QUANTITATIVE END-GRAFTING OF DNA ONTO FLAT AND NANOPOROUS GOLD SURFACES

Ling Huang[1], Erkin Seker[1], Marcel Utz[2], Matthew R. Begley[2], James P. Landers[1,2,3]

[1]Department of Chemistry, [2]Department of Aerospace and Mechanical Engineering, [3]Department of Pathology, University of Virginia, USA

ABSTRACT

The adsorption of end-thiolated single- and double-stranded DNA on gold surfaces has been studied with particular attention to the dependence of the adsorption density on the DNA base pair length. Quantitative understanding of probe immobilization and target binding is crucial for improving the performance of hybridization-based sensors and microarrays.

KEYWORDS: DNA grafting, nanoporous gold, DNA hybridization, microarray

INTRODUCTION

Single-stranded DNA (ssDNA) grafting and its hybridization on planar gold surfaces has been studied qualitatively [1]. Using gold (Au)-thiol chemistry, we extend the study of single-stranded oligonucleotides grafting on planar gold to double-stranded DNA (dsDNA) binding, and further to the investigation of binding events on nanoporous gold (np-Au) structures. Nanoporous gold [2] is a promising material for high-density DNA microarrays and biomolecular sorting tools. Along with our modelling work, the findings provide a platform for rational design of DNA sensors and DNA microarrays with higher probe binding capacity and hence higher sensitivity.

EXPERIMENTAL

In order to create the planar gold surface, glass plates were cleaned with piranha solution, subsequently rinsed with deionized water and dried with N_2 gas flow. Holes were punched out on a 300 μm-thick PDMS film. The PDMS layer and the glass were treated in oxygen plasma, and permanently bonded together. The exposed glass surface was sputter-coated with 6.5 nm-thick Cr and 30 nm-thick Au layers. The plastic mask above the PDMS layer was peeled to create gold patterns encircled by the punched PDMS layer (Fig. 1a).

A microfabrication protocol was developed to produce samples with highly repeatable pore morphology (Fig. 1b). An array of gold-silver ($Au_{0.4}Ag_{0.6}$) alloy spots was photolithographically patterned on silicon wafers. The substrates were then scored with a dicing saw to produce identical chips that contained a AuAg spot, but not yet broken into individual pieces. The AuAg patterns were dealloyed in concentrated nitric acid (65%) for 10 min at 85ºC followed by a deionized water rinse. Finally, the substrates were broken into individual pieces. The np-Au spot thickness was measured to be 355±6 nm with a low-force stylus profilometer. The average porosity was 35.1±2.4% and the average pore diameter (with circular pore assumption) was 68±3 nm.

978-0-9798064-1-4/μTAS2008/$20©2008CBMS

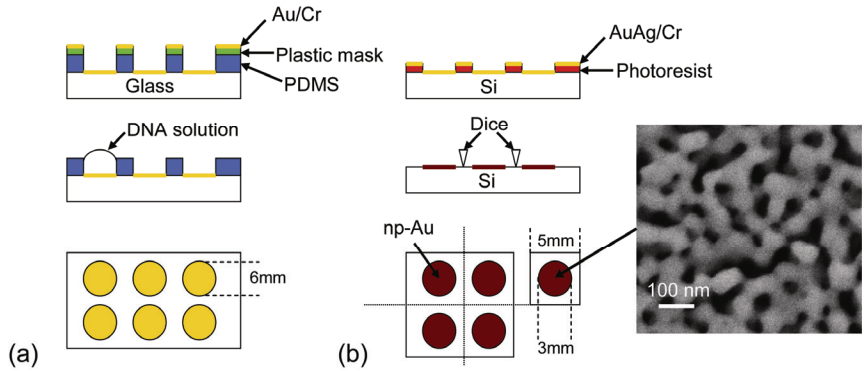

Figure 1. Sample fabrication for: a) planar gold and b) nanoporous gold surface.

Following the sample preparation, grafting experiments were carried out on planar gold and np-Au samples using both thiolated ssDNA and dsDNA solutions. The effect of DNA strand length on grafting density was investigated by repeating the experiment for various DNA probe lengths (10, 20, 30, 40 base pairs). In addition, hybridization experiments were performed with the grafted ssDNA to demonstrate accessibility of the grafted DNA strands for hybridization.

RESULTS AND DISCUSSION

Due to its inherent stiffness, adsorption of dsDNA leads to vertical ordering, and, hence, to a length-dependent repulsion between the molecules. A corresponding decrease in adsorption density with increasing base pair length has been observed. The effect is stronger than a simple Onsager-type excluded volume model of the vertical ordering process would predict (Fig. 2).

