodies have been performed using sol–gel immobilized parasite cells. First experiments have been performed *via* the so-called ELISA (Enzyme Linked Immuno-Sorbent Assay) method, using *Leishmania* cells as antigens. This parasite, transmitted by the sand fly, is responsible for the leishmaniasis disease that affects mammals. Whole cells are immobilized in a porous silica matrix within the micro-wells of a usual titration plate. They are then incubated in the presence of sera from infected patients. Antibodies bind specifically to the trapped cells and are detected *via* a usual colored reaction.[46] Immuno-fluorescence and immunoperoxidase assays have been performed recently with *Trypanosoma cruzi* epimastigotes and *Leishmania guyanensis* promastigotes trapped within a silica film. Homogeneous, ready to use, long lasting coated slides were then obtained, which are appropriate for making tests in field conditions.[47]

Silica biohybrids could also be used for cell transplantation. Following the so-called 'biosil' process, whole cells are covered by a sol–gel silica layer deposited from alkoxide vapors on the cell surface. Artificial pancreases have been made with Langerhans islets trapped within such gel layers. *In vitro* experiments show that the insulin secretion capacity of trapped cells is maintained upon sol–gel encapsulation. *In vivo* experiments have been performed *via* the transplantation of encapsulated islets into a diabetic mouse. The cells continue to function and mimic the behavior of the natural organ. The porous silica membrane protects transplanted islets against antibody aggression while nutrients are allowed to reach the cell and by-products to escape.[48]

3.2 Viability within a silica matrix

The main challenge for whole cell encapsulation remains to be able to preserve the viability of trapped cells in a good physiological state and as long as possible.[49] *Escherichia coli* bacteria for instance have been shown to retain their bio-catalytic activity after encapsulation but the number of viable bacteria decreases quite fast, mainly when alkoxide precursors are used.[50] Dead or damaged bacteria may still exhibit some enzymatic activity. They just behave as a cell-bag of enzymes, but are no longer able to maintain activities that would require whole cell integrity.

Aqueous silica precursors offer an interesting alternative for cell encapsulation.[51] Silica is formed *via* the acidification of

sodium silicate aqueous solutions around pH $\approx$ 7. Very dilute solutions have to be used in order to avoid fast precipitation and silica colloids are added to improve the mechanical properties of the matrix. In such a medium implemented with glycerol (Fig. 3), an osmoprotecting agent, *Escherichia coli* bacteria have been shown to retain, in both aerobic and anaerobic conditions, their glucose metabolic activity that actually depends on the cell wall integrity. They even keep the ability to synthesize new proteins. These results show that encapsulated bacteria can be kept alive in the gel in a really good physiological state.[52] In such conditions about half of the trapped bacteria are still viable one month after encapsulation.[53] The *in situ* visualization of trapped cells by cryo-SEM experiments shows that they are surrounded by an additive layer that protects them from the inorganic silica matrix and prevents water evaporation.[54] With such additives, sol–gel encapsulation can be extended to other inorganic matrices and long-term viability was also reported for *E. coli* in alumina gels.[55]

Amphiphilic phospholipids have also been used to improve the viability of trapped cells. Upon drying, the living cells organize phospholipid chains into a sort of multi-layered membrane that protects the cells and behaves as a template to direct the formation of ordered mesophases.[56] Living cells are then surrounded by a fluid consisting of multilayered lipid vesicle that interfaces coherently with the silica matrix (cell-directed assembly). This bio–nano interface prevents drying and maintains cell viability yet providing accessibility to small molecules.[57]

Another limitation of sol–gel encapsulation for making bioreactors is that cells do not have enough space to divide. They cannot be cultured in order to improve the production of metabolites. Diatoms actually appear to behave in a special way. Indeed, a void progressively forms between living cells and the silica matrix suggesting that diatoms are able to dissolve silica from the sol–gel matrix. As a result, they keep the possibility to divide and long-term viability, beyond 3 months, was observed.[58] Hybrid bio-reactors have been made by Bilmes *et al*. Cells, trapped within alginate beads, are embedded within a silica gel. Alginate is then dissolved by removing the cross-linking Ca^{2+} ions with citric acid. Large cavities, several millimetres in diameter, are formed inside the silica matrix in which trapped cells can then divide.[59] The silica gel appears to behave as a barrier against external biological contamination and plant cell proliferation was observed over a 6-month period.[60]

A main challenge in nanobiomedicine is the engineering of nanomaterials that can efficiently encapsulate drugs at high load, cross cell membranes, and controllably release their cargo at target sites. Liposomes are currently used for such a purpose. These synthetic vesicles made of lipid bilayers can be filled with drugs and used to deliver drugs inside the cells. However, they are not very stable and drug release cannot be easily controlled. Porous silica nanoparticles are also being used to deliver drugs but their aggregation under physiological conditions and their nonspecific binding in protein-containing solutions limit their applications in biomedicine. An alternative design would be to combine silica nanoparticles with a lipid coating in order to mimic cellular envelopes. New nanostructured biohybrids are made of a porous silica core and a lipid bilayer shell, associated *via* the electrostatic-mediated fusion of positively charged liposomes on negatively charged silica cores.[61] These 'protocells' mimic the cell's protective lipid membranes and combine advantages of both porous inorganic nanoparticles and liposomes. The porous silica can be loaded with drugs while the lipid layer provides biocompatibility. It can be designed with target species (peptides, antibodies, aptamers, PEG,...) in order to enter living cells and deliver drugs. These targets are inserted within the fluid lipid layer where they can move around and gather at the point of interaction with another cell creating a binding effect through multivalency. Just a few peptides are then required to affect targeted delivery. One protocell is sufficient to kill a cancer cell, an efficiency 100 times greater than current liposomes.[62]

3.3 Quorum sensing in confined nanopores

Bacteria are able to communicate with one another *via* chemical signal molecules called 'quorum sensing' (QS). This cell–cell communication relies on the principle that when a single bacterium produces signaling molecules, the extracellular concentration is below a certain threshold. However, when the cell density increases, a critical concentration can be reached that allows the signaling molecule to be sensed and enables the bacteria to respond in order to modify their metabolic activity.[63] Using these signal–response systems, bacteria synchronize particular behaviors on a population-wide scale and thus function as multicellular organisms. It is now clear that cell–cell communication is the norm in the bacterial world and that understanding this process is fundamental for industrial and clinical microbiology. Encapsulation may modify the cell activity in many ways. Cells are randomly dispersed within the silica matrix that prevents the formation of colonies. Bacteria remain isolated from each other. It becomes then possible to study the behavior of a single bacterium rather than the collective behavior of a whole colony.

