Introduction

The assembly of organized macromolecular and nanoparticle has becoming increasingly important. The methods used for the assembly of ultrathin films with various degrees of molecular order and stability include: spin coating and solution casting, thermal deposition, polyion layer-by-layer assembly, chemical self-assembly, Langmuir-Blodgett technique and free-standing films (in
this paper we will not discuss the methods of inorganic film growth). The optimal combination of molecular order and stability of films determines the practical usefulness of these technologies [1].

The most ordered macromolecular films are free-standing liquid crystalline films, but they are very unstable. Every year one can find hundreds of new papers on Langmuir-Blodgett (LB) method, which allows one to construct amphiphile multilayers with a thickness ranging from 5 to 500 nm, but it does not have any industrial applications, because only small and flat substrates can be covered by LB-film, and it has intrinsic defects at the lipid grain borders. Another method that can be applied to surface modification (especially in biomaterials) is monolayer self-assembly, based on thiol or silane compounds. By this method, one can achieve self-assembly of 2-5 nm thick monolayers on silicon or gold surfaces, but there is no simple means for thicker film construction with this approach. Other widely used methods for the industrial manufacture of thin films are spin coating and thermal deposition of macromolecules onto a substrate. Unfortunately, unlike the methods considered above, these methods do not allow one to control the molecular order in the films.

Finally, there is yet a newer method for film self-assembly that makes use of the alternate adsorption of oppositely charged macromolecules (polymers, nanoparticles and proteins) [2-9]. The assembly of alternating layers of oppositely charged linear or branched polyanions and nanoparticles is simple and provides the means to form 5–500 nm thick films with monolayers of various substances growing in a pre-set sequence on any substrates at a growth step of about 1 nm. These films have a lower molecular order than LB or free-standing films but they have the advantage of high strength and the easy preparation. T. Mallouk [4] has called this technique “molecular beaker epitaxy,” meaning by this that with simple instruments (exploiting the materials self-assembly tendency) one can produce molecularly organized films similar to the ones obtained with highly sophisticated and expensive molecular beam epitaxy technology used for metals and semiconductors.

1. Description of the Polycation / Polyanion Assembly

1.1 General Procedure.

A cleaned substrate of any shape and dimension is immersed into a dilute solution of a cationic polyelectrolyte, for a time optimized for the adsorption of a single monolayer (ca 1 nm thick), and then it is rinsed and dried. The next step is the immersion of the polycation-covered substrate into a dilute dispersion of polyanions or negatively charged nanoparticles (or any other nanosize charged species) also for a time optimized for the adsorption of a monolayer, then it is rinsed and dried. These operations complete the self-assembly of a polyelectrolyte monolayer and monoparticulate layer sandwich unit onto the substrate (Fig. 1). Subsequent sandwich units are self-assembled analogously. Linear polycation / polyanion multilayers can be assembled by similar means. Different nanoparticles, enzymes and polyanions may be assembled in a pre-planned order in a single film.
The forces between nanoparticles and binder layers govern the spontaneous layer-by-layer self-assembly of ultrathin films. These forces are primarily electrostatic and covalent in nature, but they can also involve hydrogen bonding, hydrophobic and other types of interactions. The properties of the self-assembled multilayers depend on the choice of building blocks used and their rational organization and integration along the axis perpendicular to the substrate.

Schematic picture of polycation / polyanion multilayer. Neighbor layers interpenetrate on about 30%, so that only first and third layers are well separated.
The sequential adsorption of oppositely charged colloids was reported in a seminal paper in 1966 by Iler [2]. The electrostatic self-assembly was subsequently “rediscovered” in the nineties and extended to the preparation of multilayers of polycations and phosphonate ions, as well as to the layering of linear polions, proteins and nanoparticles by Mallouk, Decher, Möhwald, Lvov, Rubner, Fendler, Hammond, Kunitake, Tsukruk, Schlenoff, Caruso, and others. This self-assembly is now employed in the fabrication of ultrathin films from charged polymers (polions) [5-47], dyes [48-51], nanoparticles (metallic, semiconducting, magnetic, insulating) and clay nanoplates [52-75], proteins [76-95], and other supramolecular species [23, 79]. That any of these species in any order can be adsorbed layer-by-layer is the greatest advantage of this self-assembly. The oppositely charged species are held together by strong ionic bonds and they form long-lasting, uniform and stable films. Self-assembly is economical and readily amenable to scaling-up for the fabrication of large-area defect-free devices on any kind and shape of surfaces.

The main idea of this method consists of the resaturation of polyion adsorption, which results in the alternation of the terminal charge after each layer is deposited. This idea is general and implies that there are no major restrictions in the choice of polyelectrolytes. It is possible to design composite polymeric films in the range of 5 to 1000 nm, with a definite knowledge of their composition. For the successful assembly of nanoparticle or protein multilayers the alternation with linear polyion layers is important. Flexible linear polyions penetrate between nanoparticles and act as electrostatic glue. The concept of “electrostatic polyion glue”, which keeps together neighboring arrays of nanoparticles, is central to this approach [77, 79]. The self-assembled film contains amorphous polyion interlayers, and this organization “heals” defects that arise as a result of the introduction of foreign particles during the process of film formation (dust, microbes) [13, 79].

**Standard assembly procedure.** As a standard approach to film preparation we employ the following steps: 1) Take aqueous solutions of polyion, nanoparticles or protein at a concentration of 0.1 - 1 mg/mL and adjust the pH in such a way that the components are oppositely charged. 2) Take a substrate carrying a surface charge (e.g., plates or polymer films covered by a layer of cationic poly(ethylenimine) which may be readily attached to many surfaces). 3) Carry out alternate immersion of the substrate in the component’s solutions for 10 min with 1 min intermediate water rinsing. To rinse a sample use a solution with pH that keeps the polyions ionized. 4) Dry the sample using a stream of nitrogen (note: drying may hinder the assembly process, and it is not necessary for the procedure).

Polyions predominately used in the assembly are as follows: polycations - poly(ethyleneimine) (PEI), poly(dimethyldiallylammonium chloride) (PDDA), poly(allylamine) (PAH), polylysine, chitosan; polyanions - poly(styrenesulfonate) (PSS), poly(vinylsulfate), poly(acrylic acid), dextran sulfate, sodium alginate, heparin, DNA (Chart 1). One can grow polymer nanocomposite films by means of the sequential adsorption of different material monolayers that employ hundreds of commercially available polyions. The only requirement is that there be a proper (positive / negative) alternation of the component charges.
1.2 Kinetics of Polyion Adsorption

For the time-dependent control of adsorption and monitoring of the assembly in situ, the quartz crystal microbalance method is quite suitable [23, 46, 86]. The kinetics of the adsorption process could be delineated by the QCM-technique, which is indispensable for establishing proper assembly conditions (e.g., a saturation adsorption time).
The multilayer assemblies are characterized by means of quartz crystal microbalance technique in two ways: 1) after drying a sample in a nitrogen stream we measured the resonance frequency shift and calculated an adsorbed mass by the Sauerbrey equation; or 2) by monitoring of the resonator frequency during the adsorption process onto one side of the resonator which was in permanent contact with polyion solutions. While performing experiments in permanent contact with the polyion solution, we touched the surface of solutions with one side of the resonator, while the upper electrode was kept open to air and the upper contact wire was insulated from the solution by a silicone paint covering.

Typical scheme of LbL-deposition on glass slide (consecutive PSS/PAH adsorption).

Next, Fig. 2 depicts the typical kinetic profile for two consecutive steps of the assembly of a polyion concentration of 1-3 mg/mL. The fitting of adsorption to an exponential law yields a first-order rate of adsorption for poly(styrenesulfonate) (PSS) $\tau = 2.5 \pm 0.2$ min and for polyallylamine (PAH) $\tau = 2.1 \pm 0.2$ min. This means that during the first 5 min ca 87 % of the material is adsorbed onto the charged support and $t = 8$ min ($t = 3\tau$) gives 95 % of full coverage. Typically, in most publications on polyion assembly, adsorption times of 5 to 20 min are used. One does not need to maintain an adsorption time with great precision: a minute more or less does not influence the layer thickness if we are at the saturation region.

Interestingly, 5 - 20 min is essentially greater than the diffusion-limited time (mass transport limitation), which is necessary for complete surface covering (for the used polyion concentrations it is a few seconds). For other species, poly(dimethyldiallylammonium chloride) (PDDA),
polyethylenimine (PEI), montmorillonite clay, myoglobin, lysozyme, glucose oxidase, the first-order rate of adsorption onto an oppositely charged surface was found to be 2, 3, 1.8, 3, 4 and 5 min respectively. Only for 45-nm silica / PDDA assembly do we have an example when 2 s time corresponds to the diffusion limited time for the SiO₂ monolayer adsorption (Fig. 2)

In solution we see QCM frequency shifts of about 800 Hz for every adsorption cycle, which is more than that (ca 300 Hz for one side electrode) detected for the dried PSS/PAH film. This difference was ascribed to the strong hydration of the layer that had just been adsorbed. The bound water was recognized by the resonator as being included in the film: during drying this water disappears. Polyanion films swell by 60 % before drying, but only 5-10 % of the water remains in polyanion films after drying. The high hydration of adsorbed polynions (ca 50 wt %) as compared with the dried film was measured in solution by the light guiding attenuation technique [25]. Six water molecules per ion pair for PSS / PDDA multilayer at 100 % humidity were found by Schlenoff et al [38], which is close to 8 water molecules per ion pair at high humidity as determined in neutron reflectivity experiments [19, 21]. These values correspond to a 40-50 % film hydration.

It was shown [46] that 1 - 2 min intermediate sample water-washing (between subsequent adsorption cycles) removes ca 10 % of weakly attached material from a resaturated polyanion layer. This explains why the step of alternating polyanion assembly is precise: It is not of great importance that during one cycle we deposited 95 % or 99 % of a saturated layer. An intermediate washing will bring them both to the level, which is intrinsic to the assembly process parameters. The importance of intermediate washing was analyzed in [34, 46].

