Nature is an inspirational source of silica structures that possess unique optical properties. A few classes of organisms fabricate metal oxides with nanoscale features by a bottom-up self assembly process. In particular, diatoms are a prolific class of single-celled algae that possess silica shells or “frustules” with intricate submicron scale features, including two-dimensional pore arrays. During frustule development, membrane-bound transporters actively take actively up the soluble silicon in the form of Si(OH)$_4$. Once inside the cell, Si(OH)$_4$ is converted to nanostructured silica by protein-mediated condensation reaction within the silicon deposition vesicle, an organelle which serves as the mold for frustule development. Recently, it has been shown that intact diatom frustules can act as 2D photonic crystals and optical sensor platforms for the detection of organic vapors. Techniques to assemble and pattern these microorganisms at large scale is needed to take advantage of these nanostructured silica for device applications such as sensors, photonic crystals and electroluminescence devices. In this work, individual shells of the diatom Coscinodiscus self-assembled into a rectangular array on a glass surface which
possessed a polyelectrolyte multilayer patterned through inkjet printing. This patterned thin film possessed hierarchical order with nanostructure provided by the diatom biosilica. The process used two polyelectrolytes with opposite electric potentials to control the surface charge of the substrate. The fine features of the diatom frustules were perfectly preserved due to the mild conditions of the deposition process. This technique has the potential to enable large-scale device applications which harness the unique properties of functionalized diatom biosilica.
Selective Self-Assembly of Biogenic Silica Assisted by Layer-by-Layer Deposition and Inkjet Printing

by

Wei Wang

A THESIS

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degree of

Master of Science

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APPROVED:

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Wei Wang, Author
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CHAPTER 1

Introduction

Diatoms are single celled microalgae that possess cell walls called “frustules” that are composed of amorphous silica nanoparticles patterned into intricate nano- and microstructures. These small dimensional features render diatoms with large surface to volume ratio and micro to nanoscale structures, which are essential for sensor, light emitting, light harvesting, and catalytic applications. For example, D.-H. Lee et al. utilized diatom frustules as silica source that reacted with Zinc and Yttrium precursors to form phosphor materials whose intensities were enhanced by the effectiveness of the fine structure of diatom frustules. Via reduction of Silica to Silicon by magnesium, Z. Bao converted porous frustules into silicon frustule sensors which were used to detect low concentration of NO(g). Both of the sensitivity and response speed of the biosensor exceed the ability of planar porous silicon sensor due to the open, porous, 3D structure with such high surface area. The growth of diatoms is a process containing different reactions by which silica shell and other organisms are formed. via controlling the culturing condition of diatoms, the composition and morphology of diatoms can be tailored to fit specific purposes to some extent. C. Jeffryes et al. have successfully fabricated an electroluminescence device using diatom frustules where Germanium was inserted by a two-stage cell cultivation process. Due to the
advantage of biogenic silica, frustules have found potential use as sensors, filtration media, photonic crystals, and abrasive materials\[^6, 7, 8\].

In terms of fabricating devices, patterning is always a significant factor for functioning properly. Compared with conventional photolithography, printing technology can directly deposit desired patterns on substrate in a low-cost and time-saving fashion. Nearly all kinds of materials ranging from organic compounds to inorganic chemicals can be formed by printing process, while photolithography is limited to photosensitive materials. For decades, the versatility of Inkjet Printing has been applied in various scientific fields. Recently, printing electronics have attracted a great deal of efforts. For example, D.-H. Lee \textit{et al.} have successfully printed high-mobility transparent amorphous oxide semiconductors to fabricate high-performance TFT\[^9\]. H. Sirringhaus made use of Inkjet Printer to fabricate all-polymer transistor circuits with a 0.02 cm\(^2\)/V\(\cdot\)S mobility and a 10\(^5\) on-off current switching ratios\[^10\]. Besides, metals like Au and Al have already been printed by Inkjet Printer\[^11, 12\].

In this work, individual shells of the diatom Coscinodiscus self-assembled into a rectangular array on a glass surface which possessed a polyelectrolyte multilayer patterned through inkjet printing. This patterned thin film possessed hierarchical order with nanostructure provided by the diatom biosilica. The process used two polyelectrolytes with opposite electric potentials to control the surface charge of the substrate. The fine features of the diatom frustules were perfectly preserved due to the mild conditions of the deposition process. This technique has the potential to enable
large-scale device applications which harness the unique properties of functionalized diatom biosilica. Figure 1.1 shows a schematic of this process.

Figure 1.1 Schematic of selective self-assembly process of biogenic silica shell
CHAPTER 2

Literature Review

2.1 Diatom

Diatoms are ubiquitous organisms living in a variety of habitats and contributing at least 25% of the world’s net primary production of organic carbon. The dimension of diatoms is in the range of 1 micron to 500 micron with characteristic rigid amorphous silica shells. The most interesting feature of diatoms is the nanometer-scale structure of silica shells (see Figure 2.1), which is fabricated at mild ambient temperature and pressure and exceeds the capabilities of present-day human engineering.

Figure 2.1 Scanning Electron Microscopy images of *Pinnularia sp.*

Diatoms are usually divided into two main groups depending upon the symmetry of their frustules. Centric diatoms tend to be radially symmetrical, while pinnate diatoms
tend to be elongated and generally have parallel striae arranged normal to the long axis. Observing diatoms carefully one can find the diatom frustule consists of two almost equal halves stick together like petri dish. Each half consists of a valve and a girdle band.

There are two major areas of research of diatoms: one focus on application of the organic or inorganic compositions as raw materials for the synthesis of other functional compounds; the other one is the study of biological mechanism, for example bio-silification. In decades the study of diatoms in these two fields has achieved considerable accomplishment. Aizenberg et al. utilized Scanning Electron Microscopy to reveal structural hierarchy of diatom demonstrating nature’s ability to improve inherently poor building materials. The genome sequences of different kinds of diatoms have been described in several groups. Based on these genome sequence research, several novel silicic acid transporters and genes involved in the formation of silica-based cell walls were found, as well as genes encoding polyunsaturated fatty acid biosynthetic enzymes and a complete urea cycle. In addition to genes, other active compounds like proteins and fatty acids have been proposed for a variety of biotechnological applications, such as bioremediation of water contaminated with heavy metals and synthesis of polyunsaturated fatty acids.

