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Assembling nanoparticles and biomacromolecules using electrostatic interactions*

Murali Sastry‡

Materials Chemistry Division, National Chemical Laboratory, Pune 411 008, India

Abstract: Nanotechnology is witnessing impressive advances on many different fronts. One of the key areas with important commercial implications concerns the assembly of nanoparticles to form thin films and superstructures by what is commonly known as the "bottom-up" approach. This paper covers some of the more recent developments in this fascinating field with particular emphasis on the work from the author's laboratory on assembly of nanoparticles using electrostatic interactions. The use of electrostatic interactions enables extension of the assembly protocols to the immobilization of biomacromolecules such as proteins/enzymes and DNA with exciting application potential.

INTRODUCTION

There is much excitement in the study of nanoscale matter with respect to their fundamental properties and organization to form superstructures and applications. The unusual physicochemical and optoelectronic properties of nanoparticles are primarily due to confinement of electrons within particles of dimensions smaller than the bulk electron delocalization length, this process being termed "quantum confinement" [1–3]. The exotic properties of nanoparticles have been considered in applications such as optoelectronics [4], catalysis [5], reprography [6], single-electron transistors (SETs) and light emitters [7], nonlinear optical devices [8], and photoelectrochemical applications [9]. Magnetic nanoparticles are being viewed with interest from a fundamental point of view (superparamagnetism in the nanoparticles) [10] as well as in applications such as magnetic memory storage devices [11], magnetic resonance image enhancement [12], and magnetic refrigeration [13]. The ability to tune the optical absorption/emission properties of semiconductor nanoparticles (the so-called "quantum dots") by simple variation in nanoparticle size is particularly attractive in the facile band-gap engineering of materials [14] and the growth of quantum dot lasers [15]. More recently, nanoscale matter has been looked at with interest for potential application in nanocomputers, synthesis of advanced materials, energy storage devices, electronic and optical displays, chemical and biosensors as well as biomedical devices [16].

How exactly does one define nanotechnology? The ultimate definition would have to be the manipulation and control of individual atoms and, thereby, the programmed formation of superstructures. However, such a definition may be extended to the organization of objects having nanodimensions such as molecules/biomacromolecules and other nanoscale matter such as quantum dots, buckyballs (also known as fullerenes), and nanotubes, etc. The scope of this definition may be further enlarged to encompass the generation of nanoscale matter from bulk objects using lithographic techniques as well as the synthesis and manipulation of materials grown in nanoscale reactors and cavities

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‡Tel.: +91 20 5893044; Fax: +91 20 5893952/5893044; E-mail: sastry@ems.ncl.res.in

occurring either naturally or synthesized by design. The two major approaches currently being used for the generation of organized nanoscale assemblies, viz. the "top-down" (or "engineering-down") and "bottom-up" ("engineering-up") methods. The top-down method wherein patterned structures are grown by suitable lithographic and ion-implantation techniques are the cornerstone of silicon-integrated chip technology [18]. If one extrapolates into the future using the current rate of miniaturization in silicon memory technology as a benchmark [18], very soon the physical limits of device dimensions realizable by ultraviolet, electron/ion beam, and soft X-ray lithographic techniques will be reached. It is, thus, clear that an alternative approach based on possibly completely different principles would be required to overcome this barrier toward further miniaturization. Incidentally, in addition to the already stated application potential of nanotechnology, another major driving force in this area is the so-called Moore's law [18]. In the context of the current very large scale integration (VLSI) silicon technology scenario, the bottom-up approach, which assumes considerable importance and research from the author's laboratory dealing with self-assembly of nanoscale objects, will form the subject matter of this paper.

While the exotic properties of individual (noninteracting) nanoparticles is well documented [4], in many situations it would be desirable to assemble the nanoparticles on suitable substrates and link them in some manner and make them "talk" to each other. In more scientific terms, the *collective properties* of nanoparticle ensembles is also an important element of nanotechnology research, and this article primarily concentrates on the methods used to organize nanoparticles on suitable substrates by different methods falling under the umbrella of *self-assembly* techniques. The ultimate goal, insofar as the growth of nanoparticle thin films is concerned, is the realization of crystals of nanocrystals wherein both the size of the nanoparticles and the interparticle separation in the film may be tailored at will and, thus, enable one to tune the collective properties of the ensemble [4].

BOTTOM-UP APPROACH TO NANOPARTICLE THIN FILMS

In his lecture, "There's plenty of room at the bottom", Richard Feynman discussed the problem of controlling and manipulating objects on small length scales [19]. In the lecture, he makes the intriguing statement, "If I could only train an ant to do this!" in connection with repairing very small parts as would, for example, occur in a woman's wrist-watch [19]. While it would be exciting to have a living microorganism to do such intricate work for us, extremely microscopic robots would also fit the bill. And indeed, the ultimate in nanoscale manipulation, atom-by-atom assembly has been demonstrated.

