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Applications of Super Resolution Microscopy: Imaging Nanoscale Particles and Phenomena

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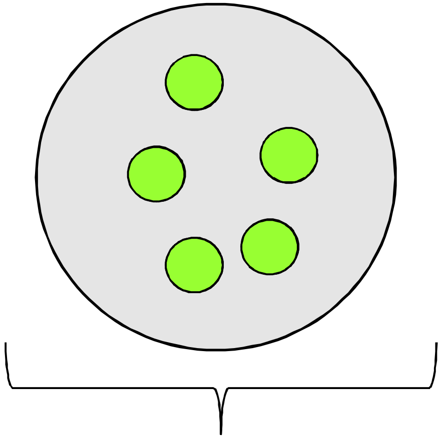
Outline

- Introduction to Super-resolution Microscopy
  - Methods Overview
  - Advantages & Challenges
- Applications:
  - Receptor reorganization during early immune response
  - Cellulase enzyme dynamics
- On the horizon

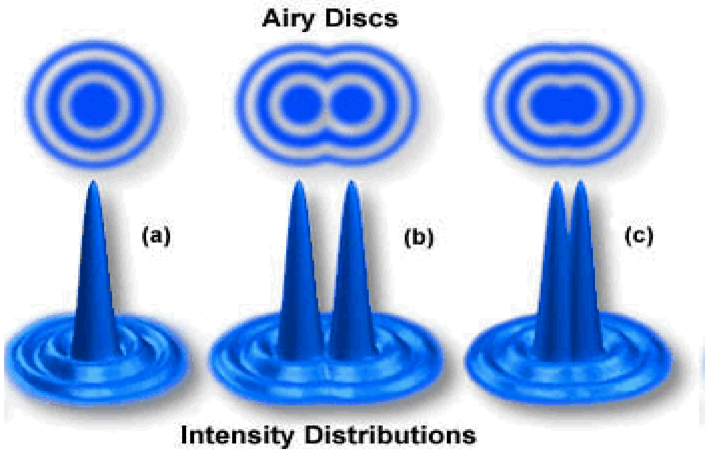
Background

- Optical microscopy is powerful tool for live cell interrogation
  - Interactions
  - Dynamics
  - Kinetics
- Fundamental biological processes occur on a spatial scale below that of optical microscopy



Breaking the Diffraction Barrier

1873: Abbe's discovery Objects in a microscope, closer than ~1/3 wavelength of light can't be distinguished



Light doesn't focus to a point, but instead forms an Airy disk and this limits resolution

$$D = 1.22\,\lambda N$$

1994: Enter Stefan Hell with landmark paper, pioneered a "practical" method to reduce the effective point-spread-function of the microscope to smaller than the dimensions set by diffraction

Current: Probe and illumination-based methods for imaging at resolutions below the diffraction of light *in the far field*

=> Super-resolution (SR) microscopy

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Super-Resolution Methods

Probe-Based

- Like pointillism
  - Stochastically activate & deactivate single molecules
  - Fit each measurement to a Gaussian (high precision)
  - Reconstruct image molecule-by-molecule
- Many different (yet highly similar) subtypes (STORM, PALM, PALMIRA, fPALM, dSTORM)
- Basic premise for all of these methods: photoswitching or photoactivating a probe molecule

Illumination-Based

- Alter the properties of the illumination light to either
  - Permit excitation of signal from sub-diffraction area
  - Permit reconstruct with sub-diffraction resolution
- Stimulation-Emission-Depletion (STED), Ground State Depletion (GSD) and Structured Illumination microscopy (SIM)

Super-Resolution Methods

Illumination-Based

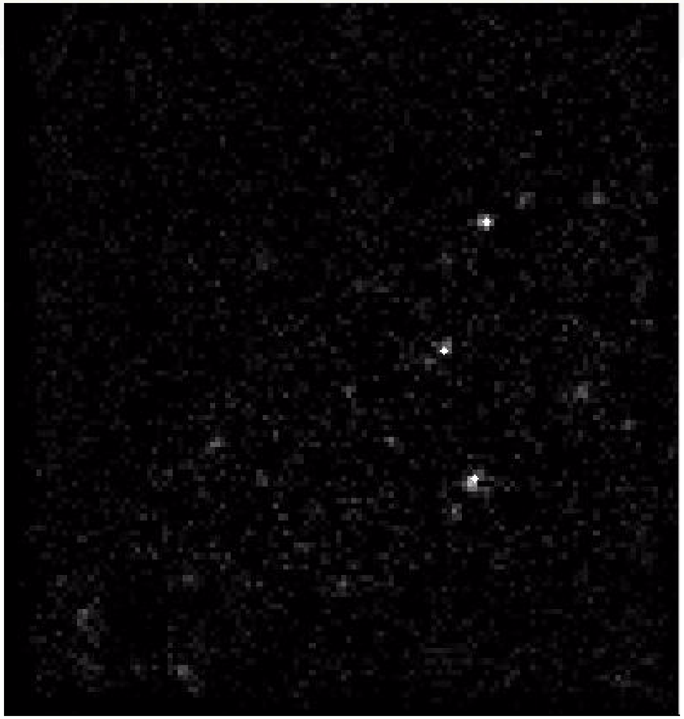
STED Principles

- Live cell compatible, speeds on the order of confocal
- High excitation powers
- Difficult alignment, can be expensive