Diatomite is porous and lightweight sedimentary rock resulting from the accumulation a compaction of diatom remains over geological time scales. It is of low density, high porosity, low thermal conductivity, high melting point and chemical inertness. All of
its features make itself suitable for filtration, insulation, absorption, building materials, mineral filler, and etc. Due to the potential application into photonic crystals and sensors based on the fine porous structure of diatom frustules, the optical properties of biosilica have attracted lots of attention. Besides, through chemical deposition processes, scientists have converted silica to other crystalline materials without damaging the three-dimensional diatom shells.

2.2 Inkjet Printing

2.2.1 Principles behind inkjet printing

Printing is always an attracting technique for scientists and engineers owing to its high efficiency, mechanization, precision and ability to pattern. One type of printing technique is inkjet printing which uses piezoelectric actuators to eject inks onto a substrate.

Figure 2.2 (a) Schematic diagram of a piezoelectric inkjet print head. (b) Schematic representation of wave propagation and reflection in a piezoelectric tubular actuator.
Figure 2.2(a) show a schematic of a typical piezoelectric drop-on-demand (DOD) inkjet print head. Usually the size of nozzle is much smaller than that of the cavity, so the nozzle end can be considered as being closed according to the acoustic wave theory. Whereas the supply end can be described as being open since the inside diameter of the supply tube is much larger than that of the cavity\textsuperscript{16}. The PZT piezoelectric actuator is driven by voltage to transfer kinetic energy outwards. A droplet is ejected when the amount of transferred kinetic energy is larger than the surface energy needed to form a droplet.

Figure 2.2(b) gives a more detail explanation about the droplet ejection process. The right and left sides of the figure stand for the ink supply end and nozzle end, respectively. Initially, increasing voltage on the piezoelectric actuator forces it to move radially outward, resulting in a negative pressure wave in the liquid (intake of liquid) (Figure 2.2 b(1)). This pressure wave splits into two with the resultant waves travelling in opposite directions with half amplitude (Figure 2.2 b(2)). When these two pressure waves travel to the ends, the one at supply end is reflected with its phase consistent, whereas the one at nozzle end is reflected with its phase reversed (Figure 2.2 b(3)). When the two reflected pressure waves travel back, meeting in the middle, the voltage across the piezoelectric decreases and causes the actuator to move radially inward, resulting in a positive pressure wave in the liquid (outlet of liquid) (Figure 2.2 b(4)). The newly formed positive-pressure meets the reflected waves and subsequently
interacts with them. The result is that the negative pressure wave is neutralized whereas the positive wave is doubled (Figure 2.2 b(5)). Finally the positive pressure wave approaches to the nozzle and pushes liquid outwards.

2.2.2 Essential parameters

In the process of printing there are several factors that can influence the performance of printers, such as the amplitude and frequency of voltage across the piezoelectric, the size of nozzle, diameter of cavity, the viscosity and surface tension of ink and etc. The effect of these variables will be discussed briefly here.

The volume of a droplet is linearly dependent on the driving voltage. For a rectangular input pulse, the voltage amplitude and its speed of variation determine the amount of droplet volume change. As the piezoelectric displacement is proportional to the applied electric field, it is easy to gain that the volume of a droplet rises with the increasing of voltage amplitude.

The frequency of electric field influences not only the quality of printing but its efficiency. At high frequencies the pressure wave is generated so fast that the previous wave interferes with the next one resulting in chaotic droplet ejection. On the other side, low frequency decreases the ejection rate.

To some extent, the dimension of a print head also controls the printing process. We can easily imagine how the nozzle size determines the size of droplet. Also the length of cavity ($l$) combined with the speed of sound ($c$) in liquid plays an important role in
the successful droplet ejection. From experiment\textsuperscript{16} it is obtained that the optimum pulse width is equal to $l/c$.

The properties of ink determine the amount of kinetic energy needed to form droplet and its velocity. Fromm\textsuperscript{18} used the Z number grouping of fluid properties, which is equivalent to the inverse of the Ohnesorge number (Oh), to provide a dimensionless analysis of the mechanics of drop formation in DOD print head:

$$Z = \left(\frac{d \rho \gamma}{\eta}\right)^{1/2} = \text{Oh}^{-1}$$

where $\gamma$, $\eta$, and $\rho$ are the surface tension, viscosity, and density of the liquid, respectively, and $d$ is a characteristic length, which in the case of piezoelectric DOD print heads is the diameter of the nozzle aperture. Fromm predicted that drop formation of in DOD systems was only possible for $Z>2$ and that for a given pressure droplet volume increases as the value of $Z$ increases\textsuperscript{18}.

All the discussions above mostly related with the formation of droplet are just the first step of printing. After that, droplet falls off on the surface of substrates where another issue is coming out, how does droplet behave as approaching the surface? Van Dam and Le Clerc divided the process of impaction into three basic steps\textsuperscript{19}. In the first step, the droplet hits the substrate. The main concern in this step is the splashing which may destroy the resolution of printing. One way to avoiding splashing is to reduce the pressure of surrounding gas above substrates\textsuperscript{16}. The second, the radius of the droplet-substrate interface expands to an order of magnitude greater than that of the in-flight
droplet radius, with an accompanying swift fluid flow, which is radically outwards. In the third step, the fluid comes to rest after an initial rebound and a series of inertial oscillations that are dampened by viscous dissipation.

Most of the substrates used in printing electronics are non-absorbing. So the phenomenon that solute tends to deposit at the boundaries of a printed feature affects the quality of printing. The consequence is non-uniform coating of solute, which may result in the failure of devices. However there are several methods to diminish its effect. The use of a binary mixture of solvents can eliminate the formation of ring stains\(^{20}\), if one of the solvents has a much higher boiling point than the other. Higher contact angle of ink depresses the spreading of the droplet and also the replenishing flow of solute.

