

BIOE Department of Bioengineering
University of Washington

The Sweet Science of Glyco-Nanotechnology

Freshman Seminar
Gen ST 197 D
Nanotechnology & Molecular Engineering

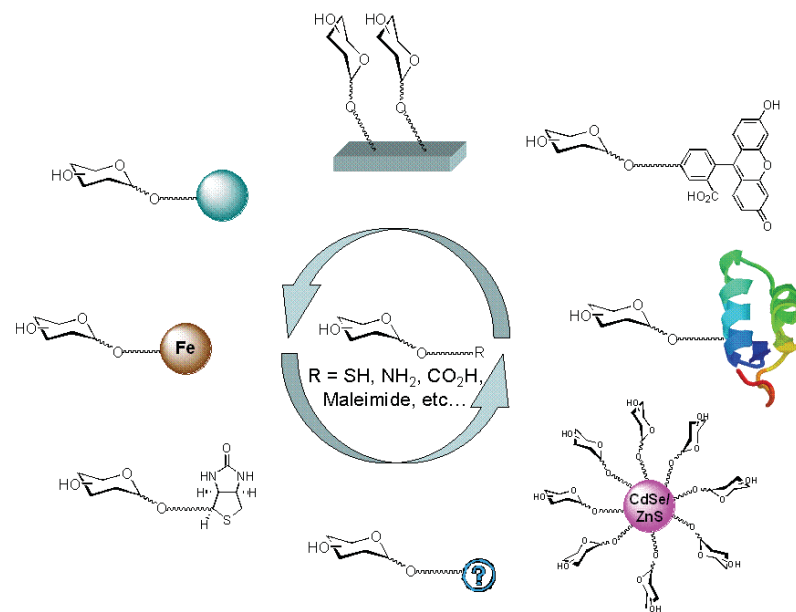
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Dept. of Bioengineering
University of Washington

The sweet surface of a cell - The outer 100 nm

The diagram illustrates the structure of a cell membrane and its surrounding layers. On the left, thick purple fibers represent the extracellular matrix. The glycocalyx, or 'sweet surface', is shown as a layer of purple and green structures extending from the membrane into the extracellular fluid. This layer consists of glycoproteins (purple proteins with green carbohydrate chains) and glycolipids (purple lipids with green carbohydrate chains). The cell membrane itself is a phospholipid bilayer with cholesterol molecules (small orange spheres) interspersed. Integral proteins (purple) are embedded in the bilayer, while peripheral proteins (purple) are attached to the surface. Below the membrane, orange filaments represent the cytoskeleton. The space above the membrane is labeled 'EXTRACELLULAR FLUID' and the space below is 'CYTOPLASM'. A vertical double-headed arrow on the right indicates the thickness of the glycocalyx layer is approximately 100 nm.

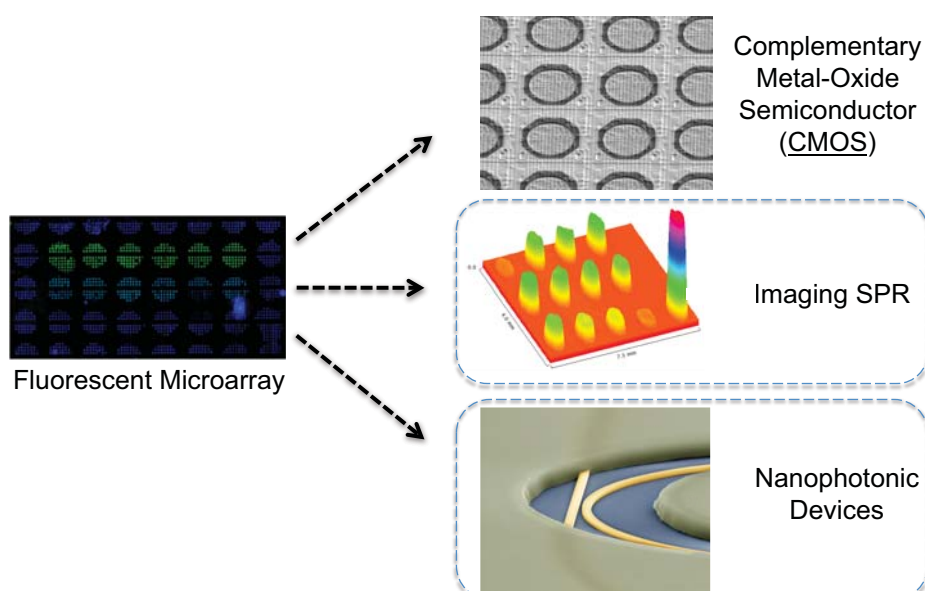
From "Biochemistry," Voet and Voet

Synthetically-Derived Tools for Glycomics

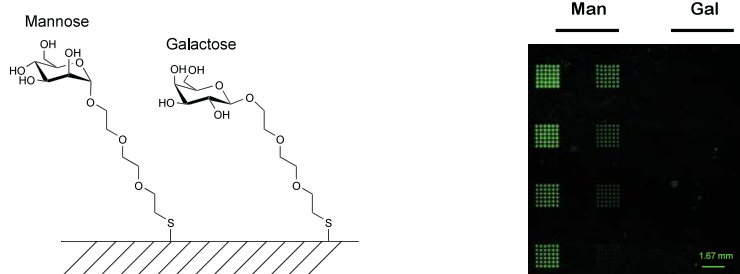


Ratner, Adams, Disney and Seeberger. *ChemBioChem*. 2004.