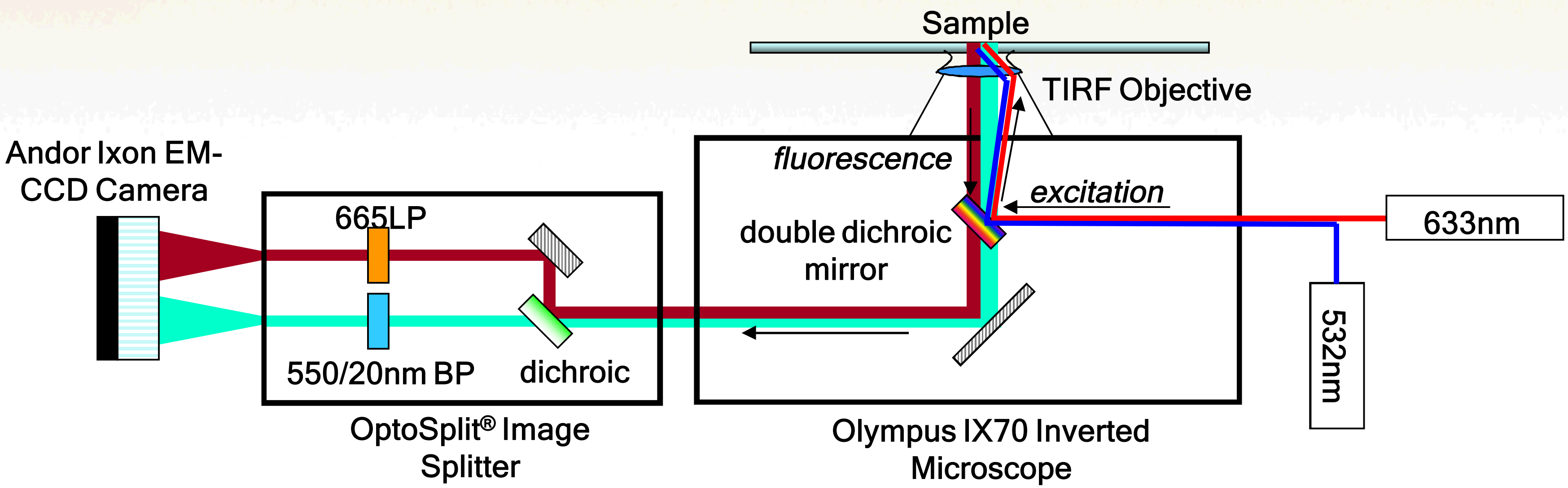
Super-Resolution Methods

Probe-Based

- Very good for interactions occurring at interfaces
- Compatible with single molecule applications
- Straightforward, relatively inexpensive implementation
- Repetitive imaging / reconstruction can be slow
- Limited compatibility with live cells, cytoplasmic processes
- Success is often dependant on the dye



Imaging Setup



- Olympus IX-71, 60x, NA 1.45 TIRF objective
- Capable of up to four excitation wavelengths (choose among 405, 488, 532, and 633nm), variable angle
- Optosplit® image splitter projects multiple emission wavelengths simultaneously onto EMCCD (Andor iXon)
- Capable of >50fps over 30µm x 30µm FOV

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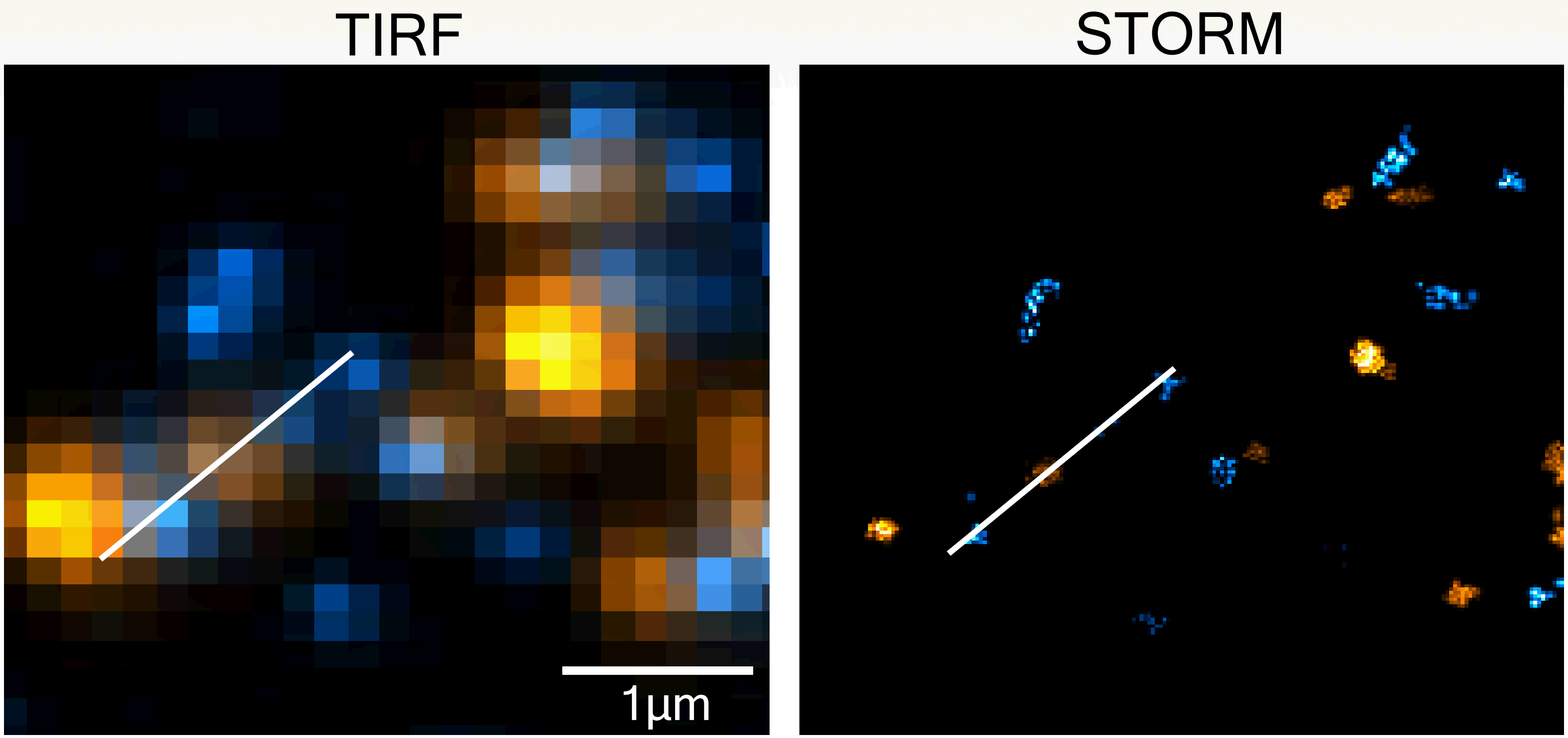
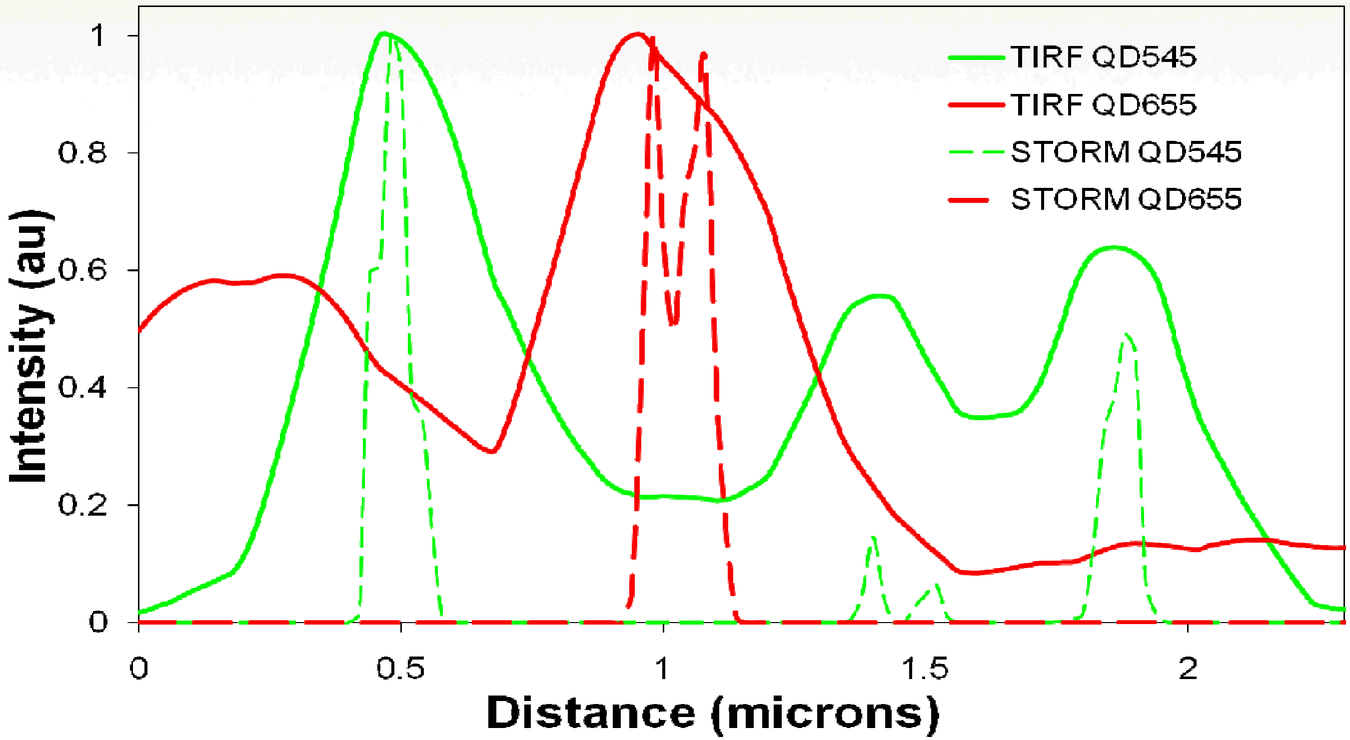
Multi-Color Validation with Quantum Dots