### 2.2.3 Comparison with other fabrication techniques

The main advantage of inkjet printing is the mask-less patterning that lowers the cost of fabrication and simplifies the process of patterning compared with other patterning techniques. Typically the traditional approach to pattern materials on surfaces is to coat surfaces with desired chemicals and then etch the coated surfaces with UV light or chemical gases. Actually before etching a polymer resist or mask layer is coated on the surface to protect the desired area. At least there are three steps involved in each patterning. So the cost of traditional patterning always stands at a high level. Also in the traditional patterning techniques, patterning is limited by the properties of chemicals on surfaces. Often times it is impossible to find a proper way to etch on
certain chemicals. In contrast printing can accomplish patterning in just one step or at most two steps with another simple evaporating or sintering process.

The operation condition for printing is usually moderate. Rigorous operation conditions like vacuum and high temperature are required for conventional patterning techniques. However, printing can work properly in a mild environment, commonly in room temperature and atmosphere. That makes it easier to access and be industrialized.

In fact, there are several shortcomings to overcome if inkjet printing will be used in a precise purpose. The first one is the resolution of printing. For inkjet printing the common resolution is in a micro scale which is non-comparable with other techniques such as photolithography. In the modern technology, the dimension of devices goes down to submicron scale and the requirement of the accuracy increases dramatically. For example, in an active matrix display application composed of over a million TFTs, a single printing error could result in electrical short and render the whole display useless. So the weakness of inkjet printing urges it to collaborate with other techniques.

### 2.2.4 Recent advancement in printing electronics

For decades inkjet printing has been used to pattern different materials ranging from organic to inorganic chemicals. Recently, a great deal of efforts has been devoted to printing electronics. Sirringhaus et al.\(^1\) have utilized inkjet printing, photolithography, and spin-coating to fabricate all-polymer TFTs with a mobility of 0.02 cm\(^2\)V\(^{-1}\)s\(^{-1}\). In this case, Sirringhaus et al. made use of surface energy variation
(hydrophilic/hydrophobic), which was formed by photolithography, to control the width of the channel between source and drain in TFTs. Later Sirringhaus et al. used a bottom-up, self-aligned inkjet printing technique to deposit polymers for source and drain of TFTs with hundred nanometer resolution\(^2\). On the other hand, Doo-Hyoung Lee et al. used simple metal chloride solution to fabricate oxide semiconductor transistor via inkjet printing\(^9\). Examples above exhibit very positive potential to utilize inkjet printing to fabricate electric circuits in a comparable scale.

In addition to printing functional materials to fabricate devices, Schubert et al. applied inkjet printing to etch polymer films as an alternative method for lithography\(^2\). The whole process includes printing of droplet, spreading of solvent, and evaporation of solvent. Thus the size of etched holes is several times larger than the dimension of droplet, which is controlled by three factors, the spreading of the solvent on the chosen substrate, the rate of evaporation of the solvent, and the rate of dissolution of the polymer. Rubner et al. ejected water to dissolve hydrogen-bonded polyelectrolyte film made from polyacrylamide and poly(allylamine hydrochloride) to fabricate light emitting devices via inkjet printing\(^2\).\(^3\)

Inkjet printing as a versatile patterning technique has been applied in the field of sol-gel materials, organic light-emitting diodes, organic transistors, conducting polymers, structural polymers, ceramics, metals, and nucleic acid and protein arrays\(^2\).\(^4\). The cost-saving and vacuum-free property renders bright prospects to inkjet printing as an alternative for lithography.
2.3 Layer-by-Layer Deposition

2.3.1 What is Layer-by-Layer Deposition?

Since early 1990s, Decher’s group has developed a technique for the construction of multicomposite films of rod-like molecules equipped with ionic groups at both ends, polyelectrolytes, or other charged materials through layer-by-layer adsorption from aqueous solution. The process, which is extremely simple, is depicted in Figure 2.3 for the case of polyanion-polycation deposition on a positively charged surface\textsuperscript{25}.

In the deposition process, the positive charges on the substrate surface attract the negative charges on polymers in breaker 1, which results in the adsorption of polyanions on the substrate. After a washing step, the charge-reversed substrate is
immersed into a polycation solution in step 3. This step similarly covers the substrate surface with cations. Another washing step is provided before depositing another polyanion film. These four steps are the basic buildup sequence for multilayer architectures.

The first issue is about the driving force. The major driving force for the formation of polyelectrolyte complexes (PECs) could be attributed to the electrostatic interaction between the polycations and polyanions. The study of PECs can be traced back to the early twenty century\textsuperscript{26}. Russian scientist V. Kabanov\textsuperscript{27} has applied himself to the theoretical study of PECs for decades. Based on his theoretical model of PECs, he gave a thermodynamic explanation for driving force of formation of PECs. In the equation below, the quantity \( \Delta pH(\Theta,a) \) is a measure of the free energy, \( \Delta G_{cs}(\Theta,a) \), providing the driving force for complexation and characterizing multisite interaction of the oppositely charged repeating units at a specified \( \Theta=a \).

\[
\Delta pH(\Theta,a) = \frac{[\Delta G(a) - \Delta G(\Theta)]}{2.3RT} = \frac{\Delta G_{cs}(\Theta,a)}{2.3RT}
\]

Here, \( \Theta \) and \( a \) are the degree of conversion from polyelectrolytes to interpolyelectrolyte complexes and the individual degree of ionization of polyelectrolytes respectively. \( \Delta G(a) \) and \( \Delta G(\Theta) \) are the free energies of ionization of a weak polyelectrolyte in the absence and in the presence of the oppositely charged polyanion respectively. Ref. 27 provides a typical \( \Theta \) versus pH plot calculated from experimental titration curves of weak polyelectrolytes in the presence of oppositely charged polyanion counterparts. From the graph, it was observed that
interpolyelectrolyte complexes were easier to dissolve in strong acidic or basic condition.

The free energy decrease responsible for the formation of PECs is basically due to an entropy gain caused by release of counterions originally localized in the vicinity of the polyelectrolyte coils. However, depending on the polyelectrolye composition and structure other attraction factors such as hydrophobic interaction, hydrogen bonding, charge-transfer etc. may also contribute\textsuperscript{27}.