Developing Label-Free Glycoarray Platforms



OEG-Thiol Linking Chemistry

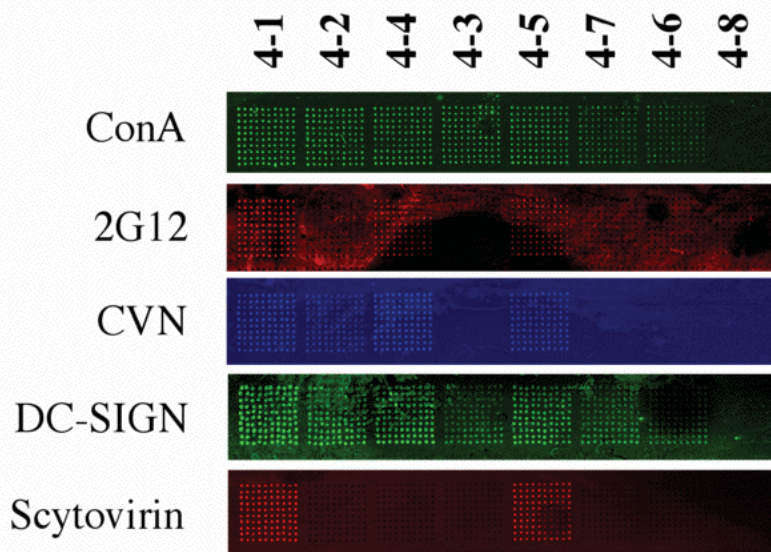


OEG – Ensure carbohydrate ligand is sufficiently solvated

Thiol – Amenable to synthesis, versatile for immobilization

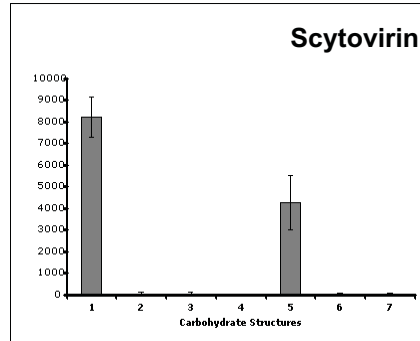
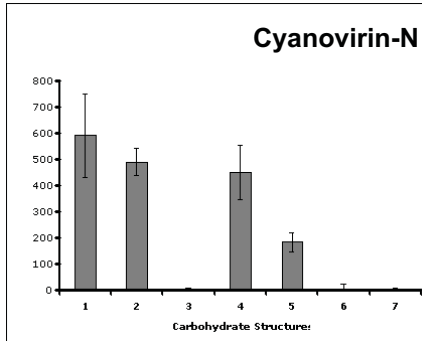
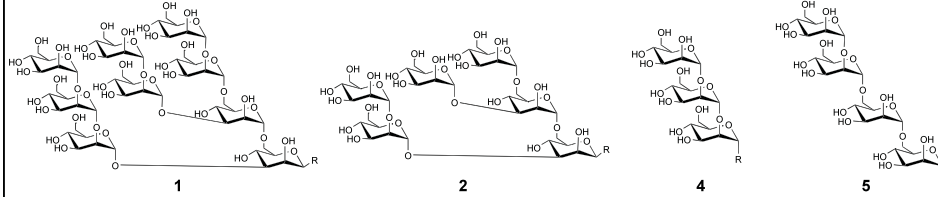
Ratner, D. M.; Adams, E. W.; Su, J.; O'Keefe, B. R.; Mrksich, M.; Seeberger, P. H. Probing Protein-Carbohydrate Interactions with Microarrays of Synthetic Oligosaccharides. *ChemBioChem*. 2004, 5, 379-383.

Screening the High-Mannose Array



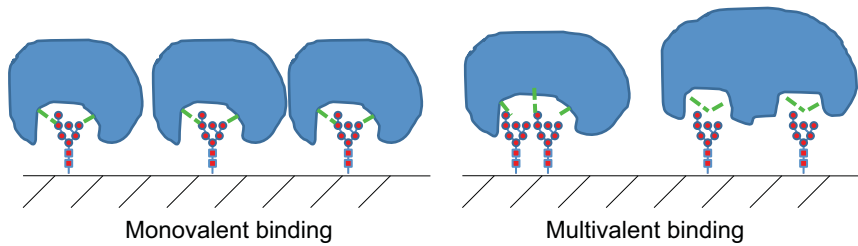
Ratner, Adams, Disney and Seeberger. *ChemBioChem*. 2004.

