

LAYER BY LAYER (LbL) SELF-ASSEMBLY STRATEGY AND ITS APPLICATIONS

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ABSTRACT – This report reviews the technique of layer-by-layer (LbL) self-assembly, in particular cases involving electrostatic interactions between thin film monolayers. LbL self-assembly is used in a variety of different applications, two of which discussed in the report are LbL MEMS and LbL protein multilayers. In the fabrication of LbL MEMS, multilayers of polymer-clay-magnetite nanocrystal are deposited via photolithographic steps on a wafer. The final product is a free-standing cantilever that responds to a magnetic field. The LbL technique, applied to MEMS, allows the precise tailoring of surface interactions, and hence is useful for the fabrication of surface-based devices. In the application of trapping active proteins, polyamidoamine dendrimers and heme proteins are integrated in a multilayer thin film structure that allows the retention of intact biological activity. The pH of the system can significantly affect the interaction strength, and although oppositely charged species are favourable for electrostatic interactions, it is not a requirement for multilayer formation. Finally, electroactivity is explored in relations to bilayer number. It is concluded that electroactivity decreases as the bilayer number increases, and it is important to control film thickness for effective electron exchange in devices.

1. INTRODUCTION

Self-assembly is a process that occurs due to the spontaneous and uninstructed structural reorganization that forms from a disordered system. Such processes are reversible and held together by non-covalent intermolecular forces. The area of study pertaining to non-covalent molecular interactions is referred to as supramolecular chemistry, and it has important implications with regards to the formation of thin film technology. Self-assembled surface monolayers have very unique properties that are useful for the fabrication of various devices. For instance, if the monolayer is conductive, it results in a two dimensional conductive sheet. This concept can be extended to three dimensional structures if a stack or a collection of monolayers can be achieved. This is where layer-by-layer (LbL) self-assembly plays a role.

This paper discusses some interesting properties and fabrication methods using LbL self-assembly and specifically focuses on the LbL electrostatic self-assembly strategy. This technology takes advantage of the charge-charge interaction between substrate and monolayers to create multiple layers held together by electrostatic forces. There are two main areas of application that are presented in this paper. First, a LbL approach is taken to fabricate flexible cantilever arrays for chemical sensing applications. Instead of traditional top-down methods involving silicon micro-machining, the LbL method makes use of a bottom-up technique resulting in multilayers that enable cantilever actuation and stability [1]. Next, the role of LbL self-assembly in the trapping of intact and functional proteins for biological usages is explored. The LbL method exploits the properties that many proteins bear charged groups that are able to interact with each other to form polyelectrolyte multilayers. This phenomenon allows biological activity to be retained in the incorporation of synthetic materials [1].

2. FABRICATION METHODS

LAYER-BY-LAYER MEMS

The cantilever must be flexible enough to display synchronized movements in response to the application of an external magnetic field. As such, the material from which the cantilever is made must be chosen wisely. The desired flexibility was achieved by using nanometer thickness, alternating charge layers of cationic poly diallyldimethyl ammonium cholride (PDAC) and anionic delaminated montmorillonite clay sheets [1].

A magnetic over-layer of iron oxide magnetic nanocrystals deposited on the surface of the cantilevers enable the actuation of the cantilevers [1].

A sequence of photo-lithographic patterning and layer-by-layer deposition steps was used to create an array of clay-polymer-magnetite ultra-thin cantilevers. Each of these cantilevers is individually anchored to a silicon substrate. Upon application of an external magnetic field, these cantilevers were able to display synchronized movements.