- QD "blinking" acts as efficient photoswitch
- 2 QD types: 585nm (blue) & 655nm (green) emitting

Multi-Color Validation with Quantum Dots



- FWHM <40nm (95% confidence) in STORM vs. 400-500nm for conventional imaging

Imaging Receptor Reorganization During Early Immune Response

APPLICATIONS

Jesse Aaron, Bryan Carson, Jerilyn Timlin
SNL NM: Bioenergy & Defense Technologies

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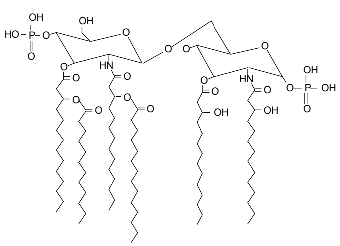
TLR4 Receptor Clustering

***Escherichia coli* (control)**

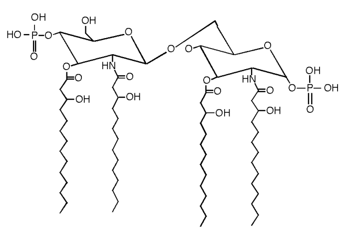
Smooth
O-polysaccharide



Bind Surface
+
↑Stimulatory

***Yersinia pestis* (37°)**

Rough
O-polysaccharide



Bind Surface
+
↓Stimulatory

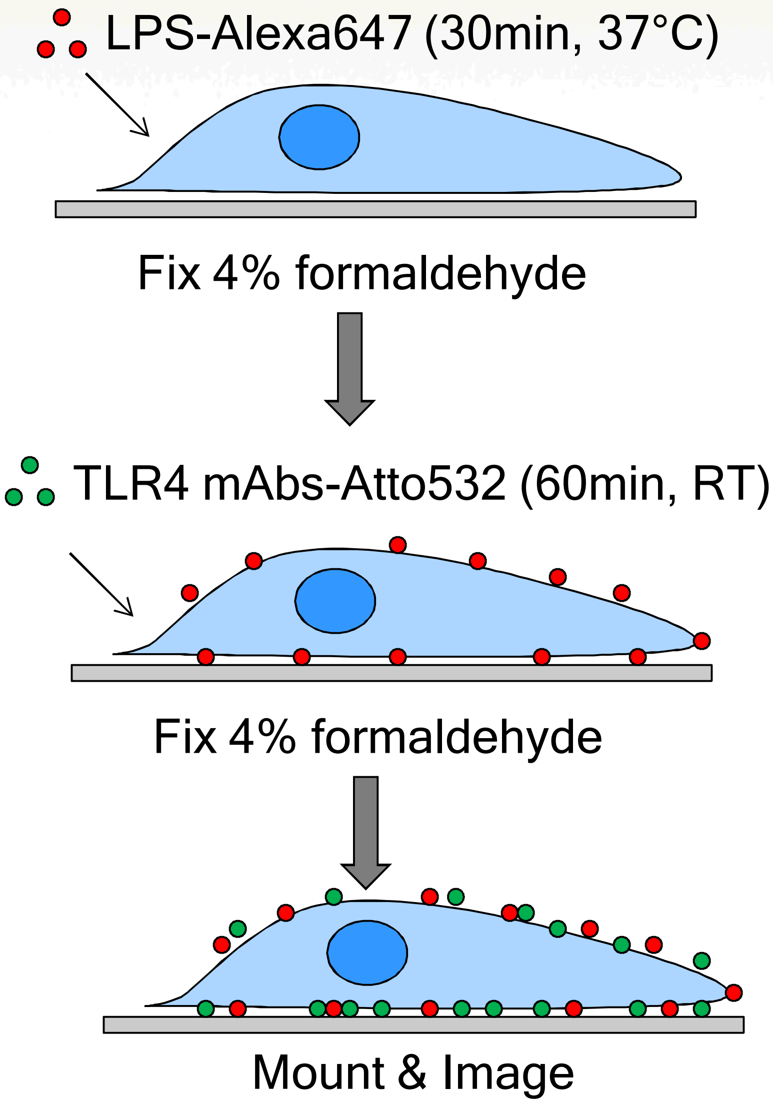
- Bulk assays have suggested that TLR4 molecules aggregate in lipids rafts within the cell membrane after LPS binding