One could suppose that polyanion adsorption occurs in two stages: quick anchoring to a surface and slow relaxation. To reach a surface charge reversion during linear polyanion adsorption one needs a concentration greater than 10⁻⁵ M [23]. The dependence of polyanion layer thickness on concentration is not great: thus, in the concentration range of 0.1 - 5 mg/mL poly(styrenesulfonate) / poly(allylamine) (PSS / PAH) pair yielded a similar bilayer thickness. A further decrease in polyanion concentration (using 0.01 mg/mL) decreases the layer thickness of the adsorbed polyanion. An increase in the component concentrations to 20-30 mg/ml may result in the non-linear (exponential) enlargement of the growth rate with adsorption steps, especially if an intermediate sample rinsing is not long enough [47].
1.3 First Layers and Precursor Film

At the very beginning of the alternate assembly process one often sees non-linear film growth [13, 23, 41]. At the first 2 - 3 layers, smaller amounts of polyion are adsorbed as compared with further assembly, when the film mass and thickness increase linearly with the number of adsorption cycles. Tsukruk et al [41] explained this as an island-type adsorption of the first polyion layer on a weakly charged solid support. In the following two-three adsorption cycles these islands spread and cover all the surface, and further multilayer growth occurs linearly. If a substrate is well charged then a linear growth with repeatable steps begins earlier.

In studying the possibility of using new compounds in the assembly, we used a precursor film approach [6, 13, 23]. On a substrate (silver electrode of QCM resonator or quartz slide) we deposited 2 - 3 layers of polyanions, and then on this “polyion blanket” with a well defined charge of the outermost layer, an assembly of proteins, nanoparticles or other compounds was produced. In a typical procedure, precursor films were assembled by repeating two or three alternate adsorptions of PEI and PSS. The outermost layer became “negative” or “positive”, respectively.

QCM monitoring of multilayer growth was often the first stage of the assembly procedure elaboration. Initially, we estimated the time needed for a component’s saturated adsorption in a kinetic experiment. Then, we performed the assembly typically with 10 min alternate adsorption. After every other adsorption step, a layer was dried by a nitrogen stream and the QCM resonator frequency was registered. The frequency shift with adsorption cycles gave us the adsorbed mass at every assembly step. A linear film mass increase with the number of assembly steps indicated a successful procedure.

The following relationship is obtained between adsorbed mass \( M (g) \) and frequency shift \( \Delta F \) (Hz) by taking into account the characteristics of the 9 MHz quartz resonators used [23]:

\[
\Delta F = -1.83 \times 10^8 \frac{M}{A},
\]

where \( A = 0.16 \pm 0.01 \text{ cm}^2 \) is the surface area of the resonator; and \( \Delta M (ng) = -0.87 \Delta F \text{ (Hz)} \). One finds that 1 Hz change in \( \Delta F \) corresponds to 0.87 ng, and the thickness of a film may be calculated from its mass. The adsorbed film thickness at both faces of the electrodes \( d \) is obtainable from the density of the protein / polyion film (ca 1.3 g/cm\(^3\)) and the real film area:

\[
d (nm) = -(0.016 \pm 0.02) \Delta F \text{ (Hz)}.
\]

The scanning electron microscopy data from a number of protein / polyion and linear polycation / polyanion film cross-sections permitted us to confirm the validity of this equation. Another powerful method for polyion film characterization was small-angle X-ray and neutron reflectivity.

Electrode of Quartz Crystal Microbalance:

![Electrode of Quartz Crystal Microbalance](image)

Picture of UV-vis spectrophotometer (left) and QCM-instrument (right) used to monitor the layer-by-layer assembly.
1.4 Multilayer Structure

UV-vis spectra give simple method to control LbL-assembly on glass or quartz slides. After every other layer one can measure the sample UV-spectra and using Beer’s law (absorbance is proportional to the material mass) to judge on amount of adsorbed polymer, and is the assembly process linear with number of adsorbed layers. Below, we give UV-spectra for 3, 5, 7, and 9 bilayers of PSS/PAH deposited on quartz slide. One can see that we have linear increase of absorbency with number of layers, therefore, the mass of the film was increasing linearly too. Maximum at 225 nm corresponds to absorbency of benzyl rings of PSS.
More detail structural information can be obtained from X-ray and neutron reflectivity data. X-ray or neutron reflectivity measurements of polyion films show patterns with profound intensity oscillations, as demonstrated in Fig. 3 [96]. They are so-called Kiessig fringes, due to the interference of radiation beams reflected from interfaces solid support / film and air / film. From the periodicity of these oscillations one can calculate the film thickness (with the help of the Bragg-like equation and taking into account refraction phenomena which are essential at small-angles). Growth steps for a bilayer of 1.1 - 2.0 nm are typical for alternate linear polyion assembly, and a thickness of one layer often equals to half of this value [6-14]. These values correspond to a polyion cross-section and show that in one cycle of excessive adsorption we have approximately one monolayer coverage of the substrate. The nanoparticle / polyion bilayer thickness is determined by the diameter of the particle. Model fitting of X-ray data gives a surface roughness of the polyion film of order 1 nm. Atomic force microscopy and scanning electron microscopy data revealed a surface roughness of 1 - 2 nm [42]. Polyion films are insoluble in water and in many organic solvents and are stable to 250-280°C [38, 95].

X-ray reflectivity curves (wavelength 0.154 nm) following the assembly of PVS /PAH multilayer film, a step of growth for the bilayer is 1.3 nm. Component concentration is 2 mg/mL, in aqueous solution at pH 4, adsorption time 15 min.

![X-ray reflectivity curves](image)

Neutron reflectivity analysis of the films composed of alternate layers of deuterated PSS and hydrogen containing PAH has proved that polyanion / polycation films possess not only a high uniform thickness but a multilayer structure, too [19-22]. The interfaces between layers in polion films are not sharp and partial interpenetration (30-40 % of their thickness) between neighbor
polymeric layers takes place [21-22]. A distinct spatial component separation may be reached between the first and the third or fourth neighboring polycation layers. In the neutron reflectivity experiments with the selectively deuterated component (usually d-PSS), it was possible to observe 1-3 Bragg reflections in addition to Kiessig fringes. This was not possible in the X-ray reflectivity experiments because of a small scattering contrast of neighboring polycations and polyanions, and because of their large interpenetration. X-ray Bragg reflections from the alternate gold nanoparticle / poly(allylamine) multilayers were observed by Schmitt, Decher and others [55]. They demonstrated that in order to have good spatial separation between gold layers in the film, one needs to make a thicker polycation interlayer (of 3-4 PSS / PAH bilayers). In a similar approach we formed the four-step unit cell multilayers of myoglobin, and deuterated and “usual” poly(styrenesulfonate): (myoglobin / deuterated-PSS / myoglobin / PSS)$_9$ [96]. A Bragg-reflection in the neutron reflectivity curve of this four-step unit cell multilayer was observed (Fig. 3b). The film’s total thickness was calculated at 94.0 nm, and the four-unit cell thickness was 11.1 nm.

**Fig. 3b**

![Graph showing Bragg peaks and neutron intensity](image_url)

**Scheme of interpenetration of neighbor layers in polycation (blue) / polyanion (red) multilayer (cross-section direction) derived from neutron reflectivity data.** An average (physical density) gave constant value. It is why we did not see Bragg reflections on X-ray reflectivity curve. But when every other layer is deuterated, it strongly scatters neutrons and we can judge on interpenetration of deuterated and non-deuterated polyions, $q = 4\pi\text{sin}\Theta/\lambda$, where $2\Theta$ is a scattering angle, and $\lambda = 0.2$ nm.
Fig. 4 demonstrates that poly(dimethyldiallylammonium chloride) / poly(styrene- sulfonate) (PDDA / PSS) alternate adsorption from solutions with different ionic strength resulted in a bilayer growth step variation of 1.6 nm up to 6 nm. It was proposed that in adsorption from water (at low ionic strength solutions) one deals with strongly charged polyions, which form a well-attached monolayer. In adsorption from high ionic strength solutions (at salt concentrations of the order 0.1 - 1.0 M) one observes partially neutralized polyion chains, which provide adsorption with major loops and the step of film growth becomes much greater [13].

**Fig. 4 Film thickness in dependence on layer number for:**
1- PDDA / PSS deposited from pure water, pH 6.5; 2- PDDA(0.5M NaCl) / PSS, pH 6.5; 3) PDDA(0.5M NaCl) / PSS(0.5M NaCl). Steps of growth were, correspondingly, 1.6, 3.2, and 6 nm. Increase in the thickness of adsorbed layers is due to polymer coil formation. This coil formation was induced by added salt, and the coil radius of gyration is proportional to the square root of the solution ionic strength.
The polycation / polyanion bilayer thickness depends on the charge density of the polyions. It was shown that more than 10% of polyion side groups have to be ionized for a stable reproducible multilayer assembly via alternate electrostatic adsorption [34]. High ionization of polyions results in a smaller step of film growth (1 - 2 nm) and lower ionization gives a larger growth step (3 - 6 nm). It can be reached either by adding salt to a polyion solution (as discussed above for strong polyelectrolytes, such as PDDA and PSS), or by varying the pH for weak polyelectrolytes (e.g., polyacrylic acid (PAA) and poly(allylamine) (PAH), as was analyzed by Rubner et al [30]).

Direct zeta-potential measurements confirmed a symmetric positive / negative alternation of the polycation / polyanion multilayer’s outermost charge with adsorption cycles [34]. This corresponds to the proposed scheme of the electrostatic layer-by-layer assembly, which is given in the Fig. 1. Below we give a typical image of polyion multilayer film.

SEM image of \{PSS(0.5 M MnCl₂)/PAH(1 M NaBr)\}_17 . The film coats silver electrodes attached to rough quartz surface. The film thickness is 85 nm.
1.5 Stoichiometry of the Components and Polyion Molecular Weight

A multilayer film has to be totally neutral, i.e., a stoichiometry of charged groups in neighboring polycation and polyanion layers has to be 1 : 1. In many cases we confirmed this (for PSS / PDDA, PSS / PEI, PSS / chitosan, DNA / PAH multilayers), but for PSS / PAH a deviation from 1 : 1 stoichiometry was found [34]. This is probably due to the incomplete dissociation of the polynomials. It is difficult to control polyion dissociation, because it depends on a concentration and on the presence of oppositely charged compounds in solution [97-98]. The dependence of the polycation / polyanion bilayer thickness on ionic strength and on variation of pH for weak polyions can be explained by partial neutralization of polyion side-groups which results in more coily conformation of polymer chains. The complex stoichiometry did not depend on the ionic strength of polynion solutions, which indicates an absence of low molecular weight ions (K⁺, Na⁺, Cl⁻) in the films.

