Lipid Bilayer Based Binding Surfaces for Nucleic Acids

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Major: Biology
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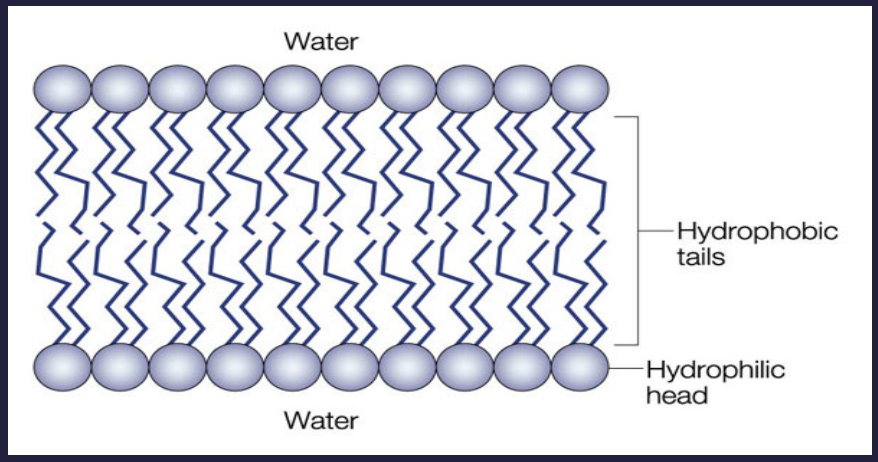
Funding By: National Institutes of Health
July 28th, 2005

Main Objectives

- Develop a mobile, semi-fluid, Atomic Force Microscopy suitable surface capable of supporting and binding nucleic acids
- Study the interactions between these surfaces and nucleic acids (NA should be able to move on the surface, but not leave it)
- Improve our control over the self-assembly of nano-structures from nucleic acids, and form nano-structures in precisely controlled, ordered environments