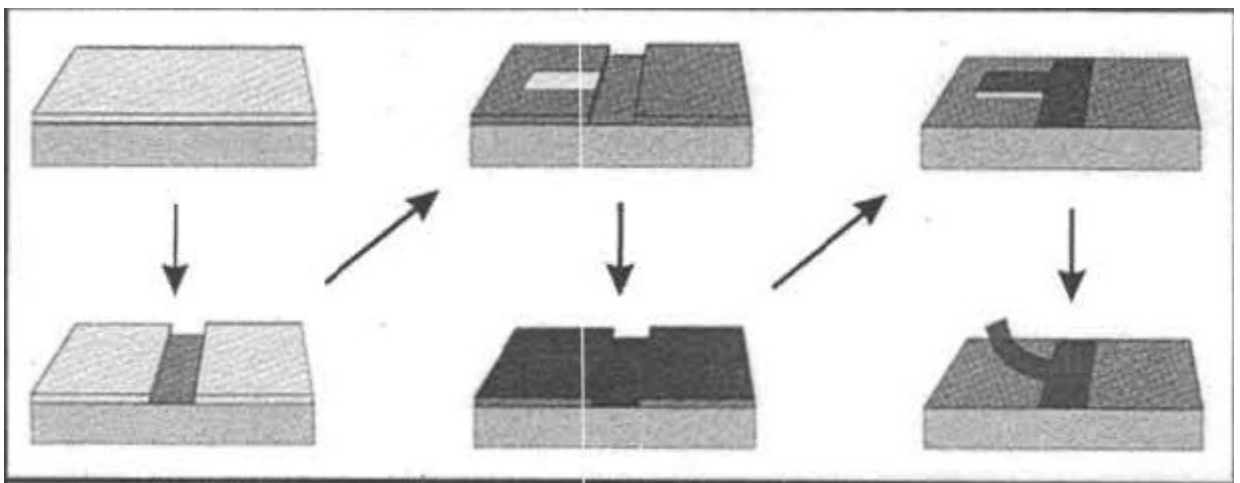


Figure 1 – Steps in the fabrication of magnetic cantilevers [1]

The photo-lithography steps are shown visually in the above schematic (Fig. 1). The following photo lithography steps are performed on the wafer:

1. Spin-coat a layer of positive photo resist on a silicon wafer.
2. Photo-lithographically create a channel in the photo-resist coating.
3. Expose the entire photo resist to UV light except for the area that will define the cantilevers. This will be a perpendicular line which remains insoluble.
4. Deposit layer-by-layer the polymer-clay-magnetite nanocrystal multilayer over the entire area of the photo resist. This layer-by-layer process is carried out over the whole sample.
5. Remove the UV exposed photo resist with developer and then the unexposed photo resist with acetone to create freestanding cantilevers.

PROTEIN INTEGRATION

The LbL mechanism is also used to deposit protein thin films on solid substrates. Most proteins bear multiple charge groups due to the large amount of amino acids from which they are assembled. As such, polyelectrolyte thin film layers can be self-assembled by the LbL adsorption of oppositely charged segments. The following section will outline the film assembly techniques associated with one particular system of integration as well as discuss the effects of pH on adsorbate assembly and the effects of film thickness on electro-activity.

A supramolecular system is studied by Li Shen and Naifei Hu of Beijing Normal University that entails the self-assembly of polyamidoamine (PAMAM) and a series of heme proteins. These heme proteins include hemoglobin (Hb), myoglobin (Mb), and catalase (Cat). PAMAM is a spherical dendrimer with 64 amine groups on its surface. It is used for the purpose of LbL protein integration due to its biocompatibility that arises from its globular structure that mimics biomolecules [2].

Film assembly begins with the preparation of basal plan pyrolytic graphite disk (PG) electrodes that were processed with metallographic sandpaper and ultrasonicated in water. The electrodes are alternatively dipped in a PAMAM solution and a heme protein solution at specific concentrations. Water rinsing and nitrogen stream drying are performed in between each dip. Each cycle of PAMAM/protein dip forms one bilayer and the process is repeated until the desired number of bilayers is reached. This process ensures the regular and linear growth of protein multilayer films [2].

The self-assembly of the PAMAM/protein structure depends fundamentally on the pH of the system, which must be precisely controlled for the process. Due to the isoelectric points of the different heme proteins, their interactions with PAMAM will vary at different pH values. For example, Hb is negatively charged at pH 9.0 while PAMAM is positively charged. At this pH value, Hb and PAMAM readily engage in LbL self-assembly because of the Coulombic attraction between them. From this observation, one would naturally assume that opposite charges are required for successful self-assembly. However, further investigation indicates that at pH 5.0, where both Hb and PAMAM are positively charged, thin films still form yet adsorption is shown to be much less than the former case [2]. This observation implies that in LbL self-assembly, substrates with opposite charge are not a requirement, but only a favourable condition.

