

# Probing 2-D Reaction Coordinates of Amino Acid / Surface Binding BE 442 Research Proposal

David Appleyard

December 18, 2003

## 1 Abstract

Binding interactions between oligo-molecules and surfaces have become important in many research fields. Specifically, these interactions can be extended to semiconductor self-assembly (1) for materials that are difficult to produce industrially. A gradient force optical trap (optical tweezers) offers an intriguing method to investigate these interactions at a molecular level by allowing the extraction of a two dimensional reaction coordinate diagrams through elucidation of the force interactions between a probe molecule and a surface. These reaction coordinate diagrams can provide the strength and directionality of the interactions and can extend and expand research in the area of semiconductor self assembly as well as a broad range of other fields.

## 2 Motivation

Gallium Nitride (GaN) has applications for solid state lighting, data storage, communication devices as well as many others. GaN can be used to make blue light emitting diodes, a much more energy efficient lighting source(2). Unfortunately pure, defect-free, GaN is very difficult to produce. However, new methods for crystal production using viruses for guided self-assembly are under investigation (1). Yet, the specific molecular interactions between the crystal structure and the self-assembly promoting amino acid chains provided by the virus are not well defined.

The ability to precisely control, monitor, and manipulate the interactions between a surface and a protein is invaluable in quantifying the reaction coordinate and can lead to determination of molecular mechanisms and chemical transformations involved in the reaction. Current exploration in the areas of silicon-biology and biologically directed nano-fabrication would benefit from knowledge of these interactions. The recent developments in optical

tweezers and 2-D force clamping has opened the door to analytical measurement and determination of these quantities.

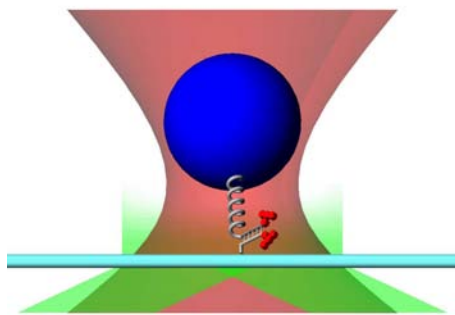
Possessing exact knowledge of the reaction coordinate of biological molecular reactions and the comprehension of the binding forces involved is instrumental in understanding the biological processes that are at the forefront of current research. This knowledge can be applied to GaN as well as a myriad of other semiconductors and other materials.

## 3 Background

### 3.1 Equipment and Technology

Laser-based, gradient-force optical traps, also known as optical tweezers, allow the positioning and control of objects whose characteristic dimensions are similar to the wavelength of light (3). These traps can place forces on the order of 200 pN with high, sub pN force resolution on objects of interest and when combined with appropriate position sensing devices can detect sub-nanometer movement. This technology offers the ability to position or pull appropriately modified proteins on a controlled, transparent surface.

The first experimental examination of photon force applied on a small particle was completed by Ashkin (4). A laser source was found to be able to provide a dense photon stream which could impart both a radial and axial force on a small particle in solution. The ability to generate a force in the radial direction, drawing the particle into the waist of the laser beam, was instrumental in the development of optical traps and tweezers. These devices are capable of trapping a small bead or particle in a tightly focused beam.



Courtesy of Jorge Ferrer. Used with permission.

Figure 1: A cartoon of a bead handle trapped in the waist of a laser beam (the red color) and attached to a double stranded nucleotide sequence.

The combination of sensitive stage positioning and beam steering devices with an optical trap adds a great deal of power to an experimental setup. The combination of these parts allows an optical tweezers setup to provide both position clamping and force clamping to

an experimental particle (usually a glass or polystyrene bead with a diameter of 100 to 500 nm). Force clamping provides a constant load to the particle in a selected direction, whereas position clamping locks the particle into a single position. Coarse laser beam steering can be accomplished using mirror pairs. Fine steering, intensity adjustment, and multiple beam control can be completed with acoustical optical deflectors (AOD) which are computer controlled. Positioning is completed using a piezo stage with three dimensional movement and control.

In order to provide the force and position clamping, a computer controlled feedback loop must be supplied with data about bead location. One way this can be accomplished is through a highly sensitive photon detector called a quadrant photo diode. This determines position by monitoring deflections in a detection beam (another laser) caused by bead motion.

Figure removed for copyright reasons.

Source: Lang, M. J., P. M. Fordyce, et al.

"Combined optical trapping and single-molecule fluorescence." *J Biol* 2, no. 1 (2003): 6.