Antimicrobial specificities

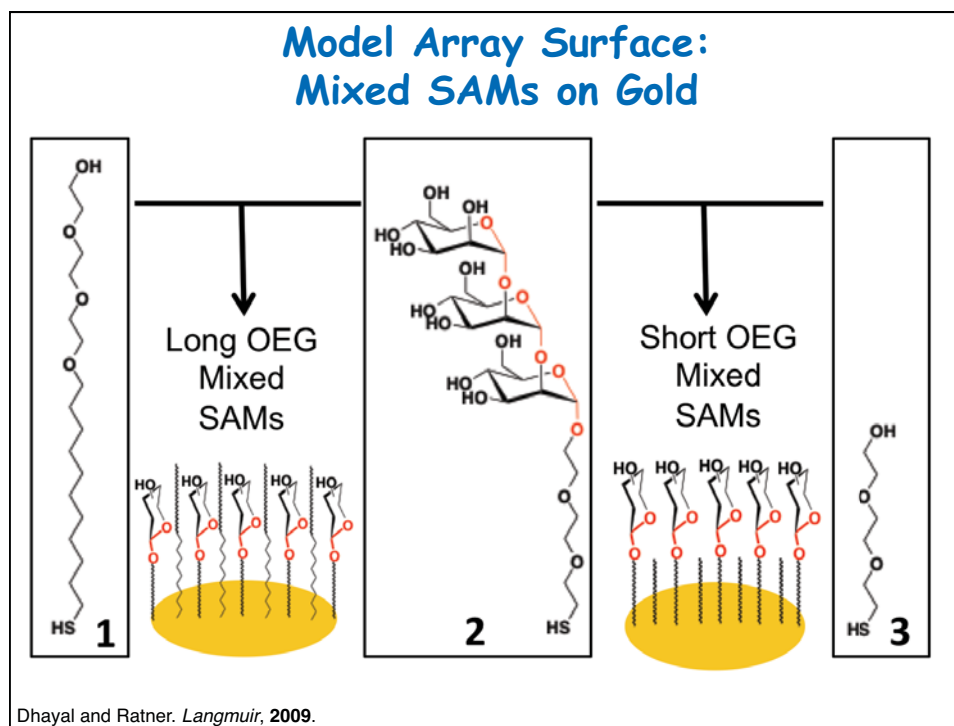


Adams, Ratner, Seeberger et al. *Chem. Biol.* 2004.

Significance of Glycan Surface Density



- Multivalency / Cooperativity
- Antigenicity
- Pathogen binding/inhibition

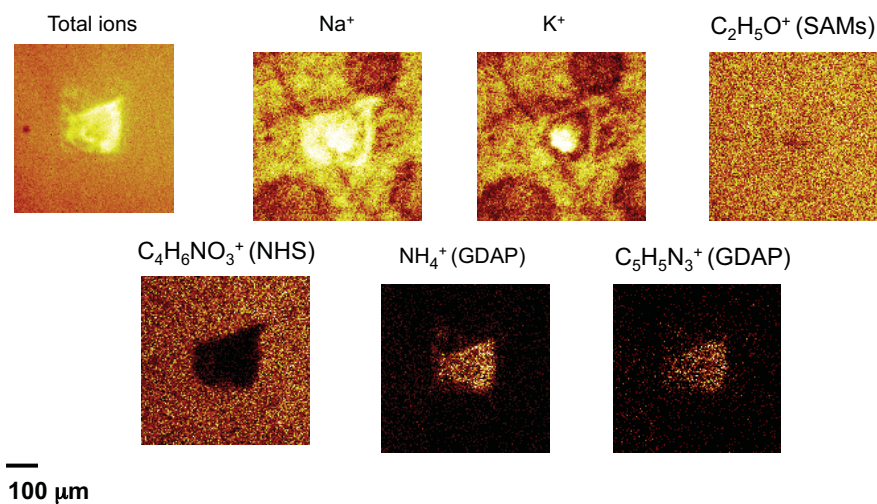


ToF-SIMS

- Provides fragment ion mass spectrum of a surface
- Exquisitely surface sensitive (top 15 Å)
 - High resolution depth profiling
- Able to image features down to ~5 μm
- Can distinguish immobilized sugars

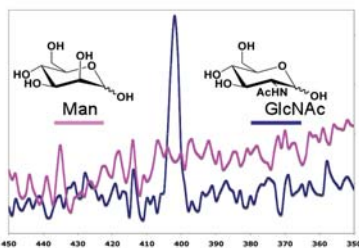
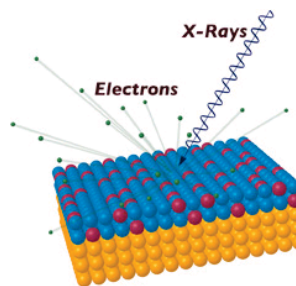
NESAC/BIO

Everything you wanted to know about your surface chemistry, and more...

