SAND2012-1580C

Visualizing Early Immune Response: Bacterial Specific Reorganization at the Nanoscale

Jesse S. Aaron, Quinton B. Smith, Bryan D. Carson, Jerilyn A. Timlin

Sandia National Laboratories
Albuquerque, New Mexico

Presented at:
ASM Biodefense, Washington, DC
Feb 26-29, 2012

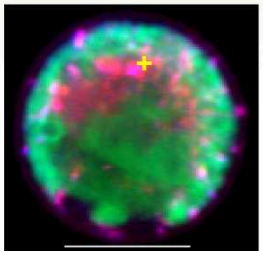
Sandia National Laboratories is a multi-program laboratory operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

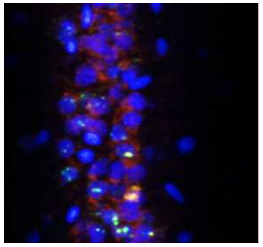
NNSA National Nuclear Security Administration

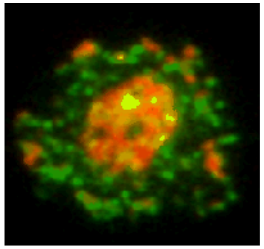
Sandia National Laboratories

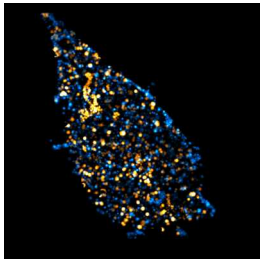
Research Focus

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







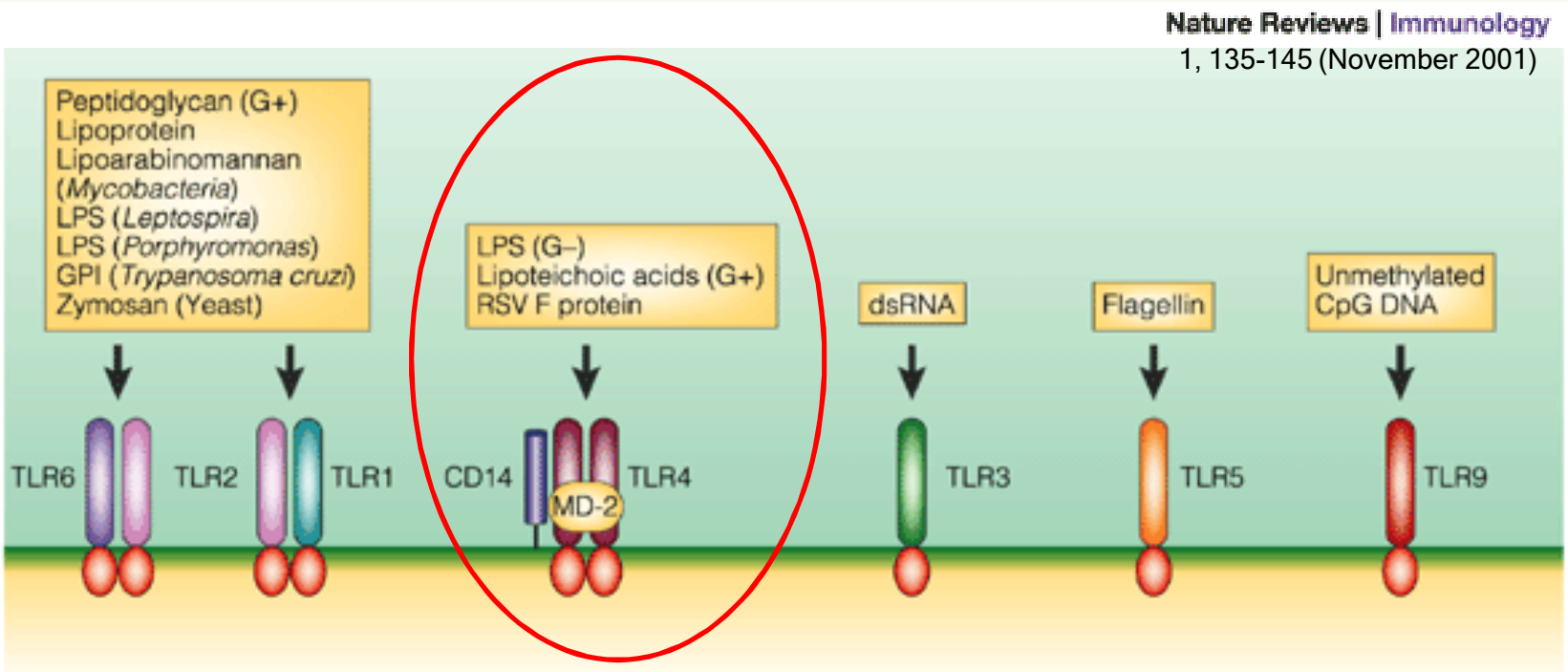


- Advanced spectroscopy
- Innovative imaging technologies
- Chemometric data analysis tools
  - Multidisciplinary
  - Cell biology, immunology, and microbiology
  - Multicomponent biological systems
  - Biodefense and Bioenergy

- *Multiplexed super resolution microscopy for deciphering complex cellular events*
  - Within the cell: *Work in progress…*
  - At the plasma membrane: *Receptor clustering at the nanoscale*

Sandia National Laboratories

TLRs: Important in Pathogenesis, Biodefense



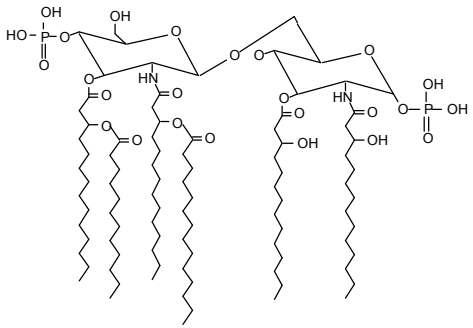
- Important element in mammalian innate immunity
- LPS recognition by TLR4 is aided by accessory proteins
- Close tie between receptor cluster formation, actin cytoskeleton rearrangement, and immune response
- Different chemotypes of LPS generate distinct immune responses