In the perspective of multilayer formation, there are basically three major phases\textsuperscript{28}. The first one is the first layer of the assembly which is obtained by adsorption of polyelectrolyte chains on an oppositely charged surface. The first layer must overcompensate the charge of the surface which allows the succeeding adsorption. Simultaneously the layer must be ‘strongly anchored to the surface. The second phase is the formation of PECs discussed above. The differences between PECs and polyelectrolyte multilayers (PEMs) are that the former is self-assembly, while the latter is template assisted assembly. An important step in the formation of PEMs is the rinsing step between two consecutive adsorptions. This step is aimed at eliminating the non-adsorbed (or weakly adsorbed) polyelectrolyte. Many experiments have pointed out that the properties of the multilayer have a deep relationship with rinse step. In reality the formation of multilayers is not an equilibrium process. However, at the present time no kinetic theory for rinse seems to be available. So a thermodynamic quasi-equilibrium theory is assumed in order to simulate this process. At most times,
we assume that polyelectrolyte adsorption is irreversible even in contact with pure solvent during the rinsing step\textsuperscript{29}.

Although there is no model that can depict the real films perfectly right now, a three-zone model\textsuperscript{30, 31} can still inspire us with a virtual representation. There are three distinct zones in polyelectrolyte films. Zone I is composed of a few polyelectrolyte layers close to the substrate. In this zone the properties of polyelectrolyte layers, such as refractive index, are influenced dramatically by substrates. Zone III is composed of a few polyelectrolyte layers close to the surface of the film. Zone II located between these two zones shows a neutral status, while zone I and II are charged.

Figure 2.4 The zone model for polyelectrolyte multilayer formation

Figure 2.4 describes a film developing process using the zone theory. When the number of polyelectrolyte layers is small, only zone I exists. After zone I reaches the final layer number and thickness, the surface of polyelectrolyte films will form into zone II until the final thickness of zone is approached. Afterwards polyion pairs that form a 1:1 complex will form a zone II gradually, while the thicknesses of zone I and II keep their respective values. There is no sharp transition between zone I and II and
between zone II and III. Actually they intermingle to some extent. Zone II will swell when exposed to salt solutions, since zone II should be zwitterionic and the salt will break ionic bonds between polycations and polyanions. In contrast, the layers in zone III should show classic polyion-like behavior. This means that they should swell in pure water and collapse in salt solutions owing to the screening of the electrostatic repulsion between charges of equal sign.

Besides the three-zone model, there are other theoretical models derived from various theories that can quantitively describe the formation of polyelectrolyte layers. Assuming the complexation between consecutive layers is the driving force, Castelnovo and et al.\textsuperscript{32} developed a theory for flexible polyelectrolytes at high ionic strength. The observation of experiments also showed that the structure within PEMs was very fuzzy and that adjacent layers strongly interpenetrated. But this model is limited in weakly charged polymers. In the limit of strongly charged polymers, Zeldovitch and et al.\textsuperscript{33} provided another theory based on ion pairs to explain the formation of PEMs. Netz and Joanny\textsuperscript{34} gave another model for rigid polymers that is different from the flexible polymers used in Castelnovo’s model.

\subsection*{2.3.2 Charge balance in multilayers}
There are two scenes, see in Figure 2.5, to maintain net charge neutrality. In one case, termed intrinsic compensation, polymer positive charge is balanced by a negative charge, also on a polymer. Alternatively, polymer charge is balanced by salt counterions derived from the bathing solution used to construct the multilayers (extrinsic charge compensation)\textsuperscript{35}. We can assume as-made polyelectrolyte multilayers contain no salt ions and that it is possible to introduce salt ions by increasing external salt concentration. In the following equilibrium\textsuperscript{36}, polyelectrolyte/polyelectrolyte ion pairs are replaced by polyelectrolyte/salt ion pairs.

\[ \text{Pol}^+ \text{Pol}^- \text{aq} \rightarrow \text{Pol}^+ \text{Cl}_m^- + \text{Na}_m^+ \]

where Pol\textsuperscript{+} and Pol\textsuperscript{-} are respective positive and negative polyelectrolyte repeat units. m refers to components in the multilayer phase. The equilibrium constant may be written as\textsuperscript{36}
\[ K' = \frac{\gamma^2}{(1-\gamma)[\text{NaCl}]_{aq}^2} = \frac{\gamma^2}{[\text{NaCl}]_{aq}^2} \rightarrow 0 \]

where \( \gamma \) is the fraction of the multilayer in the extrinsic form and \( 1-\gamma \) is the intrinsic fraction. It is clear, from this equilibrium, that \( \gamma \) is proportional to the salt concentration, that is, higher the salt concentration, larger the fraction of extrinsic compensation.

Logically one would expect multilayers to expand as ions enter the film. Direct measurements of swelling have demonstrated the thickness of multilayers increases, see ref. 37, when they are exposed to external salt solutions. Different types of polyelectrolyte pairs own various swelling properties. However, there is a limit of swelling to maintain the combination of polyelectrolyte pairs in multilayers. In ref. 37 one can obtain that it is possible to reverse polyelectrolyte association at sufficiently high salt concentrations. At certain critical salt concentration, the extrinsic compensation is dominant while the intrinsic compensation, polyelectrolyte/polyelectrolyte pairs, is negligible and no longer capable of holding the PEMs together. After this critical point, multilayers will decompose into complexes in solutions.

Why do we need salt or extrinsic compensation in the process of multilayer developing? The fundamental requirement is extrinsic compensation can make excess charges on top layers which can adsorb opposite-charge polyelectrlyte to form PEMs. Actually it is impossible for layers with completely mixed intrinsic materials. In the
bulk multilayer, there is no extrinsic compensation, in contrast to the region near the surface, where salt counterions balance excess polymer charge. As the number of layers grows, the external salt ions will not stay in the bulk of multilayer but move together with solid/liquid interfaces, which has been verified via XPS\textsuperscript{37}. Also extrinsic compensation may help build up multilayers if layering with periodicity greater than the width of a polymer chain.

Using radiolabeled counterions\textsuperscript{35} one can trace that the external labeled salt ions in the top layers of multilayers would self-exchange with the unlabeled ions in solution. However, the same thing does not happen on polyelectrolyte polymers. The adsorption of polymer is irreversible. That prevents the multilayer polymers from being stripped off from the surface on exposure to a solution of its oppositely charged partner. In other words, salt mediates the removal of surface polyelectrolyte by loosening the interactions between polymers via competitive ion exchange.