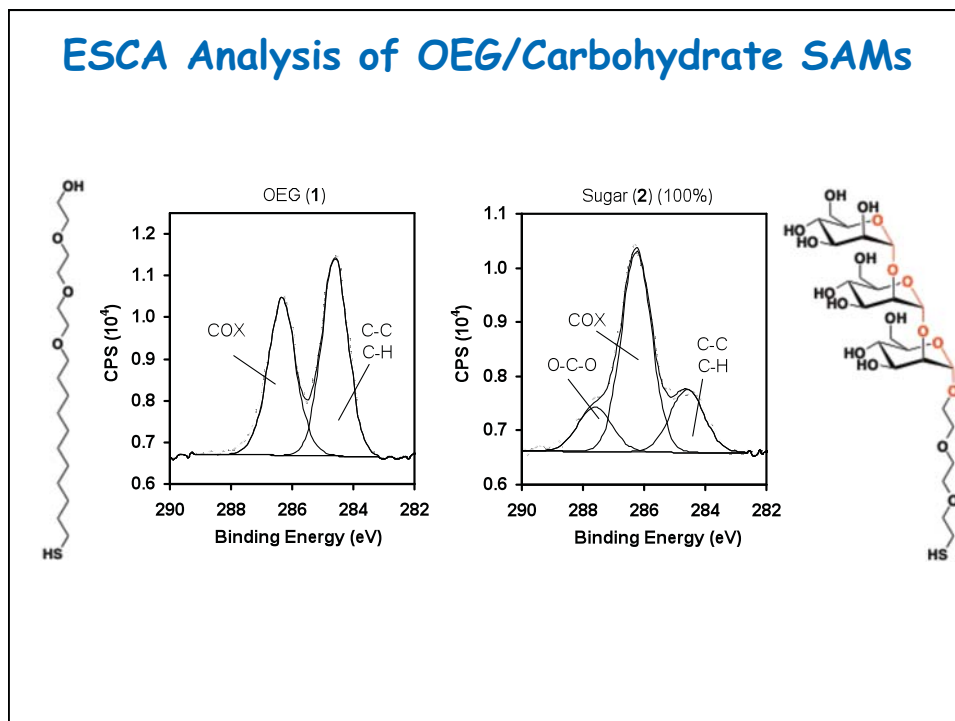


XPS / ESCA

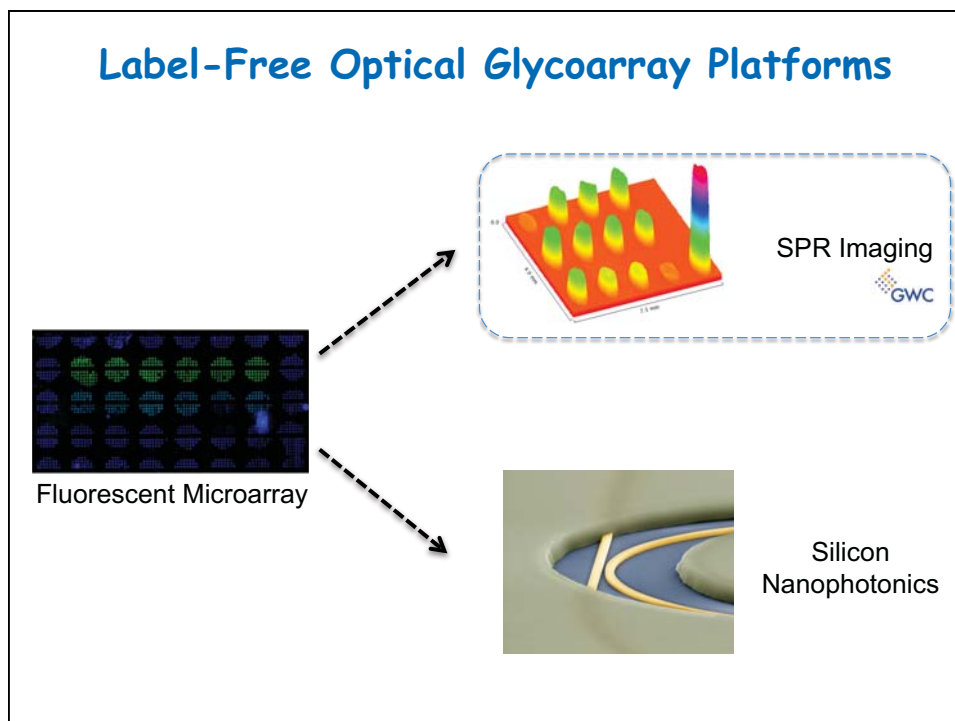
- Establish reference for glycan surface modification
- Composition of glycoarray surface
 - Quantify mixed SAMs



ESCA Analysis of OEG/Carbohydrate SAMs

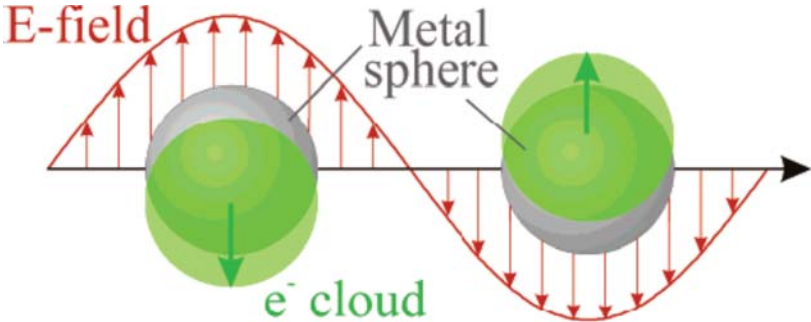


Label-Free Optical Glycoarray Platforms



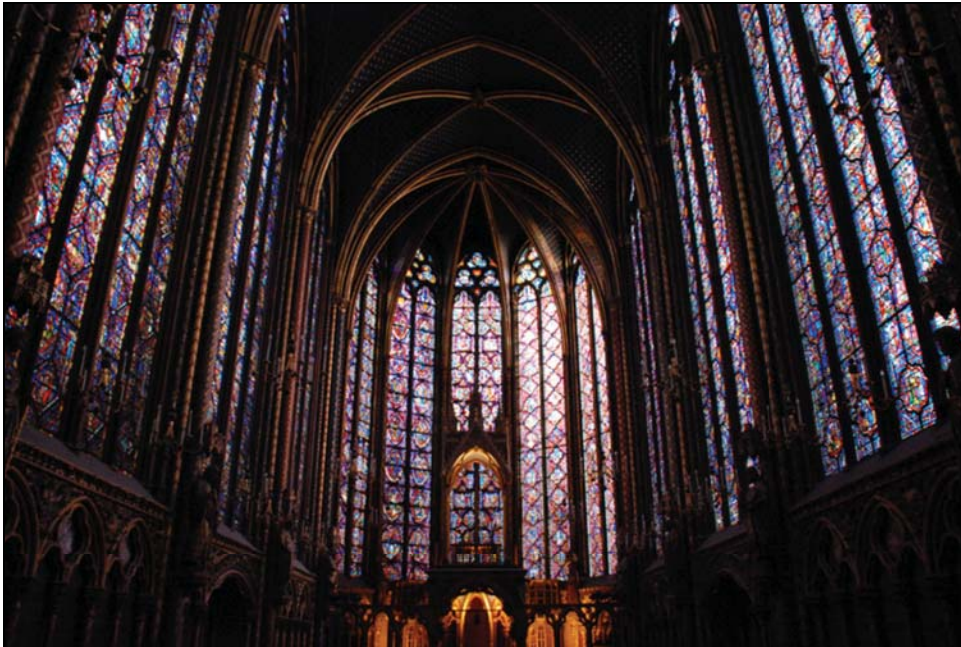
Plasmon Resonance

- Incident light leads to an e^- density wave, or surface plasmon
- Due to sinusoidal electric field



Where have we seen SPR before?