For deposition from water solutions the PSS / PAH growth step was similar for PSS molecular weights varying from 80,000 to 1,000,000 [21]. The influence of the polymerization degree on the formation of stable interpolyelectrolyte bulk complexes was analyzed by Kabanov [97]. He found that such complexes were stable when a polyion contained more than 20 charged groups in the sequence. We used polyions, such as PSS, PVS, PDDA, PEI with molecular weights of 50,000 - 200,000. They contain hundreds of ionized groups in the pH range from 3 to 9. A cooperative electrostatic interaction between the polycations and polyanions used is strong and prevents the dissolution of the multilayers even in high ionic strength solvents.

1.6 Non-Equilibrium Growth and Admixed Polyions Complexes.

When the polynion assembly was performed at equilibrium conditions, a saturated adsorption was achieved at every deposition step, and a careful intermediate sample washing removed all non-specific polyion adsorption. Then one has the permanent increment of the multilayer growth (i.e., the film mass and thickness increased linearly with the cycles of adsorption). In some cases, we encountered the situation when the film growth step exponentially increased with the number of adsorbed layers. We recognized such conditions as non-stable and, usually, reached a stable growth-step by optimizing the solution pH or by decreasing the polyion concentration.

Recently, an exponential increase of the growth step was used for quick assembly of relatively thick polylysine / alginate films for biocompatible coverage [47]. Such a mode of assembly was achieved by using a brief time of intermediate rinse. In this way non-specifically adsorbed polyions were included in the multilayer. The first five bilayers in this assembly had a thickness of 20 nm; the next five bilayers were 35 nm; the next - 100 nm, and so on. In such an assembly a stepwise formation of a complex polyion gel rather than solid multilayer film was observed.

As we discussed above, one can increase the speed of growth from 1-2 nm to 5-10 nm in 10 min by increasing the ionic strength of the polynion solutions. Another way to increase the assembly speed is by using preformed interpolyelectrolyte complexes (coacervates) [92]. The formation of water-soluble polyelectrolyte complexes has been intensively studied by means of turbidity measurements [58, 97, 98]. Aqueous mixtures of oppositely charged polyelectrolytes are usually homogenous at the stoichiometry far from the neutralization point. First, such complexes permit an increase in the size of the assembly construction blocks and, second, make it possible to immobilize
with polyelectrolyte the materials, which were not possible to use in the assembly directly (poor charged nanoparticles, dye and proteins).

We analyzed formation of charged water-soluble interpolyelectrolyte complexes of proteins and linear polyelectrolytes [79]. A protein surface charge can be altered by excess complexation with polyelectrolytes. Above and below the flocculation point, interpolyelectrolyte protein complex solutions are clear and stable, but the protein-polyion particles possess either their own or a reversed surface charge. The turbidity study of albumin-PDDA complexation at pH 9 shows the flocculation point at a mass ratio of $C_{\text{albumin}} / C_{\text{PDDA}} = 10$ (the molar component ratio is 1 : 56). At a protein concentration above this point the protein preserved its negative charge, and below – the albumin globule “shelled” by PDDA was positive. Thus, we demonstrated the alternation of the protein surface charge without changing the solution pH and could use the protein either as negative or positive particles. A similar approach permitted the direct assembly of anionic glucose oxidase (GOx) with anionic montmorillonite clay. At pH 6.5 the glucose oxidase / PDDA flocculation point was at the component mass ratio $C_{\text{protein}} / C_{\text{polyion}} = 24$, and below this point the complex was positive. A higher PDDA concentration provided a cationic shell for the glucose oxidase globule and permitted an assembly with anionic clay plates.

**1.7 Polyelectrolyte Films for Biocompatible Coverage.**

Multilayer assemblies of natural polyelectrolytes, such as DNA, polynucleotides, polylsine, and polysaccharides (e.g., heparin, chondroitine, and chitosan) are interesting for biological and medical applications. DNA and polynucleotides (polyuridylic and polyadenylic acids) can be readily assembled in alternation with polycations (PEI, PAH, polylsine). The assembly of polysaccharides with oppositely charged polyelectrolytes is possible as a means of biocompatible surface preparation. A chitosan and albumin/heparin multilayer assembly for such coatings has been developed [42-44].

The polyelectrolyte assembly is possible on biological surfaces too. It overcomes two fundamental obstacles preventing the use of colloidal materials on biosurfaces: first, the self-assembly onto a proteinaceous biosurfaces is hindered by the heterogeneity of the chemical groups on such surfaces, and, second, the self-assembled material must itself be relatively biologically inert [47]. The assembly of (polylsine/alginate)$_5$ multilayer above a gelatin (or extracellular matrix) surface resulted in 200-fold drop in the adsorption of human fibroblast cells, as compared with the untreated surface. The layer-by-layer polyelectrolyte assembly makes such heterogeneous surfaces inert in biological liquids. The usefulness of such a treatment in medicine would stem from the ability to treat a limited area of tissue via multiple rinsing steps with polyelectrolyte solutions, the ability to generate thin coating on tissue surfaces, and the inherent biocompatibility and degradability of the system.

![Cross-section of biocompatible film](image)

**Cross-section of biocompatible film:** silver electrode (bright) coated with 8 bilayers of (hyaluronic acid / PDDA), film thickness 30 nm (dark).

**1.8 Multipolar Dye and Liposome Assembly**
The layer-by-layer assembly by alternate adsorption of oppositely charged molecules was applicable for multipolar dyes with symmetric charges possessing conjugated rings and other hydrophobic fragments [48-51]. Hydrophobic fragments probably enhance dye stacking. Interestingly, the dye / polyion bilayer has the same thickness in a wide range of dye concentrations. For Congo Red / PDDA films the bilayer thickness was 1.5 nm for dye concentrations from 0.01 up to 10 mg/mL, i.e. below and above the critical micelle concentration (CMC) of the dye.

The characteristic feature of the assembly of some dye multilayers was greater than the monolayer adsorption at the dye adsorption cycle followed by a depletion of the material during the following linear polyion adsorption step. In this process we have substantial non-specific adsorption at the dye adsorption step, and then the removal of non-specific bond material by complexation with an oppositely charged polyion (at the next assembly step). In the graph of the film mass against the number of adsorption steps, such a growth mode looks like “a large step up followed by a small step down”[46, 50, 99]. A similar growth-mode was observed for protein / polyion and nanoparticle / polyion assemblies, especially with a short intermediate sample washing time [58, 77].

An elaboration of the assembly with charged lipid vesicles and liposomes is important for membrane protein architecture. The first results were obtained for the assembly of anionic PSS with cationic azobenzene containing amphiphiles: \( \text{CH}_3(\text{CH}_2)_7\text{Azo}(\text{CH}_2)_{10}\text{N}^+ \) [45]. Bolaform amphiphiles (i.e., symmetrically charged amphiphiles) are not required for this assembly, since the self-assembling nature of symmetric bilayer membranes gives rise to a new charged surface after their adsorption. The present approach may replace certain aspects of the Langmuir-Blodgett technique [1] as appropriate self-assembling amphiphiles and lipids are employed.

We studied an assembly for positively charged liposomes from didodecyldimethylammonium bromide (DDAB). Using in alternate assembly liposomes whose stability depends on hydrophobic interactions between hydrocarbon tails, we include in the process forces other than electrostatic ones. Their influence on the assembly is not well understood. The alternate assembly of DNA and positively charged DDAB liposomes (concentration 10 mM) proceeded linearly; the growth step corresponds to QCM frequency shifts of 340 Hz for DDAB + 130 Hz for DNA (i.e., 5.4 nm and 2.1 nm for DDAB and DNA layers). 2.1 nm is close to DNA diameter of 1.9 nm. The length of DDAB molecule is 1.7 nm; hence, its bilayer thickness should be below 3.4 nm. Thus, complexes more complicated than a multilayer of DNA / DDAB could be formed in the assembly. When polyanion PSS was used instead of DNA, the multilayer assembly proceeded with a growth increment of \( \Delta F = 220 \text{ Hz} \) corresponding to 4.4 nm DDAB / PSS complex layer thickness. This is equal to the sum of thicknesses of the DDAB and the PSS layers.

1.9 Combination of Polyion Assembly with the Langmuir-Blodgett Technique. Hydrophobic Interaction

The Langmuir-Blodgett transfer (LB) [1] of amphiphile monolayers from the air / water interface onto solid supports is a powerful tool for creating periodic lattices in many varieties, usually composed of bilayers. The combination of LB transfer and polycation / polyanion assembly allows a precise extension of the LB lattice, and a superlattice may be formed by combining of LB and polyion units [8, 13]. The superlattice was produced in a multicomponent film, in which additional polyelectrolyte sheets are inserted between the amphiphile head groups. At first, LB films of a cationic dioctadecyldimethylammonium bromide lipid / poly(vynilsulfate) complex (DODAB / PVS) were prepared and a bilayer spacing of 4.4 nm was found. Anionic PVS was injected into the subphase under the monolayer. For the construction of superlattices, sequences of polyion layers were adsorbed between the bilayers by the following method: After the deposition of one DODAB\(^+\) /
PVS− layer, the wet substrate was removed from Langmuir-Blodgett trough and PAH+ and PVS− were alternatively adsorbed from their solutions. The resulting superlattice repeat unit consists therefore of DODAB / PVS bilayer and the inserted ensemble of polyanion layers. Samples of 1, 3 and 5 additional PAH/PVS layers per unit cell were prepared and their structure was tested with small-angle X-ray reflectivity. The variation of the repeat unit of the superlattice results in a linear increase of the superlattice spacing. The constructed film consists of two components with different melting points: for DODAB it is about 85º C; polyanion films are stable up to 250º C.

Therefore, there is the possibility of separating lipid bilayers by means of well-defined thin polymer interlayers. This opens the way to experiments where one needs to separate amphiphile molecule arrays in the range of 0.5 to 10 nm. An ion diffusion along nano-scale polyanion channels inserted between lipid bilayers may be studied. It is possible to use the electrical properties of the two component assemblies: lipid bilayers, which are a good dielectric in a transverse direction, may be combined with conductive polyanions (e.g., polyaniline, polypyrrole), which would provide a tangential conductivity in inserted layers.

Roberts et al. [100] used hydrophobic forces (a regular drying at the every other deposition cycle) to produce non-centrosymmetric chromophore orientation in the polyanion multilayer, which resulted in light second harmonic generation (SHG) by the film.