Quorum sensing should operate at the level of a single cell but up to now the ability of a single bacterium to quorum sense in a confined space has not been tested definitively. Sol–gel encapsulation provides the right conditions to study the confinement-induced quorum sensing of individual bacteria under conditions of complete chemical and physical isolation. In such conditions, self-signaling *Staphylococcus aureus* bacteria have been shown to induce genetic re-programming of the cell similar to quorum sensing. This 'discrete' quorum sensing allows *S. aureus* to sense

confinement and to activate virulence and metabolic pathways needed for survival.[64]

Trapped bacteria remain far apart so that there is no chemical communication between them. It is then possible to control their behavior by adding QS molecules.[65] This was shown with *Serratia marcescens* bacteria. They produce a red pigment called prodigiosin that exhibits some cytotoxic activity against cancer cells. We can artificially mimic their chemical communication by adding QS molecules in the nutrient medium. At the same time, the production of prodigiosin increases and almost all trapped bacteria are still viable after one month in the gel.[65]

Genetically engineered *E. coli* cells have been trapped in transparent sol–gel silica films deposited on glass plates in order to make optical biosensors. These bacteria are obtained by coupling a gene promoter sensitive to chemical or physical stress to a reporter gene coding for luminescent proteins (GFP, RFP). Silica–bacteria biohybrids have been shown to maintain their ability to synthesize GFP or RFP in the presence of chemical inducers. Disposable sensors, test-kits and even early warning devices have been made that can operate in continuous flow conditions.[66] One limitation for such whole cell sensors is that a constant number of active cells is required to obtain a constant analytical response. This is realized with trapped cells that cannot divide within the silica matrix. The ability of such cells to show a concentration-dependent fluorescence intensity was demonstrated in the presence of various specific inducers (ethanol, mitomycin C, nalidixic acid, hydrogen peroxide, phenol and methyl viologen), most of which could be detected in the mM concentration range. Moreover, as different bacteria can be encapsulated without being able to communicate, a constant population is maintained, preventing the dominance of one species over the other. Two bacteria strains, one expressing bioluminescent GFP as a response to toxicity and the other expressing fluorescent RFP as a response to genotoxicity, were co-encapsulated. The independent or simultaneous monitoring of two different kinds of inducers became then possible, opening the route to multi-functional whole-cell biosensors. Dual sensing and multistep biosyntheses could then be made with a single device.[67]

Beyond controlling gene expression on a global scale, quorum sensing allows bacteria to communicate within and between species. The ability to interfere with bacterial cell–cell communication may be crucial in preventing colonization by pathogenic bacteria that use quorum sensing to coordinate virulence. The possibility to trap simultaneously cells from different strains opens new possibilities for biotechnology. Several populations of bacteria with complementary metabolic activities can be trapped together in a symbiotic relationship. This could be used for the *in situ* bioremediation of soils contaminated with chlorine derivatives such as PolyChlorinated Biphenyl (PCB). The pollution of the soils can be cleaned up by adding PCB degrading bacteria. However, these bacteria are often not able to compete with autochthonous microorganisms and to face the toxicity of the contaminated biotope. They could then be encapsulated within porous silica matrices that would protect them against chemical and bacterial degradation.[68]

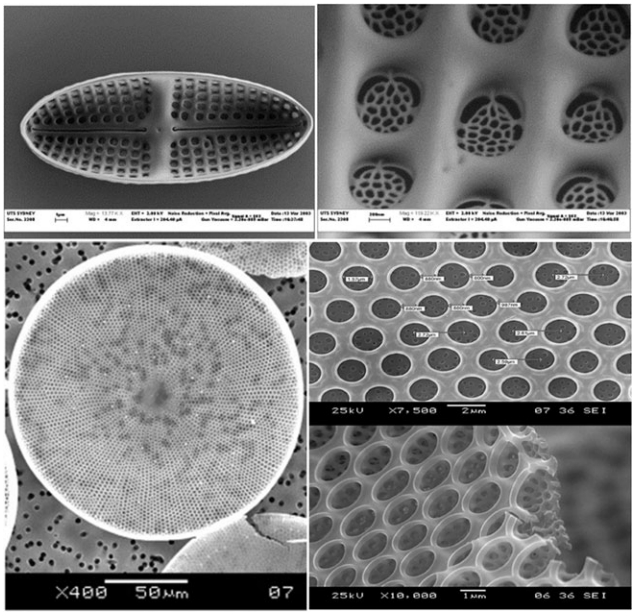


Fig. 4 Hierarchical distribution of pores in diatom frustules; Top: *Achnanthes subsessilis* (reprinted from Materials Science and Engineering C, 25, K.S.A. Butcher *et al.*, A luminescence study of porous diatoms, 659, Copyright (2010), with permission from Elsevier); Bottom: *Coscinodiscus walesii* (reprinted from ref. 77, Copyright (2010), with permission from Elsevier).

4. Towards 3D nanostructured biohybrids

4.1 Diatom nanotechnology

One of the main challenges for the development of nanotechnologies is to design materials with hierarchical 3D nanostructures.