### 2.3.3 What advantage does it have?

The layer-by-layer (LbL) deposition technique is involved in template assisted assembly. Template assisted assembly is much faster than self-assembly/chemical modification cycles whose outcome is often uncertain or difficult to predict\textsuperscript{38}. For the case of LbL-deposition, multimaterials including small organic molecules, inorganic compounds, macromolecules, and colloids, can be assembled into one multilayer film without special chemical modifications, thus giving possibility to tailor the chemical, electronic, magnetic or etc. properties of the films. Comparison with Langmuir-
Blodgett technique that is usually used in the deposition of specific organic materials in liquid phase onto solid surfaces via immersion or emersion step, the layer-by-layer deposition technique is more universal in terms of deposited materials.

The ultra-low-cost and environmentally friendly properties of PEMs deposition process make this technique competitive with other deposition processes. The mild condition of LbL-deposition can preserve the adsorbate activity and prevent adsorbates from damage.

The LbL-deposition can occur on surfaces of any kind and any shape as long as solvents can access to the surfaces. After the first deposited layers, the interaction between surfaces and adsorbates becomes independent of substrates.

2.3.4 Major achievement

Since the outset of PEMs, there have been numerous novel applications using PEMs in different field. Basically the fundamental principle underneath is the stacking of multimaterials in a certain sequence. Caruso and et al.\textsuperscript{39, 40} used PEMs to assemble silica nanoparticles on Latex particles. The strategy they used is the alternate adsorption of SiO\textsubscript{2} and poly(diallyldimethylammonium chloride) (PDADMAC) on a precursor three-layer polyelectrolyte multilayer. After the assembly Caruso removed the templated core (Latex particles) by annealing or etching. Finally hollow spherical silica shells were fabricated. Using the similar method, Caruso\textsuperscript{41} also introduced magnetic material Fe\textsubscript{3}O\textsubscript{4} into PEMs on Latex particles to make magnetic core-shell particles. Through this alternate assembly technique, others have applied it to dye
molecules, bio-molecules, nanoparticle, two-dimensional inorganic sheets, and molecular aggregates\textsuperscript{42}.

The attractive point in PEMs is to create order along the layer normal. On the other side, however, we have to use other techniques to create order within the plane of the film. Such techniques include printing, photolithography, and etc. Hammond\textsuperscript{43} used microcontact printing to deposit PEMs in a patterned area. Subsequently, the polyelectrolyte surface was dipped into an aqueous colloidal suspension of bare SiO\textsubscript{2} microparticles or functionalized Latex particles. The colloids self-organized at the surface, driven by the spatially varied electrostatic and secondary interactions between the colloid and the substrate. Nevertheless Rubner\textsuperscript{44} made use of instability of hydrogen bond in the aqueous solution to partially dissolve PEMs by using inkjet printing, resulting in patterning the film. However this method is just limited in the range of hydrogen bond. Decher\textsuperscript{38} used a very classic method, photolithography, to pattern PEMs. Also photolithography can provide higher resolution compared with microcontact printing and inkjet printing.

Besides, PEMs can be used in LED devices\textsuperscript{38,44} and encapsulation of medicines and proteins\textsuperscript{45} due to its conductivity and swelling property, respectively.
CHAPTER 3

Experimental Section

3.1 Selective self-assembly of biogenic silica

3.1.1 Fabrication of polyelectrolyte multilayer films by dipping

The procedure of the fabrication of polyelectrolyte multilayers is adapted from Ref. 46. Poly(sodium 4-stryrene sulfonate) (PSS, MW=70 000g/mol), poly(allylamine hydrochloride) (PAH, MW=15 000g/mol) were purchased from Sigma-Aldrich. PSS at 3.0mg/ml and PAH at 1.35mg/ml were prepared, respectively. Each polyelectrolyte solution was freshly prepared by dissolving respective polyelectrolyte in RO water
with 0.5mol NaCl. Micro slides (25mmX10mm) sonicated for 2min were treated by a standard acetone, methanol, and deionized water (AMD) precleaning method, and dried by spraying with compressed nitrogen from a nitrogen cylinder. The dipping process for deposition of PEMs is illustrated in Figure 3.1. Cleaned glass slides were submerged into PAH solution for 20min, to allow enough time for saturated adsorption, rinsed with plenty of water, and blown to dry using compressed nitrogen. Afterwards the glass slides were dipped into PSS solution for another 20min and followed by the same cleaning step. Each cycle creates two layers which consisting of one layer of PAH and one layer of PSS. We then repeat this deposition procedure to fabricate the desired number of layers. The last layer is normally the PSS layer.

### 3.1.2 Printing of the top PAH polymer layer

![Figure 3.2 Schematic of printing of PAH and self-assembly of diatom frustules](image)

27mg PAH and 0.295g NaCl were dissolved in 1ml RO water, 2ml Acetonitrile, and 7ml Ethylene Glycol with sonication for 5min. About 2ml PAH ink filtered by 0.2μm
PTFE filter was injected Dimatix cartridge which was then installed into Dimatix DMP-2831 Inkjet Printer. The substrate used in printing is pre-coated with PEMs using the procedure described above. Since positively charged polymer PAH will be printed, the top layer on pre-coated substrate ought to be negatively charged PSS. After printing designed pattern on substrates, it was rinsed by DI water and blowing compressed standard nitrogen. Figure 3.2 illustrates the steps of polymer printing and adsorption of biogenic silica.

The printing parameters of Dimatix printer are list here:

<table>
<thead>
<tr>
<th>Voltage/V</th>
<th>Frequency/kHz</th>
<th>Number of Nozzles</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>3</td>
<td>1-2</td>
</tr>
</tbody>
</table>

The values of these parameters are related with the printing quality, especially printing resolution. The voltage applied on piezoelectric of the cartridge must be high enough to overcome the surface tension to eject the ink drop successfully out of nozzles. If the voltage is too small, there is no ink coming out of the cartridge. However, high voltage is not always proper since tail will be formed due to high pressure in nozzles. The electric frequency applied on nozzles is usually smaller than the acoustic frequency of ink. The reason why just 2 nozzles were used is that the smaller the number of nozzles the higher the printing resolution due to the mechanical limit of the printer.
3.1.3 Self-assembly of biogenic silica shells

Biogenic silica shells were made from diatom cells treated via SDS method for the removal of organisms. The remaining silica shells were then suspended in water for the purpose of adsorption. The substrate used in this step was coated with patterned PAH layer on top. For adsorption, 0.4ml frustules suspension with 0.5M NaCl was dropped on the substrate. Because the surface of substrate is selectively positively charged and the silica shells own negative potential, via ionic bond silica can be selectively assembled on the PAH layer which was printed on a PSS layer (see Figure 3.2). After 20min, the suspension was washed away by water, and the substrate was blown to dry using compressed nitrogen gas. At the end, the patterned diatom frustules were obtained on the substrate.

