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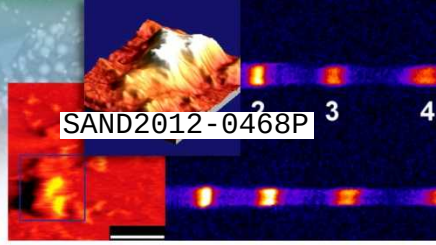

Advanced Imaging for Biodefense and Emerging Infectious Disease

Jerilyn A. Timlin
Principal Member of Technical Staff
Bioenergy and Defense Technologies
Sandia National Laboratories
Albuquerque, New Mexico

Presented at:
Bioscience Advisory Panel Meeting, Livermore, CA
Jan 25, 2012

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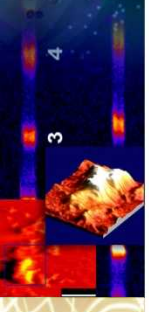
Outline

- Overview of Bioanalytical Imaging for BEID
- Project highlight:

  “Visualizing TLR4 Distributions at the Plasma Membrane with Nanoscale Resolution”
- Conclusions & Future vision



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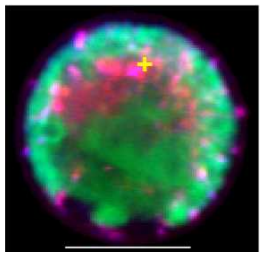
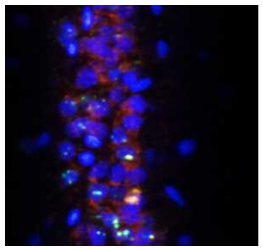
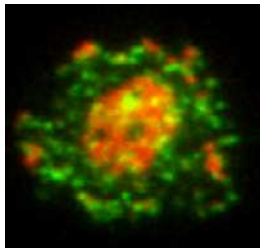
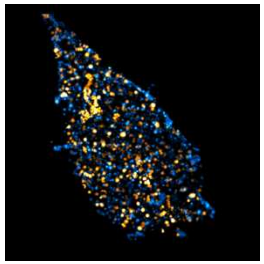
Research Focus

http://bio.sandia.gov/people/timlin.html



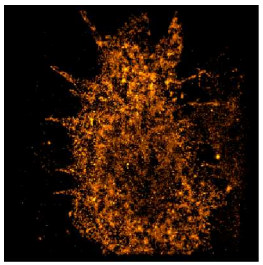


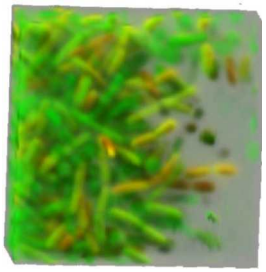




Unraveling Spatial-Temporal Relationships in Complex Multicomponent Biological Systems at Multiple Scales

- Advanced spectroscopy
- Innovative imaging technologies
- Chemometric data analysis tools
  - Multidisciplinary
  - Cell biology, immunology, and microbiology
  - Biodefense and Bioenergy





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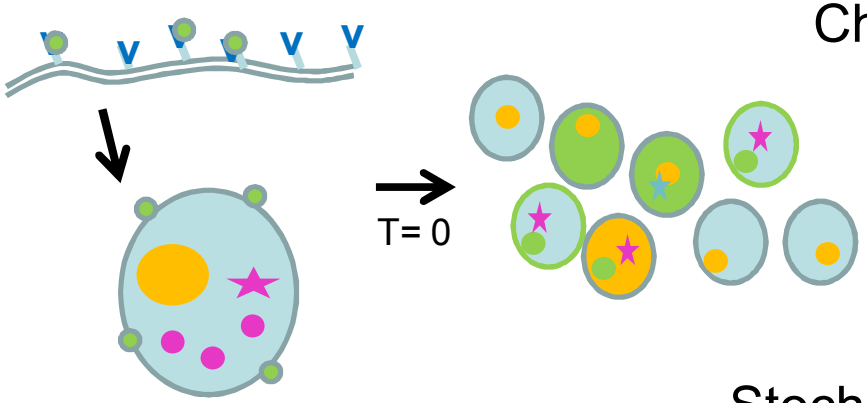
It's No Secret … Space and Time are Important in Cell Response



Changes occur on multiple scales

- Individual molecules
- Subcellular organelles
- Cell populations

Stochasticity of response is important

- Space
- Time
- Population diversity

Multiplexed measurements are key

- Temporal efficiency
- Interactions

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SNL Imaging Technologies are Well-Matched to These Questions

Hyperspectral Fluorescence Imaging
- -Confocal
- -Line scan
- -Multi-photon
- -Lifetime