Sandia National Laboratories

Chemotypes of LPS Exhibit Differential Immune Response

***Escherichia coli* (control)**

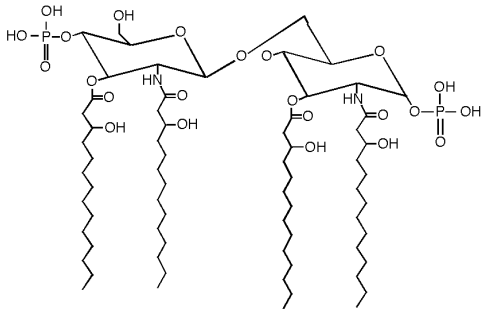
Smooth O-polysaccharide



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**

Sandia National Laboratories

Receptor Clustering Can be Necessary Component of Immune Response

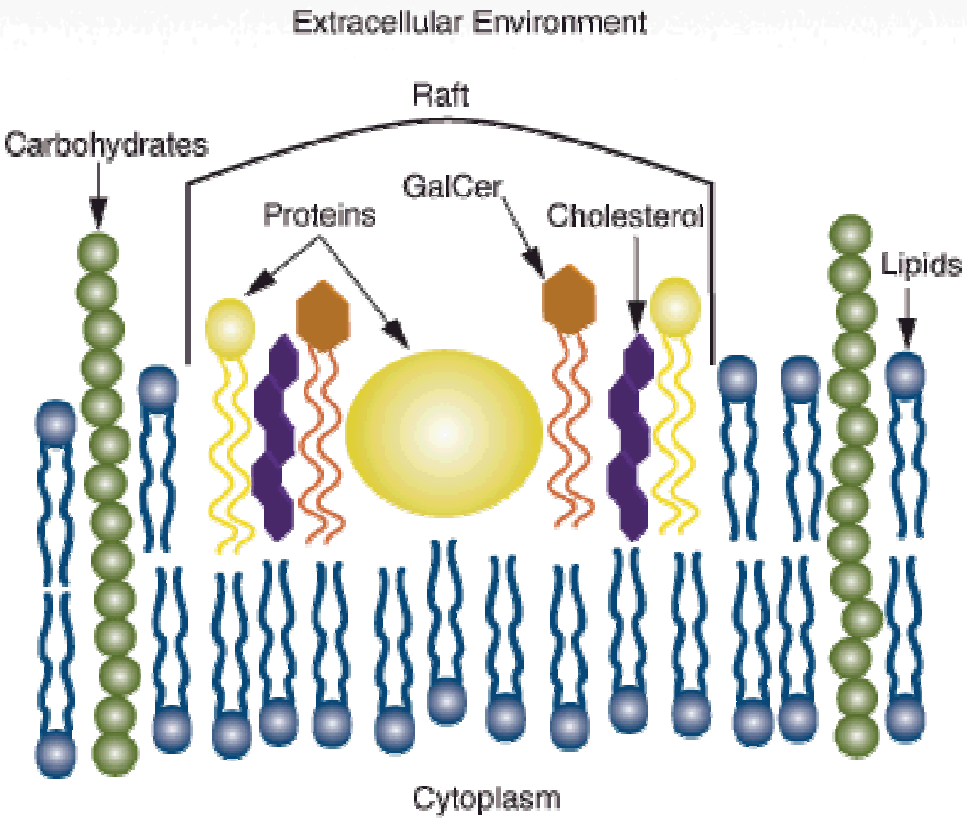


Image Courtesy of Tim Ratto, Lawrence Livermore National Labs

- Domains act as assembly areas
- Aggregation of receptors often follows activation/ligand binding
- Bulk assays have suggested that TLR4 aggregates in lipids rafts within the cell membrane after LPS binding*
- Link between receptor cluster formation and actin cytoskeleton rearrangement
- Visualization at the single cell level has been limited by optical diffraction

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

Sandia National Laboratories

Hypothesis

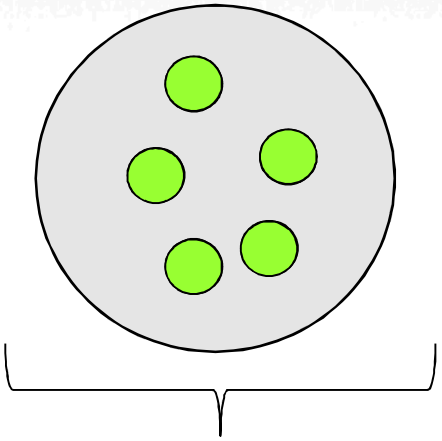
TLR4 receptors cluster at the membrane and the characteristics of this cluster are

1) *dependant on the properties of the LPS engaged, and*
2) *tied to downstream signaling events*

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

Sandia National Laboratories

Stochastic Optical Reconstruction Microscopy (STORM)



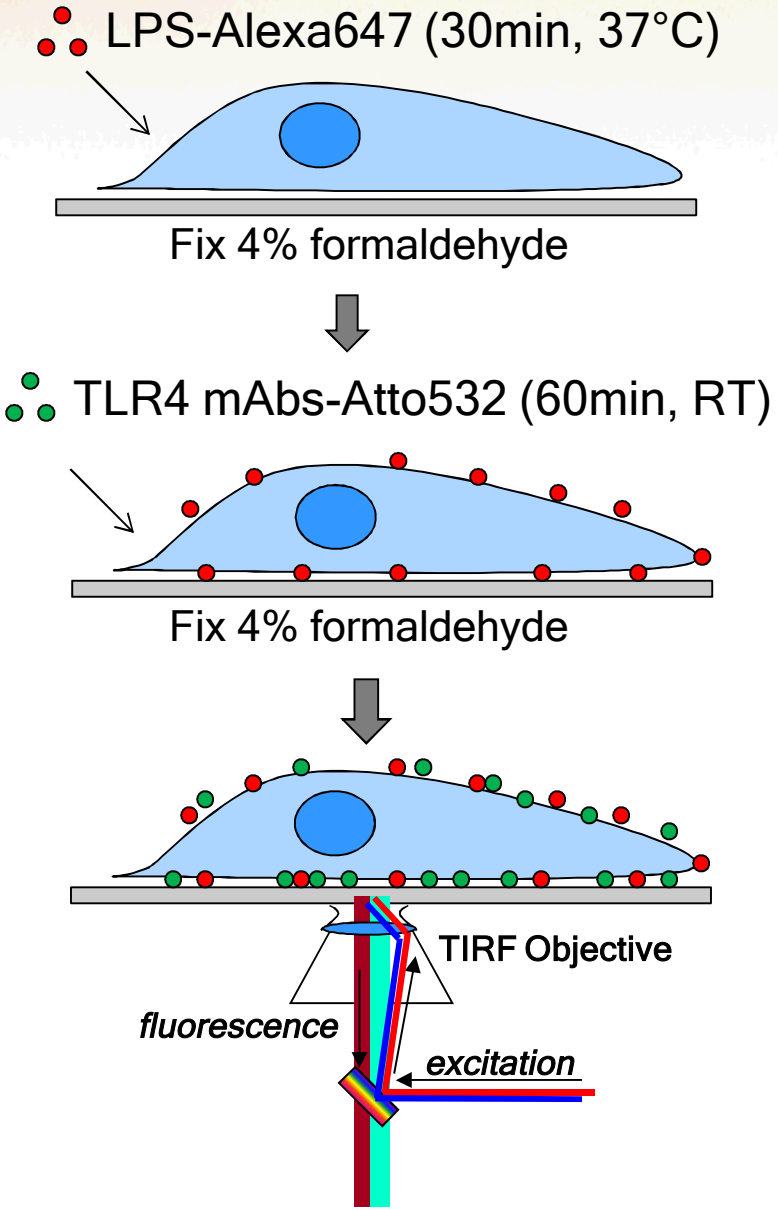
diffraction-limited spot size (~300 nm)

