NIRT: Probing Viral Adhesion with Nanoengineered Biomembranes and Quantum Dots

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This NIRT focuses on the fundamental and molecular level understanding of the adhesion process necessary for viral recognition of host cells. Using a combination of nanofabrication, lipid bilayer engineering and nanoparticle functionalization carefully designed ligand arrays and local bio-environments can be constructed and used to investigate the binding strength, stoichiometry, cooperativity, and kinetics of viral adhesion.

This project is currently focused on the HIV adhesion to host cells. HIV targets the T-cells, gaining entry through a cascade of events mediated by the viral gylcoproteins gp120 and gp41. Gp120 is a surface protein, noncovalently bound to the transmembrane protein gp41. Together these two glycoproteins are presented in trimeric form on the viral surface. As part of these efforts, we are studying the interactions of gp120 with the cellular receptor GalCer.

Recently, the Gervay-Hague group has prepared 2nm diameter Au nanoparticles containing galactosyl and glucosyl headgroups linked to the surface with a thiol. These nanoparticles have been characterized by TEM in the Kauzlarich group, AFM in the Liu group as well as NMR spectroscopy. A biotin-NeutrAvidin adhesion assay was used to evaluate the relative ability of carbohydrate disulfides and Au glyconanoparticles to displace rpg120 from plate-bound GalCer. The data indicate that the Au glyconanoparticles were greater than 300 times more active than the disulfide ligand of the glycosyl moieties and at least 20 times more active than biotinylated GalCer.

The Kauzlarich group has been optimizing the synthesis of Si nanoparticles as a viable quantum dot probe. We have been able to surface functionalize these nanoparticles with siloxanes. These are water and air stable for several months. Separation and further characterization of Si nanoparticles is being carried out with a joint student between the Kauzlarich and Liu groups. We are currently optimizing this synthetic method to provide a quantum dot with an amine termination for further linkage with GalCer.

Longo's goal is to use nanometer scale domains of GalCer in supported fluid phase lipid bilayers to study the multivalent binding affinity of gp120 to GalCer. This system allows us to vary parameters, believed to influence multivalent binding in a biomembrane environment, such as number of binding sites (by domain size), binding density (by spacing between GalCer molecules), variation in GalCer structure (in collaboration with Gervay-Hague) and GalCer mobility within the domain (by including cholesterol which fluidizes the GalCer within the domains). Domains containing GalCer, GalCer and gel phase lipid, and GalCer and cholesterol in fluid phase supported lipid bilayers have been formed and studied by AFM and their size (as small as 40 nm) have been uniformly varied using a temperature quench technique (see figure 1,

A and B). An annealing protocol which couples both monolayers of the supported bilayer has been developed to assure that domains are accessible to the gp120 solution and substrate. The coupling phenomenon has been characterized by AFM (see figure C) and fluorescence recovery after photobleaching (FRAP). The next step in this work is characterization of the binding of gp120 to the GalCer-containing domains using total internal reflection fluorescence (TIRF) and AFM force spectroscopy in collaboration with Lawrence Livermore National Lab.