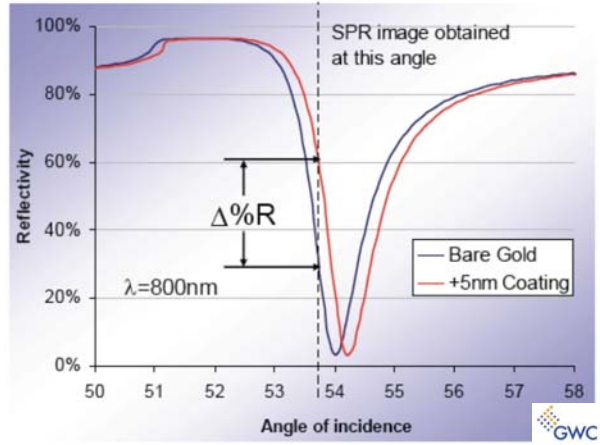
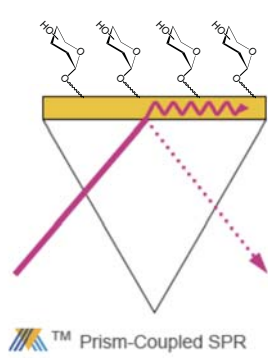
NanoToday, 2007, Vol. 2, No. 1, p18- [Polarized Light: http://mrsec.wisc.edu/Edetc/nanolab/gold/gold03.html](http://mrsec.wisc.edu/Edetc/nanolab/gold/gold03.html)