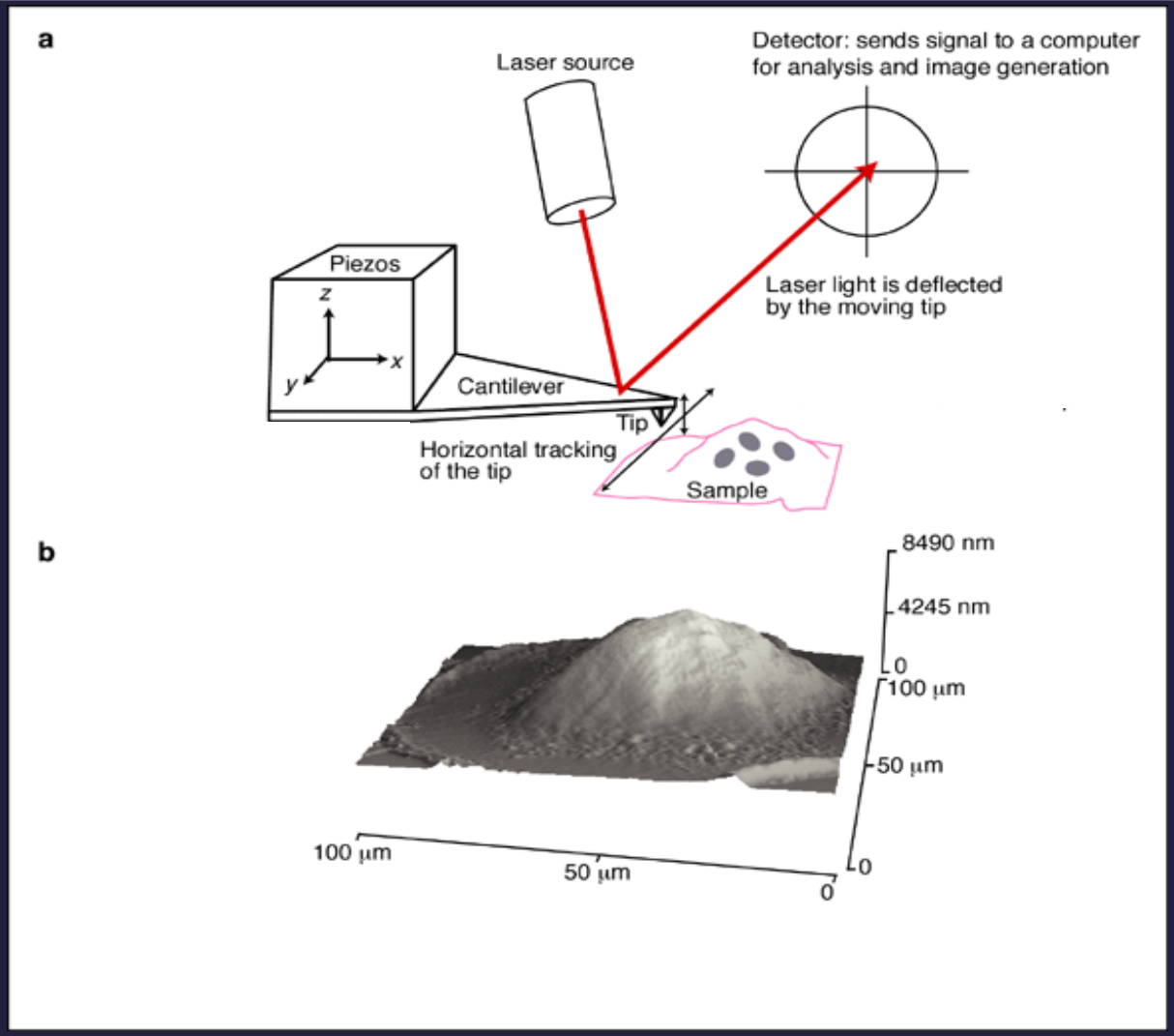
Research Goals

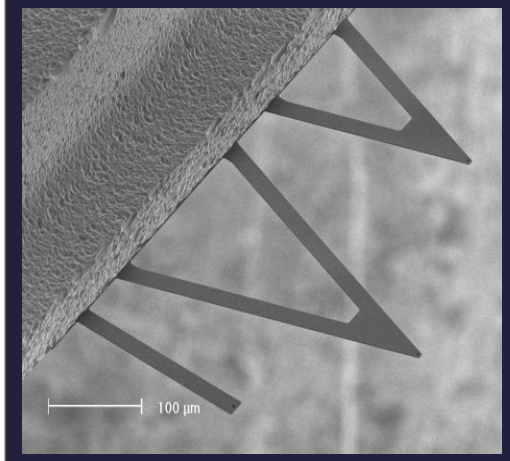
- Discover a lipid, or combination of different lipids that create optimum surfaces for AFM imaging
- Study the stability and quality of lipid bilayer surfaces under different conditions
- Bind DNA and/or RNA to these surfaces and examine their binding properties and appropriateness for nucleic acid nano-structure assembly

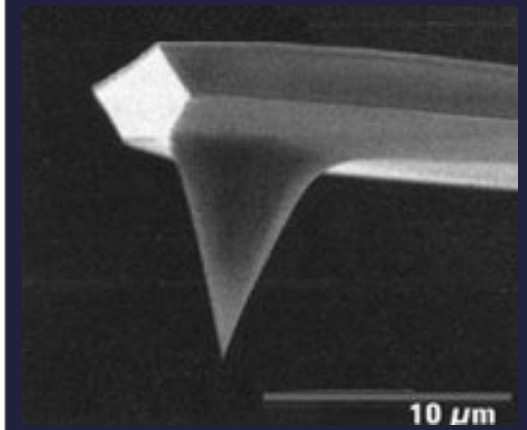


www.nature.com/.

The Atomic Force Microscope (AFM)