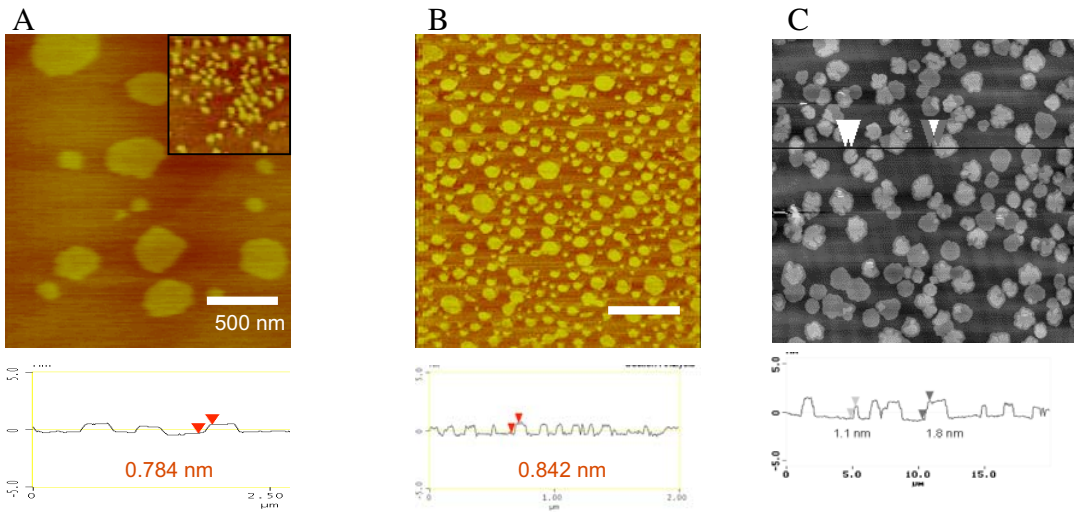

Figure 1. Contact Mode AFM images of nanometer scale domains (lighter shade) in fluid phase (DLPC) supported lipid bilayers. Domains contain **A)** GalCer (inset: demonstrates our ability to control of the size of these domains); **B)** GalCer and Cholesterol (50:50); **C)** DSPC, a gel phase phosphatidylcholine (at the 1.1 nm height the domain is uncoupled - contained in only one monolayer and at the 1.8 nm height the domain is coupled - in both monolayers)

The Liu group focuses on engineering nanostructures of HIV binding ligands to mimick the Cellular membranes, and the regulation of the gp120 binding to these artificially engineered structures by changing the geometry, local environment and functionality. Approaches include (1) production of nanostructures of ligands using scanning probe lithography and advanced nanofabrication methodologies developed in Liu lab; (2) in situ and real time monitoring of gp120 binding to the engineered structures to correlate the binding behavior with the geometry, local environment and functionality of the nanostructutres and (3) binding of HIV viruses with the nanostructures of ligands.

Recent progress includes: (1) successful production of nanostructures of carbohydrate ligands (Gal and GalCer) using nanografting and self-assembly; (2) immobilization of viral protein rgp120 onto Gal and GalCer terminated self-assembled monolayers (SAMs); and (3) preliminary success in adhering rgp120 to nanostructures of ligands.

Using nanografting, an AFM-based nanofabrication method developed in the Liu group, nanostructures of Gal-terminated nanostructures are successfully produced and imaged, as shown in Fig. 1. The AFM topographs and height measurements reveal that Gal-terminated alkylthiols can be successful grafted in alkanethiol matrix. The molecules are closely packed although the

long-range order was not observed for Gal-terminated areas. Rgp120 proteins are found to selectively bind to Gal- or GalCer-nanostructures over the methyl-terminated areas

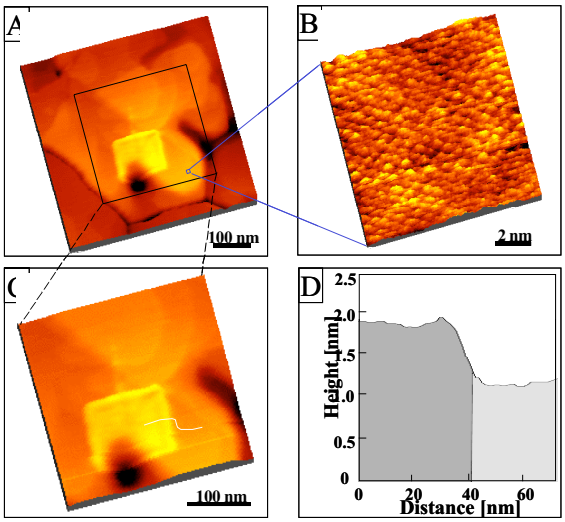

Figure 2. Nanostructures of Gal-terminated alkyl thiols inlaid in octanethiol matrix. [A] A 500 x 500 nm^2 AFM topographic image including a 130 x 110 nm^2 Gal pattern. [B] A 10 x 10 nm^2 high-resolution view of the matrix area. [C] A 300 x 300 nm^2 zoom-in of [A]. [D] Cursor profile as indicated in [C].

The Dandekar group is charged with testing the various derivatives of galactosyl ceramide ligated nanoparticles in cell culture. Specifically, we aim to test HIV neutralization by these nanoparticles while also focusing on their cytotoxicity in cell culture using both lymphocyte and gut epithelial cell lines. We are presently testing the neutralization of GalCer ligated gold nanoparticles with various ratios of carbohydrate against the HIV strains: IIIB, LAI, and BAL. Our experiments have currently ruled out two of the derivatives of the nanoparticles as increasing the viral infectivity in culture. In addition, we have grown large batches of IIIB virus to plate into ELISA assays allowing us to test the optimal ratio of nanoparticle to virus to use. We will be using a competition Elisa with a neutralizing antibody (NIH) against the same site on the V3 loop of gp120 that binds GalCer. It is our hope that this assay will help us determine the optimal conditions to achieve our goal of neutralizing HIV before it has a chance of binding and entering gut epithelial cells.