Figure 2: A diagram of an optical tweezers setup incorporating TIR, a quadrant photodiode (QPD), and single molecular fluorescence.

Further information can be extracted from experimental systems with optical tweezers by combining single molecule fluorescence. This introduces yet another excitation laser beam into the sample area (Figure 2) In order to provide the high level of signal to noise required for single molecule fluorescence, total internal reflection (TIR) is used. TIR redirects the excitation beam into the glass coverslip such that it travels "parallel" to the sample plane. This produces an evanescent wave which only penetrates to around 500 nm into the sample. This greatly reduces the noise from fluorophores located deeper in the sample which are not of interest.

Significant knowledge of optics and microscope design is necessary to integrate all of these parts into one device. Trapping, detection, and excitation lasers must be run at different

wavelengths, and also not overlap the fluorophore emission wavelength. Computer control for experimental automation and data collection is essential in any research setup.

## 4 Supporting Studies

An article by Kawaguchi (5) provides a pertinent example of the use of an optical gradient trap in extracting force and binding data. Here, optical tweezers were used to deduce the binding states of kinesin as it progressed both forward and backward along a microtubule under different nucleotide states. Various ATP analogues were used to halt kinesin at some of its progressive steps. At each step the kinesin molecule was pulled from the microtubule (both with the direction of kinesin motion and against kinesin motion) using an optical tweezers setup. This directional force data helped to elucidate the kinesin motility cycle along a microtubule.

Stout (6) was able to probe a single immunoglobulin G (IgG) - *Staphylococcus* Protein A (SpA) bond by holding a bead with a single IgG molecule a precise distance away from a surface with SpA bound to it. The bond was able to form and then was broken through addition of force from the optical tweezers. The binding data produced could be compared to earlier flow detachment experiments for the same IgG-SpA pair.

## 5 Proposed Experiment

Initial experimentation for silicon-biological or other semiconductor-oligo interaction must start with a well described model such that appropriate ground work is laid for further studies. Mica, silicon, or Gallium Nitride offer excellent starting surface materials. Because each of these materials is used in industry, or has commercial potential, they are well characterized.

Due to the crystal structure along certain surface planes, the atomic interactions encountered when travelling along different axis will be fundamentally different (see the perpendicular directions of travel on silicon in Figure 3). Because the surface is attached to the microscope sample stage, a specific orientation of the atomic surface is held constant to the laboratory frame of reference. Thus a force exerted by the optical tweezers in the laboratory frame can be related to unbinding in the atomic frame. This allows for the investigation of different molecular interactions depending on the direction of force exerted by the optical trap.

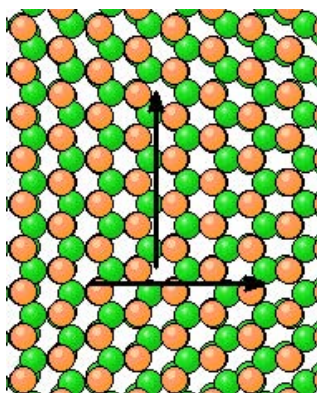


Image by Dmitri Petrovykh (<http://nanowiz.tripod.com>).  
Courtesy of Dmitri Y. Petrovykh. Used with permission.

Figure 3: The (111) surface of Silicon with arrows indicating two possible different reaction coordinate directions. (Initial image from [nanowiz.tripod.com/sisteps/si111.htm](http://nanowiz.tripod.com/sisteps/si111.htm))

Many options exist for molecules to manipulate on the surface, the M13 virus stands out as one possibility. It has been heavily investigated, its DNA sequence is known, and it is relatively easy to modify. Various peptide sequences can be expressed on its coat protein pIII (7). A library of amino-acid sequences on pIII of M13 has been created (8) and would offer an excellent source. Furthermore, the Belcher laboratory at the Massachusetts Institute of Technology has already selected optimal binding viruses for a multitude of surfaces from this library (1), and thus collaboration with this research group could bare fruitful results.

A challenge that would be faced with use of the M13 virus is the morphology of the virus itself. It has five pIII chains which would display the amino acid sequence, Thus, when put in contact with a surface, the chains could land in multiple directions. This eliminates the ability to investigate a single orientation on the surface. However, if it is found that all the side chains line up (thus, the force required to unbind in two perpendicular directions are significantly different) then there is evidence for a preferred binding orientation on the surface.