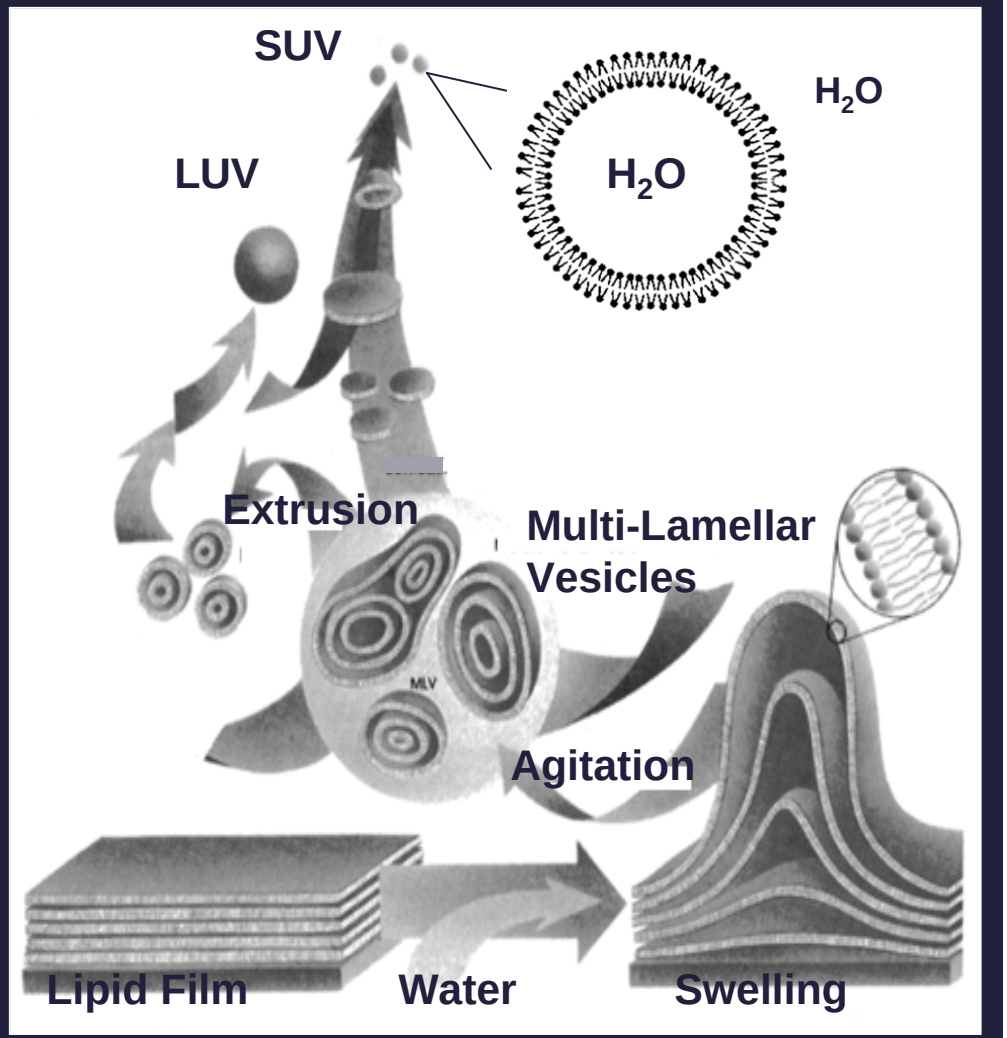




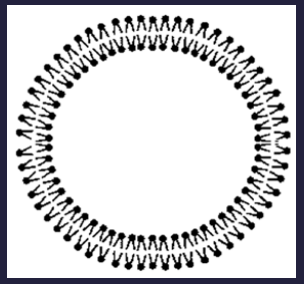
www-ermm.cbcu.cam.ac.uk/ nfig001mhu.gif www.nanotechweb.org

Preparing Liposomes

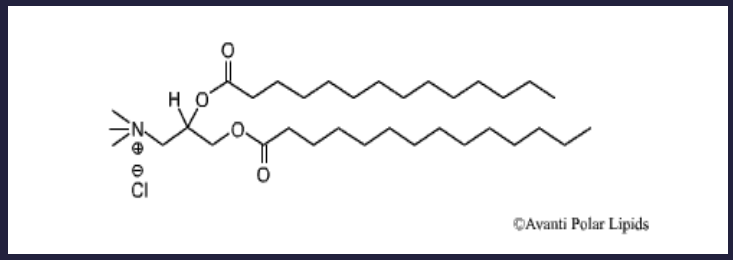
- Lipids
  -Type
- Dilute
  -Concentration 10-20 mg/mL
- Dry
  -N_2 Stream
  -Vacuum Pump
  -Lipid “Cake”
- Hydrate
  -HEPES KOH Buffer pH 7.4
  or Ultra Pure H_2O
  -Freeze/ Thaw Cycles
  -Multi-Lamellar Vesicles
- Extrusion
  -Membrane and Supporting Filters
  -Small Uni-Lamellar Vesicles
  (100-150 nm in diameter)