There are several ways to explain the phenomenon of the adsorption of species with like charges. One of them has to do with the residual amino acids found on the Hb surface. It was observed that a large amount of glutamic acid and aspartic acid (having pKa values at around 4.0) was found near the surface of Hb. Due to the low pKa values, these amino acids are negatively charged at pH 5.0, where PAMAM and Hb are both positively charged. These residual amino acids add up to an amount significant enough to contribute to the electrostatic interaction with PAMAM, forming self-assembled layers held together weakly [2].

3. EXAMPLE RESULTS

LAYER-BY-LAYER MEMS

Once the photo-lithography steps have been performed, the structure of the layer-by-layer magnetic cantilevers will be like those seen in the figure below (Fig. 2).

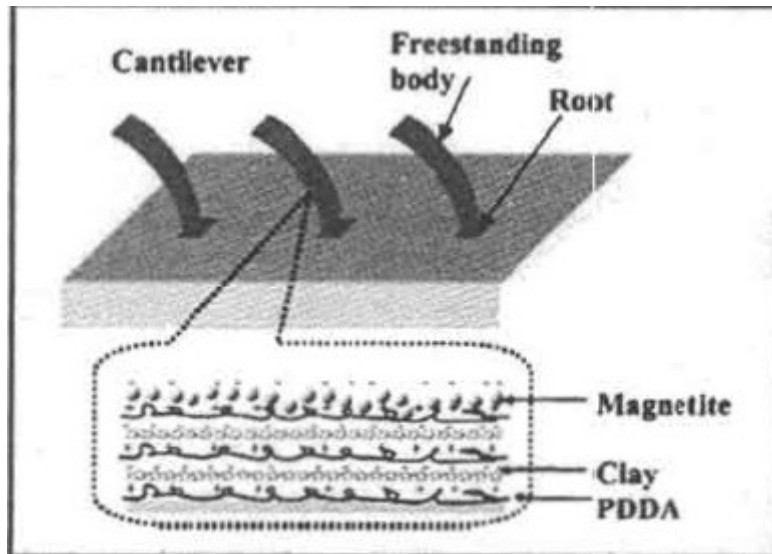


Figure 2 – Structure of the LbL magnetic cantilevers [1]

Below (Fig. 3), we detail some of the optical images of the cantilever before and after applying a magnetic field.

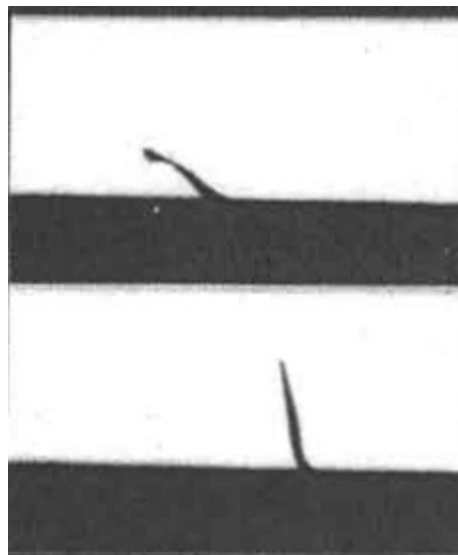


Figure 3 – Optical images of the cantilever before and after application of magnetic field [1]

LbL deposition can be used to perform what is known as template assisted assembly (TAA). Template assisted assembly is much faster than self-assembly/chemical modification cycles whose outcome is often uncertain or difficult to predict [3].