Vibrational Spectroscopic Imaging

TIRF Microscopy

Single-molecule Imaging

Super Resolution Microscopy

Multivariate Image Analysis

Image Correlation, Particle Tracking

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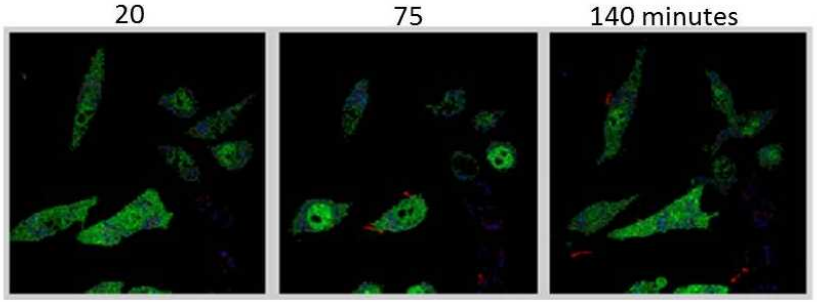
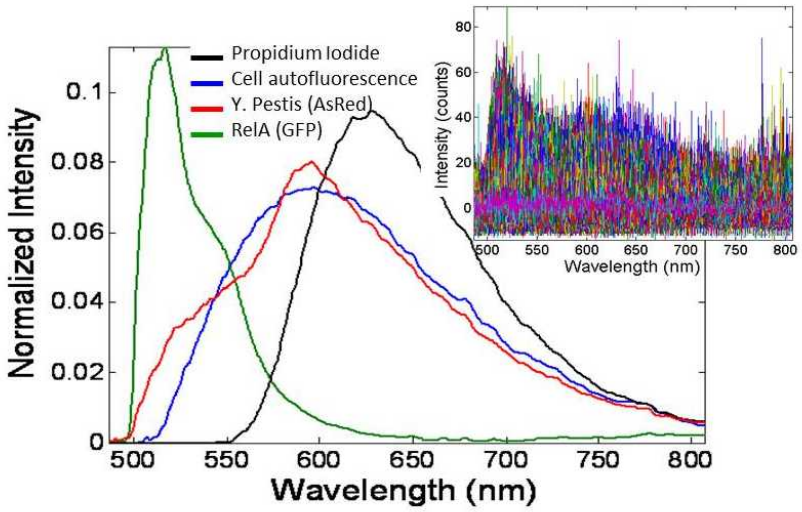
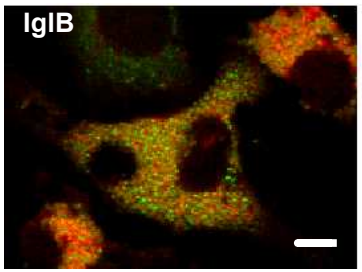
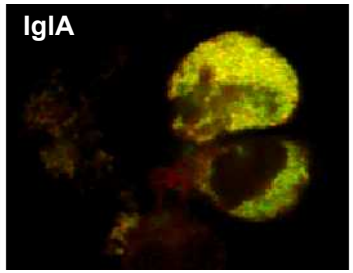
Spectral resolution provides chemical/molecular specificity, enables multiplexing









Davis, RW, et. al. Microscopy & Microanalysis, 2010, 16:4, 478-487.

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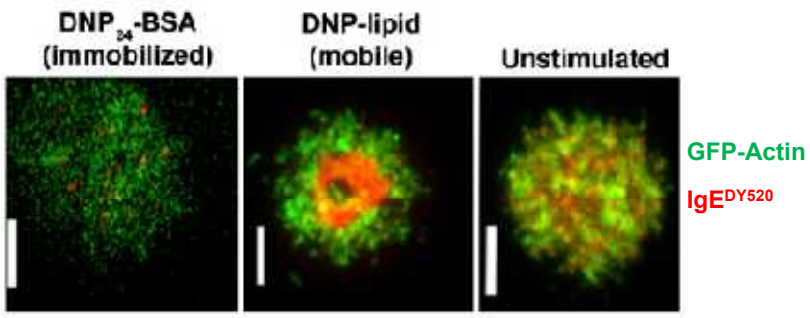

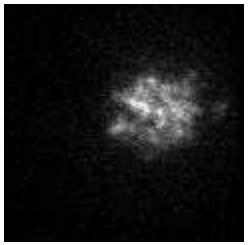

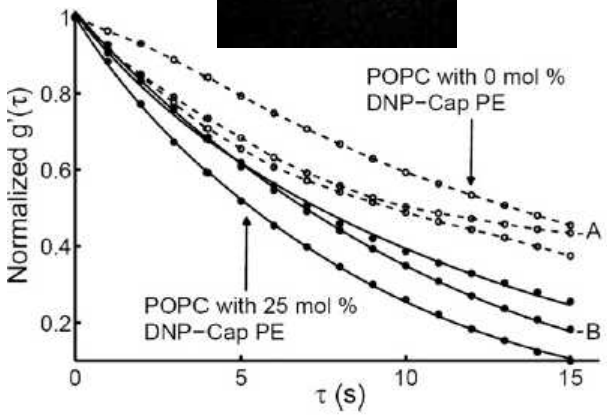

Excellent temporal resolution, and membrane specificity permits investigations of dynamic receptor behavior

Carroll-Portillo, A, et. al. *Journal of Immunology*, 2010, 184:3, 1328-1338.
Spendier, K, et. al. *Biophysical Journal*, 2010, 99:388-397.

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Single-molecule Imaging

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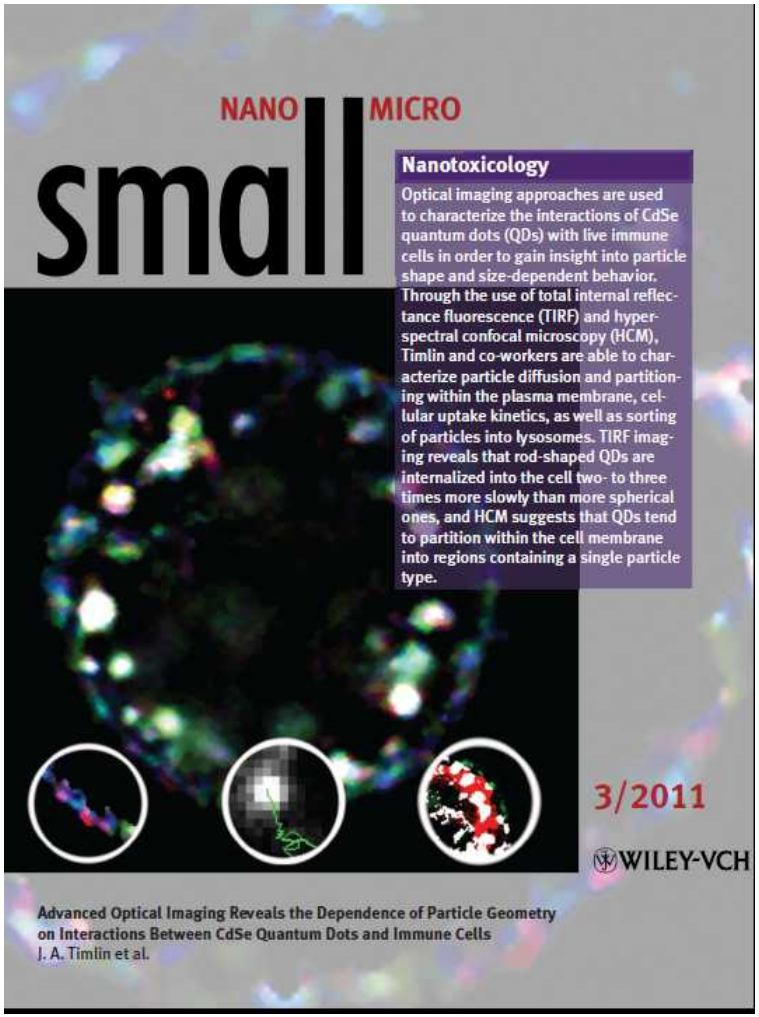
Multivariate Image Analysis