(Triantafilou, et. al, *Biochem. J.* 381(Pt 2): 527-536)

- Differential immune response observed with chemotypes of LPS is not fully understood.
  - LPS from *E. coli* binds & produces an immune response
  - LPS from *Y.* pestis (plague @ 37 °) binds, but does not

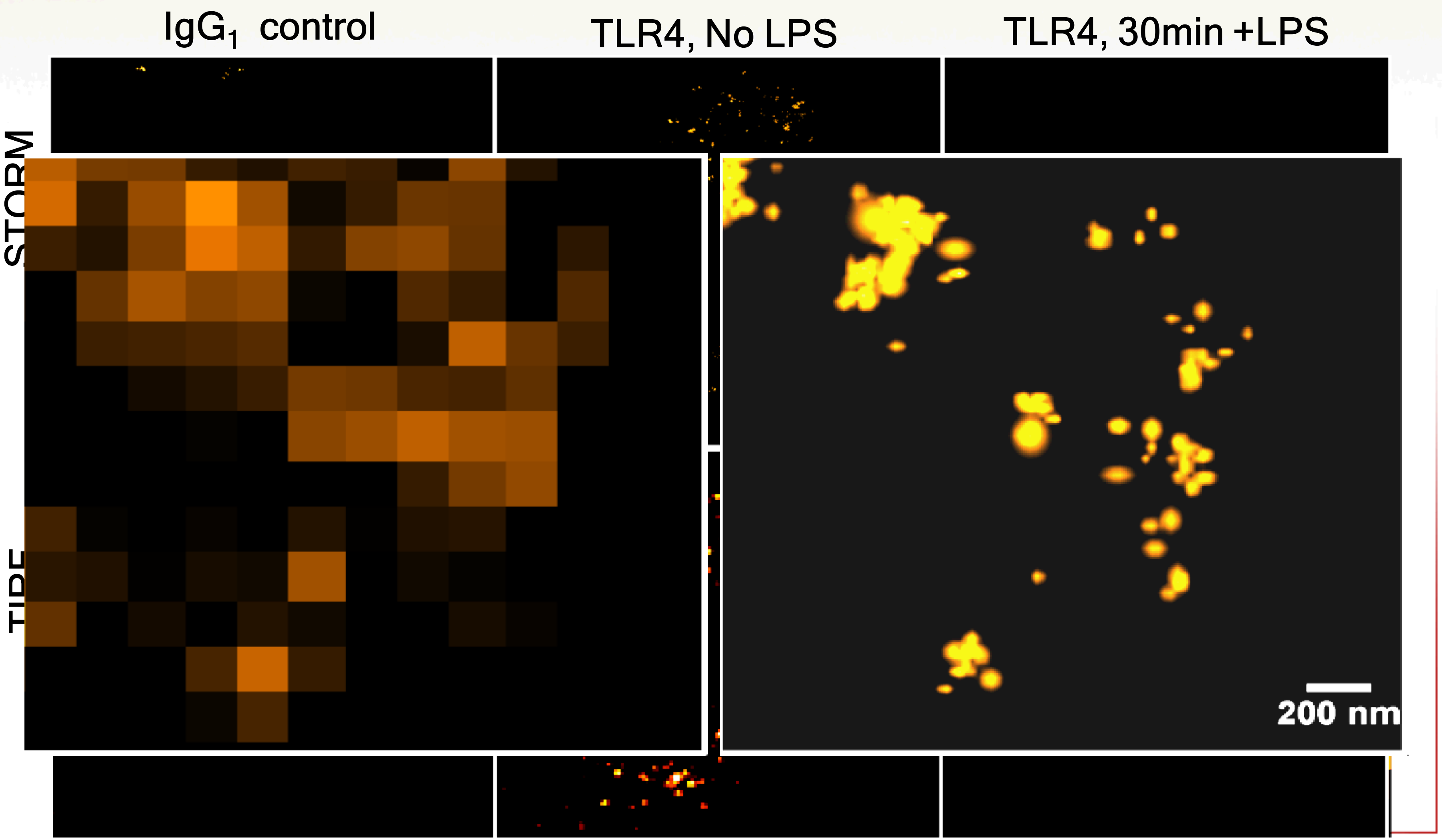
Are there clues in the nano-scale arrangement of the early immune response at the membrane interface?