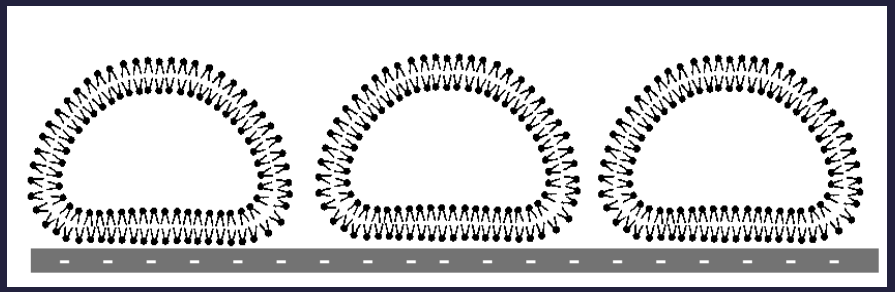
Creating the Bilayer



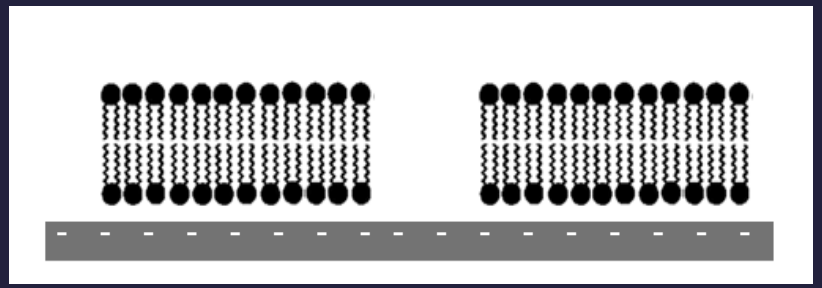
Uni-Lamellar Vesicles Composed of a Cationic Lipid (Positively Charged Head Group)



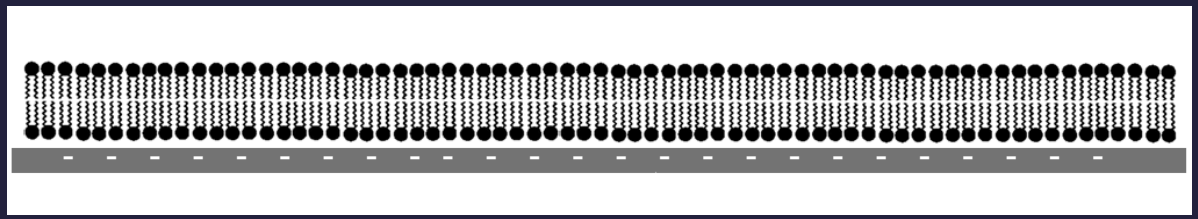
Trimethylammonium-Propane Cationic Lipid (TAP) Varied Chain Length



Accumulation and Fusion of Vesicles to Negatively Charged Mica Surface



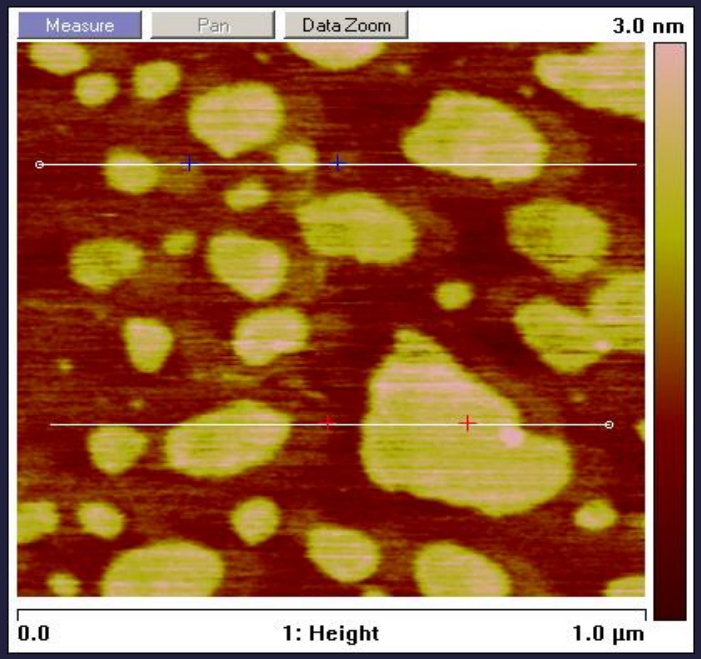
Partial Fusion of Vesicles to Mica Creates Patches of Bilayer