Image Correlation, Particle Tracking

Multiple bioanalytical imaging modalities elucidate membrane trafficking, uptake, and ultimate fate of nanoparticles within cells



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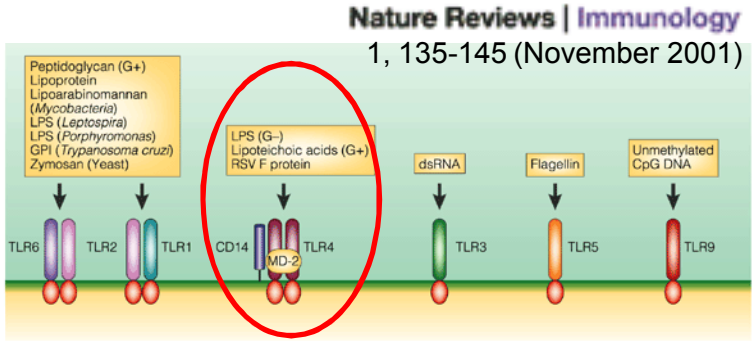

- Important element in mammalian innate immunity
- LPS recognition by TLR4 is aided by accessory proteins
- Different chemotypes of LPS generate distinct immune responses

TLRs: Important in Pathogenesis, Biodefense

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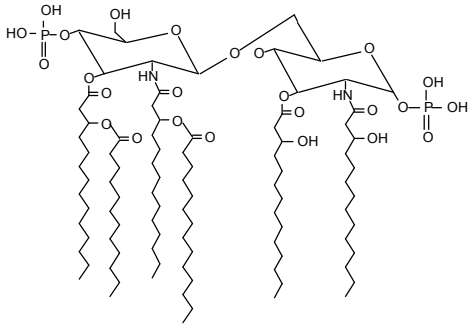
Chemotypes of LPS Exhibit Differential Immune Response

***Escherichia coli* (control)**

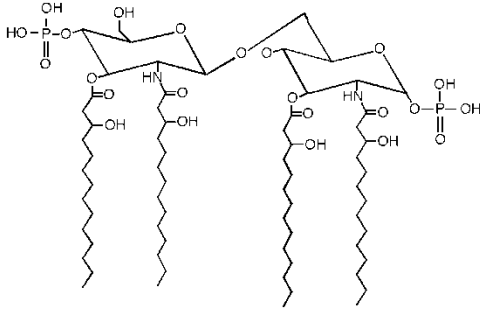
Smooth
O-polysaccharide



Bind Surface
+
↑Stimulatory

***Yersinia pestis* (37°)**

Rough
O-polysaccharide



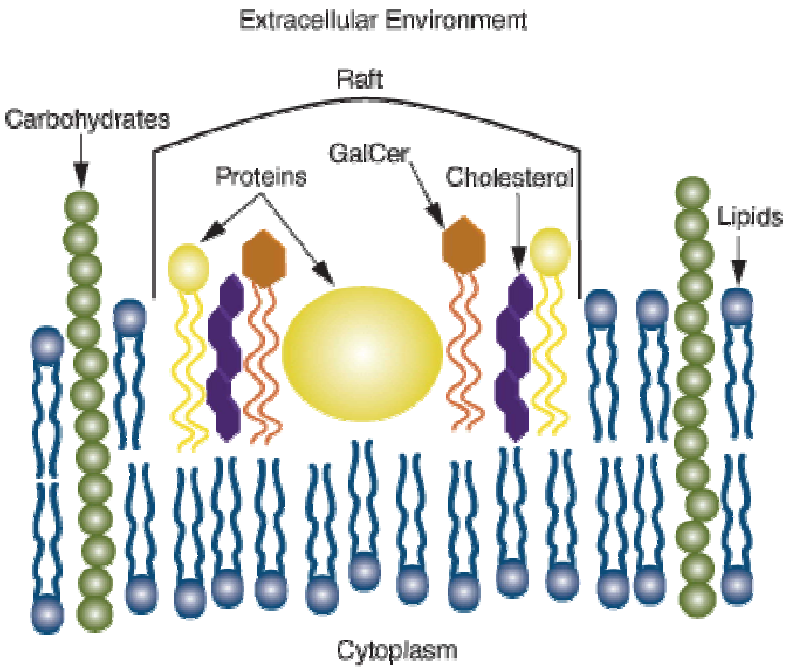
Bind Surface
+
↓Stimulatory

Differential immune response observed is not fully understood.