Experimental Details

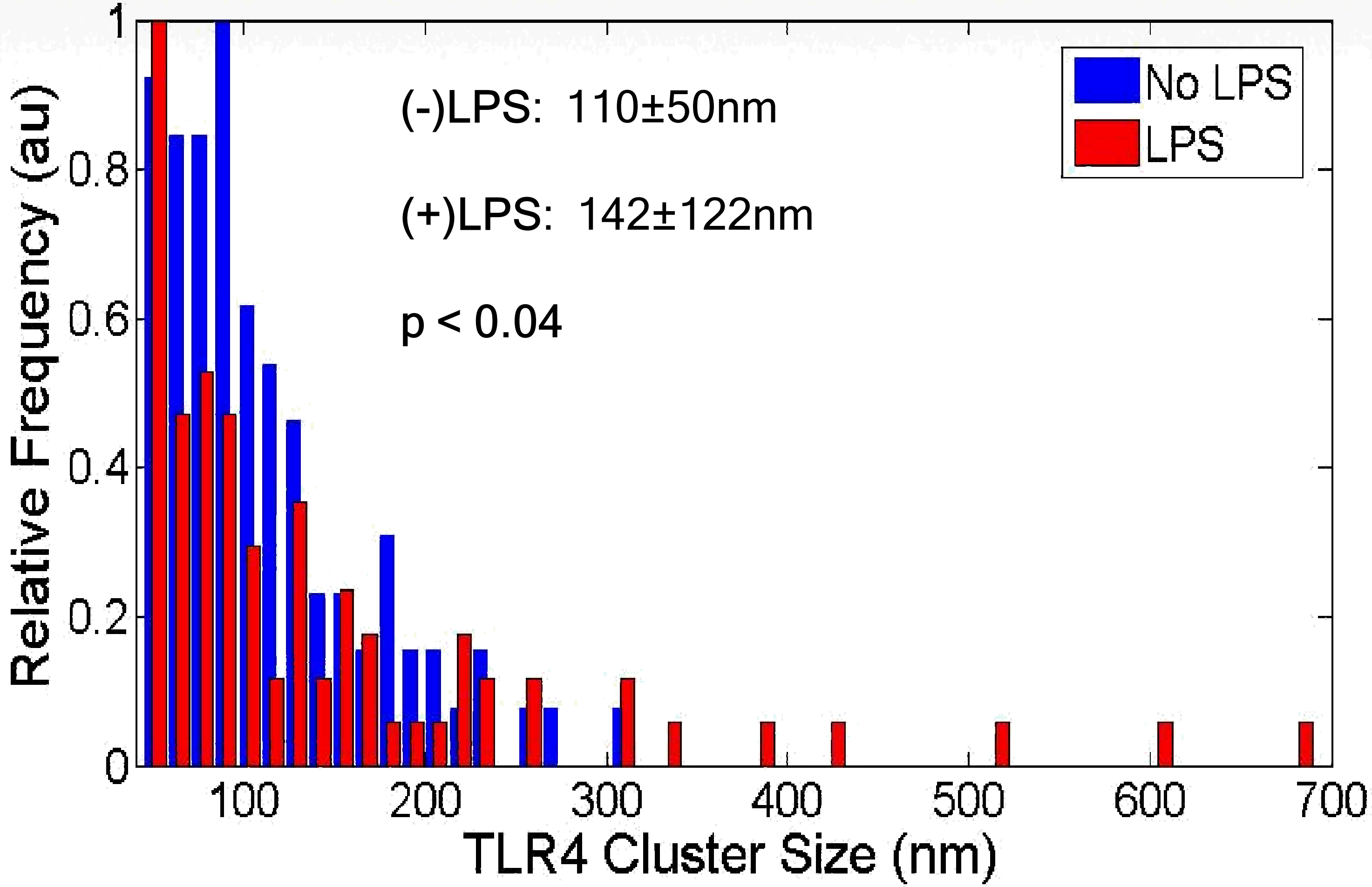


- Mouse macrophage cells (P388D1) incubated with 100nM *E. coli* or *Y. pestis*-derived LPS for 30 min at 37°C and formaldehyde fixed. LPS are labeled with Alexa Fluor 647-hydrazide via linkage with core-polysaccharide
- TLR4 receptors visualized via 1^0 antibodies labeled with Atto532
- Cells imaged in O_2-scavenging buffer containing β-mercaptothiol using dSTORM

