The wettability and topography of self-assembled protein monolayer linked by alkanethiols

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***Abstract*—Protein interactions have great significance in biomedical and bioengineering research. It is a challenge to form an ideal protein surface. Self assembled monolayers (SAMs) is a rather new chemical method which is easily handled to fabricate a well defined protein surface. In this work, we performed alkanethiol monolayers with carboxyl end groups, and mixed alkanethiols monolayers with carboxyl and methyl end groups. Self assembled alkanethiols films with carboxyl end groups were activated by 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC), and *N*-Hydroxysulfosuccinimide (NHS), then immersed into human IgG solution, formed protein layers. Protein layers were investigated by atomic force microscopy (AFM) to acquire high resolution 2-D and 3-D topography images, and contact angle goniometry to characterize the wettability of the surfaces. AFM images of bare gold and protein layers showed significant differences in topography and heights. It suggested that protein was uniformly linked with the alkanethiols films. This was also supported by comparing the contact angle of alkanethiols films and protein surfaces. This work could be extended to microarray.**

Keywords-self-assembled; protein; monolayer; wettability; topography

I. INTRODUCTION

Self assembled monolayers (SAMs) have been developed over two decades, early research mainly focused on different chemical compounds onto different metal surfaces, numerous results showed that alkanethiols self assembled onto the gold surface formed well defined and controlled films. The mechanism of SAMs is simple [1]: sulphur containing molecules (thiols, sulfides and disulfides) have a strong affinity for gold and interact with it in near covalent manner. The reaction is spontaneous, and thiol-based SAMs are obtained by simple immersion of the gold surface into a solution of the selected thiols. The SAM will be ideally composed of tightly packed and well ordered chains, although several factors may lead to the formation of defects and irregularities. SAMs formed by alkanethiols consist of different end groups (amino, carboxyl, maleimide, etc.). Since the carboxyl groups can activated by the 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC), and *N*-Hydroxysulfosuccinimide (NHS), SAMs have great implications for the directed conjugation/incorporation of biocomponents and, therefore, surface functionalisation [2]. Thus, SAMs expanded its application to biomedical and bioengeering research field, and reached distinguished achievements. The mechanism of protein linked on the alkanethiols/Au(111) film is shown in Fig 1.

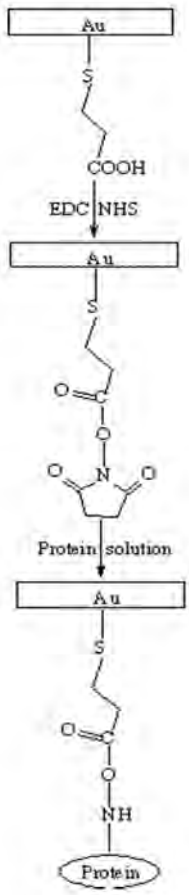

Figure 1. Reaction mechanism of protein immobilization on 16-Mercaptohexadecanoic acid self assembled monolayers/Au(111)

Atomic force microscopy (AFM) is capable of imaging biological samples either in air or physiological environment. Because of free sample preparation and subnanometer resolution, ever since its invention [3], AFM has been broadly used in biomedical and bioengeering research, including imaging, force mapping, and biological interaction sensing [4]. Water contact angle measurement is a traditional method to

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characterize the surfaces [5], it provide the hydrophiliciy/hydrophobicity of the surfaces by determining the contact angle θ between the surfaces and water. AFM and contact angle measurements are extensively used in the characterization of biological surfaces.