- LPS from *E. coli* binds & produces an immune response
- LPS from *Y.* pestis (plague @ 37 °) binds, but does not

Triantafilou, *J Cell Sci* **2002**
Triantafilou, *J Cell Sci* **2004**
Triantafilou, *Biochem J* **2004**
Netea, *Trend Immunol* **2002**

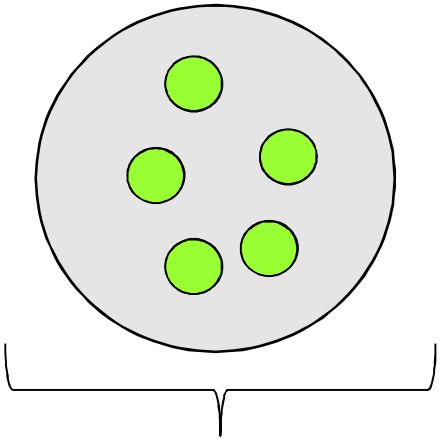
Receptor Clustering Can be Necessary in Immune Response



- Domains act as assembly areas
- Aggregation of receptors often follows activation/ligand binding
- Bulk assays have suggested that TLR4 molecules aggregate in lipids rafts within the cell membrane after LPS binding*
- Visualization at the single cell level has been limited by optical diffraction

Optical super-resolution gives us a way to differentiate TLR4 clustering at a much finer scale than conventional imaging.

Stochastic Optical Reconstruction Microscopy (STORM)



diffraction-limited spot size (~300 nm)

- The Abbe resolution limit can effectively be broken if the fluorophors in a sample can be imaged *independently* from each other.
- Assuming <1 fluorophor per diffraction-limited area, it's position can be determined with nanometer precision.
- In STORM, this means incorporating stochastic "photoswitching"
- Photoswitching for organic dyes can occur in buffer containing small thiol (i.e. BME) and oxygen scavenging system. (dSTORM)

Rust, et. al, Nat. Meth. 3: 793 - 796 (2006)

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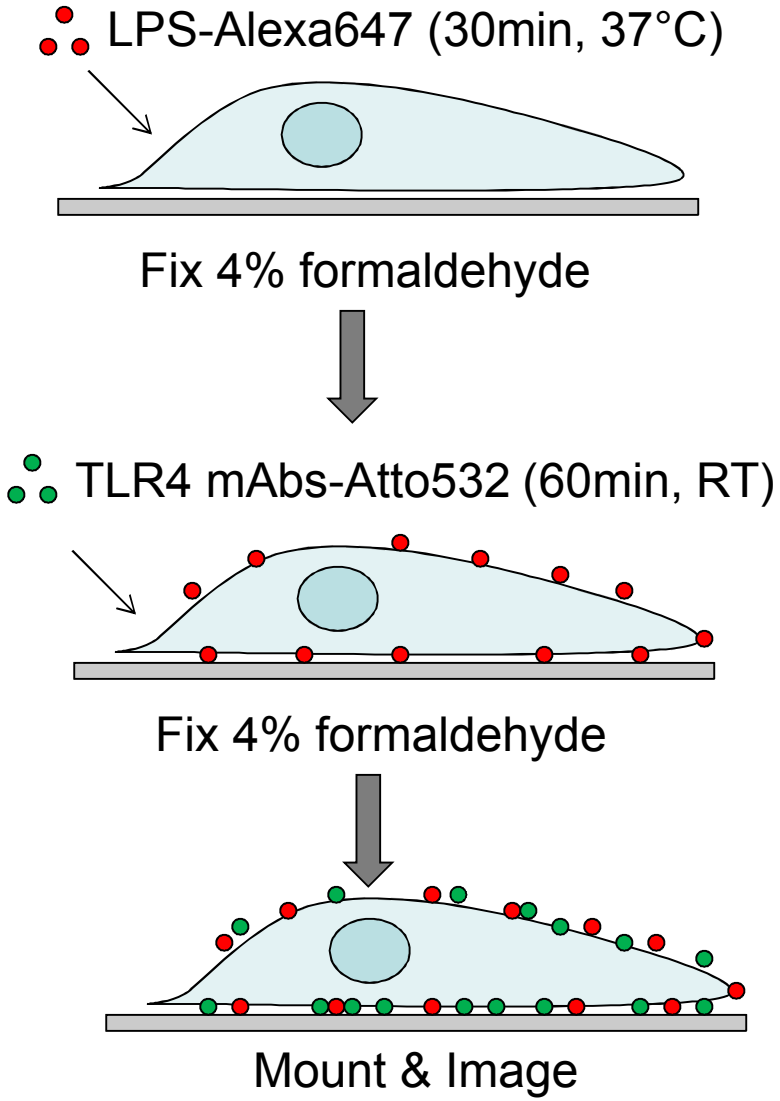
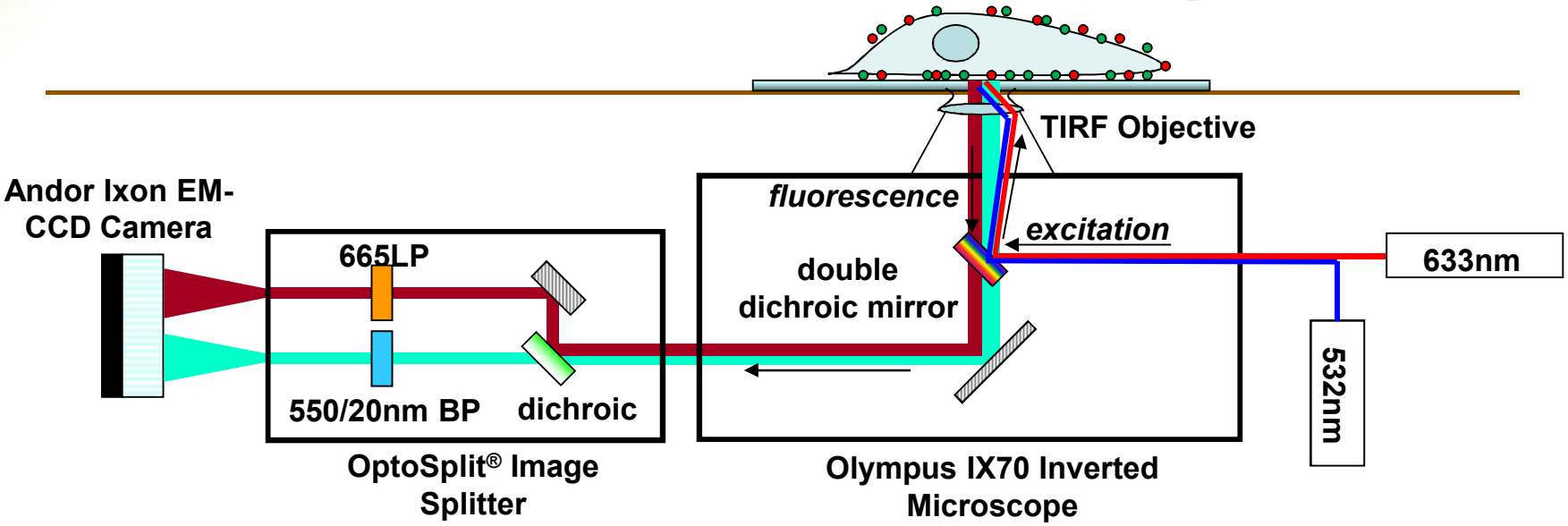
Experimental Design