1.10 Surface Patterning with Linear Polyanion Films.

It is possible to perform not complete surface coverage, but to grow the polyanion multilayers on micropatterns, as was demonstrated by Hammond, Whitesides et al [31-32]. First, they patterned a gold surface using a thiol microprinting technique that resulted in the formation of 2-µm width charged strips separated by hydrophobic regions. Second, they performed the layer-by-layer assembly of linear polycations and polyanions on this substrate with careful intermediate sample washing in an ultrasonic bath. The polyanion film was growing only onto the charged strips at moderate ionic strength of solutions (0.1-0.4 M NaCl) and without intermediate drying. A method for assembling multilayers selectively, only on charged patterns and then on uncharged regions (“reverse deposition”), is under consideration [32]. In more details this will be discussed in the next lecture.

1.11. Fabrication of Microporous Films

A simple process to convert multilayers of weak polyelectrolytes (e.g., poly(acrylic acid) and poly(allylamine)) into uniform microporous films has been developed by Rubner et al. [104]. These multilayers were immersed briefly into acidic solution (pH 2.4) to effect an irreversible transformation of the film morphology. The resulting microporous structure thickness is 2-3 times less than the thickness of the originals film, and possesses reduced relative density of 1/2 to 1/3. The interconnected pores are ranging in size from 100 to 500 nm. A refractive index of the porous PAA/PAH film was n = 1.18 ± 0.01. It is much less than the origin n = 1.54 for the untreated polyanion multilayer. Correspondingly, a dielectric constant of the porous PAA/PAH film dropped to ε’ = 2 from the initial ε’ = 5-7.
2. Nanoparticle / Polyion Multilayers.

2.1. Silica Multilayers.
2.2 Latex Assembly.
2.3 Compact MnO2 / Poly(ethyleneimine) Multilayers.
2.4 Open TiO2 / Poly(styrenesulfonate) Films.
2.5 Organized Gold / Polycation Multilayers.
2.6 Layered Ceramic (Plates).
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2.10 Polyoxometallates.
2.11 Multilayer Nanoreactors for Metallic and Semiconducting Particles.
2.12 Lithographic Approach to Pattern Self-Assembled Nanoparticle Multilayers

The construction of organic / inorganic nanostructured materials is an important goal of modern materials research. An alternate adsorption procedure was used for the following charged nanoparticles: clay and ceramic plates, nanotubules, 10, 20, 45, 75-nm silica spheres, 50, 150, 300-nm latex, 15-nm gold, 30-nm magnetic Fe₃O₄, 50-nm CeO₂, MnO₂, ZrO₂, 30-nm TiO₂ particles [23, 52-71], as well as 34-nm diameter spherical plant viruses [12]. Many oxide particles have an isoelectric point at pH 4-5. They are negatively charged at pH 7-8, and can be readily assembled by means of alternate adsorption with such polycations as PEI or PDDA (Fig. 5). The number of particle monolayers in such “sandwich” multilayers is exactly known, and any profile across the film can be constructed with a resolution of 5-10 nm. Our experience shows that using a "soft" polymeric interlayer was important for the composite multilayers formation: flexible linear or branched polions optimize electrostatic attraction. Nevertheless, after the assembly is completed, organic interlayers can be removed by a 2-hour thermal treatment above 300°C on air (calcination process), which results in direct contacts between the nanoparticles needed for improved electric or magnetic properties. Nanoparticles, such as gold, silver, and fullerenes may be “sandwiched” in multilayers with proteins providing electrically or optically induced electron donor-acceptor properties. Semiconductor nanoparticles, such as PbS, CdS, CdSe, were used in this assembly [54, 67-69]. An assembly of core-shell nanoparticles, such as Ag/TiO₂ or Fe₃O₄/Au, was also possible [71, 73].

Fig. 5
2.1. Silica Multilayers.

As an example of the nanoparticle architecture, let us analyze a 45-nm silica assembly by alternate adsorption with polycation poly(dimethyldiallylammonium chloride), (PDDA) [58-59]. In situ quartz crystal microbalance (QCM) monitoring of alternate PDDA and SiO₂ adsorption gave the kinetic of the assembly process. In the first step, PDDA was adsorbed onto Ag-electrode. The QCM frequency decreased during the first 60 s, after which a slower change was observed as adsorption saturation set in. Then, the resonator was immersed in pure water (black dots) for washing. Next, the film was immersed in SiO₂ dispersion and silica adsorption saturation occurred within several seconds. After subsequent water rinsing, the film was immersed again in a PDDA solution, and so on (Fig. 6a). Each growth step was reproducible, and the adsorption process reached 90% saturation in 10 s for SiO₂ and 30 s for PDDA. Fig. 6b shows that the film assembly was not possible simply by the multiple immersion of the substrate in the silica solution. An alternation with an oppositely charged polyion was necessary. At every assembly step the component monolayers were formed, as was recorded by QCM, scanning electron microscopy (SEM) and ellipsometrical studies (Fig. 6-7).

Fig. 6a-b

The average density of SiO₂ / PDDA multilayers is $\langle \rho \rangle = 1.43 \pm 0.05$ g/cm³. SiO₂ / PDDA film volume composition is: 60 % SiO₂ + 10 % polycation + 30 % air-filled pores. These pores are formed by closely packed 45-nm SiO₂ and have a typical dimension of 20 nm. The films have controlled pores, which can be varied by the selection of the nanoparticle diameter.

Fig. 7 Cross-section images of two different films of (45-nm diameter silica/PDDA)₂₄-₂₈ on silver electrodes. The right one – at larger magnification.
We estimated the diffusion limitation for surface coverage $A(t)$ by adsorption from solution of particles with the diffusion coefficient $D$ from $A(t) = \frac{2}{\pi} C \sqrt{D t}$. For $t = 2$ s, $C = 10 \text{ mg/cm}^3$ and assuming for 45-nm silica $D = 1.1 \times 10^{-7} \text{ cm}^2/\text{s}$, $A \approx 3 \times 10^{-6} \text{ g/cm}^2$ and the layer thickness: $L = A(t)/\langle \rho \rangle \approx 21$ nm. This is reasonably close to the experimental silica monolayer thickness of 24.6 nm. Thus, 2 s corresponds roughly to the diffusion-limited time for the SiO$_2$ monolayer adsorption; this time is the fastest nanoparticle monolayer formation rate that we have achieved.

2.2 Latex Assembly.

Charged latex is a good building block for an electrostatic layer-by-layer assembly. Positively or negatively charged monodisperse lateses with diameters of 30, 40, 45, 50, or 75-nm and with different colors are commercially available, for example, from Seradyn Inc or IDC-Ultraclean Uniform Latex Inc. For the first time a multilayer assembly of negative latex spheres (carboxyl-modified or sulfate polystyrene) in alternation with positive latex (amidine-modified polystyrene) was reported by Bliznyuk and Tsukruk [57]. They have described a strong tethering of charged nanoparticles to the surface, which prevents surface diffusion and the rearrangement required for formation of perfect lateral ordering. This situation is different from the one with the formation of the ordered 3D-mesocrystals by slow crystallization of the monodispersed aqueous colloids [72]. In the nanoparticle / polycation multilayers one looses the crystal-like ordering, but gains control of the process, preparing multilayers of close-packed nanoparticles with precisely known number of monolayers. We demonstrated a regular layer-by-layer assembly of carboxylated 45-nm diameter Seradyn latex in alternation with poly(ethyleneimine) (PEI).

QCM monitoring on negative 40-nm diameter sulfonated polystyrene latex in alternation with polycation-PEI. Kinetic adsorption measurements.
Our experience in nanoparticle / polyion assembly has also shown the importance of the balance of electrostatic forces between nanoparticles. On one hand, we need a strong electrostatic attraction to anchor particles to the surface and to prevent the adsorption of more than one monolayer. On the other hand, a strong particle charge results in their repulsion and prevents the close packing of the particles in the monolayer. It was possible to obtain close-packed multilayers of silica and latex spheres by means of increasing the ionic strength of their solutions (which decreased the nanoparticle surface charge) (Fig. 7). For the assembly from silica or latex dispersion in water without salt we obtained less ordered, porous multilayers. Nanoparticle films with an “open” structure may be useful for catalysis, and in section 2.4 we will describe one of such films.

2.3 Compact MnO₂-nanoparticle / Poly(ethyleneimine) Multilayers

Fig. 8 shows quartz crystal microbalance (QCM) frequency shifts during the assembly of nanoparticle MnO₂ / PDDA films with 10 min adsorption times and drying before every measurement [60]. Here we used a precursor film consisting of PDDA / PSS / PDDA layers on the solid substrate. Then, on this “polyion blanket” with positive charge of the outermost layer, a layer of negative MnO₂ nanoparticles followed by subsequent PDDA / MnO₂ layers were added (steps 4-18). The frequency shift with an increasing number of adsorption cycles was proportional to the adsorbed mass at every assembly step. UV-vis spectral monitoring of (MnO₂ / PDDA)_x films showed a linear absorbance increase of strongly overlapped bands in the wavelength range of 250–400 nm with the number of MnO₂ layers x = 2 to 11. This agrees with QCM measurements suggesting regular film growth. Fig. 9 shows scanning electron micrographs of film cross-sections and, partially, a top-view of a film with architecture PDDA / PSS / PDDA + (MnO₂ / PDDA)_9. This film has a remarkably smooth surface and uniform thickness. The total frequency shift by QCM was 11,000 Hz and film thickness from scanning electron microscopy (SEM) was 170 nm. Dividing the film thickness by the number of manganese oxide/PDDA bilayers we obtain values slightly less than the 23 nm bilayer thickness found above from QCM because the first two layers of manganese oxide are thinner. We cannot detect separate layers in the film by SEM, but individual structures of about
20 nm dimensions are clearly visible and may be attached to nanoparticle. The top-view shows what seems to be a porous surface with grains 15-20 nm in diameter.

**Fig. 8 – 9.** QCM monitoring of \((\text{MnO}_2 / \text{PDDA})_9\) film, and its SEM cross-section image. The film coats silver electrode.

We analyzed the chemical composition and the chemical state of Mn and N atoms on the surface of a film with architecture PDDA / PSS / PDDA + \((\text{MnO}_2 / \text{PDDA})_9\) by XPS. Spectra revealed a surface rich in carbon and oxygen with manganese and nitrogen present in concentrations less than 7 %, and trace amounts of chlorine. The Mn (2p) region consists of a spin-orbit doublet with a Mn (2p1/2) binding energy of 653.30 eV and Mn (2p3/2) binding energy of 641.63 eV. This doublet can be assigned to a mixed valent manganese system, most likely Mn (4+) and Mn (3+) since the average oxidation state of Mn in the nanoparticles is 3.7. The N 1s region showed two peaks at 402.04 eV and 399.12 eV, indicating two different chemical environments for the nitrogen atoms. The difference in the chemical environment may reflect the formation of contact ion pairs and long-distance charge pairs between the MnO2 particles and the polycation PDDA.