- 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)

Sandia National Laboratories

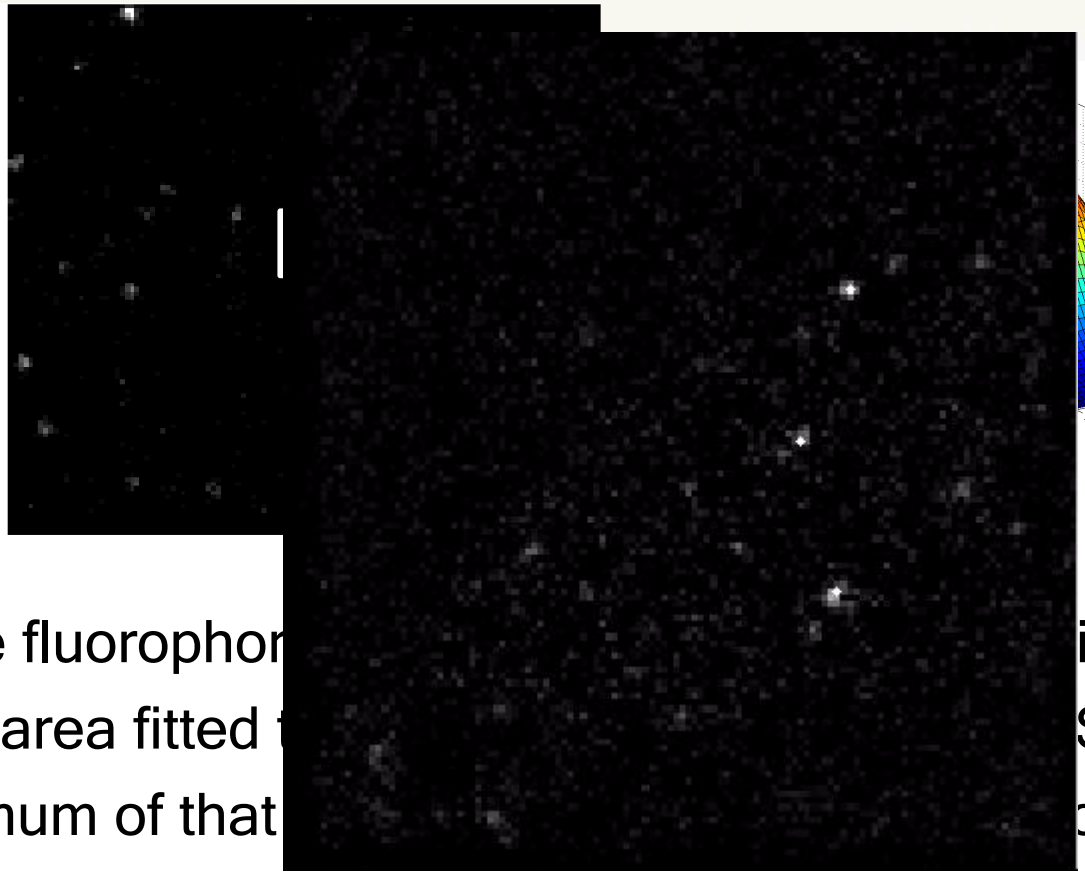
Experimental Design



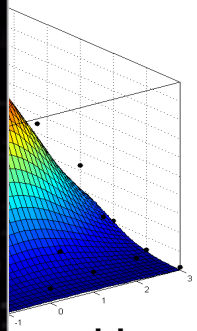
- Mouse macrophage cells (P388D1) incubated with 100nM *E. coli* or *Y. pestis*-derived LPS for 30 min at 37°C and formaldehyde fixed.
- LPS are labeled with Alexa Fluor 647-hydrazide via linkage with core-polysaccharide
- TLR4 receptors visualized via 1^0 antibodies labeled with Atto532
- Mount in O_2-scavenging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

Sandia National Laboratories

Fluorophor Localization



nm , 44±23nm)