In my experiment, there are three kinds of diatom species which are used as row materials for the self-assembly, Cyclotella, Pinnularia, and Coscinodiscus wailesii. The centric Cyclotella and Coscinodiscus have diameters of around 10µm and 200µm, respectively, whereas football-shaped Pinnularia sp. is about 20µm. The dimension of frustules influences the self-assembly dramatically. Usually smaller particles are easier to adsorb, since they have more chance to contact with positively charged surface due to Brownian motion. In order to conquer the limited Brownian motion for larger particles, the substrate with suspension on top was tilted at a small angle and spun gently during the adsorption.

3.2 The characterization of deposition and adsorption
3.2.1 Zeta potential measurement

The EKA Electro Kinetic analyzer is used for zeta potential measurement via measuring streaming potential or streaming current. An electrolyte solution flows through the measuring cell containing the sample to be measured. Depending on the flow resistance of the sample, a pressure drop is detected across the measuring cell. The electrolyte flow is generated by a pump and causes charge transportation in the flow direction along the cell. The resulting potential difference is detected by measuring electrodes connected at both ends of the measuring cell. Below is the equation for calculating of zeta potential:

\[ \zeta = \frac{dU}{dp} \cdot \eta \cdot \frac{L}{\varepsilon \cdot \varepsilon_0 \cdot Q \cdot R} \]

where \( \zeta \) is donated to zeta potential, \( \frac{dU}{dp} \) slope of streaming potential versus pressure, \( \eta \) electrolyte viscosity, \( \varepsilon_0 \) permittivity, \( \varepsilon \) dielectric constant of electrolyte, \( L \) length of the capillary system, \( Q \) cross-sectional area of the capillary system, \( R \) AC resistance of cell using electrolyte solution.

Figure 3.3 presents the zeta potential measured on glasses with different number of polyelectrolyte layers. Initially the bare glass had a net negatively charged surface. After depositing a layer of PAH, the charged was inversed to be positive. By repeating the deposition of oppositely charged polyelectrolyte on glasses, the zeta potential was inversed periodically.
Figure 3.3 Zeta potential measurement of polymer layers formed in dipping process

The parameter setting is list below.

<table>
<thead>
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<th>Rinse</th>
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<th>Duration/sec</th>
<th>Repetition</th>
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</tr>
<tr>
<td>Cell</td>
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<tr>
<td>Measure</td>
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<td>120</td>
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</table>

3.2.2 Contact angle measurement

Contact angle is used to evaluate the wettability of PAH ink on PEMs surfaces which have a PSS layer on top. Figure 3.4 shows the force balance and the definition of contact angle in a liquid/vapor/solid system. From the consideration of thermodynamic
equilibrium between these three phases, the contact angle is determined by the equilibrium of three surface tensions. The equilibrium equation is known as the Young Equation:

\[ 0 = \gamma_{SV} - \gamma_{SL} - \gamma_{LV} \cos \theta_C \]

where we denote the solid-vapor interfacial energy as \( \gamma_{SV} \), the solid-liquid interfacial energy as \( \gamma_{SL} \), and the liquid-vapor interfacial energy as \( \gamma_{LV} \), and the equilibrium contact angle as \( \theta_C \). If the liquid is very strongly attracted to the surface, the droplet will completely spread out on the solid surface and the contact angle will be close to 0°. When the surface tension between solid and liquid \( \gamma_{SL} \) increases, correspondingly the contact angle \( \theta_C \) will increase to keep the equilibrium of three phases. Commonly the difference between liquid and solid phases could induce the increase of contact angle. For example, hydrophobic surface meets hydrophilic liquid.

![Figure 3.4 Contact angle and surface tensions in a liquid/vapor/solid system](image)

In the printing process, both low and high contact angles are useful. For high printing resolution, large contact angle is needed, whereas for large thin film printing, small
contact angle is helpful. In my experiment, larger contact angle is preferred, since micron-size pattern was printed.

![Image of contact angle measurement](image)

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<tr>
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</tbody>
</table>

<table>
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<th>Theta</th>
<th>average</th>
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<tr>
<td>3</td>
<td>18.93°</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>19.13°</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.5 Contact angle measurement on PEMs formed by dipping process. (a) H2O on PEMs (PSS on top) (b) PAH ink on PEMs (PSS on top)

The result of contact angle measurement is shown in Figure 3.5. Water (as reference) and the PAH ink are used for liquid phase. The substrate coated with a 4-dilayer polyelectrolyte film (PSS on top) is chosen as solid phase. From the result one can see that the PAH ink wets the polymer surface better than water. The hydrophobic property of the polymer surface plays an important role in decreasing the contact angle of PAH ink, since both polymer layers on substrates is made from carbon chains which have strong interaction with Ethylene Glycol (EG) the solvent of ink. It is hard to find a proper solvent to replace EG, because the substitute must own hydrophilic
property and high viscosity at the same time. So the low contact angle of PAH ink restricts the resolution of printing.