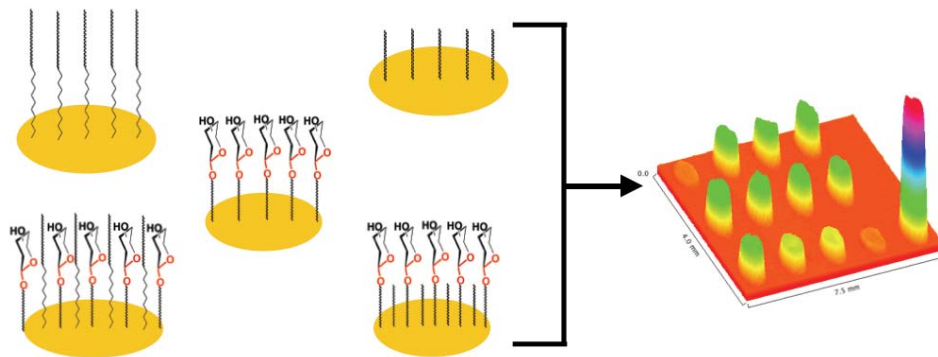
Sainte Chapelle, Paris

http://www.robertpenridge.com/blogger/uploaded_images/saint-chapelle-740112.jpg

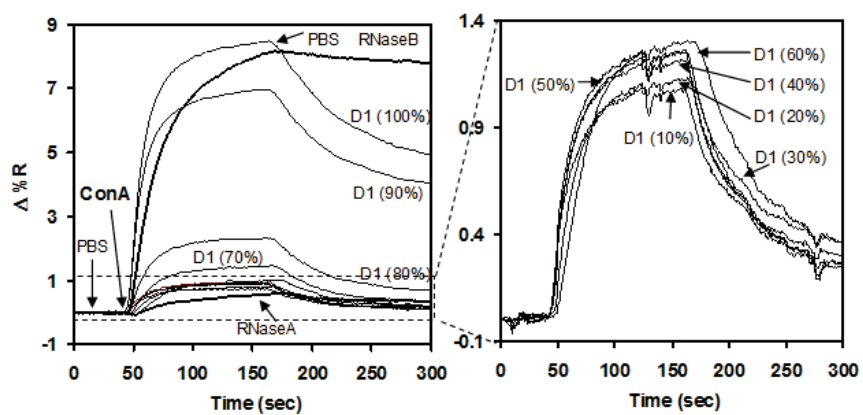
SPR Imaging on the GWC SPRImager®II



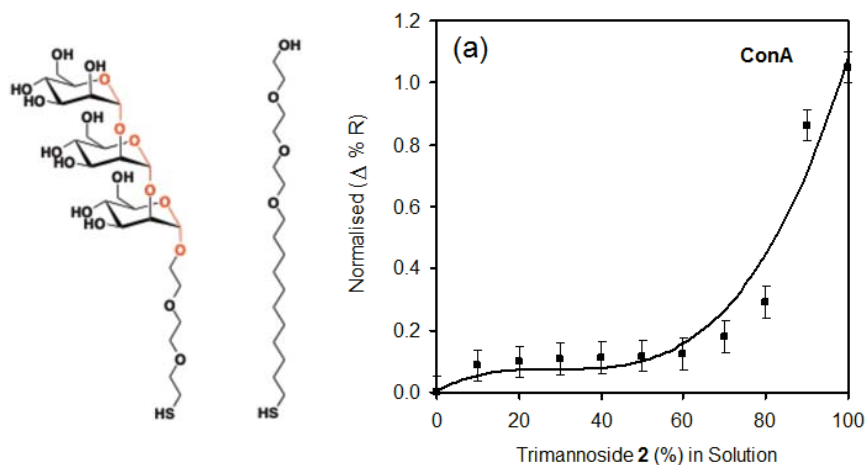
Model SAMs for SPR Imaging Analysis



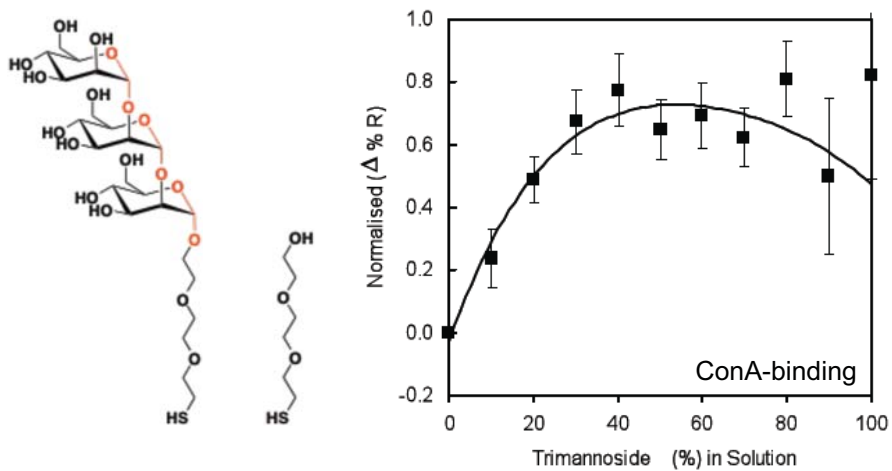
SPR of Mixed SAMs with Alkanethiolate OEG



SPR of Mixed SAMs with Alkanethiolate OEG



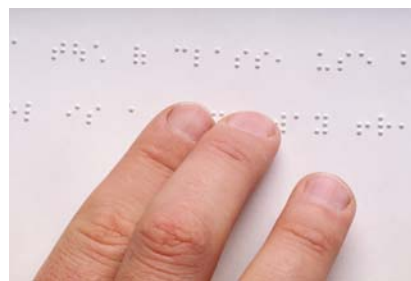
SPR of Mixed SAMs with short OEG-thiol



'Seeing' is believing
Feeling



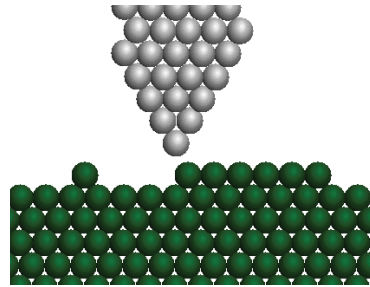
Microscopy



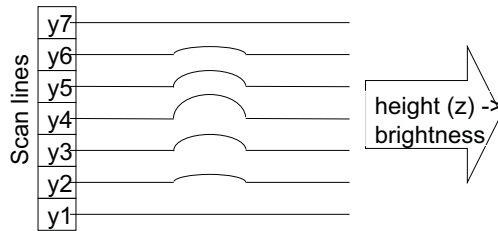
Scanning Probe Microscopy

Scanning Probe Microscopy

- Sample moved by piezoelectric actuators with nanometer resolution
- Å possible

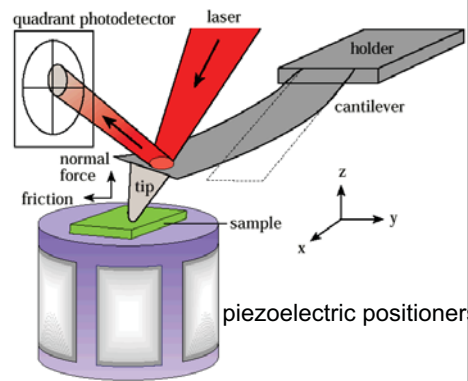


mrsec.wisc.edu



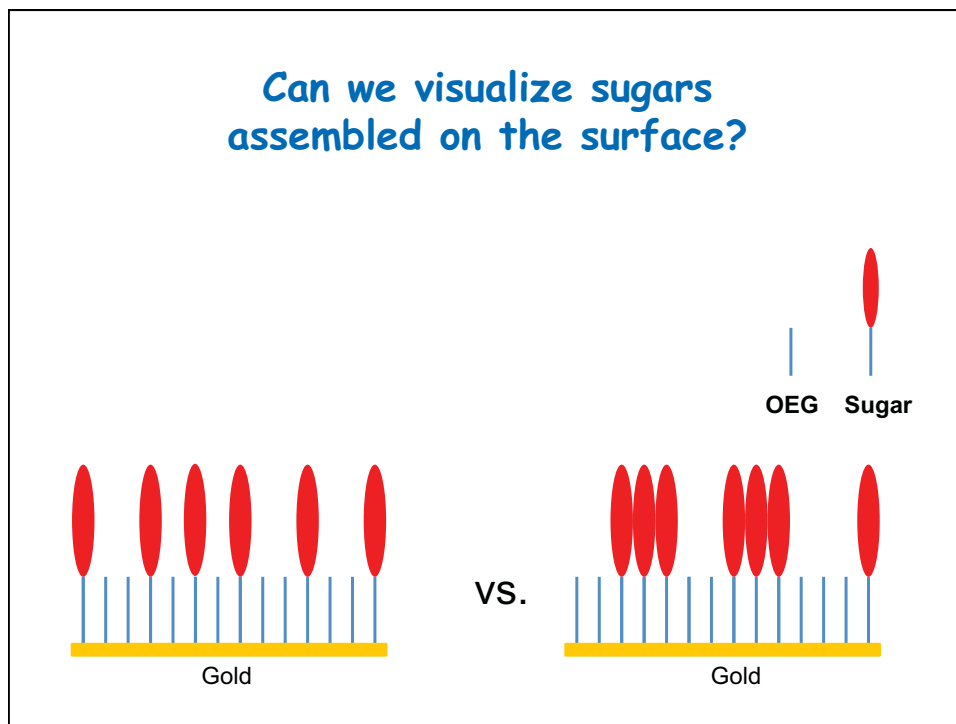
Atomic Force Microscopy

- Atomic force microscopy
 - tip displaced up/down
 - measure light reflected off cantilever