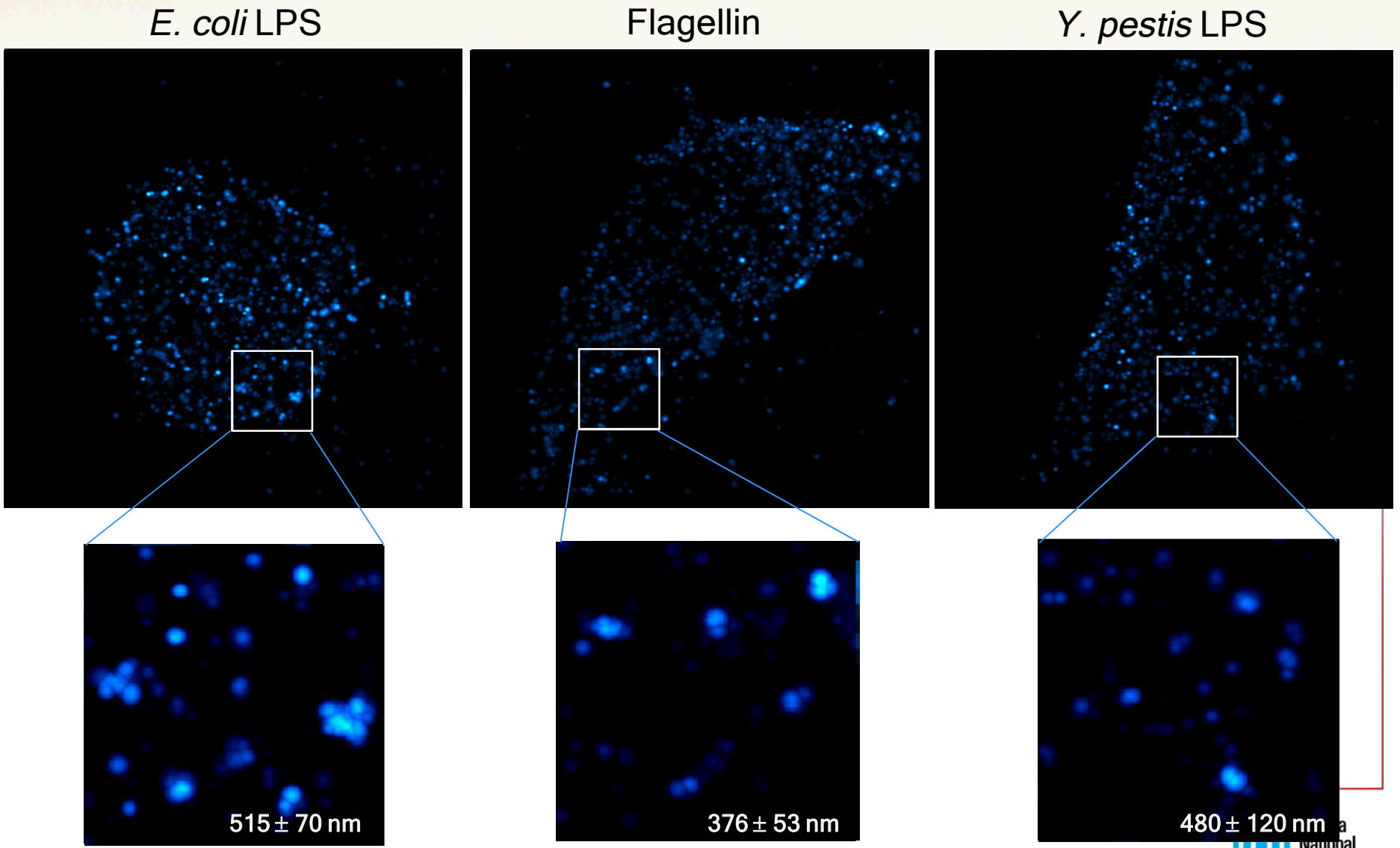


Uncertainty ~ 1/√N
(SNR)

- Single fluorophor ified in each frame
- Local area fitted t SF
- Maximum of that of the fluorophor
- Typically, location fit uncertainty 40-60nm
- Process repeated over 1k-10k frames to build STORM image