TLR4 Receptor Localization

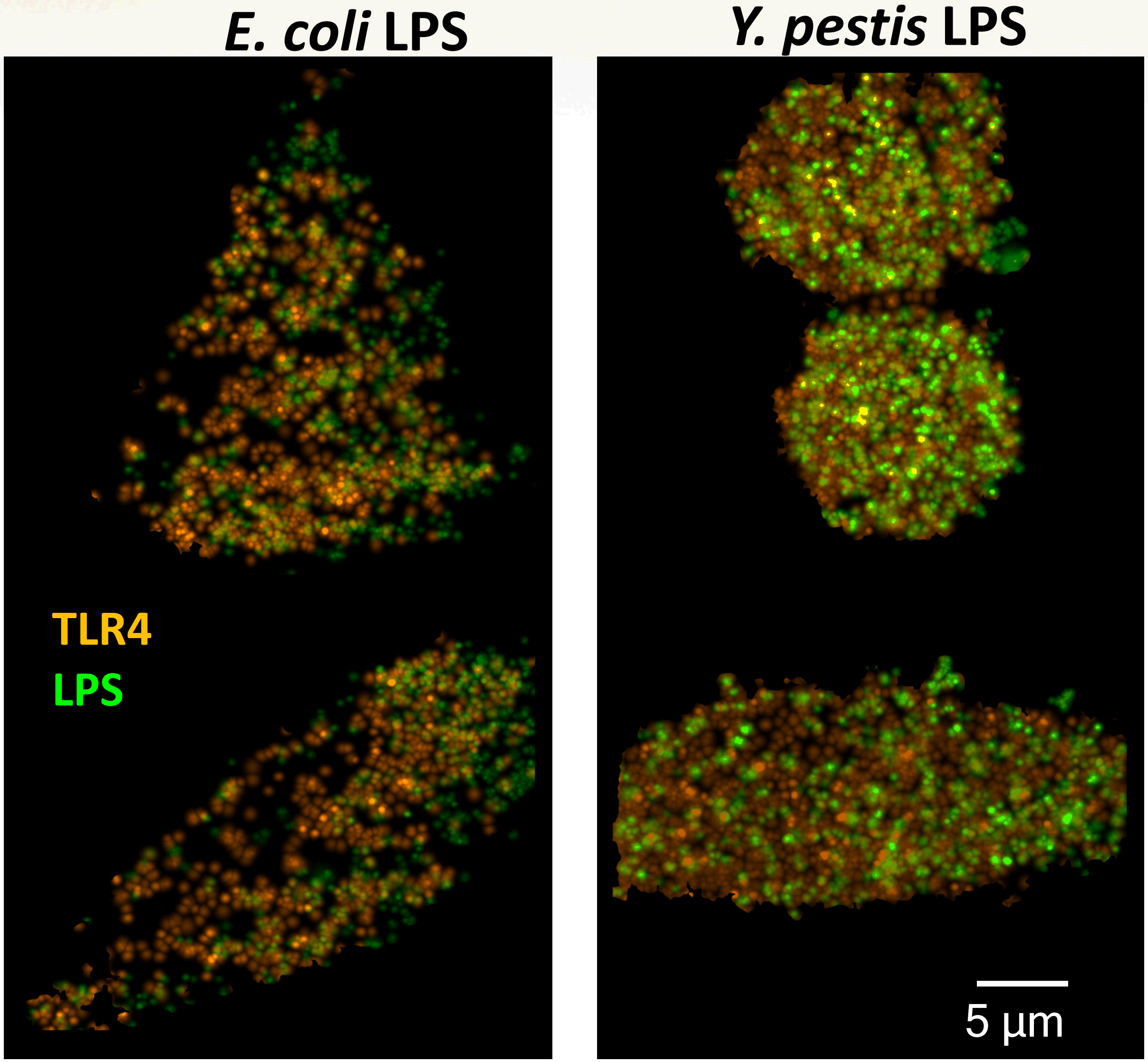


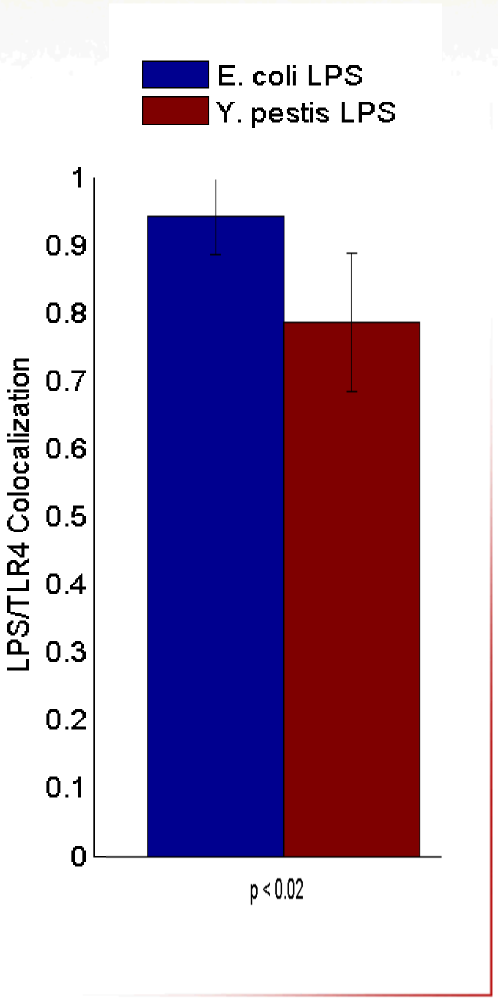
TLR4 Cluster Size



** Differences not apparent in TIRF ($p = 0.7$)

Simultaneous Visualization of TLR and LPS





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Cellulase Enzyme Kinetics

APPLICATIONS

Jesse Aaron, Michael Kent, Jerilyn Timlin
SNL NM: Bioenergy & Defense Technologies
JBEI: Deconstruction Team

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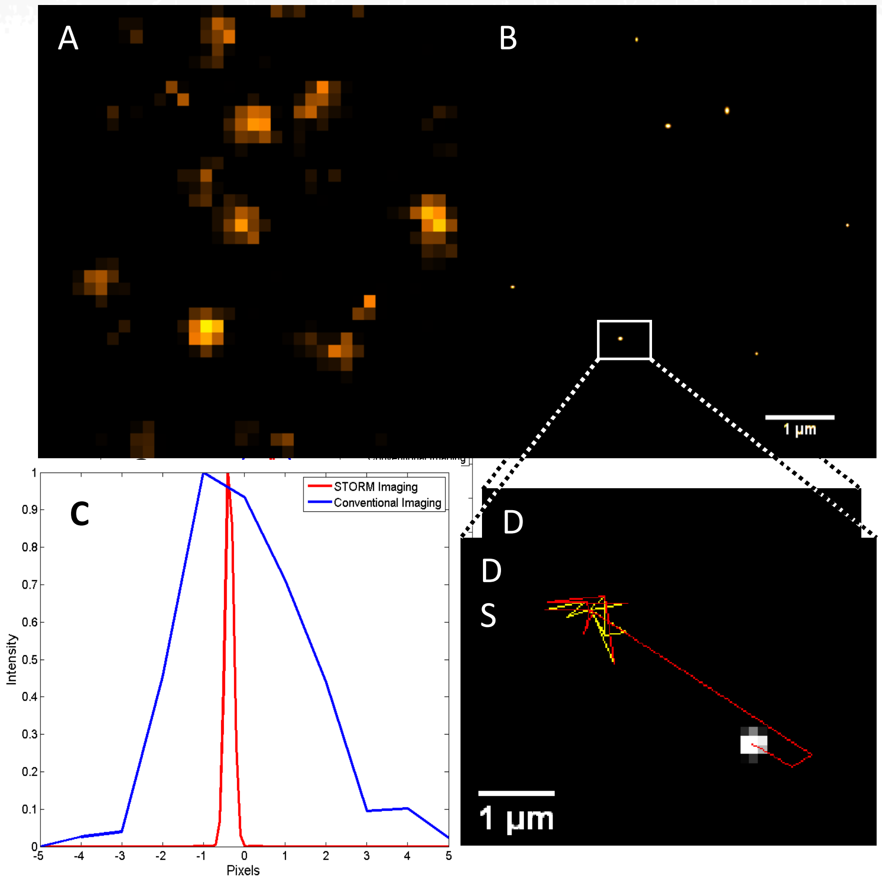
Real-time protein displacements on the nanometer scale



- Single cellulase enzymes on amorphous cellulose substrate
- Yellow and red trajectories correspond to movements over 100 ms and 200 ms, respectively
- Broad implications in many fields

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Challenges of the Future

- Probe-based methods:
  - Temporal resolution, imaging speed
  - Live cell compatibility
  - Algorithms for image reconstruction
- Illumination-based methods:
  - Cheaper, more flexible, easier to use
- Extension to multicolor
- Probes, probes, and more probes!
  - Brighter
  - More photostable
  - Easier to get in
  - Larger variety