Diatoms themselves offer a unique example of all made 3D nanostructures that could be directly exploited for the realization of novel nanodevices.[69] Indeed, diatoms exhibit an incredible variety of sophisticated nanostructures. More than 100 000 species have been described, each with its own morphology. These microalgae can be easily cultured and a large number of identical silica frustules can be obtained within few days.[8] Moreover, the recent progress on diatom genetics provide new tools to understand how silica shells are made, enlarging the possibility to make unique 3D nanomaterials with designed structures and functionalities.[70,71] Genetically Engineered Micro/nanodevices (GEMs) have been made from diatom silica shells.[72]

Porous diatomaceous earths are already widely used as filters, adsorbents or thermal insulators. For instance, the well known dynamite invented by Alfred Nobel was made *via* the impregnation of nitroglycerine into diatomite. Highly porous diatom frustules exhibit a large solid–gas interface. Thus they offer promising possibilities for the realization of gas sensors. The photoluminescence emission of silica frustules is affected by even small modifications of the gas atmosphere. A detection limit of few tenths of ppm was obtained in the case of NO_2.[73] The photoluminescent properties of silica frustules can be modified by introducing foreign elements in the culture medium. Germanium for instance can be metabolically

inserted into the frustule biosilica of *Pinnularia* sp. by a two-stage cell cultivation technique. Some Si atoms are replaced by tetravalent Ge resulting in germanium doped frustules that exhibit both photoluminescent and electroluminescent properties in the blue range.[74,75]

The hierarchical 3D periodic distribution of pores provides some specific optical properties (Fig. 4). Porous silica frustules behave like photonic crystals. Irridescence is observed under a light microscope and diatoms have been described as living biophotonic crystals.[76,77] Such photonic crystals can be described as dielectric structures with periodically modulated refractive indices. They exhibit unsuspected optical properties such as light focussing. The incoming light of a laser beam is confined by the regular pore pattern of the diatom surface into a spot of few microns. This focusing ability of the diatom's frustule should have a biological function. It could provide an effective way to concentrate the light inside the diatom's protoplasm. Diatom frustules could also find applications as photonic micro-components. The single valve of the centric marine diatom, *Coscinodiscus wailesii*, for instance could fit the top of an optical fiber to make a lensed fibre without modifying the glass core.[78,79]

Diatom frustules could also find applications in many other fields such as microfluidics and drug delivery.[6,69] But in all such cases, the biological components have to be removed and only the silica nanostructure is used.

Silica frustules can be used as templates for the production of 3D nanostructured porous materials (oxides, gold, polymers) *via* replica molding.[80,81] They can be biologically functionalized with antibodies for the realization of biosensors.[82,83] They can also be chemically converted into other ceramic materials. Several approaches have been used to change the chemical composition of diatom frustules without loss of their 3D nanostructure. These processes are currently called BaSIC (Bioclastic and Shapepreserving Inorganic Conversion). In such processes, silica frustules are converted into a new composition *via* a shape-preserving gas/silica displacement reaction.[72] The silica frustule for instance can be transformed into MgO upon heating in a magnesium gas at 900 °C for 4 h.[84] Many other nanostructured oxide materials such as TiO_2,[85] ZrO_2,[86] or $BaTiO_3$[87] have thus been synthesized. The silica frustule can even be reduced into silicon, opening new possibilities in the field of micro-electronics.[88] Such a synergistic combination of biological nanostructures with synthetic chemical functionalization opens the door to large numbers of 3D micro/nanostructures with chemistries and properties that can be tailored for device applications.

The chemical composition of diatom frustules can also be modified *in vivo via* the metabolic insertion of foreign elements added in the culture medium. TiO_2 was inserted into the silica frustule of the diatom *Pinnularia* by a two-stage cultivation process[89] whereas germanium was used to modify the photoluminescent properties of the diatom *Nitzschia frustulum*.[75]

4.2 Bioinspired nanostructured materials

The amazingly beautiful hierarchical patterning of the silica-based diatom cell walls attracted research interest over a long time. The pore size is controlled from the nano to the micrometre range scale.[6,76,90] They are becoming increasingly important from the materials science point of view. However many aspects of diatom cell wall formation and patterning are still not fully understood. It would be important to know how they build their silica shells in order to be able to design better nanostructured materials. Novel developments in the synthesis of bioinspired materials rely on both a better understanding of the mechanisms governing the formation of bio-structures and an in-depth characterization of those three-dimensional networks.

Biominerals always contain an organic fraction intimately bound to the skeletal structure. This organic matrix, mainly made of biopolymers, is supposed to control the deposition of the inorganic materials. Three kinds of biomolecules have been extracted from the silica walls. They have been shown to induce the rapid precipitation of silica nanospheres from silicic acid solutions and should be involved in the formation of the frustules. These are phosphoproteins known as silaffins, long-chain polyamines (LPA) and acidic proteins called silicidins (Fig. 5).[4,5,91–93]

Over the last decade, numerous strategies including the use of organic templates (polymers, surfactants, organo-gelators,...) have been set for the *in vitro* generation of shaped and nanostructured silica-based materials.[1] Most organic molecules are based on long-chain cationic polymers, mainly polyamines, in order to mimic the positively charged sequences of silaffins.[5,29] They are described to interact, concentrate and stabilize locally solute precursors. However, it is still an open question whether the overcharged structure of such natural extracted proteins is ideal to behave as a mould to structure the mineral.

Tailored synthetic polypeptides and polyamines appear to favor the condensation of silica. They control the size and morphology of the silica particles but usually give rather simple shapes such as spheres, rods or even hexagonal platelets.[94,95] Poly-L-lysine (PLL) for instance was shown to promote the condensation of silica from a silicic acid solution. The shape of the resulting materials depends on the molecular weight of the peptide. Spherical nanoparticles are obtained with a low-molecular weight PLL while hexagonal silica platelets are formed with a large molecular weight PLL. Electrostatic interactions between positive PLL and anionic silica precursors could promote the formation of intramolecular

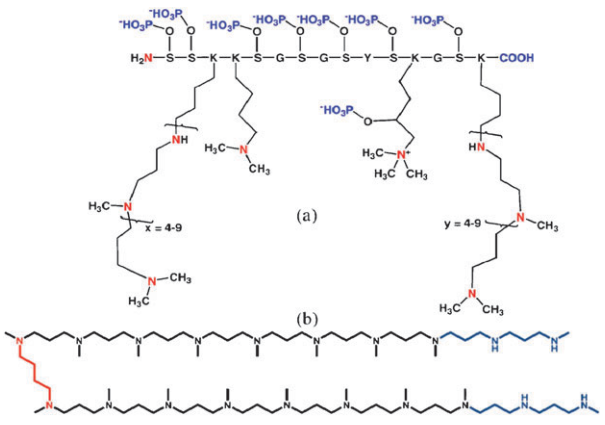


Fig. 5 Molecular structure of (a) silaffins and (b) LPA.