3.2.3 Fluorescence images of printed PAH layer with Rhodamine-123

![Fluorescence images of printed PAH layer with Rhodamine-123](image)

Figure 3.6 Visualization of the printed PAH pattern on the (PAH/PSS)$_4$ PEM. (a) (b) Epi-fluorescence of images at 530 nm emission. (c) (d) Line Scan of image intensity in (a) and (b) respectively

Rhodamine-123 is one dye which has a fluorescence emission at 530nm using 480nm for excitation. Following the procedure described above, PAH ink mixed with Rhodamine-123 was injected into cartridge for the purpose of printing evaluation. Figure 3.6 shows the images of epi-fluorescence emission under the excitation of 480nm wavelength using Leica microscope system coupled with a fluorescence source. From
this figure, one can obtain that the width of stripes is about 150µm, while the diameter of dots is about 300µm. The targeted sizes of stripes and dots are 50µm and 150µm, respectively. Spreading of the printed patterns was caused by the wetting of the PAH ink on the PEM. The measured contact angle for the PAH ink on the PEM (20°), was lower than that for pure water, due to the hydrophobicity of both the PAH ink and the underlying polyelectrolyte layer. The uniform distribution of green color across the printed PAH areas indicates the uniformity of the film in terms of thickness.
CHAPTER 4

Results and Discussions

There are many types of forces which can be used for self-assembly, such as van der Waals force and chemical force. In this work, we have used electrostatic force to assemble diatom frustules because of its longer interaction distance and universality in comparison with the chemical bond. For example BS[^3] (Bis(sulfosuccinimidyl) suberate) a homobifunctional, water-soluble crosslinker has a size about 11.4Å which is too small to anchor micron-size particles. In addition, this crosslinker can only react with specific surface groups which do not exist on biogenic silica surface naturally. On the other hand, electrostatic force can work easily at microsacle range. It is well-known that in an aqueous solution silica surface reacts with water to form a layer of silanol (SiOH) groups, thereby giving it a net negative surface charge[^47]. When they are exposed to a positively charged surface, silica particles undergo an adsorption process driven by the electrostatic attraction. This chapter will discuss the influence of processing condition on adsorption of frustules.

4.1 Adsorption of diatom frustules on different surfaces

Normally silica surface under atmosphere possesses a negative potential, which means on the surface of silica there are negative charges. So glass substrates generally repulse diatom frustules since both of them have negative charges on surface. The consequence is no adsorption occurring on bare glasses (see Figure 4.1(b)). However,
via depositing polyelectrolyte layers on glass surface, the surface potential can be inverted to be positive (see Figure 4.1(a)). Due to ionic repulsion, there is also no adsorption occurring on PSS coated glass which is negatively charged (see Figure 4.1(c)). So far we have shown that adsorption can occur only on PAH surface not on silica or PSS. This indicates that selective adsorption can successfully pattern the biogenic silica on substrate. That is very important for my proposed experiments to work.
4.1 Optical images of adsorption of diatom frustules on different surfaces (a) glasses with (PAH/PSS)\textsubscript{5}PAH (b) glasses without PEMs (c) glasses with (PAH/PSS)\textsubscript{5} layer on top

4.2 Influence of pH

In weak polyelectrolyte systems, it is possible to adjust the strength of electrostatic attraction by altering the pH of the suspension of diatom frustules. PAH is a weak polyelectrolyte and has an ionic equilibrium as \([\text{RNH}_3^+]_n + \text{OH}^- \leftrightarrow [\text{RNH}_2]^+_n + \text{H}_2\text{O}\), where R is dedicated to carbon chain and n is the degree of polymerization. Base can push the above reaction to a neutral status which results in losing positively charges on polyelectrolyte backbones. The consequence of discharge is the decrease of ionic attraction strength.

Figure 4.2 shows the result of the influence of pH on adsorption of frustules on PEMs. The amount of adsorption of frustules is higher at lower pH. The adsorption prefers to occur in an acid condition rather than in a base condition. So the ideal working
condition should be at low pH. We can simplify the ionic equilibrium as $\text{RNH}_3^+ \leftrightarrow \text{RNH}_2 + H^+$. The equilibrium constant can be described as:

$$pK_a = p \left( \frac{[\text{RNH}_2]}{[\text{RNH}_3^+]} \right) + pH$$

From reported literature one can obtain the pK$_a$ value for PAH, which is equal to 8.548. When the solution is neutral, $[\text{RNH}_2]/[\text{RNH}_3^+]$ is equal to 0.03 which means around 97% of PAH is in ionic status. So the interaction between silica and PEMs is strong and the adsorbed diatoms form a dense layer (see Figure 4.2(a)). When the pH of solution goes to 9, the ratio between $[\text{RNH}_2]$ and $[\text{RNH}_3^+]$ increases to 3.16, more than 75% of PAH is deionized to neutrality. From Figure 4.2(b), one can observe that the density of adsorbed diatoms decreases dramatically. When pH increases to 12, PAH loses its protons from carbon chain almost completely, which leads to the poor adsorption shown in Figure 4.2(c).
4.3 Influence of number of polymer layers

The thickness of each polyelectrolyte layer is about several nanometers. That is quite small compared to the surface roughness and the dimension of frustules. Since the multilayer is not continuous to some extent and the glass surface is not perfectly smooth, there have to be enough layers to suppress the influence of substrate. This
means that, after a few layers, the polyelectrolyte multilayer films are independent of the underlying substrate.

Figure 4.3 Optical images of frustules of *Pinnularia* sp. diatom attracted to (PAH/PSS)_nPAH substrate by electrostatic force at (a) n=3 (b) n=5

Figure 4.3 shows the influence of number of polymer layers on the adsorption of diatom frustules. We found that more layers results in a denser adsorption. That indicates more charges were formed on the surface which is consistent with the
The explanation described above. Normally four pairs of electrolyte layers are required for good adsorption.

### 4.4 Influence of exterior salt concentration

Adjusting ionic strength in solutions is another way to control the quality of PEMs and the adsorption of silica. Salt added in the solution can assist the development of polyelectrolyte layers via charge screen and provide extrinsic compensation of charge in films. More importantly frustules can’t be adsorbed without extrinsic salt, which was verified by experiment, due to charge screen.

We can use the theory mentioned in chapter 2.3.2 to qualitatively explain the influence of ionic strength. For a reversible reaction: \( \text{Pol}^+ \text{Pol}^- + \text{Na}^{+} \text{aq} + \text{Cl}^{-} \text{aq} \rightleftharpoons \text{Pol}^+ \text{Cl}^- + \text{Pol}^- \text{Na}^+ \), the equilibrium constant may be written as:

\[
K^\prime = \frac{\gamma^2}{(1-\gamma)[\text{NaCl}]_{aq}^2} = \left( \frac{\gamma^2}{[\text{NaCl}]_{aq}^2} \right)_{r \to 0}
\]

As discussed previously, the higher the salt concentration, the larger the fraction of extrinsic charge compensation. Commonly polymer complex is in a neutral state. Therefore more extrinsic charge compensation may produce more surface charge in the top layers of PEMs due to the weak interaction between salt ions and polymer ions. It means that salt can screen the electrostatic interaction between oppositely charged polyelectrolytes and resulting in charged groups on PEM surface. Based on this reason,
it is easier to understand why external salt can help and even manipulate the building of PEMs and adsorption of biogenic silica.