Sandia National Laboratories

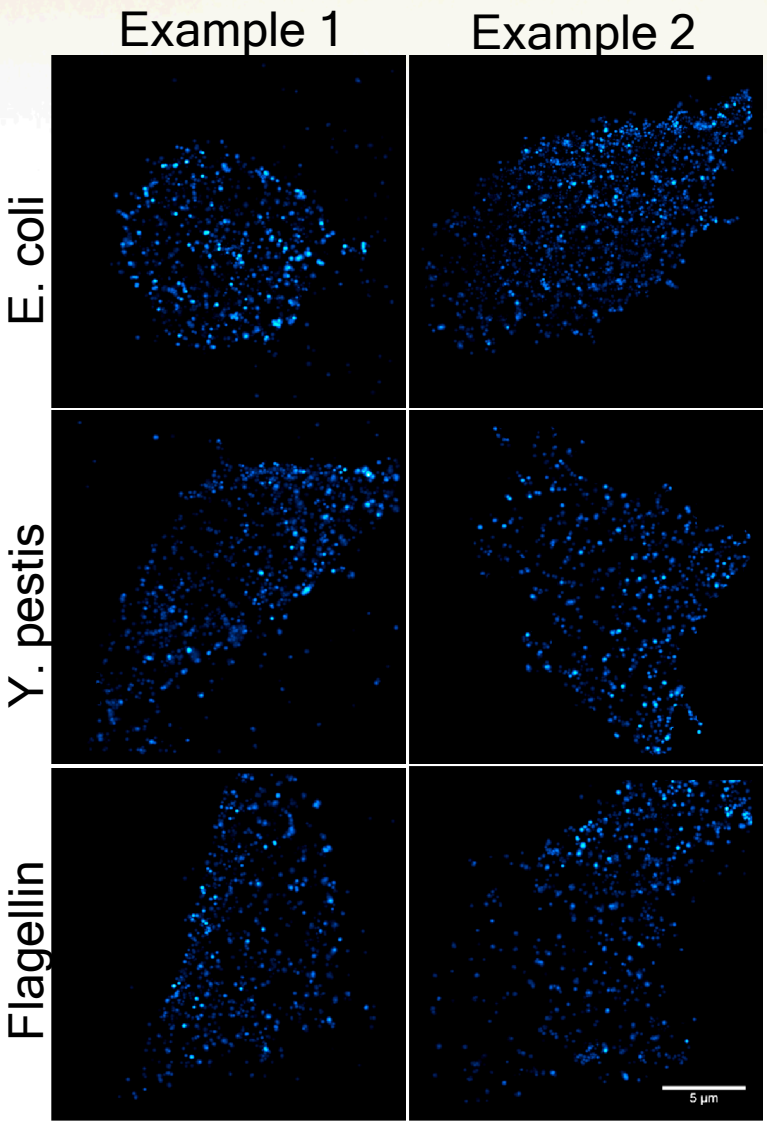
TLR4 Clustering is Specific

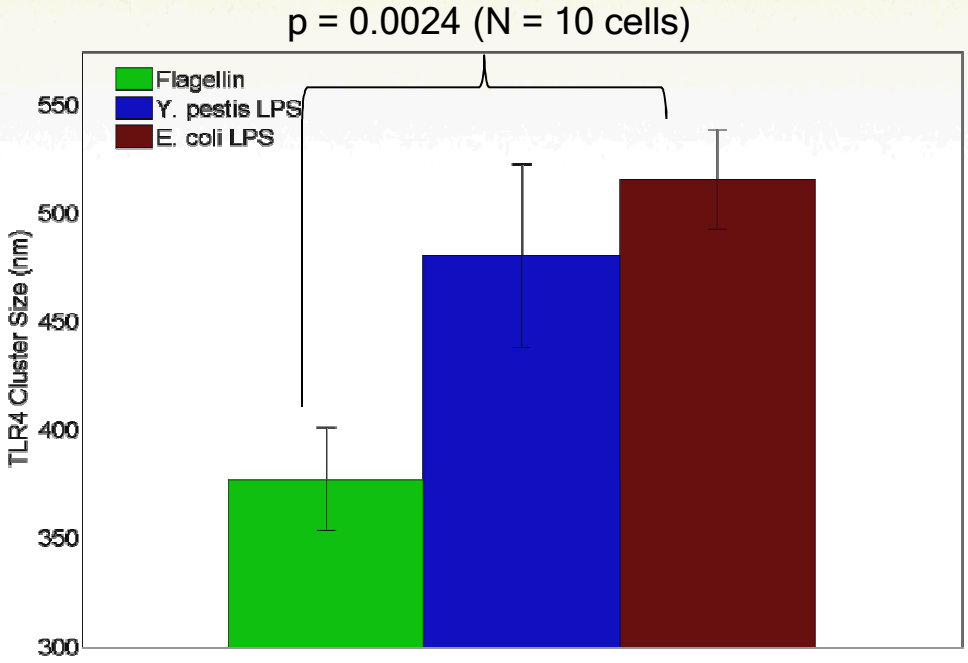


STORM image ~ 10 fold increase in resolution

TIRF images – diffraction limited

TLR4 Cluster Analysis



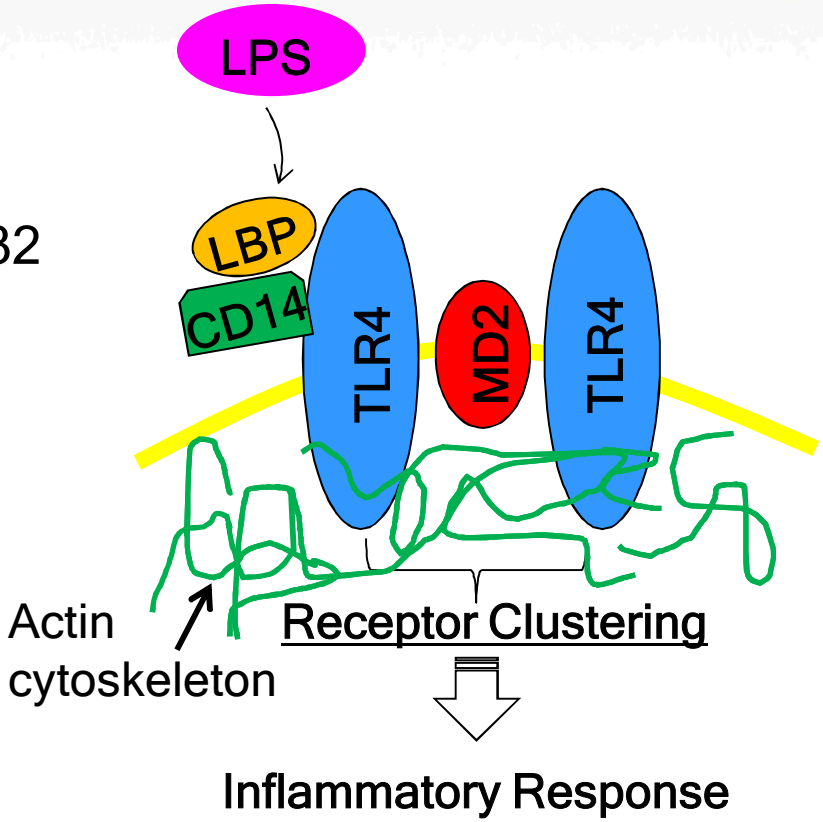


- Ripley's K-analysis indicates that *E. coli* LPS induces significant clustering over negative control (flagellin)
- Suggests that *pestis* induces less clustering, but not significant
- TLR4-LPS complex?