Interestingly, it was possible to grow MnO2 multilayers alternating with positively charged protein myoglobin (solution pH 8.5). This is the only case in our experience, when an alternate assembly was possible without linear polyions (the possibility of alternating proteins with colloidal particles described in Iler’s paper [2]). Films of myoglobin and MnO2 showed an unusual growth
mechanism featuring competition for adsorption of myoglobin and dispersed nanoparticles. Nevertheless, stable myoglobin / MnO₂ films up to 30 nm thick featuring reversible electrochemistry of the protein could be constructed. Catalysis of the reduction of oxygen suggests that these films may be useful for an enzyme-like oxidation of organic molecules [60, 101-103].

2.4 Open-Structured TiO₂-nanoparticle / Poly(styrenesulfonate) Films.

The assembly of 35-nm diameter TiO₂ (Degussa P25) was possible at pH 3.8 (when the particles were positive) in alternation with poly(styrenesulfonate), (PSS) [58]. TiO₂ assembly with polycation PDDA at this pH was not possible (Fig.11). The saturation adsorption time for TiO₂ nanoparticles was 5 min. The growth step of the TiO₂ / PDDA multilayer was stable and linear, with the mass adsorption increment corresponding to the monolayer surface coverage. The scanning electron microscopy of the TiO₂ / PSS multilayer shows a not a close-packed but an open structure of the film (Fig. 12). It was possible to vary the porosity of the film by changing the ionic strength of nanoparticle dispersions. Such porous structures can be useful for catalytic applications.

Fig. 11-12 QCM monitoring of the assembly and SEM image of the film cross-section.

In another approach, 2.3-nm diameter TiO₂ were capped with aminoundecanoic acid. Such 6.3 nm diameter building blocks were used for a multilayer assembly with PAH by the attracting amine groups with poly(allylamine) [69]. In this case the binding was realized by sharing protons between amine-groups of aminoundecanoic acid and ammonium-groups from PAH, rather than electrostatic interaction.

2.5 Organized Gold / Polycation Multilayers.

In [55-56] the self-assembly of 15-nm diameter gold particles in alternation with polycation poly(allylamine) (PAH) was demonstrated. Gold alteration with the polycation was possible because of negative nanoparticle charge (surface potential –35 mV) due to the synthesis procedure. The saturation time needed for the formation of close-packed gold nanoparticle monolayer (full
coverage) was 2 hours because of the low particle concentration in solution [55]. An approach to a spatial separation of nanoparticle layers in such sandwich-like films was elaborated: three- to five polycation / polyanion interlayers were assembled between gold layers (e.g., Au / {PAH + (PSS/PAH)_{3.5}} / Au). The gold surface (with a roughness of the order of the radius of nanoparticles) was covered and smoothed by the 6-10 nm polyion layer. Further “sandwiching” of gold nanoparticles with the thick polyion interlayers resulted in the ordered gold / polymer heterostructure. The low-angle X-ray reflectivity of these samples gave 2-3 orders of Bragg-reflections with the spacing corresponding to such complex unit-cell. We think that this is a general approach to creating ordered inorganic / organic heterostructure multilayers, and we have used it also for formation of protein / polyion heterostructures [77, 79].

2.6 Layered Ceramic (Plates).
Mica-type layered silicates can bear a natural negative charge because of the isomorphous substitution of silicon in octahedral sheets by aluminum or magnesium. The charge is generally balanced by potassium cations that reside in the galleries between layers. The intercalation of organic polymers between sheets of layered ceramics provides access to novel polymer-ceramic nanocomposites. These nanocomposites exhibit unique physical and mechanical properties attributable to the synergism of the individual components. The build-up of the multilayers in a stepwise manner rather than in the bulk “all-at-once” manner is of special interest. Kleinfeld and Ferguson [52] were first to apply the electrostatic layer-by-layer adsorption to produce multilayers of anionic synthetic silicate - hectorite and cationic PDDA. We used the polyon assembly to build up multilayers with alternating 1-nm thick montmorillonite sheets and cationic PEI or PDDA [64] (Fig. 13). The film thickness increase for the montmorillonite adsorption cycle was 1.1 nm and for PEI 2 nm and after 20 cycles the resultant film had a permanent thickness of 63 nm (Fig. 14). The construction of ultrathin ceramic films is remarkable, if we take into account that a diameter of a
montmorillonite sheet is ca 20 times larger than the film thickness. Two types of defects were visible in the film: the first is due to the adsorption of non-delaminated montmorillonite particles, and the second - to border defects connected with the overlapping of edges of the montmorillonite lamellas. Fig. 13-14

2.8 Tubule Ceramic
We have described the formation of ordered multilayers from ceramic nanotubules, inorganic spherical particles and enzymes, showing that one can “sandwich” nanoparticles of different shapes and kinds in the organized films using linear polyelectrolytes as an electrostatic glue. Halloysite (Al₄Si₄O₁₀(OH)₈·4H₂O) is a naturally occurring alumosilicate that exhibits a tubular morphology in the hydrated state. At pH above 4 halloysite is negatively charged. The tubule morphology of Halloysite exhibits great potential for manipulation in slow-release formulation [65]. The micro-cylinders of the halloysite G used are 50 nm in diameter, 300 - 500 nm in length and have 20-nm diameter hollow inner core. Halloysite assembly by sequential adsorption with poly(ethyleneimine) (PEI) or poly(dimethyldiallylammonium chloride) (PDDA) resulted in the formation of ordered multilayers containing from 2 to 20 layers of tubules kept together by polycation interlayers (Fig. 15). An alternation of anionic halloysite with a polycation was necessary for a successful assembly. Multiple immersion of the resonator only in halloysite (Hal/Hal) or in halloysite and polyanion PSS (Hal/PSS) did not

Fig. 15 and QCM monitoring of LbL-assembly by alternating the tubules and cationic PEI (left), and TEM image of cross-section of Halloysite tubules (right).
result in the film growth. The tubules in a monolayer are loosely packed; they form a network leaving ca 50 % of empty space. The total thickness of (Halloysite / PEI)14 film was found to be 720 nm and a one-layer thickness is 54 ± 5 nm (Fig. 16).

**Fig. 16** Top and side SEM images of (Halloysite / PEI)14 film.

### 2.9 Assembly of Tubule / Sphere Superlattices.

In this approach we constructed a multilayer by alternating negatively charged tubules with 45-nm diameter silica spheres, which are kept together by polycation interlayers. SEM images confirm the regular alternation of tubules and spheres in the multilayer (Fig. 17). This is the first example of the manufacture of ordered arrays from differently shaped nanoparticles [65]. The assembly of alcohol dehydrogenase (ADH) and nicotinamide adenine dinucleotide (NAD)-loaded halloysite multilayers with PEI connecting layers was also achieved. This assembly is targeted for the design of nanocomposites that provide a direct supply of the ADH-cofactor to the enzyme immobilized in polymer films.
2.10 Polyoxometallates.
We produced an alternate adsorption assembly of negatively charged multinuclear complexes Mo$_8$O$_{26}^{4-}$ with cationic PAH with QCM monitoring of the process [66]. The adsorption kinetic study indicated that a molybdenum oxide layer was formed via accelerated condensation of complexes at the positively charged PAH surface. In this architecture we combined saturated adsorption of PAH and non-saturated (time-dependent) adsorption of Mo$_8$O$_{26}^{4-}$. It permitted us to design composite films where Mo$_8$O$_{26}^{4-}$ layer thickness may be adjusted between 1 and 5 nm. 

Scheme of the assembly and SEM image of (Mo$_8$O$_{26}^{4-}$/PAH)$_{14}$ multilayer.

2.11 Multilayer Nanoreactors for Metallic and Semiconducting Particles.
This process was recently introduced by Rubner et al [75]. First, polyelectrolyte multilayers with controlled content of free non-ionized carboxylic acid groups were fabricated with weak polyions (e.g., poly(acrylic acid) via suitable pH-adjustments of the processing solutions). These groups were then used to bind various inorganic ions that were subsequently converted into 2-nm diameter particles (e.g., Ag, Pb, or PbS). The spatial control over the growth of the nanoparticles was achieved by the use of multilayer heterostructures that also contain bilayer blocks that are not able to bind inorganic ions. These nonbonding bilayers were fabricated from strong polyions, such as poly(styrenesulfonate). A density modulation with a few nanometer-resolution across the polymeric films is possible by selective synthesis of heavy atom nanoparticles.

2.12 Lithographic Approach to Pattern Self-Assembled Nanoparticle Multilayers

Traditional microlithography was combined with electrostatic layer-by-layer nanoassembly to produce 3-D structures. A 1 µm photoresist layer was first applied on a silicon substrate and a pattern was produced through a mask by UV-irradiation. Multilayers of cationic PDDA or PEI and anionic silica or 40-nm FITC-latex were assembled via the electrostatic layer-by-layer self-assembly technique. Then, the photoresist with polyions on top of it was washed with acetone accompanied with sonication, leaving the patterns, 5-µm strips of ordered nanoparticle layers. The edge roughness was better than 0.2 µm as evident from SEM micrographs. Fluorescent microscopy was also employed to analyze the 2D-patterns assembled from FITC-labeled nanospheres and polycations.

There are works on application of the layer-by-layer assembly on two-dimensional (2-D) patterns. They are based mostly on the microprinting of thiol compounds on gold and further assembly of the poliyon multilayers on charged patterns, and they were developed by Hammond et al. [1-4] This strategy is designed to produce patterns by stamping onto substrates chemicals with different functionalities, i.e. polyion adhesive or resisting. The polyions were directed only to charge “attractive” regions and were repelled from the resistant regions. Whitesides et al crystallized latex particles in capillary channels produced by PDMS micromolding and made 3-D ensembles of 450-nm spheres with resolution of ca.1 µm. In another approach, poly(pyrrole) and poly(styrenesulfonate) were LbL-assembled on the 2-D charged micropattern produced on fluoropolymer by plasma treatment. The three methods described were quite successful, but restricted in applications by substrate materials (gold, fluoropolymers) or by necessity of special plastic stamps [5-6]. We elaborated an approach to realize 2-D patterning of self-assembled multilayers by silicon based lithographical technology, which is well established industrial process.