This work was motivated by verifying the effectiveness and facility of SAMs for acquiring uniform protein surfaces. In this work, a series of alkanethiols mixed films (with the molar ratios of 16-Mercaptohexadecanoic acid ($HS(CH_2)_{15}CO_2H$) to 1-Dodecanethiol ($HS(CH_2)_{11}CH_3$) are 1:0, 0.8:0.2, 0.6:0.4, 0.5:0.5, 0.4:0.6, 0.2:0.8, respectively) were prepared by immersing the gold substrates into the alkanethiols solutions. Subsequently, alkanethiols films were activated by NHS/EDC, and immediately transferred into the human IgG solution to form protein layers. Protein layers were investigated by atomic force microscopy (AFM) to acquire high resolution 2-D and 3-D topography images, and contact angle goniometry to characterize the wettability of the surfaces. AFM images of bare gold and protein layers showed significant differences in topography and heights. It suggested that protein was uniformly linked with the alkanethiols films. This was supported by the contact angle results. This work could be extended to microarray.

II. Experimental Section

A. Materials

1-Dodecanethiol ($HS(CH_2)_{11}CH_3$), 16-Mercaptohexadecanoic acid ($HS(CH_2)_{15}CO_2H$), 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC), and *N*-Hydroxysulfosuccinimide (NHS) were purchased from Sigma Aldrich Chemical Co. and used as received. Phosphate buffered saline (PBS; 140mM NaCl, 3mM KCl, pH 7.4) and ethanol (guaranteed grade) were purchased from Merck Co.. Ultra pure water (resistivity is 18.2 MΩ • cm) was obtained by Millpore purification system. Human IgG was purchase from Biosun Co (China).

B. Au(111) Substrate

Gold substrates were prepared by the vapor deposition of gold onto freshly cleaved mica in a high vacuum evaporator at ~10^{-7} Torr. Mica substrates were preheated to 325^{o}C for 2 h by a radiator heater before deposition. Evaporation rates were 0.1-0.3nm/s, and the final thickness of gold films was ~200nm.Gold-coated substrates were annealed in H_2 frame for 1 min before use.

C. SAMs

SAMs were formed by soaking gold coated substrates in the ethanol solution of alkanethiols (1mM) for 24 h. SAMs containing methyl terminal groups were rinsed sequentially with pure ethanol, ultra pure water, and dried in a N_2 stream. SAMs containing carboxylic acid terminal groups were supersonicated in pure ethanol for two minutes to remove unbound thiols.

D. Protein Immobilization

Mixed or mono- SAMs with carboxylic acid terminal groups were activated by 2mg/mL NHS and 2mg/mL EDC PBS buffer solution for 1 before protein immobilization, then rinsed thoroughly with ultra pure water, and dried in N_2 stream. The activated SAMs were then immersed into 7μg/mL protein PBS solution at 4^{o}C for 6h. Finally, the specimens were stored in PBS solution under 4^{o}C.

E. AFM Imaging

All AFM images were acquired using Benyuan CSPM5000 scanning probe microscope (Benyuan co., China) equipped with a 26-μm E scanner. Commercial Si_3N_4 cantilevers with spring constant of 0.3N/m were used. AFM were performed with contact mode in air with typical scan rate of 2.0 Hz. Immobilized protein were thoroughly rinsed with PBS buffer solution and dried in N_2 stream before use.

F. Contact Angle Measurements

To determine the wettability, Milli-Q water was used. The static sessile drop method was employed using contact angle goniometry (Magicdroplet 200, Taiwan), measurements were performed under room temperature (20~25^{o}C) with ambient humidity. Drops with 3.2μl were deposited at random locations on the modified surfaces, and the angle between the baseline of drop and tangent at the drop boundary was measured on the both sides of the drops. The results presented here are the average of at least three measurements.

III. Results and Discussion

A. Wettability of Alkanethiols and protein surfaces

For alkanethiols, most literature refers to 1mM solution for self assembly [6]. The chain length will affect the order of self assembled alkanethiols films. Long chain length was commonly used as a spacer [7]. To increase the stability of protein, in present work, 1-Dodecanethiol and 16-Mercaptohexadecanoic acid were chosen for protein immobilization. For investigating wettability of surfaces formed by different end groups, a series of alkanethiols mixed films (with the molar ratios of $COOH:CH_3$=1:0, 0.8:0.2, 0.6:0.4, 0.5:0.5, 0.4:0.6, 0.2:0.8, respectively) were prepared by immersing the gold substrates into the alkanethiols solutions. It takes 24h to form a well defined alkanethios films. All films were rinse by ethanol and ultra water, and dried in N_2, then immediately transferred onto the sample stage of contact angle goniometry.