The ease of mutation in M13 would also allow for the attachment of a biotin-streptavidin linkage to enable binding to a  $1\ \mu\text{m}$  glass bead to allow for optical tweezers manipulation. The biotin-streptavidin linkage is not the only available method for attaching beads. DNA is commonly used to provide a tether between the probe molecule and the glass bead. Additional research into developing a PEG linker with appropriately modified ends for covalent attachment points is also under investigation.

Another method for constructing the amino acid probe would be to covalently attach a peptide to the linker molecule and then directly to the bead. This may offer a much simpler, single chain, method of detecting surface binding. However, it lacks the ease of library availability and potential of genetic manipulation that the M13 virus provides.

Another plausible way of creating a probe would be to engineer a virus which had the geometric and optical properties which would allow it to be directly manipulated with the optical tweezers. This would be the most difficult probe to construct, however, if designed,

would be an extremely useful tool for a large number of research options involving optical tweezers.

The phage or other probe can be placed on the optically transparent surface where it is allowed to bind. The optical tweezers would then be used to trap the bead handle and a directional force could then be applied and measured. This would be repeated in multiple directions to elucidate a two dimensional representation of the binding strengths and help illuminate the reaction coordinate landscape.

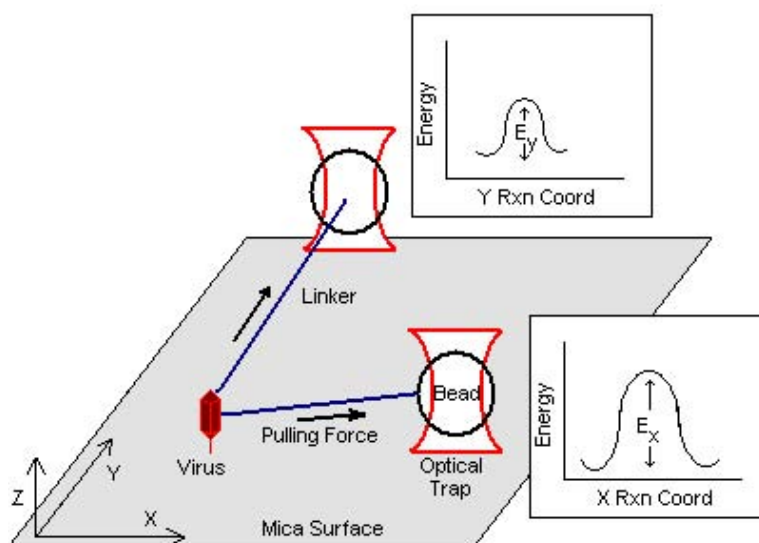


Figure 4: Translation of a virus in two directions relative to the laboratory axis using a phage probe attached to a bead handle for optical trap manipulation.

## 6 Summary

Optical tweezers setups have been used to investigate directional binding forces when probing the behavior of motor proteins like kinesin. A similar analytical setup has the potential to be applied to a simple surface binding assay for amino-acid or other oligo-molecule interactions with optically transparent surfaces.

There are multiple methods for oligo probe design such that effective tweezers manipulation can be achieved. Either direct attachment of the sequence to the bead via a linker molecule or phage display could be successful. A wealth of surfaces with industrial and commercial applications are available for study, and determination of molecular interactions could have useful implications for current and future research.

The molecular forces can be investigated by directional application of pulling force from the optical tweezers on the probe oligo-molecule. This force data can then be used to elucidate a two dimensional reaction coordinate diagram for the molecular probe - surface

interaction. In turn, this data can be applied to several areas of materials research. Additionally, this work will lay the background for more complex surface interaction studies involving more complex oligo-molecules or even larger macromolecules like proteins.

## 7 References

1. S. Whaley *et al.*, *Nature* **405**, 665 (2000)
2. M. Benaissa *et al.*, *Phys. Review. Bio.* **54**(24), 17763 (1996)
3. M. Lang, S. Block, *Am. J. Phys.* **71**, 201 (2003)
4. A. Ashkin, *Phys. Rev. Lett.* **24**(4), 156 (1970)
5. K. Kawaguchi, S. Ishiwata, *Science* **297**, 667 (2001).
6. A. L. Stout, *Biophysical J.* **80**, 2976 (2001)
7. N. Seeman, A. Belcher, *P. N. A. S.* **99**, 6451 (2002)
8. S. S. Sidhu, *Biomolecular Eng.* **18**, 57 (2001)
9. M. Lang *et al.*, *Biophysical J.* **83**, 491 (2002)