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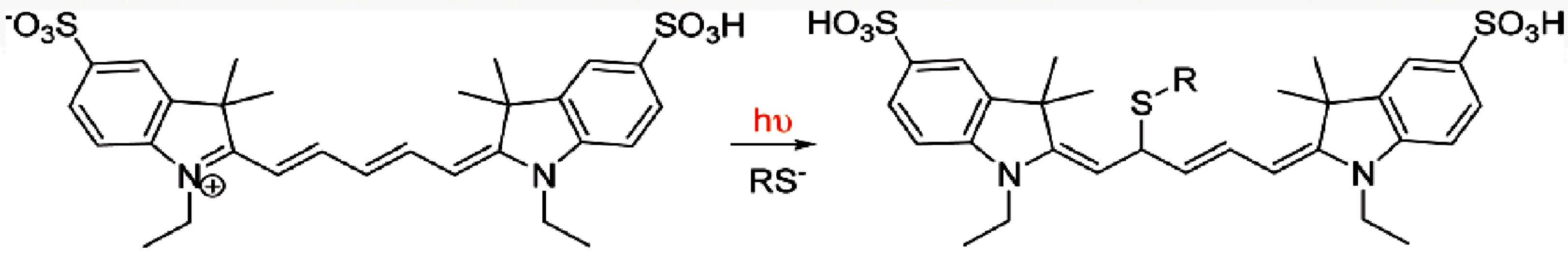
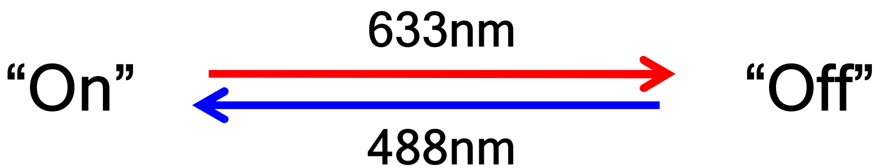
"Direct" Photoswitching

(d-STORM)





- Cyanine-based dyes have been shown to switch between "on" and "off" states in the presence of small thiol-containing molecules and an oxygen scavenging system
- Process is photon-dependent, and the rate can be adjusted via relative intensities of a probe (633nm) and activation (UV-488nm) beam

Dempsey, et. al, *J. Am. Chem. Soc.*, 131(51): 18192-18193 (2009)

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Characterization of Orthopoxvirus Protein Affinity to Chondroitin Sulfate

APPLICATIONS

Jesse Aaron, Jerilyn Timlin, Masood Hadi
SNL NM: Bioenergy & Defense Technologies
SNL CA: Biomass Science & Conversion

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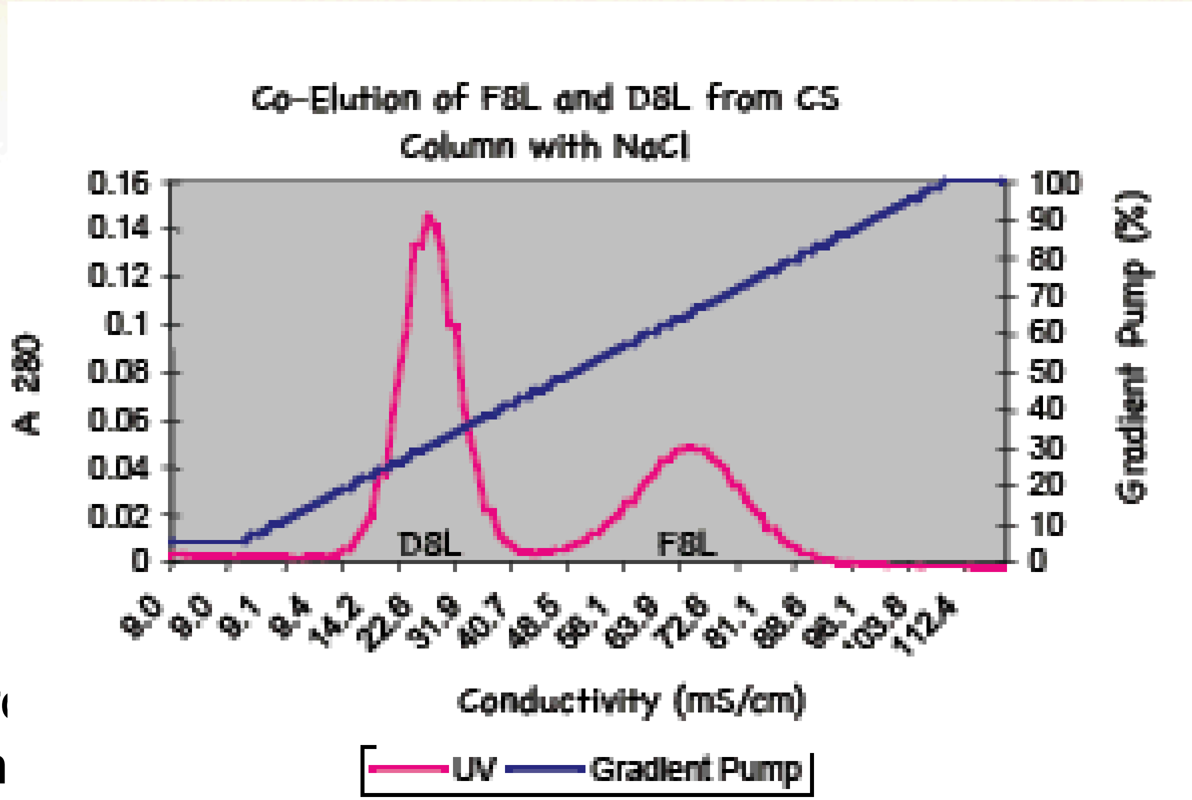
F8L-Like Proteins



- F8L-Like pr… a common an…
- Very close a… ope protein
- D8L shown to bind chondroitin sulfate on host cell surface
- D8L(-) mutants <10% infectivity of wild-type
- F8L shows higher affinity to CS than D8L