Images of contact angle measurements are shown in Fig 2. All contact angle θ values are average of at least three measurements, the results are as follows:

- The value of contact angle θ of bare gold (a), 16-Mercaptohexadecanoic acid self assembled on gold surface (b), mixed alkanethiols self assembled on gold surface, the molar ratio of 16-Mercaptohexadecanoic acid to 1-Dodecanethiol is 0.8:0.2(c), and human IgG linked on alkanethiols film (d),are 58 ^{o}C, 18 ^{o}C, 108 ^{o}C, and 62 ^{o}C, respectively.

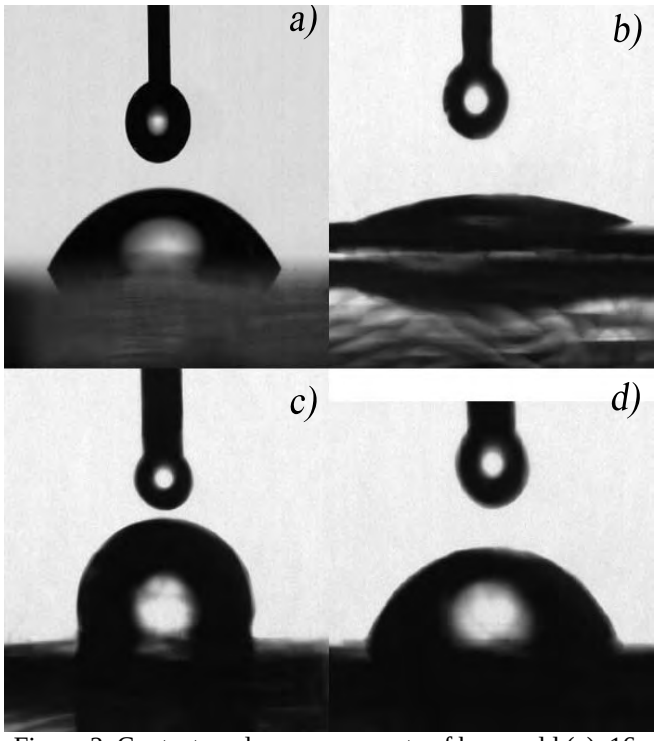

Figure 2. Contact angle measurements of bare gold (a), 16-Mercaptohexadecanoic acid self assembled on gold surface (b), mixed alkanethiols self assembled on gold surface, the molar ratio of 16-Mercaptohexadecanoic acid to 1-Dodecanethiol is 0.8:0.2(c), and human IgG linked on alkanethiols film (d).

- The θ of mixed alkanethiols films are varied with the molar ratio of carboxyl group to methyl group. Typically, the film formed by pure alkanethiol with carboxyl end group is lower than 20 ^{o}C, and that formed by methyl end group is slightly higher than 110 ^{o}C. Figure 2 (b) and (c) are consistent with the literature reports [4].
- The θ of protein surface is 62 ^{o}C, which is significantly higher than that of carboxyl end group surface, but is distinguishing lower than that of mixed surface ($COOH:CH_3$=0.8:0.2), indicated that protein were successfully linked on the alkanethiols film.
- Our experimental experience (data not shown) show that high concentrations of -COOH groups exhibit a "hump" (which means the θ of –COOH end group surfaces may exhibit higher than it should be). This phenomenon was observed by Lee et al, [5, 8], they suggested the hump probably arises from conformational changes of the carboxylate groups upon partialionization, and offer two hypothesis to rationalize the existence of the hump. The first hypothesis (the disorder hypothesis) proposes that the onset of ionization induces a sufficient degree of disorder in the monolayer to cause hydrophobic methyl groups to be exposed at the surface. In the absence of unforeseen structural of steric effects, however, induce disorder of this type should be possible for the other ionizable surfaces that they have examined. The second hypothesis proposes that the hump results from stable hydrogen bonding at intermediate values of pH, which results in the exposure at the surface of carbonyl groups or methyl groups rather than the more hydrophilic –COOH or COO-groups. Li et al, [9] reported that wash films with 10% acetic acid could effectively remove unbound –COOH groups thiol molecules. Unfortunately, in our experience, this did not work. However, we found that supersonicate sample for 1-3 minutes is an effective way to remove unbound thiol molecules.