The pioneering example of atom-by-atom growth of nanostructures is the writing of the IBM logo by placing individual atoms on a surface using an STM tip [20]. In this elegant work, Eigler and Schweizer showed that xenon atoms could be picked up using an STM tip and placed at specific sites on a Ni (110) surface [20] and thus to "write" atomic-resolution patterns on surfaces. More recently, gold nanoparticles have been manipulated in liquid environments using dynamic mode scanning force microscopy (SFM) [21], opening up the exciting application of SFM in nanorobotics [22]. Kim and Lieber have demonstrated that an AFM may be used to machine complex patterns in oxide thin films [23]. An AFM tip has recently been used to write 30-nm-resolution alkanethiol patterns on gold thin films in a manner analogous to that of a dip-pen [24]. The transfer of the alkanethiol molecules from the AFM tip to the gold surface is accomplished using capillary forces [24]. This technique opens up the exciting possibility of creating nanoscale-functionalized surfaces [24]. It is clear that there is considerable excitement in such intricate nanoscale manipulation, and the area of nanorobotics is sure to develop further in the future.

While atom-by-atom growth of nanostructures is definitely exciting, it is difficult to imagine how the technique of nanorobotics can be used to grow massively parallel structures as would be required, for example, in the silicon chip industry. Is there a viable alternative method that would be simple and also overcome the many shortcomings mentioned above with regard to the top-down method? The answer is yes, and there is tremendous excitement in the bottom-up approach of realizing nanostruc-

tured assemblies. This technique relies on the self-assembly of nanoscale objects on suitable substrates using a variety of interactions.

NANOPARTICLE THIN FILMS BY SELF-ASSEMBLY

The formation of thin films of nanoparticles, either in monolayer or multilayer form by means of the bottom-up self-assembly method consists essentially of two main steps. The first step is the synthesis of the nanoparticles and, if necessary, derivatization of the colloidal particles. The colloidal route for the synthesis of nanoparticles has been chosen for the following reasons. Fairly monodisperse particles over a range of chemical compositions (metals, oxides, and semiconductors) and sizes can be easily grown by the colloidal method [25]. Furthermore, synthesis of the nanoparticles in solution form affords one the additional degree of freedom to modify the surface of the particles through surface capping with suitable surfactants. The process of surface modification may be used to not only stabilize the colloidal particles in solution, but also to self-assemble them on suitable substrates, which is the second step in the realization of nanoparticle thin films.

A number of interactions operative in self-assembly of molecules may be used to organize the colloidal nanoparticles on different surfaces. Electrostatic interactions, hydrogen bonding, covalent bonds with surfaces, and self-assembly near templated surfaces are some of the forces that lead to organized nanoparticle thin films. In the author's laboratory, the primary focus has been on the use of electrostatic interactions for nanoparticle assembly and will be discussed below in some detail. One of the advantages of using electrostatic interactions for assembly of nanoscale matter is that the protocols developed may be easily extended to the assembly and immobilization of charged biomacromolecules such as proteins/enzymes and DNA.

LAYER-BY-LAYER NANOPARTICLE ASSEMBLY DRIVEN BY ELECTROSTATIC INTERACTIONS

The first report on the electrostatically driven layer-by-layer self-assembly of inorganic colloidal particles may be traced to the work of Iler [26]. Iler showed that oppositely charged silica and alumina particles could be electrostatically self-assembled in multilayer structures by alternatively immersing the substrate in the two colloidal solutions. However, true layer-by-layer growth was not fully established in this case [26]. Since then, the group lead by Decher has been very active in this field and have demonstrated the layer-by-layer electrostatic self-assembly of cationic and anionic polyelectrolytes as well as multilayer structures consisting of combinations of charged colloidal particles, including biomacromolecules such as DNA [27]. Many groups have now used electrostatically driven layer-by-layer assembly to realize assemblies containing proteins/polyeletrolytes [28] and inorganic nanoparticles such as magnetite [29], SiO_2, TiO_2, and CeO_2 [30], alternating layers of positively charged gold and negatively charged silver particles [31] as well as quantum dots of CdSe [32]. We have shown that gold nanoparticles rendered amphoteric using the amino acid, valine, may also be electrostatically assembled in a layer-by-layer fashion by suitably changing the pH of the gold colloidal solution [33]. An interesting recent development is the demonstration of layer-by-layer assembly of proteins on nanoscale curved surfaces (polystyrene latex spheres) using polyelectrolytes and the formation of protein multilayer structures on colloidal particle supports [34].