Sandia National Laboratories

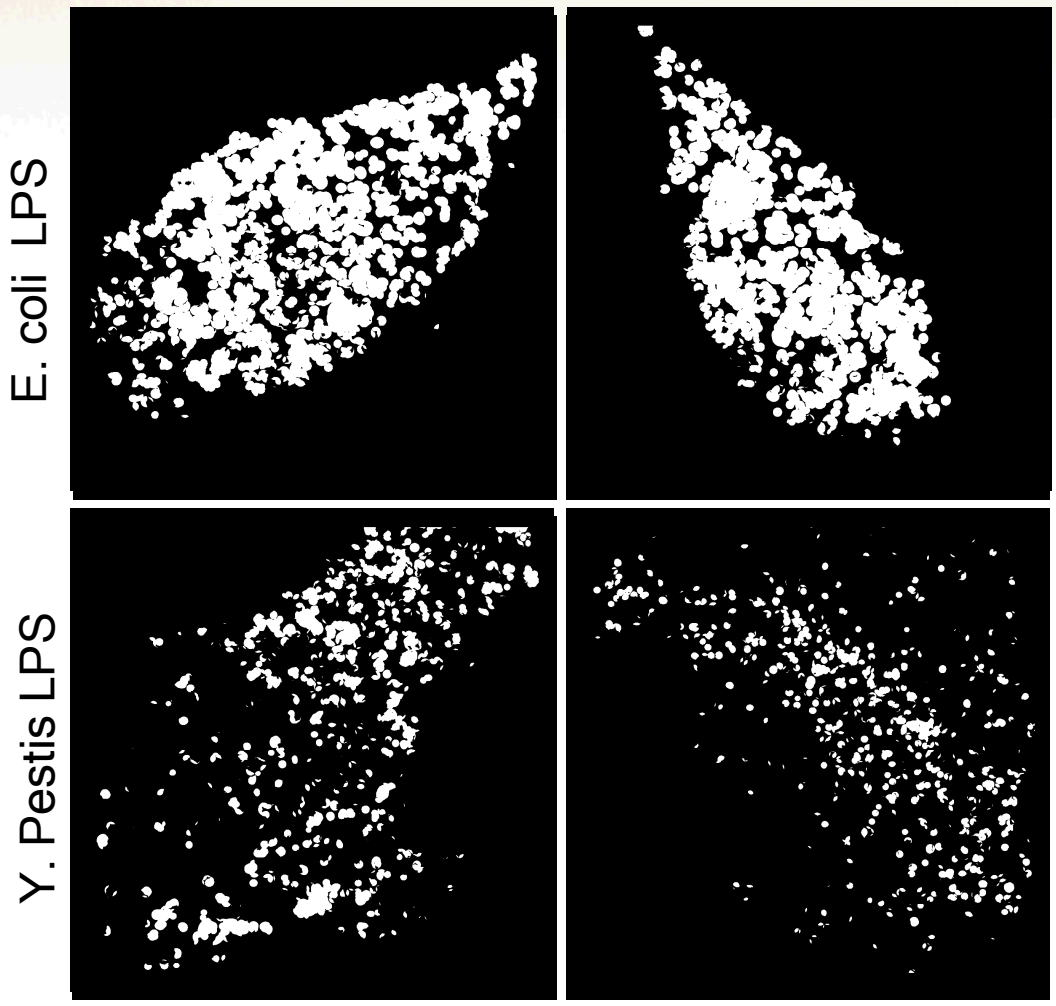
Imaging the TLR4-LPS Complex

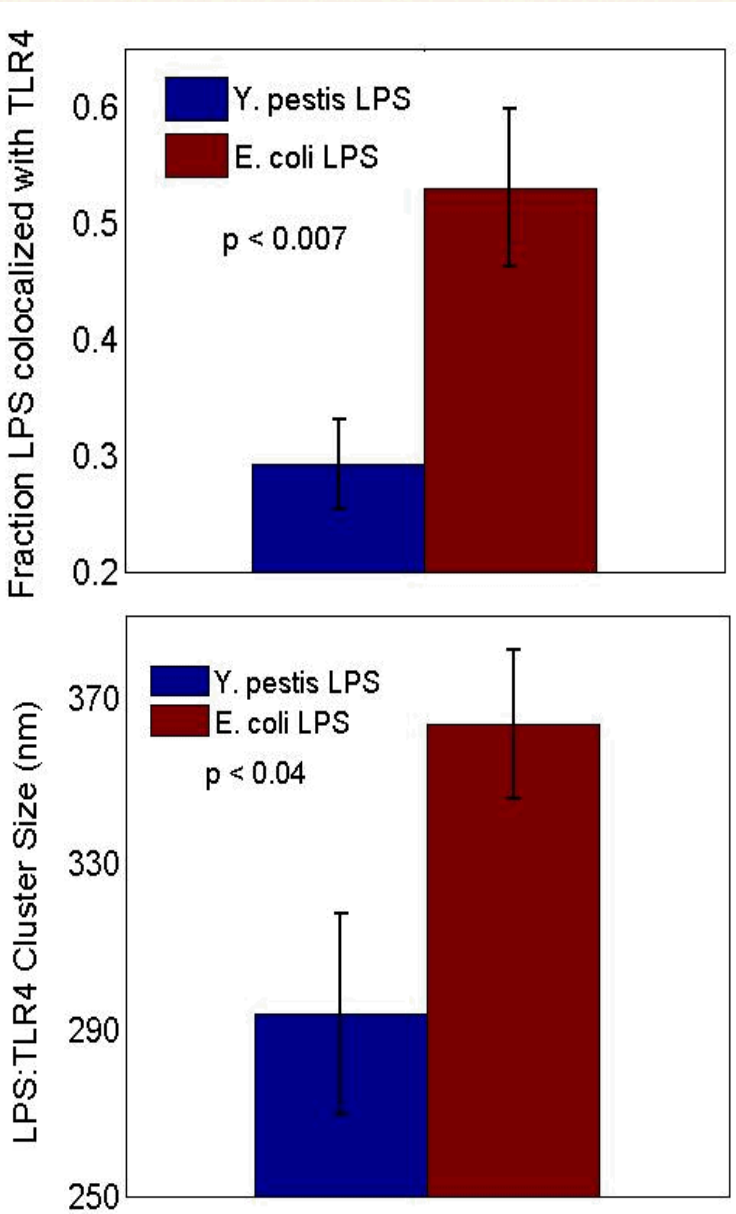
- Dual-color STORM
  - LPS-Alexa647/ TLR4-Atto 532
  - 532 & 638 nm excitation
  - Total irradiance ~10W/cm^2
  - 0.05-0.1 seconds per frame
- Image registration using multicolor beads
  - Linear transform
  - Errors <50 nm



Sandia National Laboratories

Colocalization of TLR4 & LPS





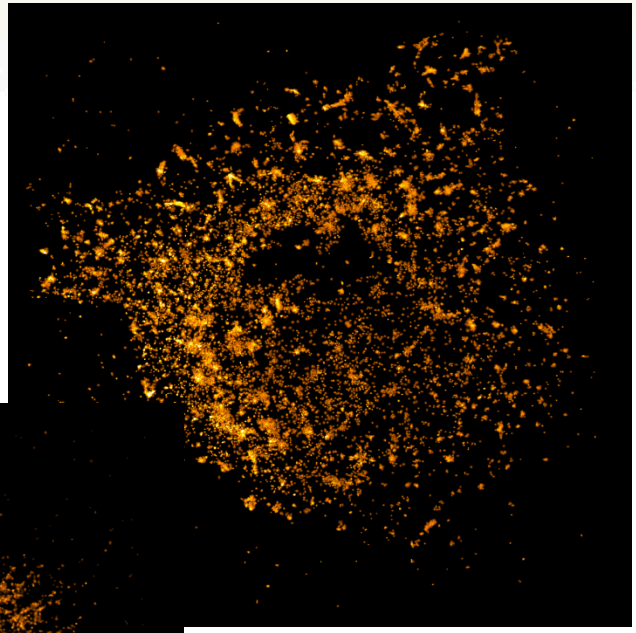
Conclusions

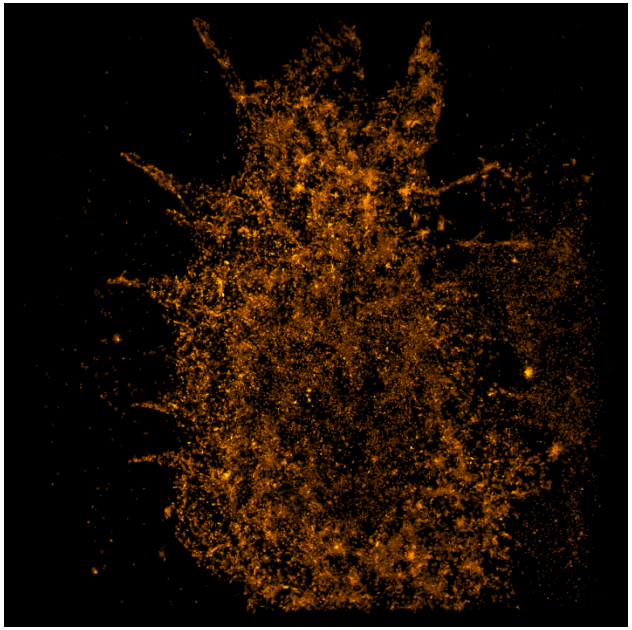
- Super-Resolution imaging allows for measuring subtle changes that aren't apparent in conventional microscopy