B. *AFM images of bare gold and protein surfaces*

The gold substrates were soaked into a hot piranha solution (v/v H_2SO_4:H_2O_2=3:1) for 30minutes for cleaning (warning: piranha solution is highly reactive and may explode on coming into contact with organic solvents, extreme precautions must be taken at all times). Immunoglobulin (~150KD) consists of three fragments, two separate and identical Fab fragments with active sites for antigen binding, and one Fc fragment [10]. The three dimensional structure is T- or Y- shaped with a dimension of 14.2×8.5×3.8 nm^3 measured by X-ray diffraction. Protein is unstable in air and at room temperature. The protein layers should keep into the PBS buffer at 4 ^{o}C if not immediately used. Protein layers should rinsed with PBS buffer solution and dried before use, then immediately transferred on the scanner of AFM [11]. All AFM images performed with contact mode. AFM images are shown in Figure 3.

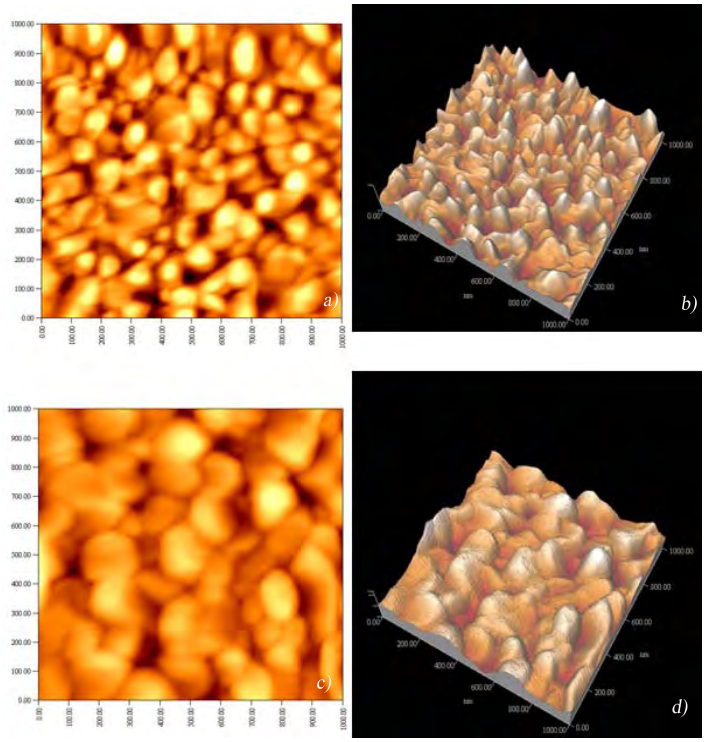

Figure 3. Contact mode AFM 2-D images of bare gold (a) and protein layer (c), (b) and (d) are their 3-D images, respectively. The scanning scope is 1μm×1μm, Z bar of (b) and (d) are 14nm and 20nm, respectively.

- 2-D images of bare gold and protein surface show different topographic structure. Figure 3 (a) shows that gold is uniformly and orderly deposited onto the mica surface. Figure 3 (c) shows large bulk structures on the alkanethiols films, indicate that protein are uniform linked on the alkanethiols film.
- The height of bare gold image is ~14 nm., while the height of human IgG linked on alkanethiols film is ~20nm, suggest human IgG is successfully linked on the alkanethiols film. Similar study was performed by He et al, [12].