ORGANIZATION/SYNTHESIS OF NANOPARTICLES AT THE AIR–WATER INTERFACE

Langmuir monolayers are excellent templates for the organization of small [35] and large inorganic ions (large ions such as polyoxometallates) [36,37] and, recently, have generated much interest in the assembly of biomacromolcules such as proteins and DNA at the air–water interface [38,39]. The air–water

interface has also been used to organize *hydrophobized* nanoparticles of metals, semiconductors, and oxides [40,41].

In this laboratory, we have concentrated primarily on the use of electrostatic interactions to drive the self-assembly of surface-derivatized colloidal particles in thin film form. Amphiphilic, ionizable Langmuir monolayers may also be used to organize charged colloidal particles at the air–water interface with the added flexibility that multilayer films of the colloidal particles may be grown on suitable substrates by the versatile Langmuir–Blodgett (LB) technique. We have demonstrated this approach in the formation of lamellar LB films of electrostatically immobilized nanoparticles of gold [42], silver [43,44], and CdS [45], as well as hetero-nanoparticle assemblies of gold and silver nanoparticles in multilayer form [46].

The process of electrostatic assembly of charged colloidal particles at the air–water interface using ionizable Langmuir monolayers is illustrated in Fig. 1. The figure shows the attractive electrostatic interaction between negatively charged colloidal particles and a positively charged octadecylamine (ODA) Langmuir monolayer and, thereby, the immobilization of the nanoparticles at the air–water interface. The charging of the nanoparticles may be accomplished by assembling a monolayer of an ionizable molecule such as 4-carboxythiophenol on the surface of the nanoparticles. The thiol group binds covalently with gold and silver nanoparticles through thiolate linkages [42,43], leaving the terminal carboxylic acid functionality free to stabilize the particles. Under slightly basic conditions of the nanoparticle solution, the carboxylic acid groups would be ionized, thereby stabilizing the particles in solution and charging the particles for further assembly onto suitable surfaces (such as a Langmuir monolayer, for example). Figure 1 also shows how the transfer of the nanoparticles complexed with the ODA monolayer onto a substrate by the LB method may be accomplished, the transfer occurring by application of pressure on the monolayer through a frictionless, movable barrier. The charges on the Langmuir monolayer and nanoparticles may be reversed; using interdigitated bilayers, we have functionalized the nanoparticles with amine groups (to render them positively charged) and immobilized them at the air–water interface with fatty acid Langmuir monolayers (which are negatively charged when ionized) [44].

The formation of multilayers of nanoparticles by the LB technique may be extended to the formation of superlattice structures of different kinds of nanoparticles and is an exciting extension of the LB method for formation of nanoparticle superlattice assemblies. Figure 2 illustrates the formation of such assemblies for silver and gold nanoparticles by sequential immersion of the substrate in the dif-

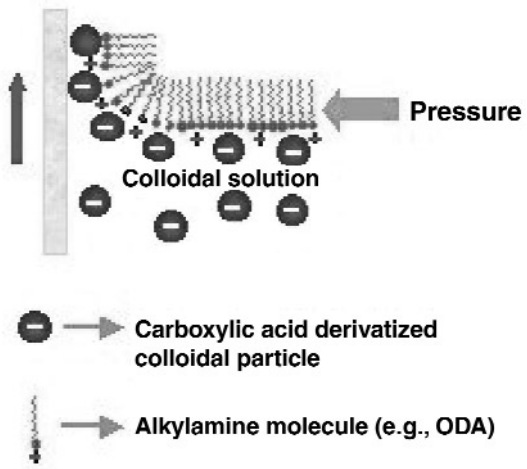

Fig. 1 Diagram showing the electrostatic assembly of negatively charged colloidal particles at the air–water interface with cationic Langmuir monolayers.

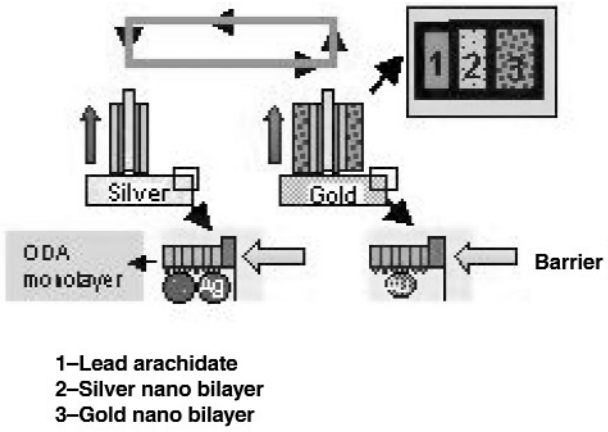

Fig. 2 Diagram showing the formation of superlattices of silver and gold nanoparticles by sequential immersion of a substrate in the different nanoparticle solutions.

ferent colloidal solutions and effecting transfer by the LB technique [46]. This process of sequential immersion would lead to alternating bilayers of silver and gold nanoparticles [46]. The number of immersion cycles in each nanoparticle solution can be programmed to lead more complicated sequences, such as the Fibonacci sequence, etc. As in the previous example, the gold and silver nanoparticles are charged negatively by surface derivatization using bifunctional molecules such as 4-carboxythiophenol [46].