- 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

Multicolor STORM Setup



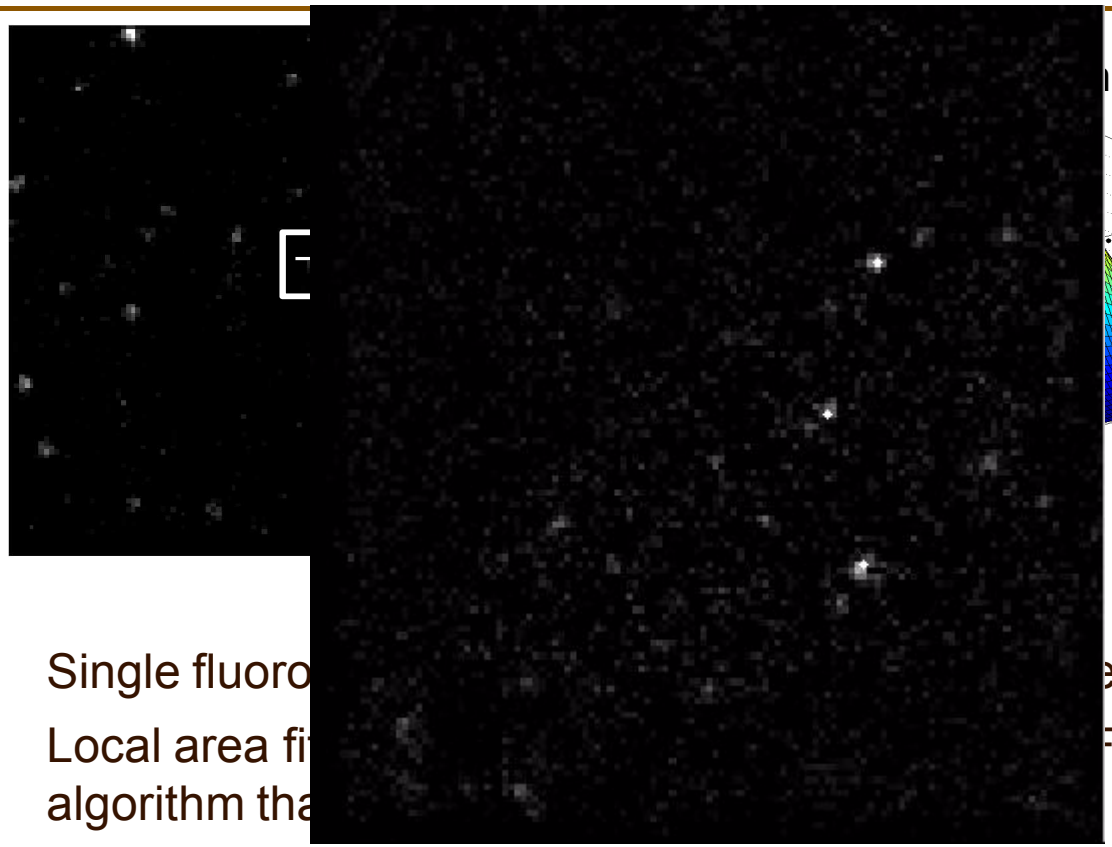
Unique capabilities:

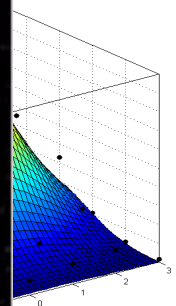
- Four excitation λ's (405, 488, 532, 633nm), variable angle
- Simultaneous dual-color emission
- Capable of >50fps over 30μm x 30μm FOV

Advantageous in:

- Receptor reorganization
- Nanoparticle-membrane interactions, uptake
  - Engineered NPs
  - Natural NPs - Viral trafficking