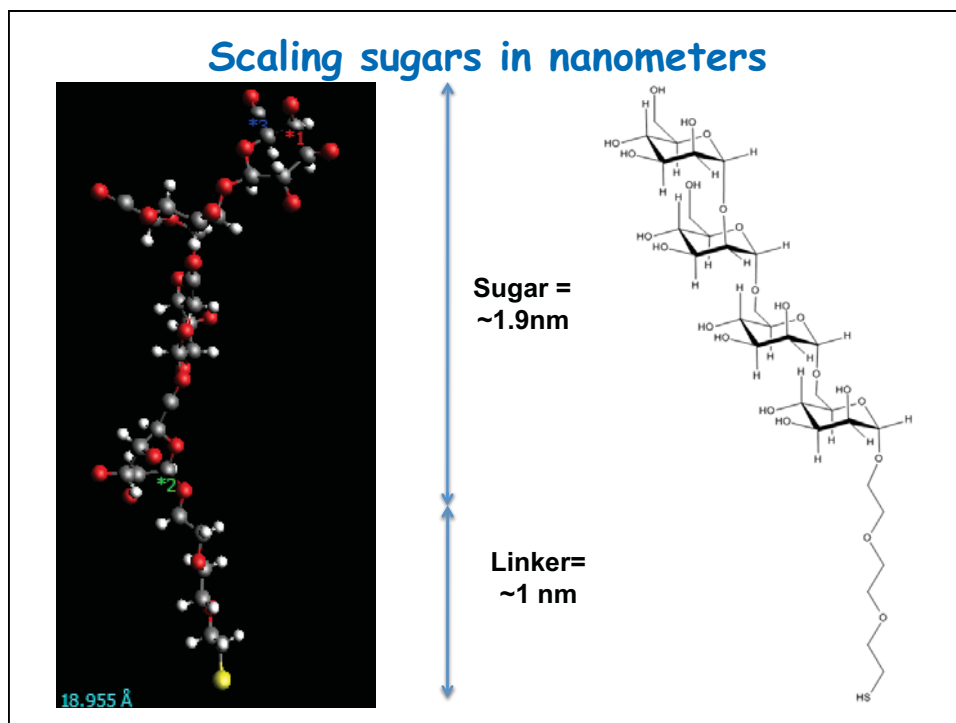


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Can we visualize sugars assembled on the surface?



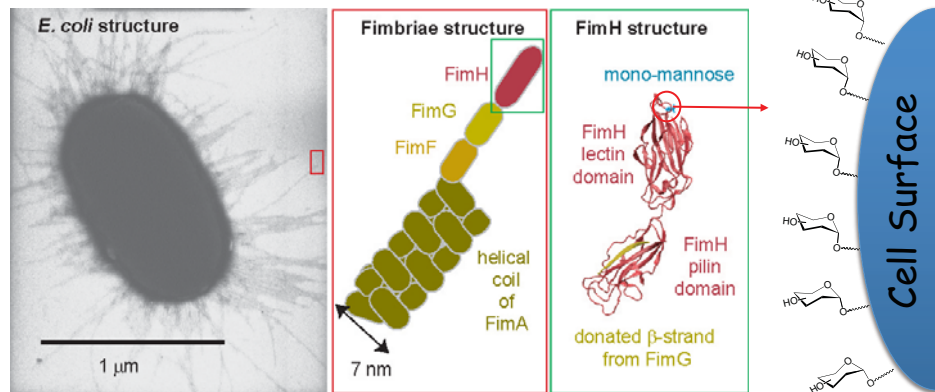
Scaling sugars in nanometers



Can we use these tools for Biology?!

Bacterial Adhesion

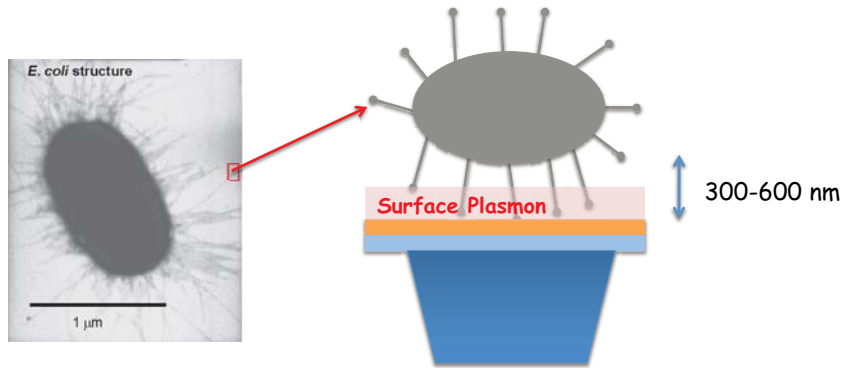
Structure of *E. coli* Adhesin FimH



> 90% of *E. coli* and other enteric bacteria have Type I Pili

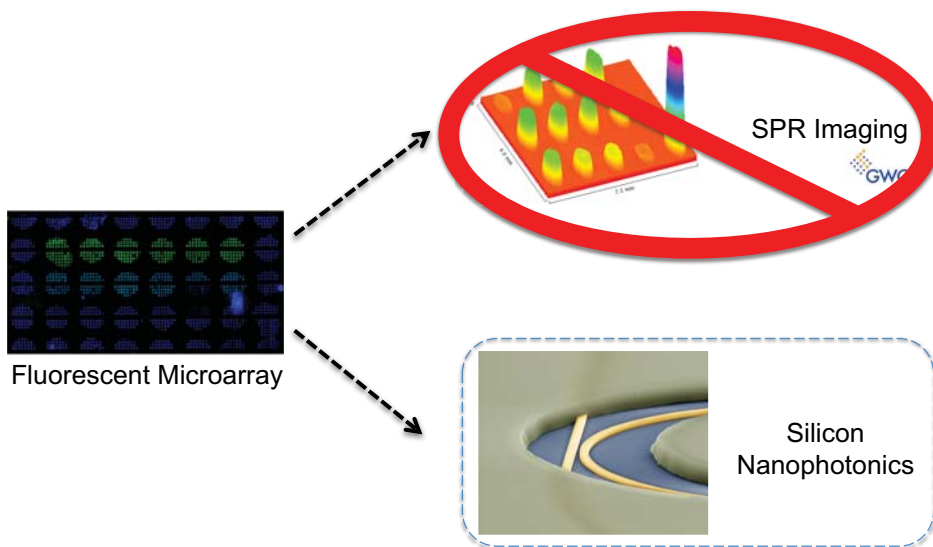
*Courtesy Wendy Thomas (UW, Bioengineering)