Nanoparticles may also be organized using electrostatic interactions at the interface between two immiscible liquids [47]. We have shown that when a biphasic mixture consisting of carboxylic acid-derivatized colloidal gold particles and a layer of ODA molecules in toluene is stirred vigorously, the gold nanoparticles are trapped by electrostatic interaction with the ODA molecules at the water–toluene interface. Furthermore, the gold nanoparticle-ODA monolayers could be transferred spontaneously onto moistened glass substrates by simple immersion of the substrate up to the interface. The gold nanoparticle monolayer was observed to climb rapidly up the glass substrate, possibly driven by the Marangoni effect [47]. While this method does not strictly classify as an LB method for organization of the nanoparticles at the air–water interface, in a sense, the mechanism, based on electrostatic immobilization with ionizable lipid molecules, renders this report not totally inappropriate for this section.

NANOCOMOPOSITES BY ELECTROSTATIC ENTRAPMENT IN THERMALLY EVAPORATED LIPID FILMS

A lipid-based protocol for the formation of nanocomposites has been developed in the author's group and has progressed chronologically from electrostatic entrapment of inorganic ions [48–50] to surface-modified inorganic nanoparticles such as silver [51,52], gold [53,54], and CdS [55] to charged biomacromolecules such as proteins/enzymes [56–59] and DNA/peptide nucleic acids (PNA) [60,61]. This simple beaker-based immersion method is illustrated in Fig. 3 for the electrostatically controlled formation of nanoparticle-lipid hybrid films. The first step in this protocol consists of deposition of a suitable ionizable fatty lipid film on a solid substrate.

This may be accomplished by resistive heating of the lipid powder in a conventional vacuum coating unit. In the figure, an octadecylamine film has been shown, which, under acidic solution conditions, is positively charged. This film is then immersed in the colloidal solution consisting of negatively charged nanoparticles, the charging of the nanoparticles arising from ionization of carboxylic acid groups from 3-D self-assembled monolayers on the nanoparticle surface [42,43]. During immersion, it

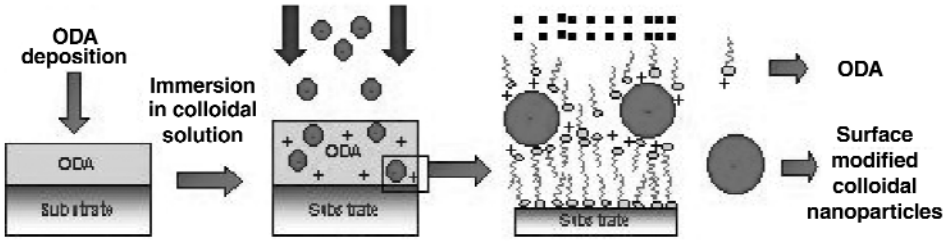

Fig. 3 Diagram showing the various steps involved in the immobilization of surface-modified nanoparticles in thermally evaporated fatty lipid films.

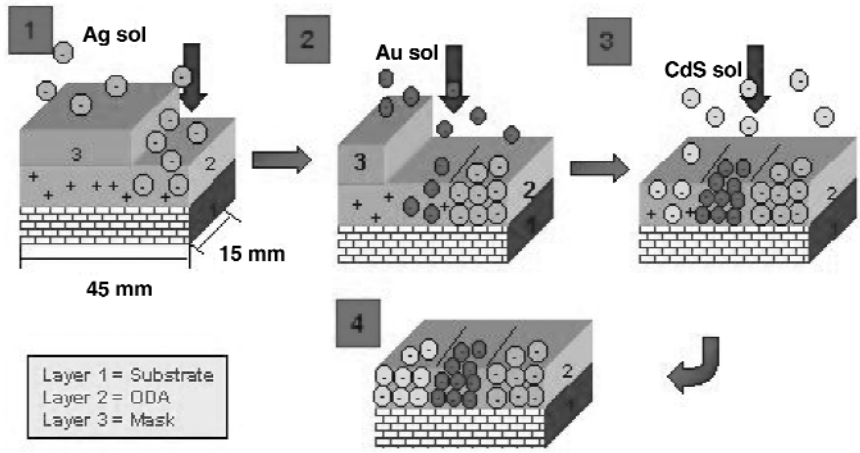

Fig. 4 Diagram illustrating the various steps involved in the formation of spatially separated hetero-colloidal assemblies on a solid surface. [Reprinted with permission from ref. 62, © American Chemical Society (2000).]