Fluorophor Localization



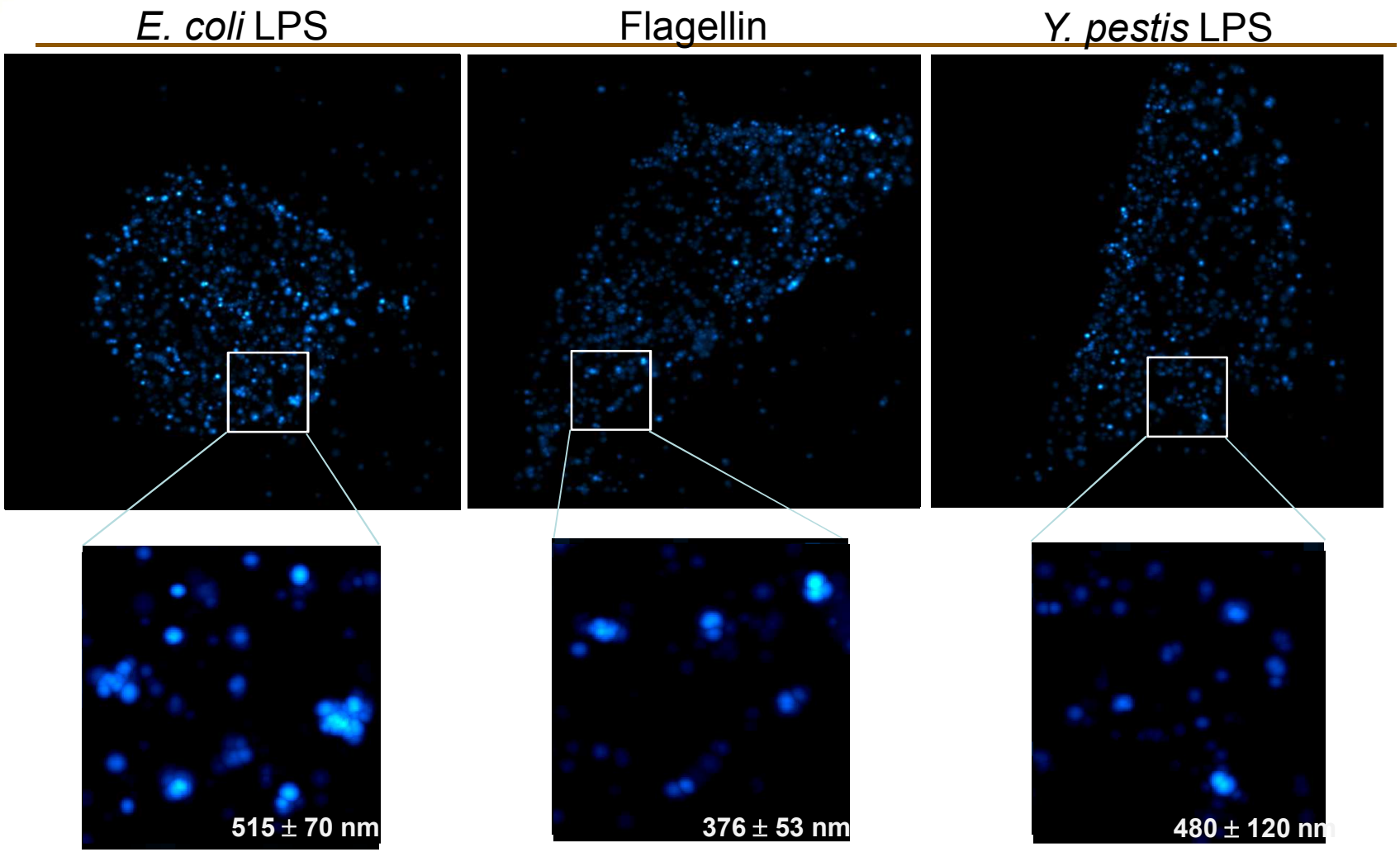


m , 44±23nm)

Uncertainty ~ 1/√N
(SNR)

- Single fluoro ed in each frame
- Local area fi F (using a novel algorithm tha
- Maximum of that surface is most likely position of the fluorophor
- Typically, location fit uncertainty 40-60nm
- Process repeated over 1k-10k frames to build STORM image

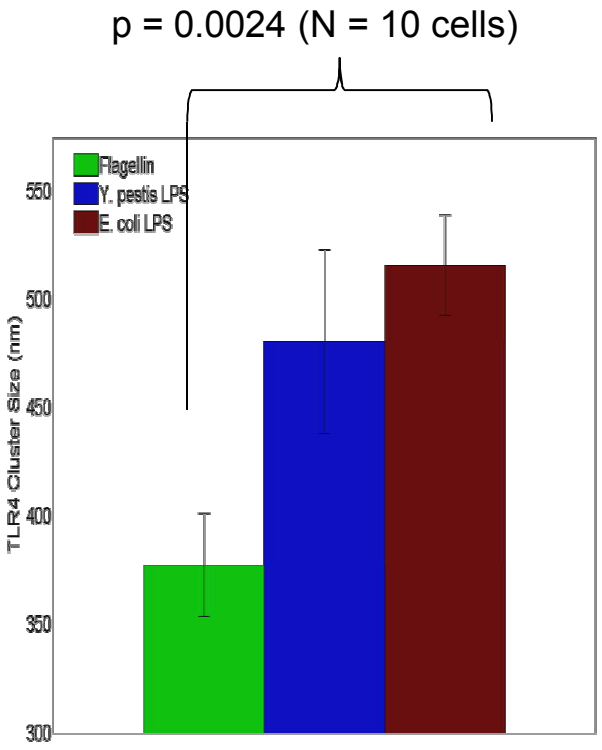
TLR4 Clustering is Specific



STORM image = 8-10-fold increase in resolution

TIRF images = diffraction limited

TLR4 Cluster Analysis



- Ripley's K-analysis indicates that *E. coli* LPS induces significant clustering over negative control (flagellin)
- Suggests that *pestis* induces less clustering, but not significant
- TLR4-LPS complex?

