

In Vivo 3-D Mapping of Nanoparticle Biomarkers in Cells and Tissues

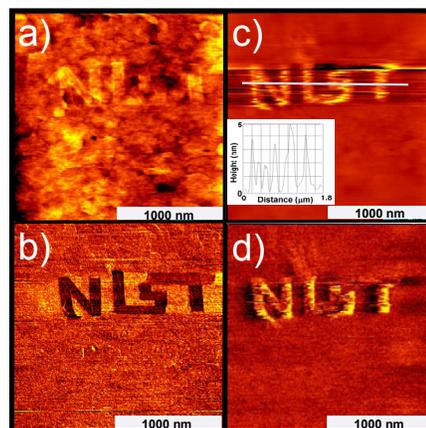
Nanoparticles comprise one of the primary building blocks in nanotechnology. There is growing interest in coupling nanoparticle systems to complex oligosaccharides. These bio-conjugated nanoparticles are promising materials for use in biosensing and disease diagnostics. Oligosaccharides are biologically important because they interact with proteins and are known to regulate many processes ranging from the adhesion of cells to the guidance of neurons during development. Central to utilizing such material as tracers in cells is the ability to track the accumulation and quantifiably assess the spectroscopic changes in nanoparticle luminescence in vivo: in vivo tracking is in fact one of the key aspects outlined in the metrology needs of the National Institutes of Health (NIH) Nanomedicine Roadmap. In this project, we have built a two-photon microscope and developed protocols to investigate the efficacy of saccharide coated Au nanoparticles for selective binding to glucose specific proteins.

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Oligosaccharides are biologically important because they interact with proteins and are known to regulate many processes ranging from the adhesion of cells to the guidance of neurons during development. Additionally, the oligosaccharide chemistry can be used in developing carbohydrate chips, similar to DNA and protein microarrays, for drug discovery and biological research. On a chip or as single particles, oligosaccharide-coated surfaces/nanoparticles can selectively target/bind cell surfaces that express sugar binding groups that are specific to a disease. Oligosaccharides have recently been shown to provide the necessary platform for the multidentate binding typically required for sugar-based recognition events. NIST has built a two-photon microscope to further investigate this finding. In two-photon microscopy, a scanning confocal optical microscope is coupled with two-photon fluorescence (using two red photons to equal the excitation energy of one blue photon) to create high-resolution, three-dimensional images of microscopic samples. This non-linear microscopy is particularly useful in biology because it can be used to probe delicate living cells and tissues without damaging the sample.

Demonstration of this metrology tool on model tissue and cellular systems will open the door to *in vivo* techniques for the screening of cellular receptors with optimal efficiency.

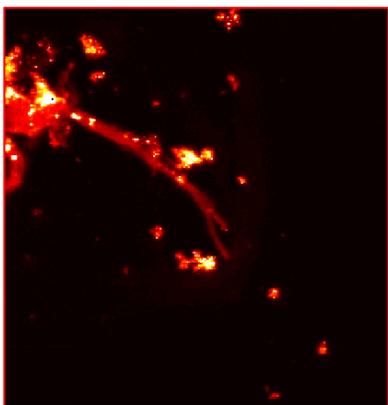
In this research, we have examined the fluorescence of Au nanoparticles (≈ 2 nm diameter) coated with glucosylamide disulfide. The oligosaccharide derivatives and coated particles were provided via collaboration with Dr. Phil DeShong at the University of Maryland. The glucosylamide disulfide layer makes these particles water soluble and affords easy incorporation with cells and proteins. Fluorescence in these particles is triggered once they aggregate in cell tissues. To test this aggregation, the glycoprotein Concanavalin A (Con A), which selectively binds glucose, was used to determine the efficacy of the glucosylamide disulfide for binding. The binding activity of the Con A for the glucose moiety was tested and confirmed by nanopatterning the glucosylamide disulfide onto a Au surface. Selective binding of the Con A to the glucose pattern was observed by atomic force microscopy, as shown in the figure below.



Glucosylamide disulfide nanopatterned onto Au using scanned probe lithography as observed in the a) topographic image and b) friction image with a background of an alcohol terminated thiol to resist non-specific protein adsorption. Con A is found to selectively bind to the pattern yielding changes in c) topography and d) friction images. The height profile of the bound structure 3nm to 4 nm is consistent with the dimensions of Con A (inset c).

Following confirmation of the selective binding of Con A to the oligosaccharide on Au, aggregates of the saccharide-coated Au nanoparticles were formed by the introduction of Con A to an aqueous solution of the particles. Upon aggregation, the particles are found to strongly fluoresce, providing an excellent test case for two-photon fluorescence microscopy. Two non-linear microscopes have been built in support of this project. One is based on an Olympus* inverted microscope coupled to an ultrafast laser that is completely computer controlled to allow it to be used by

non-laser specialists. The second is an in-house design that can be quickly modified to address changing experimental demands. A two-photon fluorescence image of the aggregates of the saccharide coated Au nanoparticle bound to Con A is shown in the figure below. The nanoparticle aggregates appear as bright features in the fluorescence image. Spectroscopic studies of the fluorescence of the individual aggregates indicate that there is some spectral diversity, but further study and analysis is needed to begin to unravel the photophysical causes of this diversity.



In this 20 μm x 20 μm image, we demonstrate the capabilities of two-photon fluorescence microscopy by tracking the accumulation and dynamic properties of oligosaccharide functionalized Au nanoparticles in a glycoprotein Con A, matrix.

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These studies will provide a means toward direct commercialization both of such agents as next-generation biomarkers, as well as establish this as a viable and reliable method for *in vivo* characterization of receptor expression in single cells. A paper on the binding of oligosaccharides on Au has been submitted to *Langmuir* (1), and details of the microscopy platform have been published *Optics Express* (2).

As the NIH Nanomedicine Roadmap has outlined, the need for the development of metrology tools for *in vivo* mapping of biomarkers in cells and tissues will continue to grow in significance. Central to this goal is the development and use of nanoparticle tags and microscopy platforms. We are currently working on determining the ultimate 3-D spatial resolution for two-photon fluorescence microscopy in nanoparticle-tagged tissues and cells. Additionally and equally important, we are working to establishing the spectroscopic detection limits and photophysical stability of nanoparticle biomarkers in tissue environments.

Publications:

1. M. Kadalbajoo, J.H. Park, A. Opdahl, H. Suda, J.C. Garno, J.D. Batteas, M.J. Tarlov, and P. DeShong, "*Oligosaccharide Based Self-Assembled Monolayers on Gold*," *Langmuir*, in press (2006).
2. M. R. Beversluis, L. Novotny, and S.J. Stranick, "*Programmable Vector Point-Spread Function Engineering*," *Optics Express*, Vol. 14, No. 7, 2656, (2006).

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