is observed that the nanoparticles diffuse into the lipid film, and in the case of gold, silver, and CdS nanoparticles, this diffusion process is easily followed by the lovely coloration that occurs in the films as they are populated by a progressively increasing number of nanoparticles. As in the case of nanoparticle assembly at the air–water interface, the sign of the charge on the surface of the nanoparticles may be controlled via interdigitated bilayers consisting of either carboxylic acid/amine functional groups in the outermost layer [44].

An important challenge in nanoscale assembly concerns the growth of 2D hetero-nanoparticle structures that are spatially separated. The formation of patterned hetero-colloidal particle composite thin films on solid supports using the diffusion technique has been shown recently [62]. The process is illustrated in Fig. 4 and consists of masking a thermally evaporated ODA film and immersing it sequentially in carboxylic acid-derivatized silver (70 Å), gold (130 Å), and CdS (45 Å) nanoparticle solutions after exposing progressively larger areas of the film surface each time (Fig. 4). In the manner illustrated in Fig. 4, it is clear that the silver nanoparticle region would be exposed to both the gold and CdS solutions while the gold region would experience an immersion in the CdS solution. However, we have observed little intermixing of the colloidal nanoparticles within any region, indicating that the trajectory of the nanoparticles in the film as they diffuse into the lipid matrix is essentially ballistic over large time scales. The edge regions were studied chemically using spot-profile energy-dispersive analysis of X-rays measurements, and an estimate of the widths of the edges separating the different nanoparticle regions was made [62].

As briefly mentioned in the introductory section, the use of electrostatic forces as a means of assembling nanoscale objects enables extension of the protocol to the assembly of biomacromolecules such as proteins and DNA. In particular, the entrapment of proteins/DNA in thermally evaporated lipid films has exciting potential for application in protein/DNA chips and will be briefly discussed below. For more complete details, the interested reader is directed to ref. 63.

Figure 5 shows the process used in the immobilization of DNA in thermally evaporated octadecylamine thin films. The fatty amine films are immersed in aqueous solutions of oligonucleotides of well-defined base sequence. Attractive electrostatic interaction between the negative charges on the DNA phosphate backbone and positive charges in the ODA matrix drives the entrapment of the DNA in the lipid film [60]. This is a surprisingly rapid process and even for 1000 base-pair DNA (calf-thymus DNA), 20 min of immersion is adequate to achieve saturation of DNA in the lipid matrix. While DNA double-helical structures can be immobilized in ODA films in this fashion, the power of this approach lies in the fact that complementary sequence DNA molecules may be immobilized in a sequential manner within the ODA matrix (Fig. 5). What is interesting is that during sequential immersion in complementary oligonucleotide solutions, the DNA molecules hybridize to yield double-helical DNA structures *within the lipid matrix* [60]. This has since been extended to the hybridization of DNA with DNA-mimics such as peptide nucleic acids (PNA) [61]. The use of thermally evaporated lipid films in DNA immobilization may be extended to the formation of patterned assemblies of DNA of different sequences on a single surface by suitable masking procedures. This concept is currently being probed in this laboratory as a means of realizing a simple DNA chip.

DNA molecules are fairly robust, and their immobilization (and long-term stability) is relatively easy to control. On the other hand, immobilization of proteins in thin lipid matrices presents additional challenges not encountered in the entrapment of rigid entities such as inorganic nanoparticles/DNA. For example, during immobilization of proteins or enzymes in the lipid matrix, is the tertiary structure of the biomacromolecules affected? This is a crucial issue from the application point of view, since a large modification of the tertiary structure of the immobilized proteins/enzymes could lead to a significant loss in biological (catalytic) activity. In a series of studies on protein immobilization in various ther-