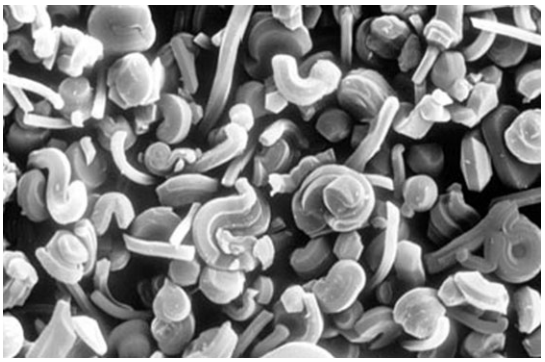


Fig. 6 Biomorph silica (reproduced from ref. 105, by permission of the Royal Society of Chemistry).

hydrogen bonds along the peptide chain leading to the formation of helical structures.[96] Native proteins are also used as illustrated by silica films of controlled thickness formed when silicatein proteins from sponges are grafted onto a flat substrate.[97]

More sophisticated shapes can be obtained by applying some physical stress (hydrodynamic field) during the condensation of silica. Silica morphology changes progressively from nanospheres to fibers and dendritic structures.[98]

Single molecules are actually far too small to direct the formation of intricate patterns. They don't lead to the formation of complex nanostructures and should be able to self-assemble into larger structure-directing aggregates in order to behave as templates.[99]

A 'phase separation' model was proposed to explain the formation of the hierarchical structured silica shells in diatoms.[100] According to this model, amphiphilic polyamines undergo a phase-separation process within the SDV giving an emulsion of microdroplets. In a close-packed arrangement these droplets form a hexagonal network. Silica precursors precipitate in the aqueous interface between droplets giving a honeycomb-like silica framework.[5,101]

Following this model *in vitro* leads to the well-known mesoporous silica MCM-41. A large variety of mesoporous shaped materials can be obtained depending on the nature of the template molecules. Chiral gelators for instance lead to the formation of helical bundles of cotton-like silica fibers.[102]

Chemists are usually looking for defect free mesoporous structures. However, it is well known that topological defects, points, lines or planes, can be observed during the formation of surfactant mesophases. The energy required to stabilize these defects is much smaller than in solid crystals and large defects up to several micrometres can be easily formed. The condensation of silica around these defects leads to particular forms of mesoporous silica (discs, tori, spirals, spheres,...) that look like biogenic silica. Each morphology can be associated with a specific kind of dislocation or disclination defect. Two defects can even combine to lead to more sophisticated morphologies (Fig. 6).[103–105]

Recent studies suggest that the cell wall morphology could in fact be controlled by the shape of the specific vesicle in which silica deposition occurs.[4] The SDV could be described as a 'cellular reaction vessel' responsible for the control of silica precipitation and pattern formation. The SDV is actually a dynamic organelle that is expanded and molded during frustule formation. The entire silica structure is then exocytosed to form the cell wall.[106] Thus, other strategies have been proposed in order to synthesize hierarchically organized silica patterns with higher-order superstructures that would mimic more efficiently biomineral architectures.[69]

Peptides are known to form α-helix or β-sheet structures that can be used as templates for the synthesis of nanostructured inorganic oxides.[107] Double-walled silica nanotubes with monodisperse diameters have been shown to self-organize around lanreotide rods into highly ordered centimetre-sized fibres. This process mimics the way some sponges make their spicule, *via* the deposition of a silica coating around a protein filament.[108] Lanreotide is a dicationic octapeptide that self-assembles in water into nanotubes with a monodisperse diameter of 24.4 nm and a wall thickness of 1.8 nm. The positively charged protonated amine groups behave as templates for the condensation of negatively charged silica precursors $[H_nSiO_4]^{(4-n)-}$. A 'dynamical template' model was suggested to explain the formation of nanotube bundles.[109] According to this model, two distinct self-assembly processes occur simultaneously: the nanotube organic template formation and the silica polymerization. This coupling occurs at the extremity of the template nanotube, where it self-assembles, at the same time that silica precursors are condensing. This leads to the formation of microfibers composed of uniaxial orientation of precipitated silica–lanreotide nanotubes. Then, the fibers alignment ordered throughout the length scales from one micron to one centimetre. Similarly, silica fibers exhibiting a distinct inner structure with six distinguishable levels of hierarchical order could also be obtained using synthetic nanotapes with a functional peptide β-sheet core and a PEO shell.[110] Such self-assembled alignments remind the structural hierarchy in tendons where the relationship between collagen molecules, fibrils, fibers, fascicles makes it up.

A parallel was already evidenced between the *in vivo* three-dimensional assemblies of organic matrices (chitin, collagen, cellulose) and molecular arrangements described in liquid crystals.[111] Therefore, such biopolymers have been used as liquid crystal-like templates for the structuration of inorganic materials. Specially, little by little, the ability to produce chitin seems to be more widespread and likely plays a more central role in diatom[112] or sponges[113] biology than previously considered.

Cellulose nanorods for instance lead to the formation of birefringent cellulose–silica composites.[114,115] Along the same line, chiral nematic liquid-crystal phases of collagen have been used for the structuration of silica over a long-range organization, from the nano to the microscale.[116] Collagen fibrils act as a template for the structuration of aggregated silica particles to form silica fibers *in vitro*.[117,118] Further use of collagen as an organic template for silicification has been explored by Heinemann *et al.* with the aim of providing new biomaterials for bone tissue engineering.[119] Mesenchymal stem cells are cultivated *in vitro* on silica–collagen hybrid xerogels derived from fibrillar bovine collagen. This procedure leads to nano- and microstructure but not to hierarchical organization as found in bones.