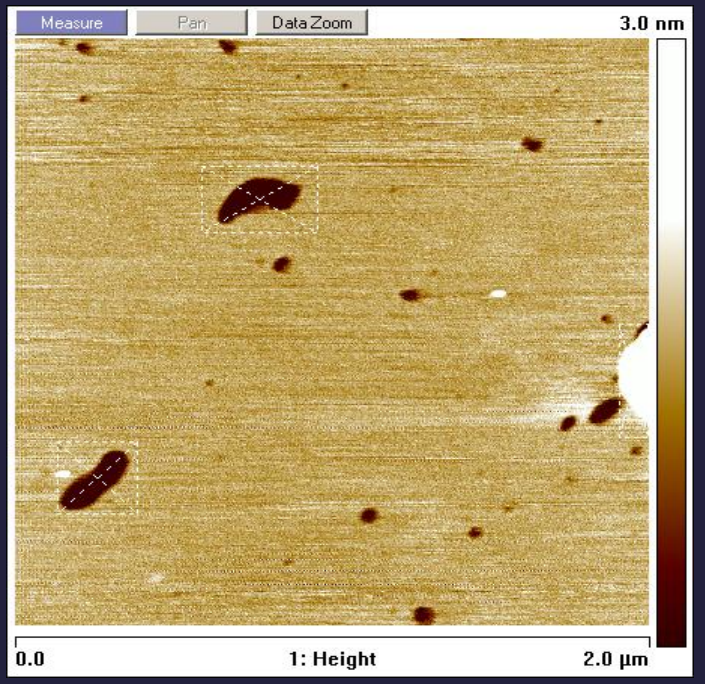
Complete Fusion of Vesicles to Mica Creates Uniform Bilayer