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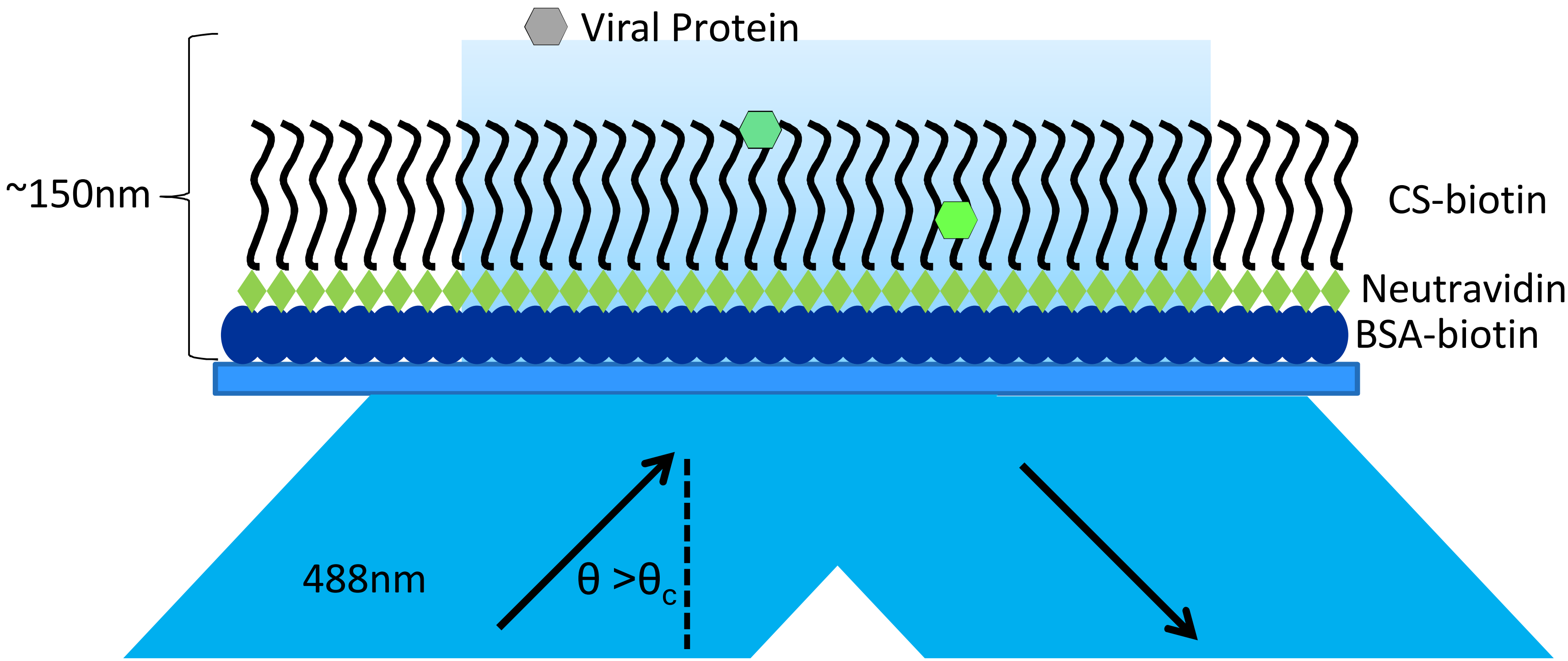
TIRF imaging of viral protein binding to CS

- Affinity column, SPR measurements give bulk/average measurements
- Total internal reflectance fluorescence microscopy =*single molecule* behavior on functionalized surface

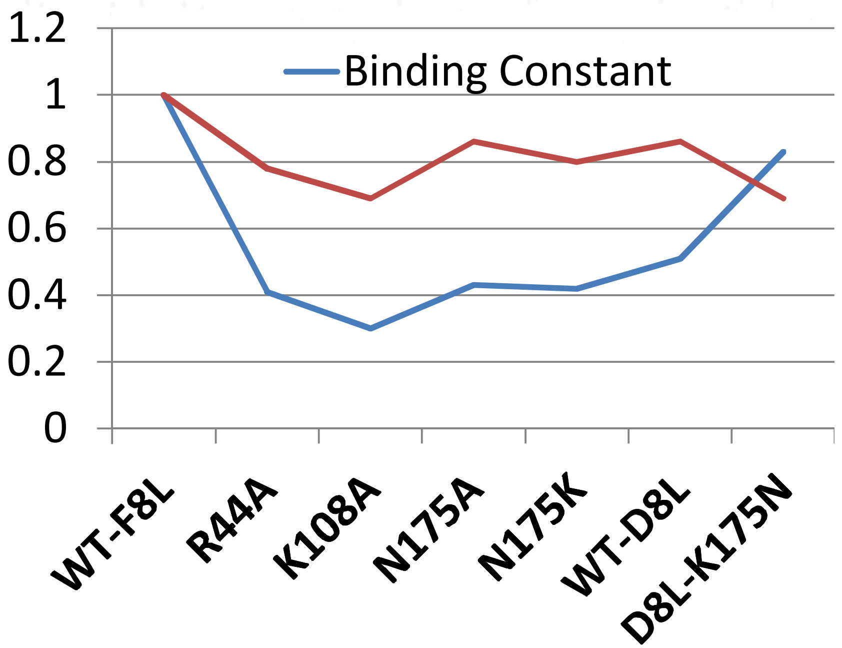


•Evanescent field decays exponentially, i.e. **I ~ exp(-z/d),** Log(Signal) proportional to axial (z) position

•Proteins not bound to CS are not visible, proteins appear brighter nearest glass substrate

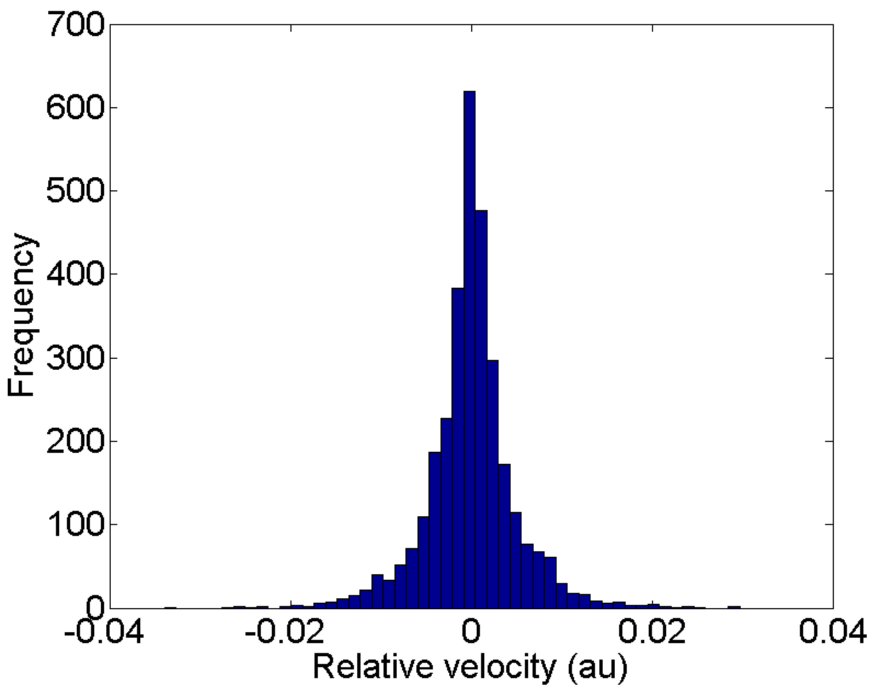
Results

Comparison of Binding Kinetics



Consistent with SPR/bulk measurements

Viral protein movement along CS molecules



Movement does not appear to be directed/unidirectional, Ave velocities centered around zero