At the beginning, a photoresist was patterned through a mask by the standard UV-irradiation procedure (Fig. 18). Then the substrate was entirely covered with polyion layers with alternate layer-by-layer method to get desired multilayer structure. Therefore, polyion film covers the entire surface (not only adsorption-promoting region as in the work with thiol microprinting) followed by removal of part of the film.

By using this strategy (Fig. 18), the deliberate selective deposition control is avoided. The photoresist was dissolved and during the dissolution, polyion multilayers were removed from the substrate at the selected areas. Nanoparticle or polyion multilayers can be micropatterned by this process. The lithographic micromanufacturing approach is widely accepted in industry, and it offers
larger versatility of the process and high pattern resolution. Since both lithography technology and the layer-by-layer thin film fabrication are well established, an industrial application of LbL ensembles organized in three directions (3D-LbL) is becoming a feasible target.

Total multilayer film architecture in this experiment was as follows: \{PEI + (PSS/PDDA)\_3 + (SiO\_2/PDDA)\_3\}. After a 7-layer precursor of PEI+(PSS/PDDA)\_3 was coated, the outermost layer was positive. Then the wafer was immersed in negatively charged SiO\_2 particles 300 nm in diameter. This layer of SiO\_2 was used to make the pattern visible. At this moment, the whole surface of the wafer becomes white due to entire cover of SiO\_2 particles. More silica/PDDA layers were deposited in a similar way. The last manufacturing step was to put the silicon wafer into acetone solution for 20 sec to strip off the photoresist together with above polyion/nanoparticle layers. For this step, ultrasonic treatment was necessary.

On resulted wafer we see clear and distinct SEM images (Fig. 19a-c). The 5-\(\mu\)m strips containing the polyion precursor with nanoparticle multilayer are well shaped and have sharp borders. Silica nanoparticles are closely packed with few vacancy-defects. An average roughness of the strip borders is less than one particle diameter (in this case 200 nm, but it could be less if smaller particles are used). There are no particles in the areas between the strips. The cross section of the image is very spectacular: strips are evenly separated and shaped and have the same height 3000 nm. The height of the strip may be controlled with LbL assembly. In the present experiment we performed three steps of silica/PDDA deposition, and every step gave 200-nm thickness increase what corresponds to triple silica layer. One layer thickness can be adjusted by lowering ion strength of silica dispersion.

These results indicate that polyion layers are permeable enough to let acetone molecules penetrate inside to dissolve photoresist and strip off the multilayers from these regions. The strip border sharpness indicates that intercalation length between neighboring molecules in polyion layer is less than 200 nm. In the control sample (Fig. 19d), during which the sample has not been sonicated during photoresist removing with acetone, the silicon wafer remains entirely covered by SiO\_2 particles. The polyion layers as well as particles above them were not lifted off and collapsed back during drying, which prevents the pattern formation. The collapse of the polyion layers and particles is due to the links not being broken at the edge of photoresist sidewall by the process. Drying at each LbL-assembly step also results in poor final patterns. Presence of particle clusters, instead of the pattern, visible at the Fig. 19d is due to incomplete removal of the polyion film located above photoresist.

The omitting of drying after rinse and ultrasonic treatment when removing the photoresist are very critical steps to the whole process. Failure to do these two steps will result in an unpatterned area where polyion chains are not completely separated. The drying step in conventional alternate adsorption will help to form stronger connections among polymer molecules. The polymer molecule chains are easy to separate if drying is omitted at the last several cycles. The internal pressure resulting from the dissolving photoresist is not sufficient to remove the polyion layers surrounding photoresist. It is the ultrasonic wave that breaks the link among polyion chains. Currently we are working on other samples based on lithography and layer-by-layer assembly combined methods, which may allow electrode fabrication directly on the polyion layers.

In another experiment, fluorescent negatively charged 45-nm diameter fluorescent nanoparticles were assembled above similar precursor: \{PEI + (PSS/PDDA)\_3 + Fluoresbright/PDDA\_3\} following the same experimental procedure. We used another mask with
wider strips to provide pattern well visible in optical microscope (Fig. 20). One can see sharp green pattern indicating a permanent coverage with width of the strips of 25-µm, and 12-µm wide dark areas without fluorescent multilayer.

In Fig. 21, the 3-D plot and surface roughness of the created U-turn pattern are shown. The root mean square roughness and average roughness are defined by equations:

\[
R_q = \frac{1}{L} \left[ \int_0^L Z^2(x) \, dx \right]^{1/2} \quad \text{and} \quad R_a = \frac{1}{L} \int_0^L |Z(x)| \, dx;
\]

where \(Z(x)\) is the difference of surface coordinate and the mean value. If the spherical particles are uniformly coated and closely packed, then the roughness should be approximately one fourth of the diameter, i.e. 75 nm. Our experimental result (87 nm) agreed with it and implies a closely packed layered structure. The surface roughness can be reduced by using smaller particles in the LbL assembly.

It is interesting to compare our approach on 2D-micropatterning of polyion/nanoparticle multilayers with thiol compound microprinting of charged patterns on gold support and further LbL assembly of multilayers developed in last six years by Hammond’s group [1-3]. Both methods give patterns of approximately the same quality with minimal elements about 1-2 µm, edge roughness about 0.1-0.2 µm, and clear support surface between the pattern features. One of the advantages of our lithographic approach is that it is compatible with existing silicon micromanufacturing technology. It means that for industrial application one can use existing silicon technology and to produce 4” diameter silicon wafer completely covered with needed patterns of nanoparticle multilayers. With the microprinting approach it is difficult to produce a perfect pattern on surface area more than few mm². On the other hand, in the microprinting approach one can assemble biological (protein, DNA) multilayers on charged patterns. It is more difficult in the lithographic approach because of need to dissolve in organic solvent photoresist underlayer at the final stage of the process. In recent development, polyion stamping on support other than gold and possibility to fill gaps between polyion/nanoparticle strips with the second component through hydrogen bonding was elaborated. In the lithographic approach it is also possible to fill gaps between LbL assembled strips with a second component using metal mask etching and oxygen plasma treatment similar to the approach used in VLSI (Very Large Scale Integrated Circuits) industry.

In conclusion, the micropatterning of LbL-self-assembled layers can be obtained by the traditional lithography technology. The advantages of the method include a simpler process with micron feature size and very good reproducibility. It is believed that the feature size can be downscaled to the submicron level. The technique can be applied to almost all charged nano-scale building blocks. One can produce a pattern of thousands of tiny identical elements on standard 4” silicon wafer. This method provides a technology for nano-devices such as nano-electronic chips or NEMS, which have varieties of potential applications. It could also allow the patterning of particle based sensors for use in biomedical applications (bioMEMS production).

References for 2.12


**Fig. 18** Scheme of patterning of nanoparticle thin film

![Scheme of patterning of nanoparticle thin film](image)

**Fig. 19** SEM pictures of self-assembly patterns, (a-b) top view, (c) cross-section view; all with SiO$_2$ particles of 300 nm in diameter at the outermost layers. The line width is 5 µm. (d) SEM picture of control sample (lift-off technology without sonication). The arrows indicate those particles which were attached on sidewall

(a) ![SEM picture of self-assembly pattern](image)  
(b) ![SEM picture of control sample](image)
Fig. 20  (a-b) Images of patterns of 25 µm width with fluorescent multilayer of \{PEI + (PSS/PDDA)₃ + (Fluoresbright/PDDA)₃\} composition produced on silicon with LbL-method (Olympus Epifluorescence Microscopy with a 530-filter and an exposure time 8 seconds).

Fig. 21 Surface characteristics measured by Wyko RST interferometric microscope (a) 3D-image of a U-turn pattern 20-µm in width which is made of (PDDA/PSS)₂ + (PDDA/300-nm latex)₅ multilayers. Blue is multilayer and yellow is silicon bare surface. (b) The multilayer surface roughness.
2.13 Selective Deposition of Nanoparticles in Microchannels

This work was initiated in collaboration with R. Besser (IfM, LaTech) to deposit layers of catalytic nanoparticles in microreactor channels.
In very narrow channels (of 5 µm) nanoparticles were deposited only inside the channels:

100-nm diameter polystyrene latex was assembled in 50-µm channels of silicon microreactor bed. SEM image shows the edge of the channel. 10-nm diameter Pt-nanoparticle was also assembled (with N.Kotov).
3. Protein Multilayers.

3.1 Generality of the Protein Assembly Procedure.
3.2 Electrochemistry of Protein / Polyion Films.
3.3 Covalent Cross-Linking of the Multilayers.
3.4 Use of Different Proteins in Alternation with Polyions.
3.5 Sequential Catalysis in Organized Multienzyme Films.

3.1 Generality of the Protein Assembly Procedure.
Multilayer films which contain ordered layers of protein species were assembled by means of alternate electrostatic adsorption mostly with positively charged PEI, PAH, PDDA, chitosan or with negatively charged PSS, DNA and heparin [76-95]. The pH of the protein solutions was set apart from the isoelectric point so that proteins were sufficiently charged under the experimental conditions. The assembly of 20 different proteins was successfully achieved (including, cytochrome, carbonic anhydrase, myoglobin, hemoglobin, bacteriorhodopsin, pepsin, peroxidase, alcohol dehydrogenase, glucoamylase, glucose oxidase, immunoglobulin, catalase, urease (Table 1)) [79]. All the proteins underwent the alternate adsorption with organic polyions for unlimited numbers of cycles as typically demonstrated in Fig. 22 - 23. The mass increment at each step was quite reproducible. Proteins immobilized in multilayers with strong polyions such as PSS, PEI, and PDDA were insoluble in buffer for a pH range between 3 and 10. The assembled proteins are in most cases not denaturated [77-78, 82, 87-88]. Moreover, in some cases the layer-by-layer immobilization with linear or branched polyions enhanced the enzymatic stability [95].

Fig. 22 QCM monitoring of LbL assembly of glucose oxidase (GOx) and myoglobin in alternation with DNA. On can see regular assembly process.
The enzymatic activity in multilayers increased linearly with the number of layers up to 10-15 protein layers, at which point the film bioactivity became saturated. This saturation was probably due to substrate diffusion limitations into the film, i.e. accessibility to the protein requires a substrate transport through the multilayer [95]. For the antigen-antibody reaction in IgG / PSS multilayers, the activity increased up to 5 IgG layers. Compactness or openness of protein multilayers may be regulated. Thus, glucose oxidase, myoglobin and albumin multilayer films were compact, but immunoglobulin IgG / PSS multilayers had an open structure with areas as large as 100 nm diameter unfilled in the upper layers of the film [87].