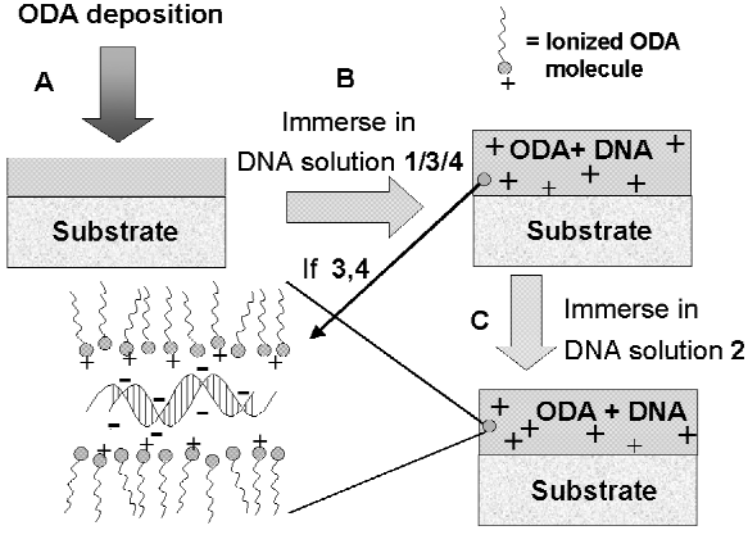

Fig. 5 Diagram illustrating the various steps involved in the immobilization of synthetic DNA molecules (single-stranded) in thermally evaporated octadecylamine films. DNA 1 and 2 are single-stranded complementary sequence oligonucleotides while 3 and 4 correspond to preformed duplex structures. The magnified view shows the expected structure of the lipid bilayer with entrapped DNA molecules.

mally evaporated lipid matrices [56–59], we have observed that the biological activity of the proteins was not reduced in any way consequent to entrapment in thin lipid films. Indeed, a large improvement in temporal, temperature, and pH stability of the immobilized proteins was observed, clearly indicating that the lipid hosts are quite forgiving of their sensitive guests [56–58,64]. As in the case of DNA, patterned assemblies of proteins may be created quite easily using thermally evaporated lipid films and a proof-of-concept paper on the realization of a 2×2 protein array has been recently presented [65].

ELECTROSTATICALLY CONTROLLED NANOPARTICLE ASSEMBLY ON BIOLOGICAL TEMPLATES

The use of biological templates in the organization of inorganic nanoparticles is another exciting area of current research. The self-assembly of nanoparticles using biological templates such as bacteria, virus molecules (the tobacco mosaic virus, TMV), DNA, and antigens/antibodies has been reported [66]. In an interesting application of biological templates in nanoparticle growth, the use of self-assembled bacterial S-layers in the synthesis and organization of quantum dots of CdS was demonstrated by Shenton, Pum, Sleytr, and Mann [67]. TMV is another biological template that has been used with success in the synthesis and organization of inorganic nanoparticles [68]. The charged amino acid residues (such as glutamate, aspartate, arginine, and lysine) on the surface of the proteins may be used as nucleation sites for inorganic particle deposition, and this was demonstrated by the growth of CdS and PbS quantum dots, iron oxide, and SiO_2 on the surface of TMV structures [68]. In a slightly different approach, Sastry and coworkers have demonstrated the assembly of colloidal gold particles capped with the amino acid, lysine, on double-helical DNA structures using purely electrostatic interactions in solution [69] and in thin film form [70]. This approach resulted in the formation of linear superstructures of the gold nanoparticles with potential application in the generation of nanowires.

To conclude, a number of different approaches for nanoparticle assembly such as organization at the air–water interface, formation of nanocomposites by diffusion, layer-by-layer assembly onto suitable substrates, and the use of biological/engineered templates for growing/organizing nanoparticles in thin film form have been discussed, with emphasis on the research efforts of the author's group. While the assembly of nanoparticles in 2D and in 3D is currently achievable (e.g., by a layer-by-layer method), a possible direction in which nanoparticle assembly could proceed is development of protocols for hierarchical self-assembly of the nanoparticles into superclusters using supramolecular chemistry concepts. It is clear that we are at the threshold of an era where nanotechnology will come into its own and play a major role in technologies spanning electronics, chemical industry, medicine and biodiagnostics, and environment protection. As we master the principles of nanoscale self-assembly and the formation of complex, interconnected, synergistic nanostructures, the promise of nanotechnology will eventually be translated into reality.

REFERENCES

1. J. H. Fendler. *Membrane Mimetic Chemistry Approach to Advanced Materials*, Springer-Verlag, Berlin (1992).
2. A. Henglein. *Top. Curr. Chem*. **143**, 113 (1988).
3. M. A. El-Sayed. *Acc. Chem. Res*. **34**, 257 (2001).
4. A. P. Alivisatos. *Science* **271**, 933 (1996).
5. T. Ahmadi, Z. L. Wang, T. C. Green, A. Henglein, M. A. El-Sayed. *Science* **272**, 1924 (1996).
6. J. F. Hamilton and R. C. Baetzold. *Science* **205**, 1213 (1979).
7. H. Weller. *Angew. Chem., Int. Ed. Engl*. **37**, 1658 (1998).
8. Y. Wang. *Acc. Chem. Res.* **24**, 133 (1991).
9. H. S. Mansur, F. Grieser, M. S. Marychurch, S. Biggs, R. S. Urquhart, D. N. Furlong. *J. Chem. Soc., Faraday Trans.* **91**, 665 (1995).