LbL deposition can be tailored to even allow multi-material assembly of several compounds without special chemical modifications thus giving access to multilayer films whose complex functionality can fall into the two following categories:

1. Tailoring of surface interactions
2. Fabrication of surface based devices

LbL deposition can also be used in the synthesis of polydisperse supramolecular objects [3]. The reagents in layer-by-layer deposition can be chosen from a wide range of materials. This is shown in Fig. 4. Many other interactions besides electrostatic interactions may be used as the driving force for multilayer build-up such as donor/acceptor interactions, hydrogen bonding, adsorption/drying cycles, covalent bonds, stereocomplex formation or specific recognition. At least one of the interactions is necessary.

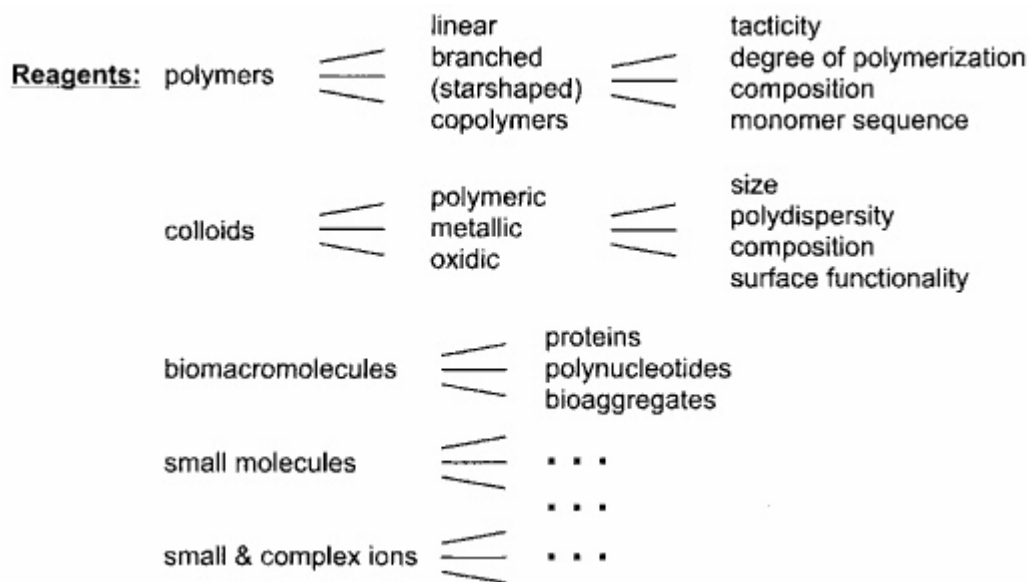


Figure 4 – Various reagents for LbL deposition [3]

Given the large set of materials which are easily incorporated into multilayer films, LbL deposition is a general approach for fabrication of complex surface coatings.

PROTEIN INTEGRATION

The most important result obtained from Shen and Hu's paper is the correlation between the number of bilayers in the protein system and the electroactivity on the Hb surfaces. This correlation is shown clearly in Fig. 5.

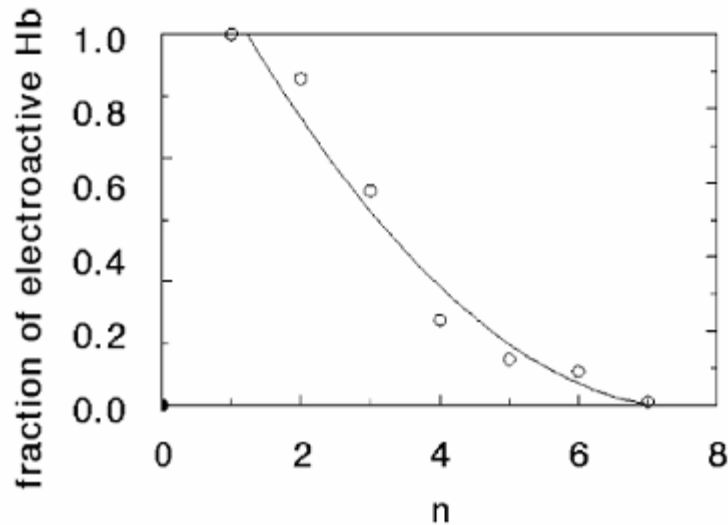


Figure 5 – Hemoglobin surface electroactivity [2]

The figure relates the n^{th} bilayer, counting from the electrode, to the fraction of the Hb layer that is electroactive. Essentially, the plot reveals that at $n = 1$ (the bilayer directly adjacent to the electrodes), almost all of the Hb molecules are electroactive. As n increases, the fraction of electroactive Hb evidently decreases. This observation has very important implications on the fabrication of devices, as the electroactivity of a device would need to be considered heavily. Therefore, film thickness and the film distance from the electrodes plays a high role in determining the efficiency of electron exchange.