Drying of polyion films exerts unclear influences on the structure. We do not need drying for the assembly process, samples were dried for control of the assembly. From the other hand, the importance of film drying (as a separate process) is still not fully understood. The assembly with a regular film drying at every other adsorption step gave polar multilayers with nonlinear optical properties [100]. A similarly prepared Photosynthetic Reaction Center / PDDA multilayer demonstrated second harmonic light generation [89]. Regular IgG/PSS assembly with the film
drying at every adsorption cycle was possible at a pH close to the IgG isoelectric point which again indicates an importance of hydrophobic interactions [87].

Table 1. Protein - polion alternate multilayer assembly [76-91].

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<th>Protein:</th>
<th>Molecular weight</th>
<th>Isoelect. point</th>
<th>pH</th>
<th>Charge</th>
<th>Alternate with</th>
<th>Protein monolayer mass coverage, mg/m²</th>
<th>Thickness of protein + polion bilayer nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Cytochrome c</td>
<td>12400</td>
<td>10.1</td>
<td>4.5</td>
<td>+</td>
<td>PSS⁺</td>
<td>3.6</td>
<td>2.4 + 1.6</td>
</tr>
<tr>
<td>2) Lysozyme</td>
<td>14000</td>
<td>11</td>
<td>4.0</td>
<td>+</td>
<td>PSS⁺</td>
<td>3.5</td>
<td>2.3 + 1.9</td>
</tr>
<tr>
<td>3) Histone f3</td>
<td>15300</td>
<td>11</td>
<td>7.0</td>
<td>+</td>
<td>PSS⁺</td>
<td>3.3</td>
<td>2.2 + 2.0</td>
</tr>
<tr>
<td>4) Myoglobin</td>
<td>17800</td>
<td>7.0</td>
<td>4.5</td>
<td>+</td>
<td>DNA, PSS⁺</td>
<td>6</td>
<td>4.0 + 2.0</td>
</tr>
<tr>
<td>5) Bacteriorhodopsin</td>
<td>26000</td>
<td>6.0</td>
<td>9.4</td>
<td>-</td>
<td>PDDA⁺</td>
<td>7.5</td>
<td>5.0 + 1.0</td>
</tr>
<tr>
<td>6) Carbonic Anhydrase</td>
<td>29000</td>
<td>5.5</td>
<td>8.3</td>
<td>-</td>
<td>PEI⁺</td>
<td>2.8</td>
<td>bilayer 2.2</td>
</tr>
<tr>
<td>7) Pepsin</td>
<td>35000</td>
<td>1.0</td>
<td>6.0</td>
<td>-</td>
<td>PDDA⁺</td>
<td>4.5</td>
<td>3.0 + 0.6</td>
</tr>
<tr>
<td>8) Peroxidase</td>
<td>42000</td>
<td>8.0</td>
<td>4.2</td>
<td>+</td>
<td>PSS⁺</td>
<td>5.3</td>
<td>bilayer 3.5</td>
</tr>
<tr>
<td>9) Hemoglobin</td>
<td>64000</td>
<td>6.8</td>
<td>4.6</td>
<td>+</td>
<td>PSS⁺</td>
<td>26</td>
<td>17.5 + 3.0</td>
</tr>
<tr>
<td>10) Albumin</td>
<td>68000</td>
<td>4.9</td>
<td>8.0</td>
<td>-</td>
<td>PDDA⁺</td>
<td>23</td>
<td>16.0 + 1.0</td>
</tr>
<tr>
<td>11) Glucoamylase</td>
<td>95000</td>
<td>4.2</td>
<td>6.8</td>
<td>-</td>
<td>PDDA, PEI⁺</td>
<td>4</td>
<td>2.6 + 0.5</td>
</tr>
<tr>
<td>12) Photosynthetic RC</td>
<td>100000</td>
<td>5.5</td>
<td>8.0</td>
<td>-</td>
<td>PDDA⁺</td>
<td>13</td>
<td>9.0 + 1.0</td>
</tr>
<tr>
<td>13) Concanavalin</td>
<td>104000</td>
<td>5.0</td>
<td>7.0</td>
<td>-</td>
<td>PEI⁺</td>
<td>8.6</td>
<td>5.7 + 0.8</td>
</tr>
<tr>
<td>14) Alkaline Phosphatase</td>
<td>140000</td>
<td>5.7</td>
<td>7.0</td>
<td>-</td>
<td>PEI⁺</td>
<td>9</td>
<td>---</td>
</tr>
<tr>
<td>15) Alcohol Dehydrogenase</td>
<td>141000</td>
<td>5.4</td>
<td>8.5</td>
<td>-</td>
<td>PDDA⁺</td>
<td>12.2</td>
<td>8.5 + 1.0</td>
</tr>
<tr>
<td>16) Immunoglobulin, IgG</td>
<td>150000</td>
<td>6.8</td>
<td>7.5</td>
<td>-</td>
<td>PSS⁺</td>
<td>15</td>
<td>bilayer 10</td>
</tr>
<tr>
<td>17) Glucose oxidase</td>
<td>186000</td>
<td>4.1</td>
<td>6.8</td>
<td>-</td>
<td>PDDA⁺</td>
<td>12</td>
<td>bilayer 8.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.5</td>
<td>PEI⁺</td>
<td>51</td>
<td>34 + 0.8</td>
</tr>
<tr>
<td>18) Catalase</td>
<td>240000</td>
<td>5.5</td>
<td>9.2</td>
<td>-</td>
<td>PEI⁺</td>
<td>9.6</td>
<td>6.4 + 0.8</td>
</tr>
<tr>
<td>19) Urease</td>
<td>489000</td>
<td>5.0</td>
<td>7.0</td>
<td>-</td>
<td>PEI⁺</td>
<td>23</td>
<td>bilayer 16</td>
</tr>
<tr>
<td>20) Diaphorase</td>
<td>600000</td>
<td>5.0</td>
<td>8.0</td>
<td>-</td>
<td>PEI⁺</td>
<td>31</td>
<td>bilayer 21</td>
</tr>
</tbody>
</table>

Picture below presents protein layer surface roughness for proteins of different molecular mass. One can see that larger proteins give more rough surface, and polion layer make this surface smoother (AFM data for ferritin –FER, glucose oxidase – GOD and peroxidase -POD).
3.2 Electrochemistry of Protein / Polyion Films.

Two methods to multiply redox signal in protein multilayers were realized, regarding to how a charge can be transported through a film when number of protein monolayers is two or more. The first one was realized by preparation of glucose oxidase (GOx) multilayers with electroactive mediator (poly(allylamine) ferrocene) transferring electrons between the layers of proteins [85]. In this case a small increase of the redox signal with the number of GOx layers was detected. A large increase of redox signal with the number of layers was observed for poly(styrenesulfonate) / poly(butyl viologen) multilayers by Laurent and Schlenoff [39]. In the second approach, multilayers of myoglobin / poly(styrenesulfonate) (Mb / PSS) on gold or rough graphite electrodes were formed (i.e., no electron mediators were used) [88, 90, 102-103] what resulted in the increase of the redox signal as compared with a single myoglobin monolayers. Electrochemistry of MB/DNA multilayers is presented below:
3.3 Covalent Cross-Linking of the Multilayers.

A fixation of protein / polyion architecture may be achieved with a glutaric aldehyde treatment or other covalent cross-linking finishing. This coating can be durable in circulating blood in vivo. An efficient passive protection of surfaces by the albumin multilayer coating from direct contact with blood was proved by the nearly complete elimination of immunoglobulin adsorption from blood plasma [44, 93].

3.4 Different Proteins in Alternation with Polyions (“Superlattices”)

An elaboration of the assembly technique for a variety of proteins makes it possible to construct multicomponent protein films (superlattices) [77, 79, 83]. We have described the formation of two types of superlattices: 1) The alternation of similarly charged proteins at identical pH conditions; both positively charged myoglobin and lysozyme in alternation with polyanion \{myoglobin^+ / PSS / lysozyme^+ / PSS\}; negatively charged glucose oxidase (GOx) and glucoamylase (GA) with polycation \{GOx^- / PDDA / GA^- / PDDA\}. 2) The combination of negative and positive proteins in the two-block film with the insertion of an additional polyion layer to change the assembly mode: \{(lysozyme/PSS)_3 + PEI + (GOx/PEI)_6\}. The protein multicomponent films are extremely interesting as novel biologically-active materials. We can arrange given protein layers according to a specific biological activity. Sequential enzymatic reactions and vectorial transfer of electrons and energy become feasible targets by preparing of anisotropic protein layers with the precise control of the distances of active layers.

Experimental results on myoglobin – lysozyme superlattice:
3.5 Sequential Catalysis in Organized Multienzyme Films.

For the first time sequential two-step catalysis was demonstrated for glucoamylase (GA) and glucose oxidase (GOx) assembled in the proper order on an ultrafilter through which a substrate (starch) solution was passed [79, 82-83]. Corresponding to the two reaction stages, the outer layer of glucoamylase was followed by a layer of glucose oxidase (Fig. 24). Between these active layers we placed a number of penetrable, but not active, polycation / polyanion layers (PEI/PSS)n (i.e., spacer layers). A 2% starch solution under 0.5 atm pressure difference moved through the multilayer. At the first enzyme layer starch was converted to $\beta$-glucose, and at the second layer, $\beta$-glucose was converted to D-glucono-$\delta$-lactone and H$_2$O$_2$. The maximal output of the two-step reaction was detected when the upper layer was GA, the bottom layer was GOx, and the distance between them was 10 nm.

Fig. 24
Another example of sequential reaction is glucose oxidase – peroxidase reaction (GOD-POD) preformed in neighbor layers of these enzymes (below)
4.3 Caps and Helices on Microtubules.

In another work we used as microtemplates for nanoconstruction 500-nm diameter lipid tubules. First, we used this technique to reveal very small underlying charge distributions on the tubules that were previously not observable. Each new layer of charged polyanion multiplies the charge of the layer below, giving a significant (~50-100) amplification process analogous to photography. Final nanoparticle treatment makes this pattern visible in an electron microscope. Second, we used this technique to build up three-dimensional sub micron structures.