As an alternative of using the lyotropic properties of molecules, aqueous sols made of silica colloids and liposomes subjected to subfreezing temperatures right after gelation led to a three-dimensional hierarchical organization of silica. The resulting biohybrids exhibit interesting properties with one colloidal entity supporting the structure (*e.g.*, silica) and the other providing functionality (*e.g.*, liposomes).[120]

Biohybrids based on microorganisms such as viruses have been recently described. Viruses are made of a genetic material (DNA or RNA) protected by a peptide coat called capsid that usually exhibits a geometrical shape (icosahedron or rod). The capsid coat is made of amino-acids such as lysine that can behave as a nucleation center for the precipitation of inorganic particles. Silica nanotubes have then been formed at the surface of the Tobacco Mosaic Virus (TMV). These rod-like viruses can form liquid crystal templates for the controlled deposition and organization of the inorganic phase. Depending on the associated inorganic phase (oxide or metallic nanoparticles), the general stability of the Tobacco Mosaic Virus liquid crystals offers a new approach to get ordered phases of unusual symmetries that are not readily achievable using synthetic templates. The corresponding structures were organized at the mesoscale. Hexagonally ordered silica–TMV mesostructures[121] or metallic (Pt, Au, Ag) tubular nanoparticles[122] were obtained.

5. Conclusions

This review paper shows how scientists could be inspired by nature to bring new developments in the field of nanostructured materials. They go further than just mimicking the structure of biomaterials like nacre to improve the mechanical properties of their composite materials. Biohybrids provide new possibilities for controlled drug delivery systems in the field of nanomedicine.[19] Bio-inspiration means that they develop novel materials that have no equivalent in nature but could find emerging applications in electronics, optics or batteries. This was very nicely illustrated by Belcher and coworkers with M13 bacteriophages. These viruses were genetically engineered in order to introduce glutamate groups in the peptide coat. They then behave as templates for the synthesis of cobalt oxide nanowires, a non-biogenic material that can be used for the realization of Li^+ ion nano-batteries. The capacity obtained with such M13–Co_3O_4 biohybrids is twice that of usual carbon anode batteries.[123]

Diatoms exhibit an astonishing variety of sophisticated nanostructures that are difficult to obtain even with most advanced synthetic procedures. Therefore, the research of biological scaffolds for the fabrication of controlled nanostructures is actively explored. However it remains a real challenge and biomaterials such as diatom frustules remain far better than our most advanced nanostructured materials. A better understanding could be reached by studying the *in vitro* formation of silica in conditions as close as possible as those occurring *in vivo* in biological systems. For example, most studies on sol–gel silica are made with alkoxides rather than aqueous solutions of silicic acid. Both routes have been compared for cell encapsulation but not for biomineralization.[124] Moreover, *in vitro* experiments are performed in diluted solutions. They do not take into account the fact that biomaterials are usually synthesized in a confined space, within vesicles. How diatoms keep high concentrations of solute silica precursors in the silica deposition vesicle remains an open question! A systematic study of the confined assembly of silica with surfactants shows that unprecedented silica mesostructures can be obtained in such conditions.[125]

Understanding the mechanisms by which microorganisms are able to construct 3D hierarchically nanostructured materials is a real challenge for the development of nanotechnologies. It took several million years for diatoms to get a real mastery of the synthesis of biogenic silica nanostructures. We could hope that we would be able to go some how faster!

Notes and references

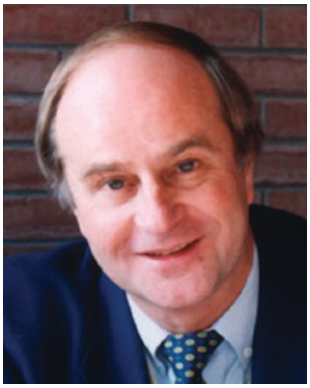
1 K. J. C. van Bommel, A. Friggeri and S. Shinkai, *Angew. Chem., Int. Ed.*, 2003, **42**, 980.
2 E. Ruiz-Hitzky, M. Darder, P. Aranda and K. Ariga, *Adv. Mater.*, 2010, **22**, 323.
3 M. Hildebrand, *Prog. Org. Coat.*, 2003, **47**, 256.
4 M. Hildebrand, *Chem. Rev.*, 2008, **108**, 4855.
5 M. Sumper and E. Brunner, *Adv. Funct. Mater.*, 2006, **16**, 17.
6 R. Gordon, D. Losic, M. A. Tiffany, S. S. Nagy and F. A. S. Sterrenburg, *Trends Biotechnol.*, 2009, **27**, 116.
7 R. Gordon and J. Parkinson, *J. Nanosci. Nanotechnol.*, 2005, **5**, 35.
8 R. W. Drum and R. Gordon, *Trends Biotechnol.*, 2003, **21**, 325.
9 M. Sumper and E. Brunner, *ChemBioChem*, 2008, **9**, 1187.
10 E. Brunner, C. Groeger, K. Lutz, P. Richthammer, K. Spinde and M. Sumper, *Appl. Microbiol. Biotechnol.*, 2009, **84**, 607.
11 L. C. Klein, *Sol-gel technology for thin films, fibers, preforms, electronics and specialty shapes*, Noyes Pub, 1988.
12 D. Avnir, T. Coradin, O. Lev and J. Livage, *J. Mater. Chem.*, 2006, **16**, 1013.
13 V. B. Kandimalla, V. S. Tripathi and H. X. Ju, *CRC Crit. Rev. Anal. Chem.*, 2006, **36**, 73.
14 H. Frenkel-Mullerad and D. Avnir, *J. Am. Chem. Soc.*, 2005, **127**, 8077.
15 D. T. Nguyen, M. Smit, B. Dunn and J. I. Zink, *Chem. Mater.*, 2002, **14**, 4300.
16 M. T. Reetz, A. Zonta and J. Simpelkamp, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 301.
17 N. Rupcich, A. Goldstein and J. D. Brennan, *Chem. Mater.*, 2003, **15**, 1803.
18 M. Mougenot, M. Lejeune, J. F. Baumard, C. Boissiere, F. Ribot, D. Grosso, C. Sanchez and R. Noguera, *J. Am. Ceram. Soc.*, 2006, **89**, 1876.
19 M. Vallet-Regi, F. Balas and D. Arcos, *Angew. Chem., Int. Ed.*, 2007, **46**, 7548.
20 B. G. Trewyn, I. I. Slowing, S. Giri, H. T. Chen and V. S. Y. Lin, *Acc. Chem. Res.*, 2007, **40**, 846.
21 K. S. Finnie, D. A. Jacques, M. J. McGann, M. G. Blackford and C. J. Barbe, *J. Mater. Chem.*, 2006, **16**, 4494.
22 C. Barbe, J. Bartlett, L. G. Kong, K. Finnie, H. Q. Lin, M. Larkin, S. Calleja, A. Bush and G. Calleja, *Adv. Mater.*, 2004, **16**, 1959.
23 I. I. Slowing, B. G. Trewyn, S. Giri and V. S. Y. Lin, *Adv. Funct. Mater.*, 2007, **17**, 1225.
24 I. I. Slowing, J. L. Vivero-Escoto, C. W. Wu and V. S. Y. Lin, *Adv. Drug Delivery Rev.*, 2008, **60**, 1278.
25 J. M. Rosenholm, A. Meinander, E. Peulhu, R. Niemi, J. E. Eriksson, C. Sahlgren and M. Linden, *ACS Nano*, 2009, **3**, 197.
26 K. T. Yong, I. Roy, M. T. Swihart and P. N. Prasad, *J. Mater. Chem.*, 2009, **19**, 4655.
27 J. Lu, M. Liong, J. I. Zink and F. Tamanoi, *Small*, 2007, **3**, 1341.
28 P. Couleaud, V. Morosini, C. Frochot, S. Richeter, L. Raehm and J. O. Durand, *Nanoscale*, 2010, **2**, 1083.
29 R. Wetherbee, *Science*, 2002, **298**, 547.
30 E. V. Armbrust, *Nature*, 2009, **459**, 185.