4. COMPETITIVE ADVANTAGES

The layer-by-layer self-assembly technique is very useful in many applications that require multilayer thin films. This report discusses multilayer systems that are formed by the electrostatic interaction between the various thin film layers. One advantage of this technique is that the process is relatively inexpensive. There are no complex reaction mechanisms – one simply needs to dip a substrate into alternating positive and negative charge containing solutions to form uniform and stable layers.

Another advantage to the layer-by-layer technique is that it is not specific to electrostatic forces. Thin film layers can also be held together by other types of non-covalent bonds, such as hydrogen bonds and hydrophobic interactions [4]. This means that there is a variety of methods to choose from when using the layer-by-layer technique, and we can choose the most appropriate method according to the conditions that are present in the problem.

A third advantage of the layer-by-layer technique, specific to protein structures, is that pH can be used as a parameter to adjust the strength of inter-layer bonding. As mentioned earlier in the report, oppositely charged species are favourable but not a required criterion for layer-by-layer self-assembly. Changing the pH of a solution will cause the charge of the solution to change, which is beneficial for some systems in which electrostatic interactions must be taken into account of. The properties of amino acids, such as aspartic acid and glutamic acid residuals, are very useful in the case where like-charge species are involved, and the experiment can be tailored to fit the way in which we want the films to be formed, achieving good outcome.

5. CONCLUSIONS

We discussed some interesting properties and fabrication methods using layer-by-layer (LbL) self-assembly and specifically focused on the LbL electrostatic self-assembly strategy. This technology takes advantage of the charge-charge interaction between substrate and monolayers to create multiple layers held together by electrostatic forces. The desired flexibility for each cantilever in the array was achieved by using nanometer thickness, alternating charge layers of cationic poly diallyldimethyl ammonium chloride (PDAC) and anionic delaminated montmorillonite clay sheets. A magnetic over-layer of iron oxide magnetic nanocrystals deposited on the surface of the cantilevers enable the actuation of the cantilevers

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The LbL mechanism is also used to deposit protein thin films on solid substrates. Polyelectrolyte thin film layers can be self-assembled by the LbL adsorption of oppositely charged segments. This report outlined the film assembly techniques associated with one particular system of integration as well as discuss the effects of pH on adsorbate assembly and the effects of film thickness on electroactivity. The self-assembly of the PAMAM/protein structure depends fundamentally on the pH of the system, which must be precisely controlled for the process. We also conclude that in LbL self-assembly, substrates with opposite charge are not a requirement, but only a favourable condition.

We attribute the phenomenon of the adsorption of species with like charges to the residual amino acids found on the Hb surface. Finally, it was observed that a large amount of glutamic acid and aspartic acid (having pKa values at around 4.0) was found near the surface of Hb.

6. REFERENCES

- [1] G. A. Ozin and A. C. Arsenault, *Nanochemistry: A Chemical Approach To Nanomaterials*, Royal Society of Chemistry, 2005, p. 104 – 106
- [2] L. Shen and N. Hu, “Electrostatic Adsorption of Heme Proteins Alternated with Polyamidoamine Dendrimers for Layer-by-layer Assembly of Electroactive Films”, *Biomacromolecules*, Jan. 2005, pp. 1475 - 1483
- [3] G. Decher and J. Schlenoff, *Multilayer Thin Films*, Wiley-VCH, 2006.
- [4] M. T. Kumara, B. C. Tripp, and S. Muralidharan, “Layer-by-Layer Assembly of Bioengineered Flagella Protein Nanotubes”, *Biomacromolecules*, Sep. 2007, pp. 3718 – 3722