Lipid tubules are hollow cylinders made up of bilayer membranes of diacetylenic lipids, with typical diameters of 0.5 µm and lengths of 10-1000 µm. For our purposes, tubules served as templates for the alternate adsorption of charged polymers and nanoparticles. By observing where these charges adsorb on the tubules, we can gain more information about the distribution of charges in tubules, and we can take advantage of tubule helicity to build novel helical structures of nanoparticles. Nanoparticle structures were assembled onto lipid tubules through the sequential adsorption of PSS⁻ and PEI⁺ and 45-nm silica spheres. For tubules of the zwitterionic 1,2 Di-(10,12-pentacosadiynoyl)-sn-3-phosphatidylcholine) - DC₈,₁₁PC, this process leads to the formation of caps on the ends of the tubules, with 50 to 100 silica spheres in each cap. For tubules of DC₈,₁₁PC mixed with 2 % of the charged lipid DC₈,₉PEOH, the sequential adsorption leads to both end caps and helices of nanoparticles winding around the interior of the tubule. Further development of this work will be targeted on the nanoconstruction of conductive and magnetic helices.

TEM image of nanoparticle caps at the ends of tubules of DC₈,₁₁PC after four-stage (PEI⁺ / PSS / PEI⁺ / 45nm SiO₂) treatment.

5.1 Determine mechanisms for nanocapsule loading via diffusion and controlled penetrability.

Nanocapsules may potentially be loaded via diffusion, where nanocapsules are suspended in a solution with molecules of interest and concentration gradients drive movement of molecules to the interior of capsules. In addition to loading by diffusion, two additional methods will be used as possibilities to control permeability of nanocapsule walls after formation. These include changing the pH and dielectric constant of the solvent.

Fabrication of hollow polyelectrolyte nanocapsules derived from different cores. The products of core decomposition might strongly influence capsule properties. The search of proper decomposable cores is planned in the project to ensure decomposition without remains. Study on polymer
segregation in multilayers is important. As one can see the polyelectrolyte segregation leading to opening and closing of capsule walls depends on different physics-chemical factors. Up to now it has been demonstrated only for one polyelectrolyte pair (PAH/PSS). Indeed, the pore formation effect is expected to occur at pK-values of the polyelectrolyte used as layer constituents. Different solvents (alcohol, acetone) might cause segregation of polyelectrolyte matrix. Such research is aimed to broaden the possibilities to control the input of macromolecules in capsules. The polyelectrolyte capsules could serve as support for a lipid membrane creating an artificial cell, which will allow using membrane proteins. For instance, there are perspectives to reconstruct channel formers into lipid bilayers supported by the polymeric shells. Design of lipid coverage on polyion shells through electrostatic adsorption of vesicles containing charged lipids is interesting.

Nanoshells will be structurally characterized with transmission electron microscopy. Loading properties of nanoshells will be studied with small fluorescent dye molecules and FITC-labeled dextran molecules, which are available in a variety of chain lengths. Thus, study of loading dependence for small and large-molecular-weight materials will be possible. The table below defines the different conditions that will be studied. In all cases, loading will be assessed by confocal fluorescence microscopic imaging of nanoshells and fluorescence spectroscopy. In the latter case, nanoshells will be isolated from fluorescent solutions via repeated rinsing or magnetic separation.

5.2 Enzyme Loading.

**Approach #1: pH controlled encapsulation and release of macromolecules from polyelectrolyte capsules of few microns in diameter.**

Capsules prepared via alternating adsorption of oppositely charged polymers, poly(allylamine hydrochloride) (PAH) and poly(styrenesulfonate) (PSS) onto decomposable melamine formaldehyde cores have been shown to be permeable to macromolecules at pH values below 6 and closed at pH above 8. The capsules undergo a structural transition at pH close to the pKa of the polymers used, which reflects possible segregation and formation of pores. An operation with opening-closing capsule walls composed of polycation - polyanion complexation in multilayers is based on a recent finding that varying solution pH can induce charge disbalance in polycation - polyanion complexation in multilayers, resulting in opening of ca. 100-nm pores [32]. Our preliminary data have shown that the threshold for pore-opening depends on: 1) pH at which an assembly was performed, and 2) involvement in the assembly of weak polycation which ionisation depends on pH. The detailed structural changes, control of pore sizes and threshold have not yet been observed or understood, and these deserve in-depth studies.

![pH-dependent pore opening/closing in PSS/PAH capsules](image)

pH-dependent pore opening/closing in PSS/PAH capsules (fluorescent images of capsules loading with FITC labeled dextran are given) [29].
**Approach #2:** Controlled encapsulation and release of macromolecules from polyelectrolyte capsules via modulation of solution dielectric constant.

Our preliminary results from experiments aimed at demonstrating encapsulation of urease in 5-µm diameter capsules with 20-nm thick walls of (PSS/PAH)₄ composition are shown below. The molecular weight selective shell permeability provided capturing of enzymes while the small substrates and products of enzymatic reactions can penetrate the capsule wall. Our results demonstrated the possibilities to encapsulate enzymes, such as α-chymotrypsin and urease, into PSS/PAH microcapsules.

Urease loading in 5-µm diameter (PSS/PAH)₄ shell (scheme and confocal images of FITC- labeled urease).

Successful encapsulation of enzymes in the shells demonstrates the possibility of their enclosure inside the capsules or in the shells as shown. It is noteworthy from these experimental results that some attachment of enzymes to walls occurs. This is unfavorable and should be avoided. The approach to separate enzyme from wall incorporation is based on the third approach (below).
Encapsulation of α-chymotrypsin. The assembly scheme (left) and confocal fluorescence Images of rhodamine-labeled α-chymotrypsin in the shells (our preliminary results [27]). (top left) Enzyme is mostly in the capsule walls (loading pH 8 and using at pH 4), and (top right) mostly inside the capsules (loading at pH 4 and using at pH 8).

6. Areas of Current and Potential Applications

Current Applications:
- Biocompatible coverage of eye lenses and implants.
- Precise dye casting on optical elements (film thickness 50-200 nm, ±5 nm).
- Increase of bioreactor efficiency by deposition of enzyme / polyion multilayers instead of immobilization of an enzyme monolayer.

Potential Applications:
**Surface Modifications.**
- Surface wettability, hardness, magnetic properties, lubrication, biocompatibility can be modified by self-assembly of ultrathin nanostructured coating (e.g., plastic hardening by coating with Al₂O₃ or ZrO₂ nanoparticle multilayer; precise λ/4 dye-coating of lenses and optical fibers, and other antireflection coating, paper processing). In biomaterials the assembly is applicable directly to tissue surfaces.
- The concept of a “smart” surface may be realized by including functional proteins in biocompatible films (e.g., lipases destroying harmful lipids in contact with biological liquids).
- Filter layer with calibrated nano-pores.
- Nanoparticle catalytic multilayers.
Sensor Layers and Bioreactors. The protein films can be made very thin and contain precisely 1, 2, 3, 4, 5, or more monolayers, which is important for biosensor elements. The deposition of enzyme / polion multilayers onto carriers of biological reactors permits an increase in their output proportionate to the number of immobilized enzyme layers. Sequential 2-3 step catalytic reactions can be realized in multiprotein films (cascade bioreactions). Enzymes in the polion films are stabilized and could work with nonaqueous substrate solutions. Polions, which keep together enzyme layers in the films, can be doped by vitamins (co-factors), specific dyes or nanoparticles of metal catalysts to mediate reaction activity.

Nanoreactors. Construction of tiny catalytic units containing ordered multilayer shells of enzymes, nanoparticles and polymers.

Electronics. •Conductive thin films. Electrical conductivity of up to 400 S/cm in 50-nm thick polypyrrole / polystyrenesulfonate film was achieved (may be used as discharging coverage). •Light emitting diodes and display devices of large area and low operating voltage. Light emitting diodes based on multilayer heterostructure of alternate poly(phenylene vinylene) and sulfonated polyaniline were made. A 20-nm thick light emitting diode device had a turn-on voltage of about 1 V and green light was clearly observed under normal room illumination [115-116]. •Electrodes for lithium storage batteries based on graphite nanoplate / polycation multilayers. •Magnetic nanoparticle mono- and multilayers, including formation of magnetic / dielectric superlattices and soft / hard magnet nanocomposites. •Optical Second Harmonic Generation layers.

Advantages and Difficulties of the Technique.

Advantages. The method permits the assembly of large area ultrathin films on any substrates with precise alternation of different component monolayers in a direction perpendicular to the surface. Commercial materials, such as polions, proteins, charged dyes and nanoparticles can be assembled in multilayers with a precision of a few nanometers. The process is environment-friendly, based on water solutions, is carried out in the open air and at room temperature. The layer-by-layer assembly can be applied to microtemplates, such as 200-500 nm diameter spheres, microcrystals, and biological cells, providing the means for encapsulation of different objects with nano-organized polymer / nanoparticle shells. There are no other methods for an assembly of ultrathin organized films (5-500 nm) from polymers, proteins and nanoparticles.

Difficulties. Growth speed: The film grows at relatively low speed; it is equal to one bilayer every 1-5 minutes. The corresponding bilayer thickness for polycation / polyanion or protein / polion pairs is 2-10 nm, for a nanoparticle / polion pair, it depends on the nanoparticle diameter, and usually is equal to 10-50 nm. Anchoring of the first monolayer to substrates depends on the surface charge and sometimes demands preliminary surface treatment (e.g., plasma, thiol or silane compound treatment). Oxidized surfaces, such as SiO2, metal oxides, usually have a good adhesion for polycations (e.g., poly(ethylenimine)). Stability: The thermostability is restricted by ca 280° C. In many cases the layer-by-layer assembly can be used as a nano-architectural method, which yields films with the stability comparable to that of a polymer. For practical devices, a final fixation of the resultant assemblies with covalent cross-linking or thermal calcination may be necessary.
Conclusions

We have described the fundamental approach to design organized films that contain different polymer, protein, dye and nanoparticle monolayers in precise locations perpendicular to the surface. The films are amorphous in plane but organized in the third direction with a precision of a few nanometers. Surfaces, porous carriers and fibers of any dimensions, curvatures or complexity may be covered by the film. Microtemplates, such as microspheres, protein nanocrystals and nanotubules, can be shelled with ordered polymer / nanoparticle multilayers. Hundreds of commercially available polylons, proteins, dye and nanoparticles may be used within the same technology. A layer-by-layer assembly is an easy and general process; it does not demand a high purity of components; it can be automated and scaled-up for mass production. This method provides a clear approach to the construction of ordered organic / inorganic nanocomposites.

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