Adopted from Dr. Ilya Reviakine, home.tu-clausthal.de/ ~ire/IMAGE/Vesicle.gif

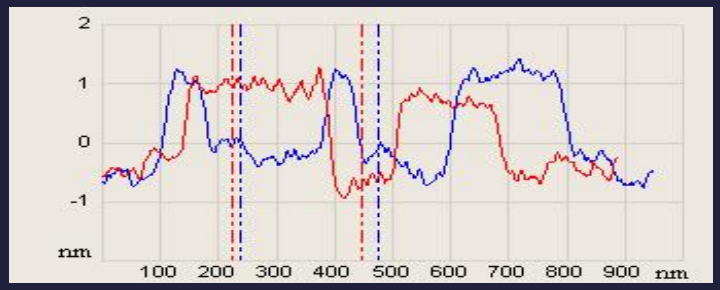
Images of Bilayers

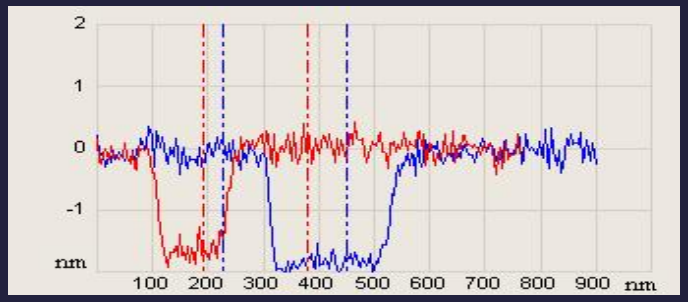


Partial Fusion- Patches of Bilayer



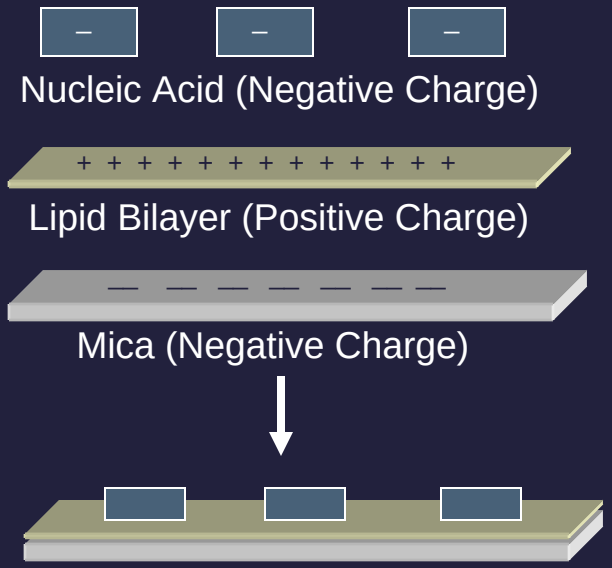
Complete Fusion- Uniform Bilayer

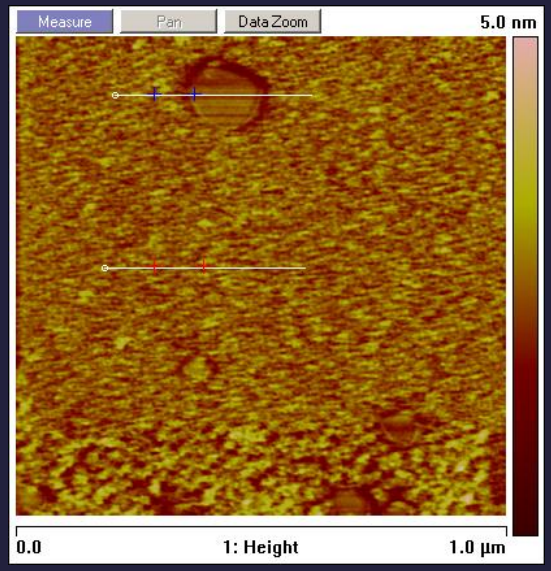


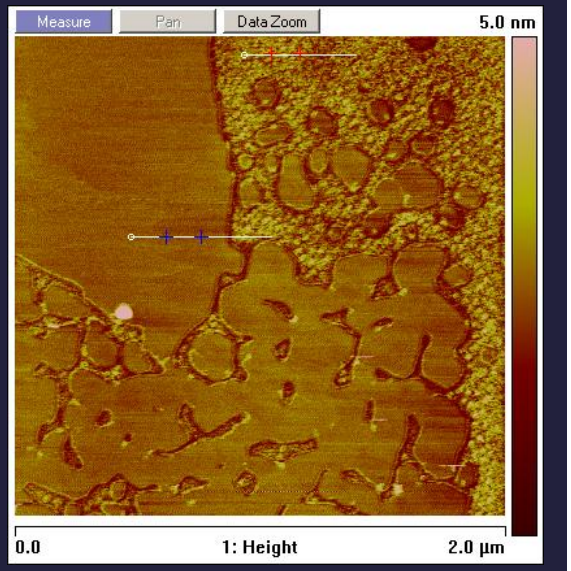


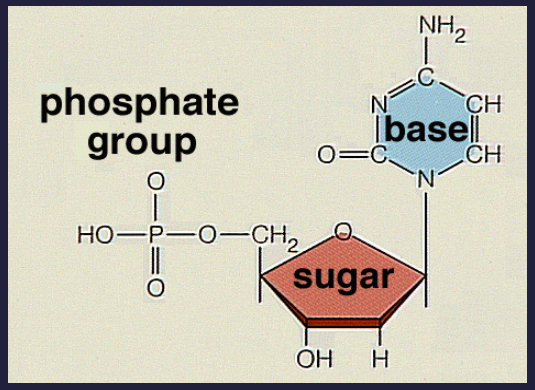
Binding Nucleic Acid

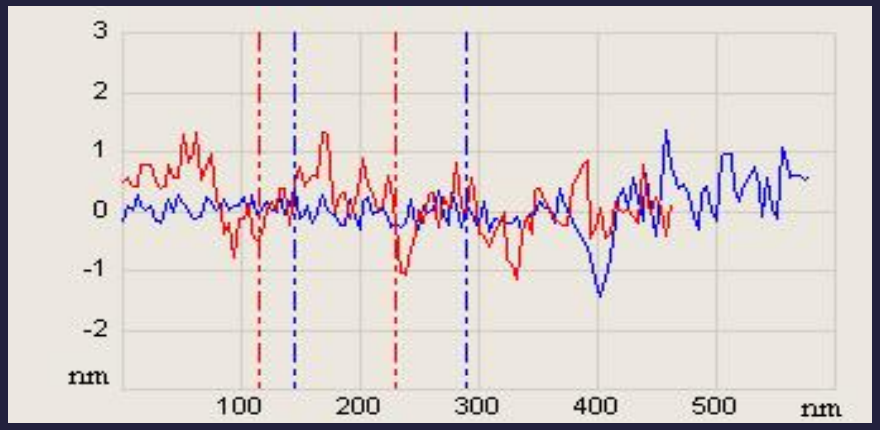
-Double Stranded DNA











nash.cbs.umn.edu/ bs101/pix/nucleotide.gif

Summary of Achievements (So Far)

- Tested Trimethylammonium-Propane (TAP) type of lipids
- Learned how to create lipid bilayers from uni-lamellar vesicles forming a relatively defect free surface
- Introduced DNA to the lipid bilayer surface and confirmed that TAP bilayer surfaces are capable of binding nucleic acids