Limited Range of Surface Plasmon (~200 nm)

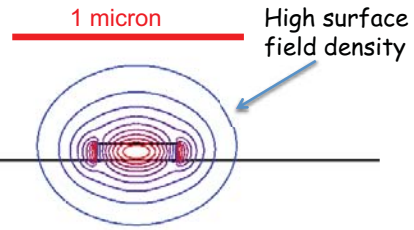
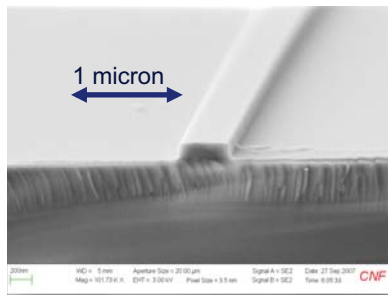


- Surface plasmon extends ~200 nm
- Pili limit effectiveness of SPR for direct detection

How can we extend the range of detection?



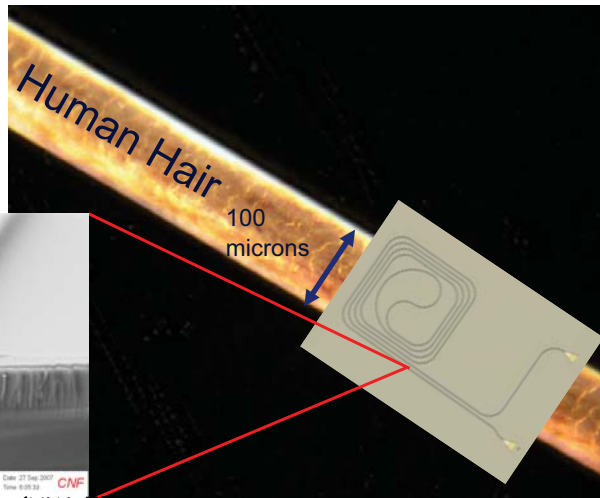
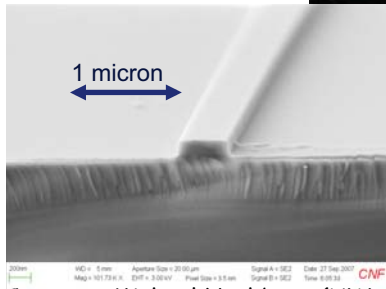
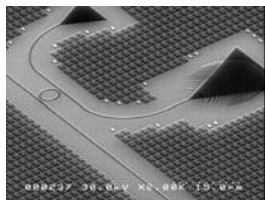
Silicon Photonics: Extending the Range of Label-Free Detection



- World's premier material for making nanostructures
- Billions of dollars of infrastructure for making silicon devices

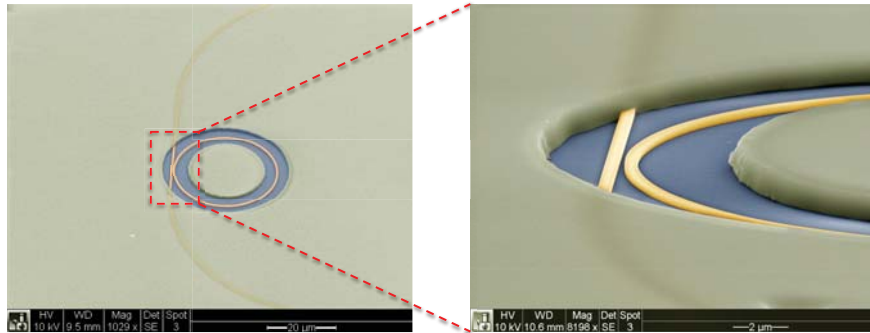
*Courtesy Michael Hochberg (UW, Electrical Engineering)

They call it Nanophotonics for a reason



*Courtesy Michael Hochberg (UW, Electrical Engineering)

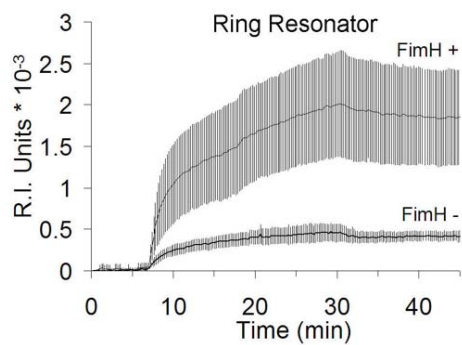
The Silicon Nanophotonic Ring Resonator



- Whispering-gallery-mode biosensor
- Evanescent wave-based detector, range 500-1000 nm

In collaboration with M. Hochberg and Genalyte, Inc.
(Photo credit, M. Hochberg)

RR vs. SPR: Pathogen Sensing



Ring Resonator Glycoarray



- Silicon photonics scales readily
- 10,000+ devices in single chip
- Limited by fluidics and sample handling