10. J. Shi, S. Gider, K. Babcock, D. D. Awschalom. *Science* **271**, 937 (1996).
11. J. L. Symonds. *Phys. Today* **48**, 26 (1995).
12. J. W. M. Bulte, T. Douglas, S. Mann, R. B. Frankel, B. M. Moskovitz, R. A. Brooks, C. D. Baumgarner, J. Vymazal, J. A. Frank. *Invest. Radiol*. **29**, 5214 (1994).
13. R. D. Shull, R. D. McMichael, J. J. Ritter. *Nanostruct. Mat*. **2**, 205 (1993).
14. J. H. Fendler and F. Meldrum. *Adv. Mater*. **5**, 607 (1995).
15. N. N. Ledentsov, M. Grundmann, N. Kirstaedter, O. Schmidt, R. Heitz, J. Bohrer, D. Bimberg, V. M. Ustinov, V. A. Shchukin, A. Yu Egorov, A. E. Zhukov, S. Zaitsev, P. S. Kop'ev, Zh. I. Alferov, S. S. Ruvimov, A. O. Kosogov, P. Werner, U. Gosele, J. Heydenrich. *Solid State Electron*. **40**, 785 (1996).
16. See the recent Feb. 28, 2002 issue of *Chem. Eng. News* and articles by R. Dagani therein for coverage of the new applications envisaged for nanomaterials.
17. Y. Xia and G. M. Whitesides. *Angew. Chem., Int. Ed. Engl.* **37**, 551 (1998).
18. In 1965, Gordon Moore (cofounder of Intel Corp.) predicted that transistor density (memory storage capacity) on an integrated circuit would double every 18 months. This has come to be known as "Moore's law", and the silicon industry has been following this trend till now.
19. The classic talk by Richard Feynman entitled "There's plenty of room at the bottom" delivered at the annual meeting of the American Physical Society at the California Institute of Technology in 1959 is possibly the first serious exposition on the problem of manipulating nanoscale objects and arguably the beginning of nanotechnology as we know it today (the talk is available on the Web at http://www.zyvex.com/nanotech/feynman.html).
20. D. M. Eigler and E. K. Schweizer. *Nature* **344**, 524 (1990).
21. R. Resch, C. Baur, A. Bucacov, B. E. Koel, P. M. Echternach, A. Madhukar, N. Montoya, A. A. G. Requicha, P. Will. *J. Phys. Chem. B* **103**, 3647 (1999).
22. C. Baur, B. C. Gazen, B. Koel, T. R. Ramachandran, A. A. G. Requicha, L. Zini. *J. Vac. Sci. Technol. B* **15**, 1577 (1997).
23. Y. Kim and C.M. Lieber. *Science* **257**, 375 (1992).
24. D. R. Piner, J. Zhu, F. Xu, S. Hong, C. A. Mirkin. *Science* **283**, 661 (1999).
25. G. Schmid. *Clusters and Colloids: From Theory to Applications*, VCH, Weinheim (1994).
26. R. K. Iler. *J. Colloid Interface Sci*. **21**, 569 (1966).
27. G. Decher. *Science* **277**, 1232 (1997) and references therein.
28. F. Caruso, K. Niikura, D. N. Furlong, Y. Okahata. *Langmuir* **13**, 3427 (1997).
29. A. Mamedov, J. Ostrander, F. Aliev, N. A. Kotov. *Langmuir* **16**, 3941 (2000).
30. Y. Lvov, K. Ariga, M. Onda, I. Ichinose, T. Kunitake. *Langmuir* **13**, 6195 (1997).
31. A. Kumar, A. B. Mandale, M. Sastry. *Langmuir* **16**, 6921 (2000).
32. T. Cassagneau, T. E. Mallouk, J. H. Fendler. *J. Am. Chem. Soc*. **120**, 7848 (1998).
33. A. Kumar, P. Mukherjee, A. Guha, S. D. Adyantaya, A. B. Mandale, R. Kumar, M. Sastry. *Langmuir* **16**, 9775 (2000).
34. F. Caruso and H. Mohwald. *J. Am. Chem. Soc*. **121**, 6039 (1999).
35. A. Ulman. *An Introduction to Ultrathin Organic Films: From Langmuir–Blodgett to Self-assembly*, Academic Press, New York (1991).
36. P. Ganguly, D. V. Paranjape, M. Sastry. *J. Am. Chem. Soc*. **115**, 793 (1993).
37. M. Clemente-Leon, C. Mignotaud, B. Agricole, C. J. Gomez-Garcia, E. Coronado, P. Delhaes. *Angew. Chem., Int. Ed. Engl*. **36**, 1114 (1997).
38. A. Riccio, M. Lanzi, F. Antolini, C. de Nitti, C. Tavani, C. Nicolini. *Langmuir* **12**, 1545 (1996).
39. M. Sastry, V. Ramakrishnan, M. Pattarkine, A. Gole, K. N. Ganesh. *Langmuir* **16**, 9142 (2000).
40. N. A. Kotov, G. Zavala, J. H. Fendler. *J. Phys. Chem*. **99**, 12375 (1995).
41. C. Damle, A. Gole, M. Sastry. *J. Mater. Chem*. **10**, 1389 (2000).
42. K. S. Mayya, V. Patil, M. Sastry. *Langmuir* **13**, 2575 (1997).