Figure 4.4 showing the optical microscopy images of adsorbed diatom frustules at different ionic strength which verifies the above argument.
4.5 Treatment of living cells

There are two ways which were used to remove the organism in living cells, Sodium dodecyl sulfate (SDS) mixed with ethylenediaminetetraacetic acid (EDTA) and Hydrogen peroxide (H$_2$O$_2$). SDS is an anionic surfactant that is commonly used for cleaners, soaps, and other industrial and household products. In biochemistry, SDS is used to separate proteins by disrupting non-covalent bonds in the proteins. EDTA commonly works as a chelating agent acting with heavy metal ions. When SDS/EDTA was used to remove, SDS disrupted the organism while EDTA coupled with Ca and Mg ions. This is a mild way to strip out organics from cells. However, H$_2$O$_2$ is an oxidant which reacts with organics to form carbon dioxide and water. To some extent, H$_2$O$_2$ can destroy the structure of silica resulting in lots of silica fragments.
Although both of the methods can get rid of organism in cells, the surface of silica cells may behave differently in terms of electrostatic interaction. In my experiment, SDS treated frustules were much easier to be adsorbed on PAH surface. In contrast, there was no adsorption if H$_2$O$_2$ was used instead. The reason for this significant difference is probably caused by the charge distribution on diatom frustules. SDS works as a detergent to physically remove organics out of silica. It preserves the surface charges on the natural silica which was from SiOH groups. However, H$_2$O$_2$ not only annihilates organics may also quench the surface OH groups which results in a low surface charge density.

### 4.6 Optical and SEM images

The epi-fluorescence and SEM images of *Coscinodiscus* frustules after adsorption are given in Figure 4.5. Figure 4.5 (a) presents a fluorescent image of an array of *Coscinodiscus* frustules. Rhodamine-123 was added into the culture solution for tracing purpose. SEM image of the same array is given in figure 4.5 (b) which shows more than one shell was adsorbed on some printed sites. Due to the spreading of ink, the diameter of printed PAH dots is around 300 µm which is about 1.5 times of the dimension of silica shells. Therefore, two or more shells may share one site. However, it is impossible to absorb more than four shells on a site based the experimental observation. That can also be explained by the dimension ratio between PAH dots and shells.
Figure 4.5 Epi-fluorescent and SEM images of *Coscinodiscus* frustules deposed on PAH-printed PEM, after coating with Au. (a) Array of Rhodamine-123 labeled diatom frustules by epi-fluorescence imaging at 530 nm; (b) array of single *Coscinodiscus* frustules by SEM, inset is the six-arm diffraction of *Coscinodiscus* frustules; (c) SEM of single *Coscinodiscus* frustules, inside surface facing up; (d) SEM of fine features on diatom biosilica surface.

For comparison, we used *Cyclotella* whose size is about 10µm for adsorption (see Figure 4.6). From the images, we can see the PAH dot has an elliptical shape and the smaller diatom frustules can cover the printed area uniformly and compactly.
Figure 4.6 SEM images of *Cyclotella* diatom frustules on PEMs coated with Au

Due to their small size, *Cyclotella* diatom shells have much stronger Brownian motion than *Coscinodiscus* frustules. That is the reason why *Cyclotella* frutules can be deposited more precisely.

In a micrometer or submicrometer scale, *Cyclotella* and *Coscinodiscus* own delicate fine structure which is beyond our mechanical limit. Diatom was cultured in a room temperature sea water system whose cost was negligible compared with modern mechanical process. Also all the reactions did not produce any harmful components which is unavoidable in our lab reactions. In a centimeter scale, we can harness these nano-structure particles to form a wafer-size patterned surface via our printing
technique which preserved those fine structures in diatom. In summary, the result presented above reveals the advantage of combination of bio-fabrication for inorganic oxide and maskless inkjet printing for patterning in terms of dimensional scales and environmental benignity.
CHAPTER 5

Conclusion and Future Work

In conclusion, I have succeeded in manipulating the distribution of surface charge and fabricating single-diatom-shell arrays through the combination of inkjet printing and Layer-by-Layer deposition process. Through controlling pH, ionic strength, and layer number, the assembly of biogenic silica was optimized. The nano- and microstructure of diatom frustules was preserved due to the mild processing condition, which renders this technique for the fabrication of large area devices such as electroluminescent, sensing, and photovoltaic devices. In comparison with microcontact printing where a specific stamp has to be fabricated before printing, inkjet printing offers a no-contact, maskless patterning process. This technique has the potential to harness the unique properties of functionalized diatom biosilica for large scale applications.

For future work, PAH ink should be optimized by changing its composition to decrease the drop size for a better printing resolution. The ink used in my experiment contains ethylene glycol and water which are very hydrophilic and congenial to PEMs. Finding a hydrophobic solvent is a good way to solve the low-resolution problem.

Three steps, including Layer-by-Layer deposition, inkjet printing, and particle adsorption, were used to fabricate patterned biogenic silica in this work. Layer-by-Layer deposition provides a continuous polymer film beneath printed layer. It is
possible to print multilayer films without the dipping process. That will provide a more controllable, better material utilization, and faster fabrication process.

Limited by print process and ink formation, the smallest size we can control in the printing process is down to 150 µm. Via increasing the resolution of printing or adjusting the composition of PAH ink, the size of printed dot can shrink to fifty micrometers or even 10 micrometers. In that case, smaller diatom shells like 10 µm *cyclotella* can be individually controlled with electrostatic force on patterned film.

In terms of application, diatom biosilica has been successfully applied in the field of gas sensors which detect harmful gases via the property of semiconductor or light emission.\textsuperscript{4, 49} With the help of this technique, it is possible to control the location of the sensor material making the sensing device more precisely and efficiently.
References