- In the process of depositing RNA nano-structures to the surface

-Not Binding so far, Why they should? Why they're not?

Future Plans

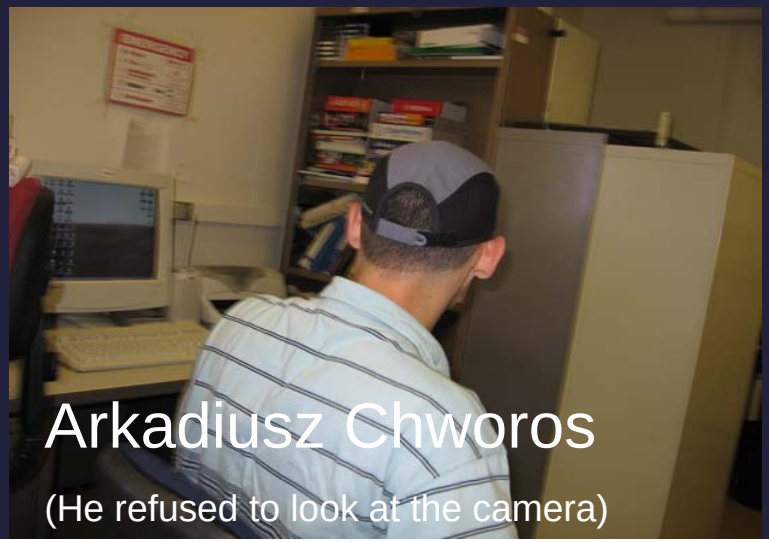
- Improve protocol for lipids with TAP head groups to achieve a reproducible, uniformly flat, AFM suitable lipid bilayer surface for imaging
- Begin testing new compounds

1. Alkyl-amines (Dodecylamine and/or Octadecylamine)
2. Alkyl-Trimethoxy Silanes

- Test these new surfaces for their ability to bind nucleic acids, and if time permits RNA nano-structures

Acknowledgements

Special Thanks To…



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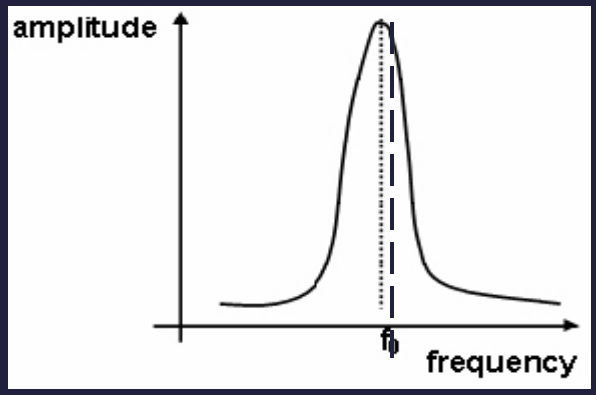
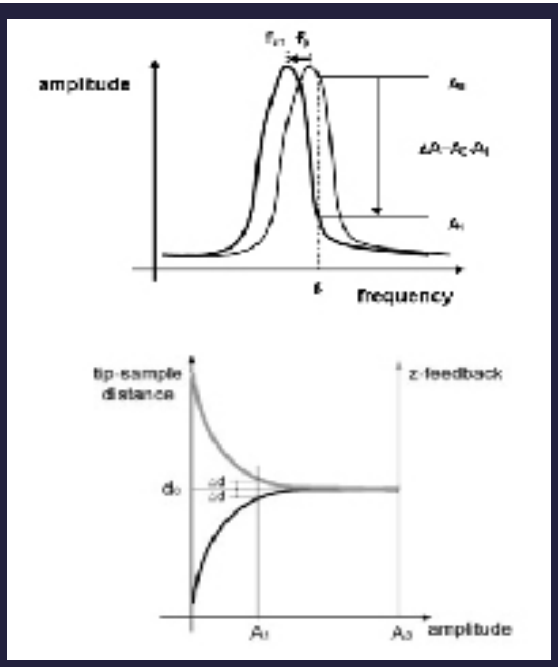
INSET
Internships in Nanosystems Science, Engineering and Technology