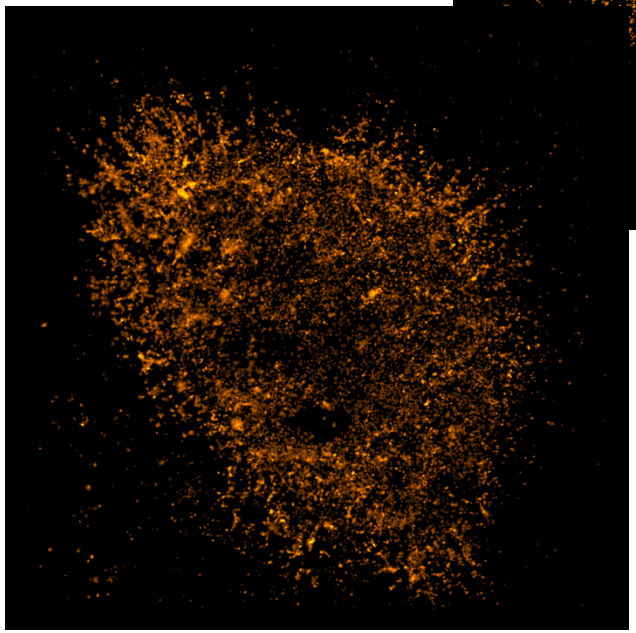
- *E. coli* LPS produces a significant increase in TLR4 cluster size within 30 minutes, as compared to a non-specific ligand (Flagellin) and non-stimulatory control
- *Y. pestis LPS* and *E-coli LPS* exhibit similar degrees of membrane binding
- *Y. pestis LPS* exhibits less co-localization with TLR4 and is less able to recruit TLR4 into clusters as compared to *E. coli* LPS → correlated with down-stream signaling response

Future Directions

- *Impact of actin cytoskeleton*
- *Fundamental properties of the membrane (e.g. temperature, curvature)*
- *Additional chemotypes of LPS*

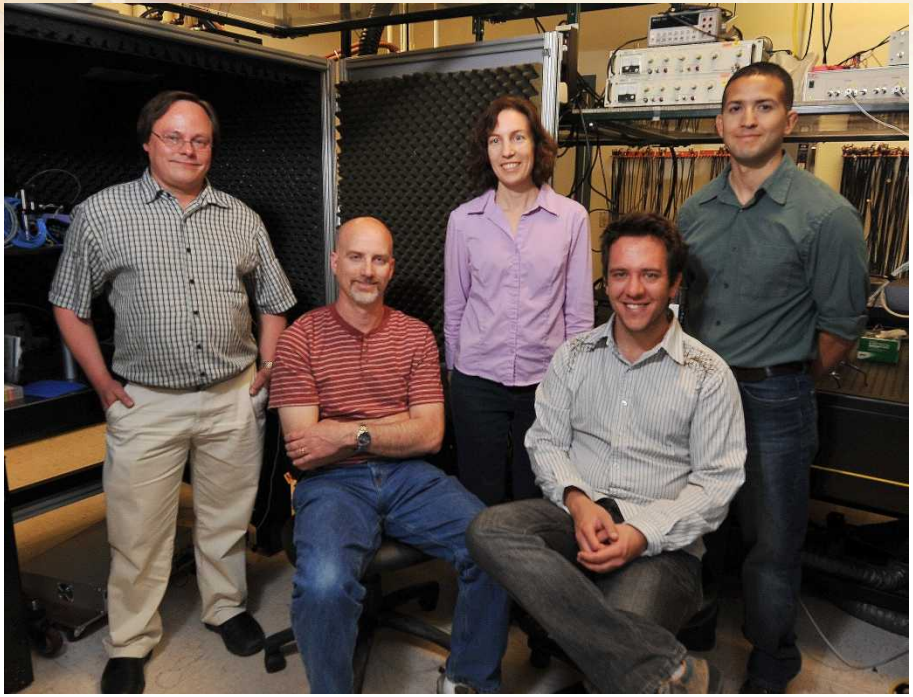






Sandia National Laboratories

Acknowledgements



Current Group Members

Dr. Jesse Aaron
Dr. Aaron Collins
Quinton Smith
Michelle Raymer
Maureen Kessler
Christine Trahan

SNL Collaborators:

Bryan D. Carson
Howland D.T. Jones
David M. Haaland
Michael Sinclair
George Bachand
Susan Brozik
Tom Reichardt
Amy Powell
Todd Lane

External Collaborators:

Oliver, Wilson, Lidke Labs, UNM Pathology
Hanson & Turner Labs, UNM Biology
Blankenship & Pakrasi Labs, WUStL
Sayre Lab, NM Consortium
Sapphire Energy
Hu Lab, ASU