31 G. Carturan, R. Campostrini, S. Dire, V. Scardi and E. Dealteriis, *J. Mol. Catal*., 1989, **57**, L13.
32 J. Livage and T. Coradin, *Med. Mineral. Geochem*., 2006, **64**, 315.
33 H. Bottcher, U. Soltmann, M. Mertig and W. Pompe, *J. Mater. Chem*., 2004, **14**, 2176.
34 C. F. Meunier, J. C. Rooke, A. Leonard, H. Xie and B. L. Su, *Chem. Commun*., 2010, **46**, 3843.
35 P. C. A. Jeronimo, A. N. Araujo and M. Montenegro, *Talanta*, 2007, **72**, 13.
36 L. Lin, L. L. Xiao, S. Huang, L. Zhao, J. S. Cui, X. H. Wang and X. Chen, *Biosens. Bioelectron*., 2006, **21**, 1703.
37 E. Pena-Vazquez, E. Maneiro, C. Perez-Conde, M. C. Moreno-Bondi and E. Costas, *Biosens. Bioelectron*., 2009, **24**, 3538.
38 M. Darder, M. Colilla, N. Lara and E. Ruiz-Hitzky, *J. Mater. Chem*., 2002, **12**, 3660.
39 D. Yu, J. Volponi, S. Chhabra, C. J. Brinker, A. Mulchandani and A. K. Singh, *Biosens. Bioelectron*., 2005, **20**, 1433.
40 J. Raff, U. Soltmann, S. Matys, S. Selenska-Pobell, H. Bottcher and W. Pompe, *Chem. Mater*., 2003, **15**, 240.
41 M. Perullini, M. Jobbagy, N. Mouso, F. Forchiassin and S. A. Bilmes, *J. Mater. Chem*., 2010, **20**, 6479.
42 D. Fiedler, U. Hager, H. Franke, U. Soltmann and H. Bottcher, *J. Mater. Chem*., 2007, **17**, 261.
43 J. C. Rooke, A. Leonard and B. L. Su, *J. Mater. Chem*., 2008, **18**, 1333.
44 C. F. Meunier, J. C. Rooke, A. Leonard, P. Van Cutsem and B. L. Su, *J. Mater. Chem*., 2010, **20**, 929.
45 D. J. Dickson, C. J. Page and R. L. Ely, *Int. J. Hydrogen Energy*, 2009, **34**, 204.
46 J. Y. Barreau, J. M. Dacosta, I. Desportes, J. Livage, L. Monjour and M. Gentilini, *C. R. Acad. Sci., Ser. III*, 1994, **317**, 653.
47 G. J. Copello, M. C. De Marzi, M. F. Desimone, E. L. Malchiodi and L. E. Diaz, *J. Immunol. Methods*, 2008, **335**, 65.
48 G. Carturan, R. Dal Toso, S. Boninsegna and R. Dal Monte, *J. Mater. Chem*., 2004, **14**, 2087.
49 A. Nieto, S. Areva, T. Wilson, R. Viitala and M. Vallet-Regi, *Acta Biomater*., 2009, **5**, 3478.
50 S. Fennouh, S. Guyon, C. Jourdat, J. Livage and C. Roux, *C. R. Acad. Sci., Ser. II*, 1999, **2**, 625.
51 T. Coradin and J. Livage, *Acc. Chem. Res*., 2007, **40**, 819.
52 N. Nassif, C. Roux, T. Coradin, M. N. Rager, O. M. M. Bouvet and J. Livage, *J. Mater. Chem*., 2003, **13**, 203.
53 N. Nassif, O. Bouvet, M. N. Rager, C. Roux, T. Coradin and J. Livage, *Nat. Mater*., 2002, **1**, 42.
54 M. L. Ferrer, Z. Y. Garcia-Carvajal, L. Yuste, F. Rojo and F. del Monte, *Chem. Mater*., 2006, **18**, 1458.
55 M. Amoura, N. Nassif, C. Roux, J. Livage and T. Coradin, *Chem. Commun*., 2007, 4015.
56 H. K. Baca, C. Ashley, E. Carnes, D. Lopez, J. Flemming, D. Dunphy, S. Singh, Z. Chen, N. G. Liu, H. Y. Fan, G. P. Lopez, S. M. Brozik, M. Werner-Washburne and C. J. Brinker, *Science*, 2006, **313**, 337.
57 H. K. Baca, E. Carnes, S. Singh, C. Ashley, D. Lopez and C. J. Brinker, *Acc. Chem. Res*., 2007, **40**, 836.
58 C. Gautier, J. Livage, T. Coradin and P. J. Lopez, *Chem. Commun*., 2006, 4611.
59 M. Perullini, M. Jobbagy, G. Soler-Illia and S. A. Bilmes, *Chem. Mater*., 2005, **17**, 3806.
60 M. Perullini, M. M. Rivero, M. Jobbagy, A. Mentaberry and S. A. Blimes, *J. Biotechnol*., 2007, **127**, 542.
61 J. W. Liu, A. Stace-Naughton, X. M. Jiang and C. J. Brinker, *J. Am. Chem. Soc*., 2009, **131**, 1354.
62 J. W. Liu, X. M. Jiang, C. Ashley and C. J. Brinker, *J. Am. Chem. Soc*., 2009, **131**, 7567.
63 C. Fuqua, M. R. Parsek and E. P. Greenberg, *Annu. Rev. Genet*., 2001, **35**, 439.
64 E. C. Carnes, D. M. Lopez, N. P. Donegan, A. Cheung, H. Gresham, G. S. Timmins and C. J. Brinker, *Nat. Chem. Biol*., 2010, **6**, 41.
65 N. Nassif, C. Roux, T. Coradin, O. M. M. Bouvet and J. Livage, *J. Mater. Chem*., 2004, **14**, 2264.
66 J. R. Premkumar, R. Rosen, S. Belkin and O. Lev, *Anal. Chim. Acta*, 2002, **462**, 11.