Amplitude/ Frequency

- The cantilever and tip oscillate vertically near the cantilever's resonance frequency
- When the tip nears and/ or contacts the surface resonance frequency of the cantilever is reduced or increased causing amplitude to rise or fall
- Drive frequency is set 5-10% away from the amplitude peak maximum on the slope because it is more sensitive to fluctuations

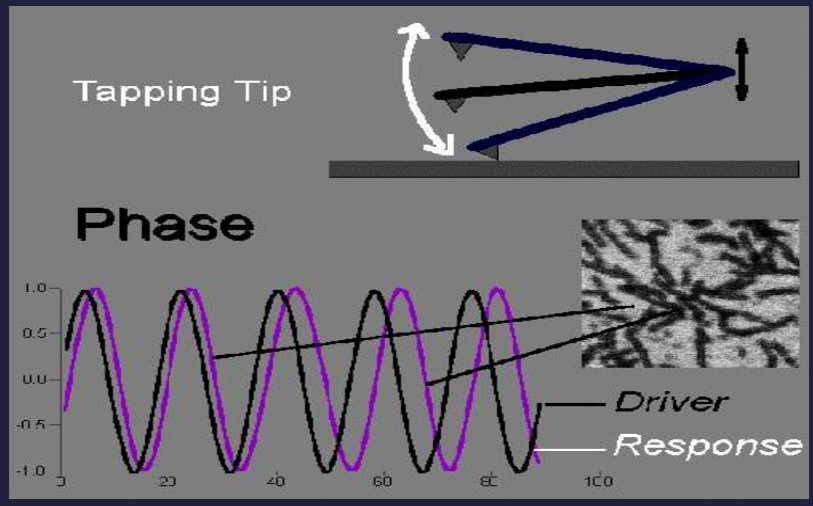


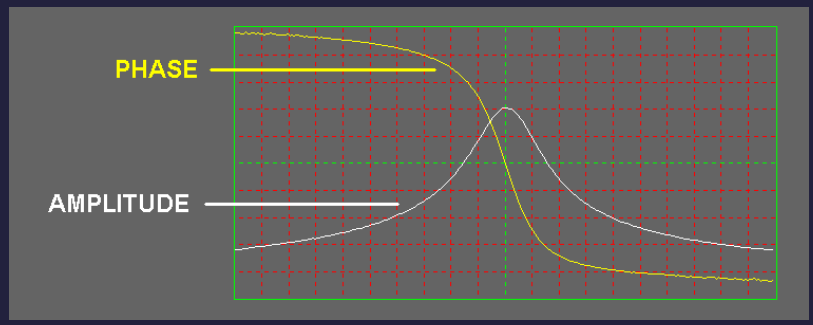


Phase Imaging

phase-*The fraction of a complete cycle elapsed as measured from a specified reference point and often expressed as an angle.*

- Measures the phase (time) lag between the wave detected from tip-surface interaction and wave sent from piezo actuator
- Phase imaging can detect changes in surface composition with high resolution, and can be better than topographical images because details are not obstructed by roughness





http://www.asmicro.com/Applications/phase.htm

Mini-Extruder

Multi-Lamellar Vesicles (MLV) ⟶ Small Uni-Lamellar Vesicles (SULV)

-**Heating Block**
(Allows Temperature Control for Lipids with High Transition Temp.)

-**Syringes** (250 uL)

-**Membrane** (.1nm)