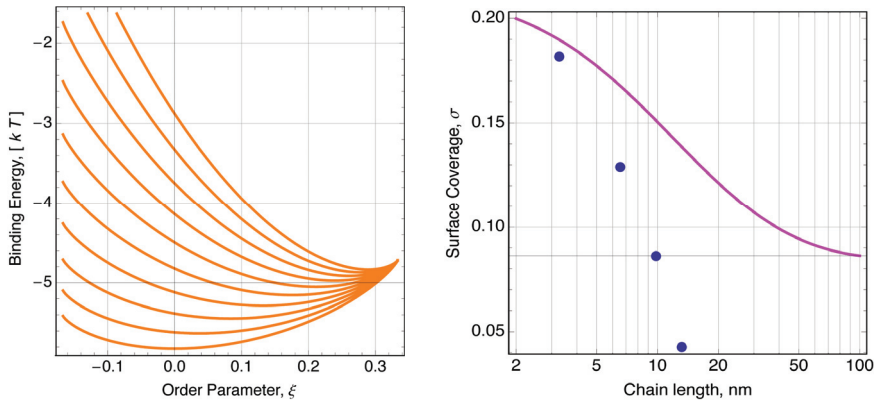

Figure 2. Free energy of adsorption predicted by an Onsager excluded volume theory as a function of surface coverage and vertical ordering parameter (left). Predicted equilibrium surface coverage as a function of dsDNA chain length (solid line) and experimental data (dots) (right).

The dependence on DNA chain length is even more accentuated on nanoporous gold (Fig. 3). Our results indicate that for short chain lengths (< 10 bp), a three-fold

enhancement in grafting density of dsDNA can be achieved over flat gold surfaces. Similar enhancements in grafting density of ssDNA and hybridization density were also observed. Generally, longer DNA reduced binding density. dsDNA molecules yielded a lower grafting density than ssDNA. While binding density on planar gold decreased steadily with increasing DNA length, np-Au samples displayed an abrupt drop above ten base pairs.

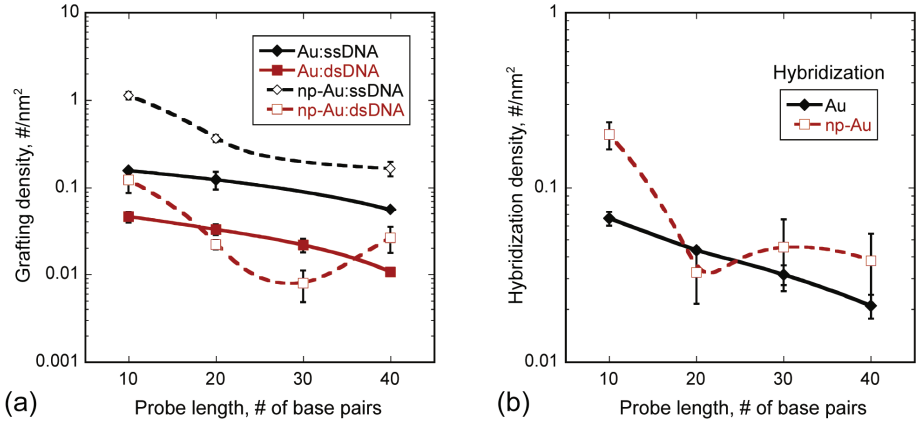

Figure 3. Experimental results for planar Au and np-Au surfaces: (a) ssDNA and dsDNA grafting densities; (b) ssDNA hybridization density.

CONCLUSIONS

The end-grafting and hybridization of oligonucleotides on both planar and nanoporous gold surfaces were quantified by fluorescence. The nanoporous gold substrate exhibits higher binding density for ssDNA and for short dsDNA. The chain length dependence of the adsorption density is more accentuated than on flat Au. These features make np-Au a promising material for use as high-capacity microarrays and biomolecular sorters.

ACKNOWLEDGEMENT

This work is supported by NSF-SST grant and funding from Carillion Biomedical Institute/UVA Fund for Excellence in Science and Technology (FEST).

REFERENCES

[1] K. Castelino, B. Kannan, and A. Majumdar, *Characterization of Grafting Density and Binding Efficiency of DNA and Proteins on Gold Surfaces,* Langmuir, 21, 1956 (2005).

[2] E. Seker, J. T. Gaskins, H. Bart-Smith, J. Zhu, M. L. Reed, G. Zangari, R. G. Kelly, M. R. Begley, *The effects of post-fabrication annealing on the mechanical properties of freestanding nanoporous gold structures,* Acta Materialia, 55, 4593, (2007).