43. M. Sastry, K. S. Mayya, V. Patil, D. V. Paranjape, S. G. Hegde. *J. Phys. Chem. B* **101**, 5954 (1997).
44. M. Sastry, K. S. Mayya, V. Patil. *Langmuir* **14**, 5921 (1998).
45. K. S. Mayya, V. Patil, P. M. Kumar, M. Sastry. *Thin Solid Films* **312**, 318 (1998).
46. K. S. Mayya and M. Sastry. *J. Nano. Res.* **2**, 183 (2000).
47. K. S. Mayya and M. Sastry. *Langmuir* **15**, 1902 (1999).
48. P. Ganguly, M. Sastry, S. Pal, M. N. Shashikala. *Langmuir* **11**, 1079 (1995).
49. S. Mandal, S. R. Sainkar, M. Sastry. *Nanotechnology* **12**, 358 (2001).
50. C. Damle, A. Kumar, M. Sastry. *J. Phys. Chem. B* **106**, 297 (2002).
51. M. Sastry, V. Patil, S. R. Sainkar. *J. Phys. Chem. B* **102**, 1404 (1998).
52. V. Patil and M. Sastry. *Langmuir* **14**, 2707 (1998).
53. M. Sastry, V. Patil, K. S. Mayya. *Langmuir* **13**, 4490 (1997).
54. V. Patil, R. B. Malvankar, M. Sastry. *Langmuir* **15**, 8197 (1999).
55. V. Patil and M. Sastry. *J. Chem. Soc., Faraday Trans.* **93**, 4347 (1997).
56. A. Gole, C. Dash, M. Rao, M. Sastry. *Chem. Commun*. 297 (2000).
57. A. Gole, C. Dash, A. B. Mandale, M. Rao, M. Sastry. *Anal. Chem*. **72**, 1401 (2000).
58. A. Gole and M. Sastry. *Biotech. Bioeng*. **74**, 172 (2001).
59. A. Gole, P. Chaudhari, J. Kaur, M. Sastry. *Langmuir* **17**, 5646 (2001).
60. M. Sastry, V. Ramakrishnan, M. Pattarkine, K. N. Ganesh. *J. Phys. Chem. B* **105**, 4409 (2001).
61. V. Ramakrishnan, M. Sable, M. D'Costa, K. N. Ganesh, M. Sastry. *Chem. Commun*. 2622 (2001).
62. M. Sastry, A. Gole, S. R. Sainkar. *Langmuir* **16**, 3553 (2000).
63. M. Sastry. *Trends Biotech*. **20**, 185 (2002).
64. A. Gole, S. Vyas, S. R. Sainkar, A. Lachke, M. Sastry. *Langmuir* **17**, 5964 (2001).
65. A. Gole and M. Sastry. *Biotech. Bioeng*. **74**, 172 (2001).
66. For a detailed recent report on nanobiotechnology and using biological templates for organization of nanoparticles see: C. M. Niemeyer. *Angew. Chem. Int. Ed.* **41**, 4128 (2001).
67. W. Shenton, D. Pum, U. B. Sleytr, S. Mann. *Nature* **389**, 585 (1997).
68. W. Shenton, T. Douglas, M. Young, G. Stubbs, S. Mann. *Adv. Mater*. **11**, 253 (1999).
69. A. Kumar, M. Pattarkine, M. Bhadbade, S. Datar, C. V. Dhamadhikari, K. N. Ganesh, M. Sastry. *Adv. Mater*. **13**, 341 (2001).
70. M. Sastry, A. Kumar, S. Datar, C. V. Dharmadhikari, K. N. Ganesh. *Appl. Phys. Lett*. **78**, 2943 (2001).