67 J. R. Premkumar, E. Sagi, R. Rozen, S. Belkin, A. D. Modestov and O. Lev, *Chem. Mater*., 2002, **14**, 2676.
68 S. Di Toro, G. Zanaroli and F. Fava, *Microb. Cell Fact*., 2006, **5**, 11, DOI: 10.1186/1475-2859-5-11.
69 D. Losic, J. G. Mitchell and N. H. Voelcker, *Adv. Mater*., 2009, **21**, 2947.
70 N. Kroger, *Curr. Opin. Chem. Biol*., 2007, **11**, 662.
71 N. Kroger and N. Poulsen, *Annu. Rev. Genet*., 2008, **42**, 83.
72 K. H. Sandhage, S. M. Allan, M. B. Dickerson, C. S. Gaddis, S. Shian, M. R. Weatherspoon, Y. Cai, G. Ahmad, M. S. Haluska, R. L. Snyder, R. R. Unocic, F. M. Zalar, Y. S. Zhang, R. A. Rapp, M. Hildebrand and B. P. Palenik, *Int. J. Appl. Ceram. Technol*., 2005, **2**, 317.
73 S. Lettieri, A. Setaro, L. De Stefano, M. De Stefano and P. Maddalena, *Adv. Funct. Mater*., 2008, **18**, 1257.
74 C. Jeffryes, R. Solanki, Y. Rangineni, W. Wang, C. H. Chang and G. L. Rorrer, *Adv. Mater*., 2008, **20**, 2633.
75 T. Qin, T. Gutu, J. Jiao, C. H. Chang and G. L. Rorrer, *ACS Nano*, 2008, **2**, 1296.
76 T. Fuhrmann, S. Landwehr, M. El Rharbi-Kucki and M. Sumper, *Appl. Phys. B: Lasers Opt*., 2004, **78**, 257.
77 L. De Stefano, P. Maddalena, L. Moretti, I. Rea, I. Rendina, E. De Tommasi, V. Mocella and M. De Stefano, *Superlattices Microstruct*., 2009, **46**, 84.
78 L. De Stefano, I. Rea, I. Rendina, M. De Stefano and L. Moretti, *Opt. Express*, 2007, **15**, 18082.
79 E. De Tommasi, I. Rea, V. Mocella, L. Moretti, M. De Stefano, I. Rendina and L. De Stefano, *Opt. Express*, 2010, **18**, 12203.
80 D. Losic, J. G. Mitchell, R. Lal and N. H. Voelcker, *Adv. Funct. Mater*., 2007, **17**, 2439.
81 E. K. Payne, N. L. Rosi, C. Xue and C. A. Mirkin, *Angew. Chem., Int. Ed*., 2005, **44**, 5064.
82 H. E. Townley, A. R. Parker and H. White-Cooper, *Adv. Funct. Mater*., 2008, **18**, 369.
83 D. K. Gale, T. Gutu, J. Jiao, C. H. Chang and G. L. Rorrer, *Adv. Funct. Mater*., 2009, **19**, 926.
84 K. H. Sandhage, M. B. Dickerson, P. M. Huseman, M. A. Caranna, J. D. Clifton, T. A. Bull, T. J. Heibel, W. R. Overton and M. E. A. Schoenwaelder, *Adv. Mater*., 2002, **14**, 429.
85 R. R. Unocic, F. M. Zalar, P. M. Sarosi, Y. Cai and K. H. Sandhage, *Chem. Commun*., 2004, 796.
86 S. Shian, Y. Cai, M. R. Weatherspoon, S. M. Allan and K. H. Sandhage, *J. Am. Ceram. Soc*., 2006, **89**, 694.
87 M. R. Weatherspoon, S. M. Allan, E. Hunt, Y. Cai and K. H. Sandhage, *Chem. Commun*., 2005, 651.
88 Z. H. Bao, M. R. Weatherspoon, S. Shian, Y. Cai, P. D. Graham, S. M. Allan, G. Ahmad, M. B. Dickerson, B. C. Church, Z. T. Kang, H. W. Abernathy, C. J. Summers, M. L. Liu and K. H. Sandhage, *Nature*, 2007, **446**, 172.
89 C. Jeffryes, T. Gutu, J. Jiao and G. L. Rorrer, *ACS Nano*, 2008, **2**, 2103.
90 M. Sumper and G. Lehmann, *ChemBioChem*, 2006, **7**, 1419.
91 M. Sumper and N. Kroger, *J. Mater. Chem*., 2004, **14**, 2059.
92 N. Kroger, R. Deutzmann, C. Bergsdorf and M. Sumper, *Proc. Natl. Acad. Sci. U. S. A*., 2000, **97**, 14133.
93 N. Poulsen, M. Sumper and N. Kroger, *Proc. Natl. Acad. Sci. U. S. A*., 2003, **100**, 12075.
94 D. J. Belton, S. V. Patwardhan and C. C. Perry, *J. Mater. Chem*., 2005, **15**, 4629.
95 A. Bernecker, R. Wieneke, R. Riedel, M. Seibt, A. Geyer and C. Steinem, *J. Am. Chem. Soc*., 2010, **132**, 1023.
96 M. M. Tomczak, D. D. Glawe, L. F. Drummy, C. G. Lawrence, M. O. Stone, C. C. Perry, D. J. Pochan, T. J. Deming and R. R. Naik, *J. Am. Chem. Soc*., 2005, **127**, 12577.
97 A. Rai and C. C. Perry, *Langmuir*, 2010, **26**, 4152.
98 F. Rodriguez, D. D. Glawe, R. R. Naik, K. P. Hallinan and M. O. Stone, *Biomacromolecules*, 2004, **5**, 261.
99 C. Groeger, K. Lutz and E. Brunner, *Cell Biochem. Biophys*., 2008, **50**, 23.
100 L. Lenoci and P. J. Camp, *Langmuir*, 2008, **24**, 217.
101 M. Sumper, *Angew. Chem., Int. Ed*., 2004, **43**, 2251.
102 Y. G. Yang, M. Suzuki, M. Kimura, H. Shirai and K. Hanabusa, *Chem. Commun*., 2004, 1332.