STMC SpatioTemporal Modeling Center

Photosynthetic Antenna Research Center PARC

NIH DIRECTOR'S NEW INNOVATOR AWARD

LDRD

U.S. DEPARTMENT OF ENERGY

Sandia National Laboratories

Extras

Sandia National Laboratories

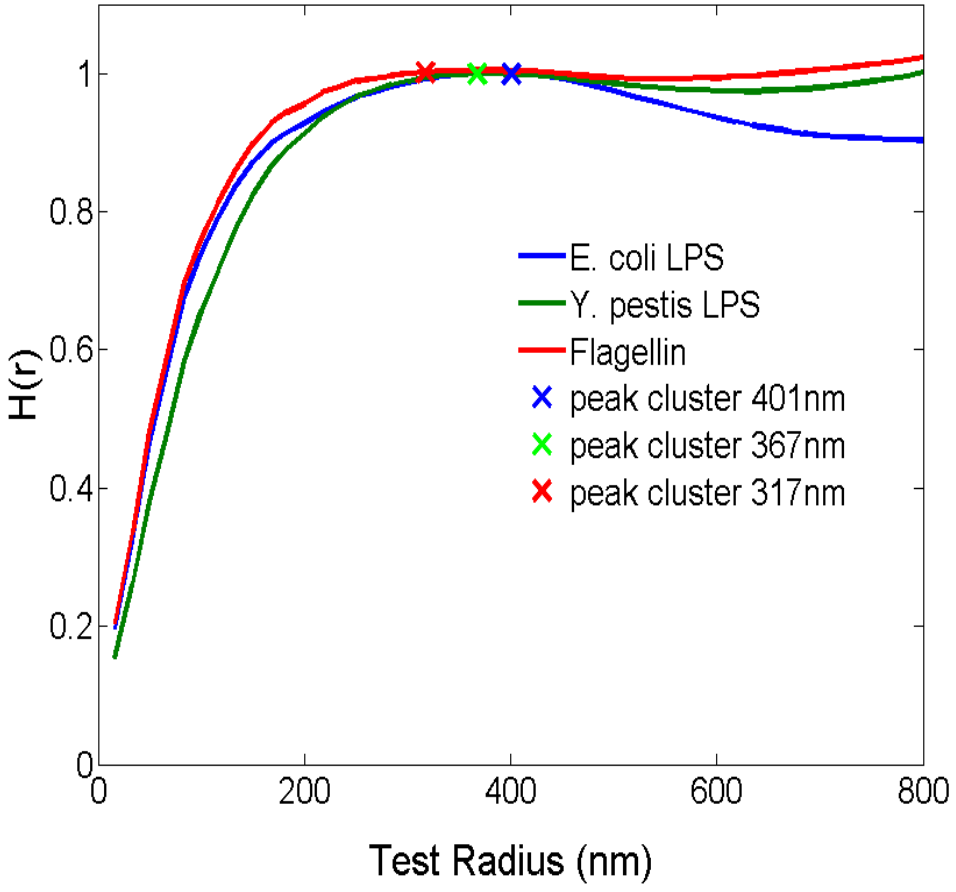
Ripley's K-function Analysis

- K-function is a normalized measure of point clustering

  Ripley, B.D. *J. R. Statist. Soc.* B41:368-374 (1979)

- Complete spatial randomness (CSR)

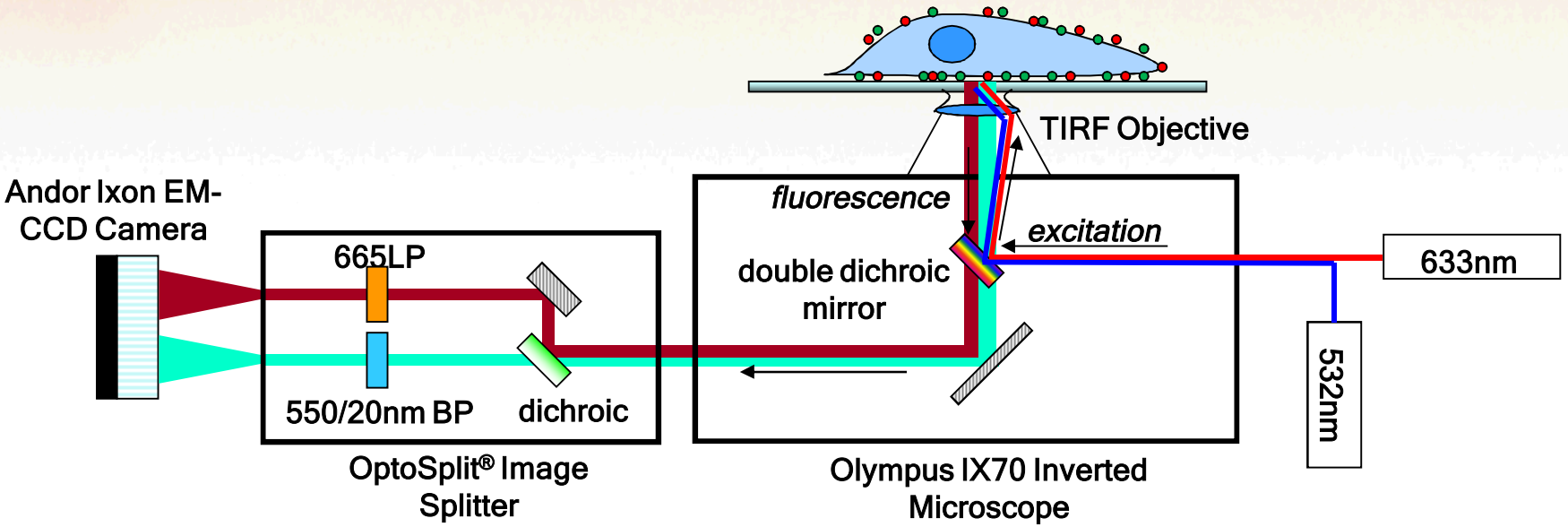
- Transform to H-function to gauge deviation from CSR at each test radius
- Peaks (or inflection points) H(r) indicate characteristic cluster sizes



Kriskowski, M.A., et al, Biophys. J. **97**(4), 1095-1103, (2009)

Sandia National Laboratories

Multicolor STORM Setup



Unique capabilities:

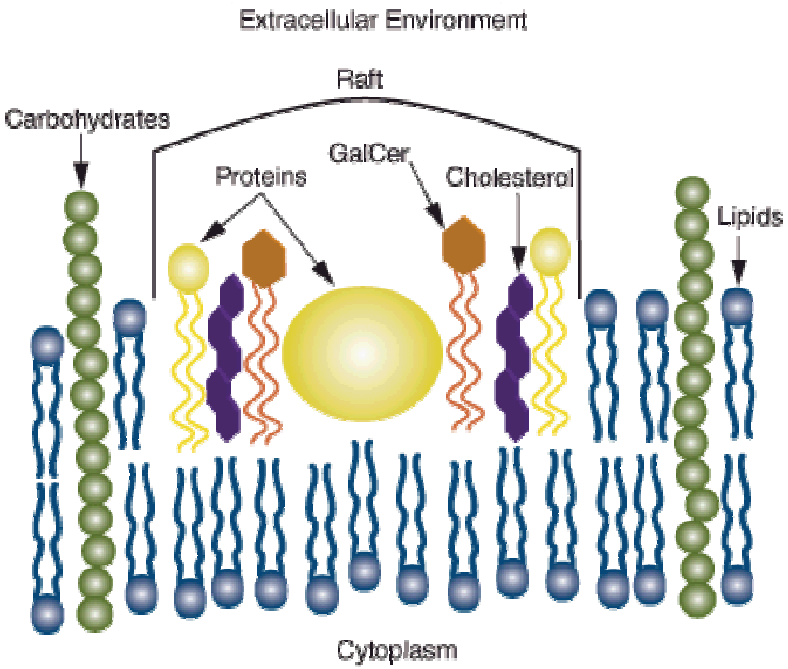
- Four excitation λ's (405, 488, 532, 633nm), variable angle
- Simultaneous dual-color emission
- Capable of >50fps over 30μm x 30μm FOV

Advantageous in:

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

Sandia National Laboratories

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.

National Laboratories