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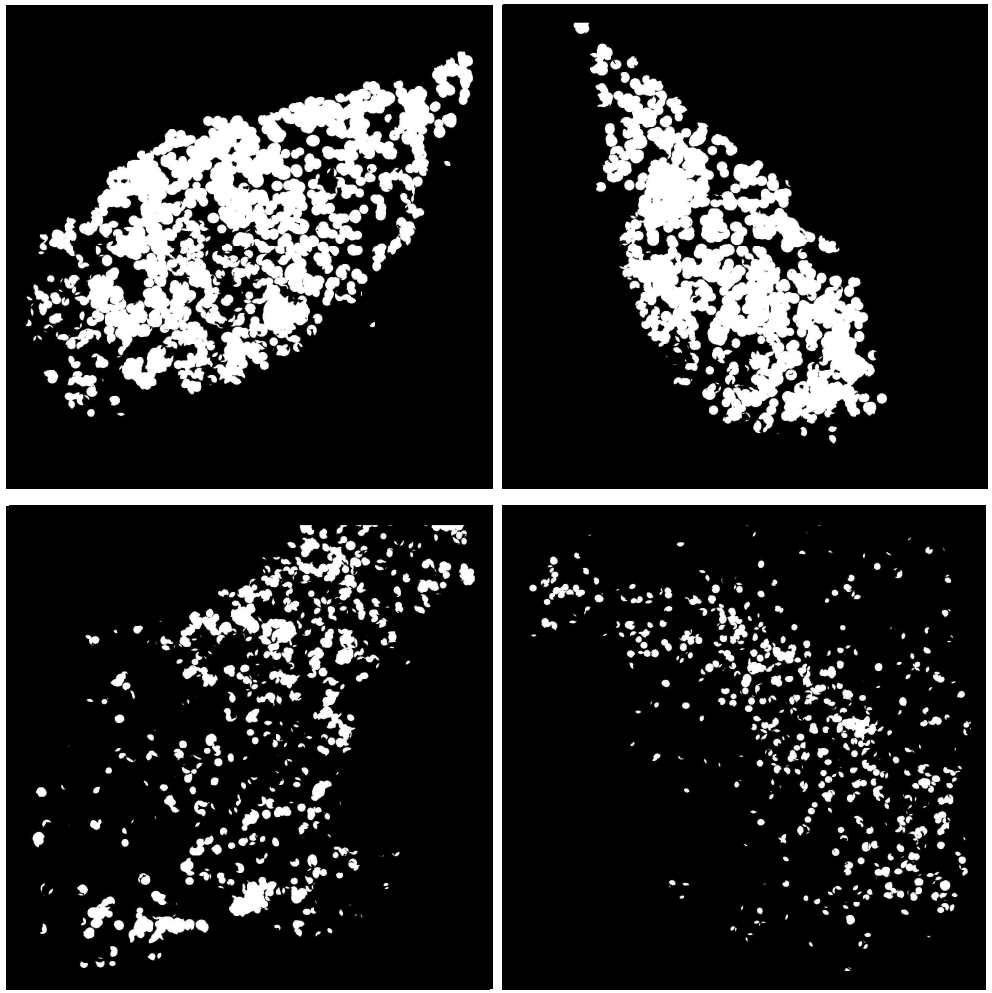
Colocalization of TLR4 & LPS



- Dual-color STORM imaging
  - TLR4 – Atto532
  - LPS – AlexaFluor647
- Image registration via multi-dye PS beads (average error ~50nm)
- Perform cluster analysis on co-localized points

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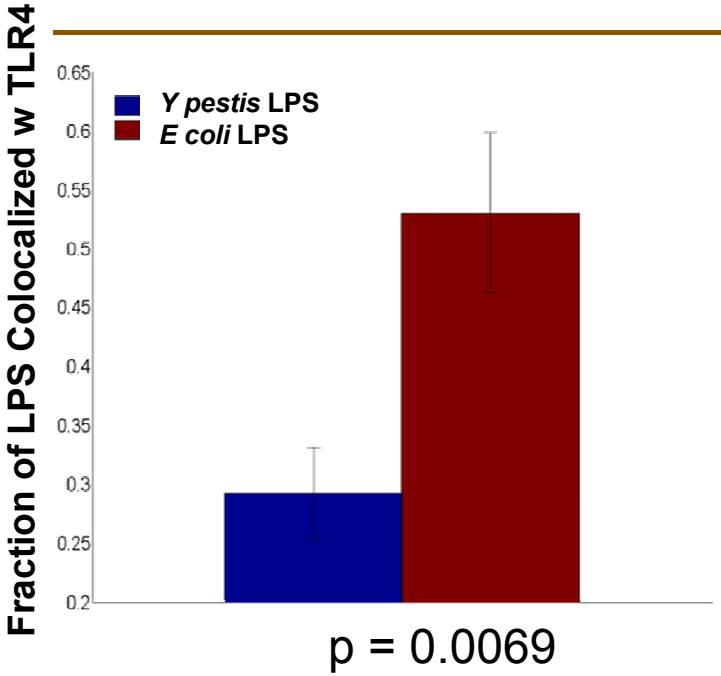
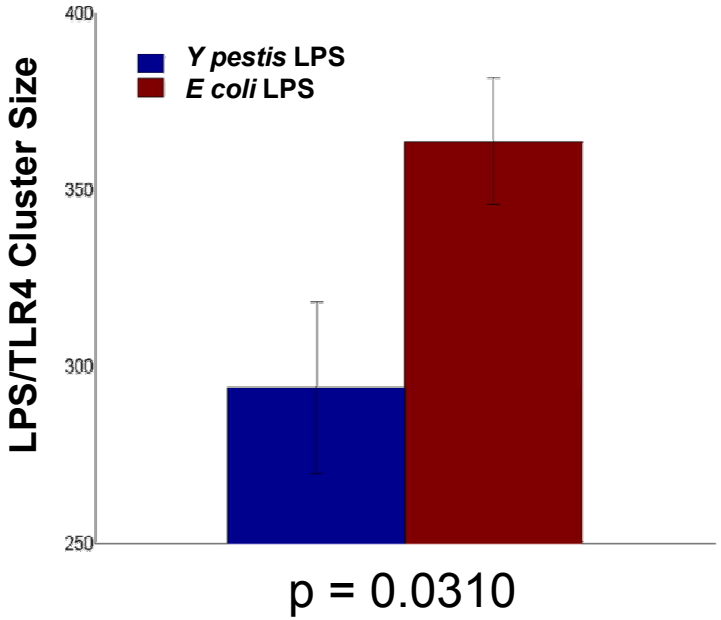
Y. pestis LPS is less Efficient at Recruiting TLR4 into Clustered Domains



p = 0.0069



p = 0.0310

Conclusions

- Significantly less co-localization of *Y. pestis* LPS with TLR4 compared to *E. coli LPS*
- Significantly smaller *Y. pestis* LPS-TLR4 clusters than *E. coli* LPS-TLR4 clusters