103 H. Yang, G. A. Ozin and C. T. Kresge, *Adv. Mater.*, 1998, **10**, 883.
104 G. A. Ozin, *Can. J. Chem.*, 1999, **77**, 2001.
105 H. B. S. Chan, P. M. Budd and T. D. Naylor, *J. Mater. Chem.*, 2001, **11**, 951.
106 L. G. Frigeri, T. R. Radabaugh, P. A. Haynes and M. Hildebrand, *Mol. Cell. Proteomics*, 2006, **5**, 182.
107 M. B. Dickerson, K. H. Sandhage and R. R. Naik, *Chem. Rev.*, 2008, **108**, 4935.
108 R. L. Brutchey and D. E. Morse, *Chem. Rev.*, 2008, **108**, 4915.
109 E. Pouget, E. Dujardin, A. Cavalier, A. Moreac, C. Valery, V. Marchi-Artzner, T. Weiss, A. Renault, M. Paternostre and F. Artzner, *Nat. Mater.*, 2007, **6**, 434.
110 S. Kessel, A. Thomas and H. G. Borner, *Angew. Chem., Int. Ed.*, 2007, **46**, 9023.
111 M. M. Giraud-Guille, *Curr. Opin. Solid State Mater. Sci.*, 1998, **3**, 221.
112 C. A. Durkin, T. Mock and E. V. Armbrust, *Eukaryotic Cell*, 2009, **8**, 1038.
113 H. Ehrlich, D. Janussen, P. Simon, V. V. Bazhenov, N. P. Shapkin, C. Erler, M. Mertig, R. Born, S. Heinemann, T. Hanke, H. Worch and J. N. Vournakis, *J. Nanomater.*, 2008, **2008**, 670235, DOI: 10.1155/2008/670235.
114 E. Dujardin, M. Blaseby and S. Mann, *J. Mater. Chem.*, 2003, **13**, 696.
115 A. Thomas and M. Antonietti, *Adv. Funct. Mater.*, 2003, **13**, 763.
116 D. Eglin, G. Mosser, M. M. Giraud-Guille, J. Livage and T. Coradin, *Soft Matter*, 2005, **1**, 129.
117 T. Coradin, M. M. Giraud-Guille, C. Helary, J. Livage and C. Sanchez, *Mater. Res. Soc. Symp. Proc.*, 2002, **726**, 79.
118 Y. Ono, Y. Kanekiyo, K. Inoue, J. Hojo, M. Nango and S. Shinkai, *Chem. Lett.*, 1999, 475.
119 S. Heinemann, C. Heinemann, H. Ehrlich, M. Meyer, H. Baltzer, H. Worch and T. Hanke, *Adv. Eng. Mater.*, 2007, **9**, 1061.
120 M. L. Ferrer, R. Esquembre, I. Ortega, C. R. Mateo and F. del Monte, *Chem. Mater.*, 2006, **18**, 554.
121 C. E. Fowler, W. Shenton, G. Stubbs and S. Mann, *Adv. Mater.*, 2001, **13**, 1266.
122 E. Dujardin, C. Peet, G. Stubbs, J. N. Culver and S. Mann, *Nano Lett.*, 2003, **3**, 413.
123 K. T. Nam, R. Wartena, P. J. Yoo, F. W. Liau, Y. J. Lee, Y. M. Chiang, P. T. Hammond and A. M. Belcher, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 17227.
124 A. Coiffier, T. Coradin, C. Roux, O. M. M. Bouvet and J. Livage, *J. Mater. Chem.*, 2001, **11**, 2039.
125 Y. Y. Wu, G. S. Cheng, K. Katsov, S. W. Sides, J. F. Wang, J. Tang, G. H. Fredrickson, M. Moskovits and G. D. Stucky, *Nat. Mater.*, 2004, **3**, 816.

Nadine Nassif

Nadine Nassif is a CNRS researcher in the Laboratory of the Condensed Matter Chemistry in Paris 6 University (LCMCP) where she gained her PhD on bacteria encapsulation in silica sol–gel matrices under Jacques Livage's guidance. She did her Postdoctoral work with Markus Antonietti and Helmut Cölfen (Max Planck Institute for Colloids & Interfaces, Potsdam) on biomimetic approaches for calcium carbonate crystallization. Presently her interest is on biomineralization and self-assembled collagen biomaterials.



Jacques Livage

Professor at the College de France and member of the French Academy of Sciences, Jacques Livage is known as the founder of 'chimie douce' or 'soft chemistry'. After his PhD in Paris and a post-doc at the Clarendon Laboratory in Oxford, he joined the University of Paris VI in 1970. Since then, he studied the electronic properties of vanadium oxide gels and the synthesis of nanostructured vanadium oxides. He is now working on bio-inspired materials obtained via the sol–gel encapsulation of enzymes, antibodies and even whole cells in silica gels.