To address the possibility of nanoparticle-induced cytotoxicity, our lab has cultured various concentrations of the gold particles with our cell lines. We have not found any type of toxicity utilizing trypan blue exclusion and examination of growth characteristics. We are currently testing the byproducts of the next generation of silicon nanoparticles in cell culture. At present, we have found no adverse effects on either the lymphocyte cell lines or the gut epithelial lines.

Publications

"**Structures of Annealed Decanethiol Self-Assembled Monolayers on Au(111): an Ultrahigh Vacuum Scanning Tunneling Microscopy Study"**
Qian, Y.; Yang, G.; Yu, J. J.; Jung, T.A.; Liu,G-Y. *Langmuir* **2003,** *19,*6056-6065.

"Synthesis of Gold Glyconanoparticles and Biological Evaluation of Recombinant Gp120 Interactions"
Notlting, B.; Yu, J. J.; Liu,G-Y.; Cho, S.-J.; Kauzlarich, S.; Gervay-Hague, J. *Langmuir* **2003**; *19,* 6465-6473.

Presentations

Adhesion of rgp120 on Nanostructures of Ligands on Surfaces.
Yu, J. J; Nolting, B; Gervay-Hague, J; Liu, G-Y. invited talk at SPIE 48th annual meeting, August 2-8, **2003,** San Diego, California.
Investigation of viral adhesion using nanoengineering approaches
Yu, J. J; Nolting, B; Gervay-Hague, J; Liu, G-Y. North California chapter AVS meeting : Nanoscience and Bionanoscience Research, June 11, **2003,** Lawrence Berkeley National Laboratory, California.
Adhesion of rgp120 on Nanostructures of Ligands on Surfaces.
Yu, J.J; Nolting, B; Gervay-Hague, J; Liu, G-Y. HIV/AIDS symposium, January 28, **2003,** University of California, Davis, California.
Recent progress in the synthesis and characterization of silicon and germanium nanoparticles. Kauzlarich, Susan M. Invited. Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United States, March 23-27, 2003 (2003), INOR-074.
Recent Progress in the Synthesis of Group IV Nanoparticles. Kauzlarich, Susan M. Invited. MRS Spring Meeting, San Francisco, April 21-25, 2003.
Synthesis of bilayer stabilized silicon nanoparticles. Verberne, Susan D.; Kauzlarich, Susan M.; Liu, Gang-yu; Gervay-Hague, Jacquelyn; Longo, Marjorie L. Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United States, March 23-27, 2003 (2003), INOR-245.
Surface functionalization of Si nanoclusters with alkoxides and NMR studies. Zou, Jing; Baldwin, Richard K.; Kauzlarich, Susan M.. Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United States, March 23-27, 2003 (2003), INOR-243.
Fabrication and characterization of gold-coated iron nanoparticles. Cho, Sungjin; Kauzlarich, Susan M.; Liu, Kai; Long, Gary J.; Grandjean, Fernande. Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United States, March 23-27, 2003 (2003), INOR-234.
Synthesis, characterization, and biological evalution of carbohydrate-functionalized nanoparticles. Gervay-Hague, Jacquelyn; Nolting, Birte; Yu, Jing-Jiang; Liu, Gang-Yu; Cho, Sung J.; Kauzlarich, Susan M.. Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United States, March 23-27, 2003 (2003), ORGN-717.
Glycoscience and the Development of Alternative Therapies to Prevent HIV Infection J. Gervay-Hague, invited seminar, Montana State University.
Development of Alternative Therapies to Prevent HIV Infection J. Gervay-Hague, Plenary Speaker at MGM Conference UC, San Diego
Design and Synthesis of Molecular Scaffolds for Ligand Display J. Gervay-Hague, invited seminar, Case Western Reserve 01/23/03.
Carbohydrates as Recognition Elements in Disease J. Gervay-Hague, invited seminar, University of Illinois Chicago 04/01/03
Chemical Innovations Facilitating Drug Discovery J. Gervay-Hague, invited seminar, Michigan State University 12/04/03.