IV. CONCLUSION

In this work, a series of alkanethiols mixed films (with the molar ratios of 16-Mercaptohexadecanoic acid ($HS(CH_2)_{15}CO_2H$) to 1-Dodecanethiol ($HS(CH_2)_{11}CH_3$) are 1:0, 0.8:0.2, 0.6:0.4, 0.5:0.5, 0.4:0.6, 0.2:0.8, respectively) were prepared by immersing the gold substrates into the alkanethiols solutions. Subsequently, alkanethiols films were activated by NHS/EDC, and immediately transferred into the human IgG solution to form protein layers. Protein layers were investigated by atomic force microscopy (AFM) to acquire high resolution 2-D and 3-D topography images, and contact angle goniometry to characterize the wettability of the surfaces. AFM images of bare gold and protein layers showed significant differences in topography structure and heights. It suggested that protein was uniformly linked with the alkanethiols films. This was also supported by comparing the contact angle of alkanethiols films and protein surfaces.

REFERENCES

[1] J.C. Love, L.A. Estroff, J.K. Kriebel, R.G. Nuzzo, and G.M. Whitesides. Self-ssembled monolayers of thiolates on metals as a form of nanotechnology. *Chem. Rev*., 105(4):1103-1169, 2005

[2] E. Baldrich, O. Laczka, F.J. Del Campo, and F.X. Munoz. Gold immuno-functionalisation via self-assembled monolayers: study of critical parameters and comparative performance for protein and bacteria detection. *J. Immunol. Methods.*, 336(2):203-212, 2008

[3] G. Binnig, C. F. Quate, and C. Gerber. Atomic force microscope. *Phys. Rev. Lett.*, 56(9):930-933, 1986

[4] N. P. Barrea, H. Ge, R.M. Henderson, W.J. Fitzgerald, and J. M. Edwardson. Automated analysis of the architecture of receptors, imaged by atomic force microscopy. Micron, 39(2):101-110, 2008

[5] T.R. Lee, R. I. Carey, H.A. Biebuyck, and G.M. Whitesides. The wetting of monolayer films exposing ionizable acids and bases. *Languir*, 10(3):741-749, 1994

[6] L. Li, S. Chen, and S. Jiang. Protein adsorption on alkanethiolate self self assembled monolayer: nanoscale surface structural and chemical effects. *Langmuir*, 19(7):2974-2982, 2003

[7] X. F. Ang, Z. Chen, C. C. Wong, and J. Wei. Effect of chain length on low tepmperature gold-gold bonding by self –assembled monolayers. *Appl. Phys. Lett*, 92:13913, 2008

[8] S. Tam-Chang, H.A. Biebuyck, G.M. Whitesides, N. Jeon, and R.G. Nuzzo. Self-assembled monolayers on gold generated from alkanethiols with the structure $RNHCOCH_2SH$. *Languir*, 11(11):4371-4382,1995

[9] L. Li, S. Chen, S Oh, and S. Jiang. In situ single-molecule dectection of antibody-antigen by tapping-mode atomic force microscpy. *Anal. Chem.*, 74(23): 6017-6022, 2002

[10] E. W. Silverton, M.A. Navia, and D.R. Davies. Three-dimensional structure of an intact human immunoglobulin. *Proc. Natl. Acad. Sci.*, 74(11):5140-5144,1977

[11] J. Wakayama, H. Sekiguchi, S. Akanuma, T. Ohtani, and S.Sugiyama. Mr\ethods for reducing nonspecific interaction in antibody-antigen assay via atomic force microscopy. *Anal. Biochem.*, 380(1):51-58, 2008

[12] X. X. He, R. Jin. L. Yang, K. M. Wang, W. Li, W. H. Tan, and H. M. Li. Study on the specific interaction between angiogenin and aptamer by atomic force microscopy (AFM). *Chin. Sci. Bull.*, 53(2):198-203, 2008