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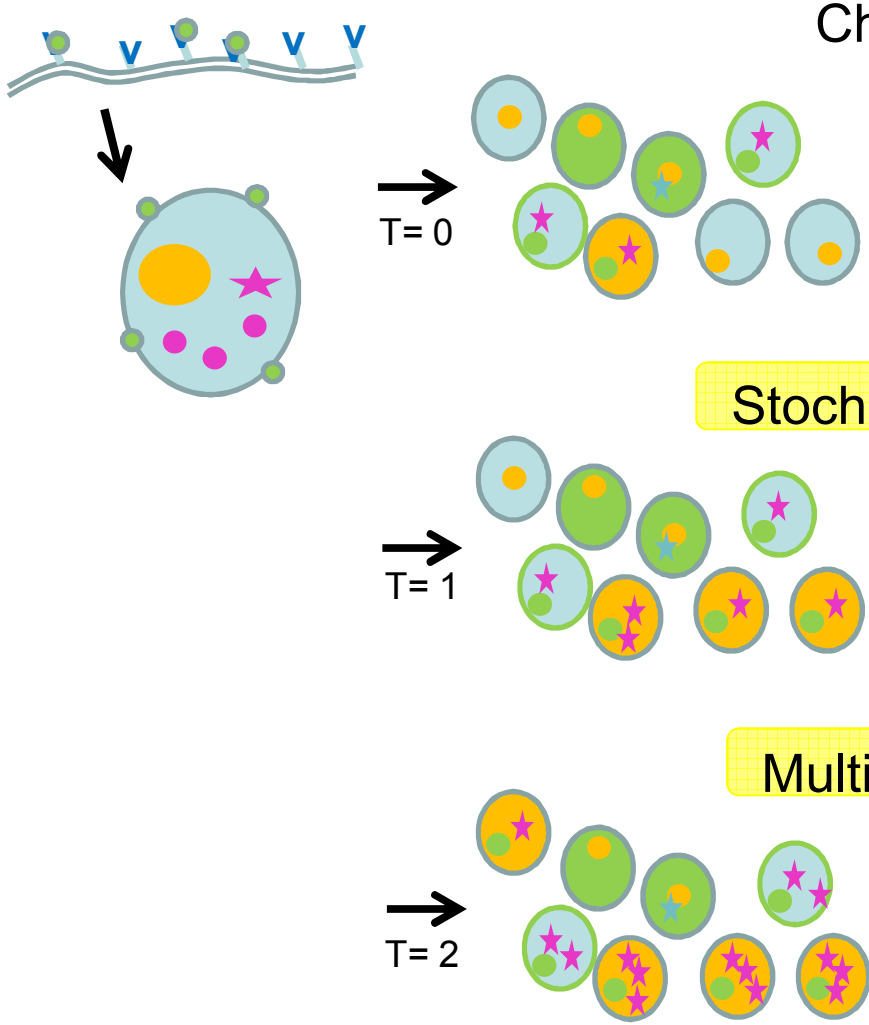
Future Vision - Multidimensional Bioanalytics



Changes occur on multiple scales

- Individual molecules
- Subcellular organelles
- Cell populations

Stochasticity of response is important

- Space
- Time
- Population diversity

Multiplexed measurements are key

- Temporal efficiency
- Interactions

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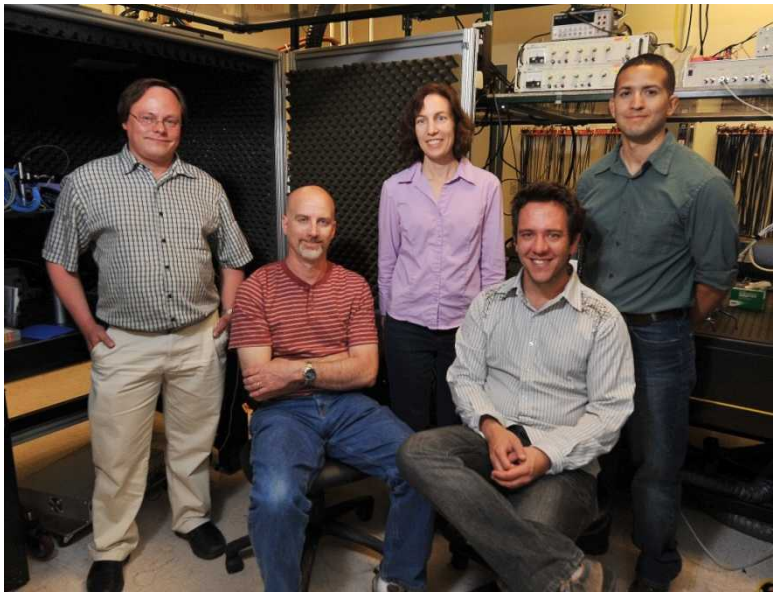
Acknowledgements



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Susan Brozik
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Hanson & Turner Labs, UNM Biology
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Sayre Lab, NM Consortium
Sapphire Energy
Hu Lab, ASU

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