



SAND2008-4905C

Transcription Factor Translocation in Response to Different Biothreat Agents LPS Chemotypes in Macrophages

Jens F Poschet, Ph.D.
Sandia National Laboratories

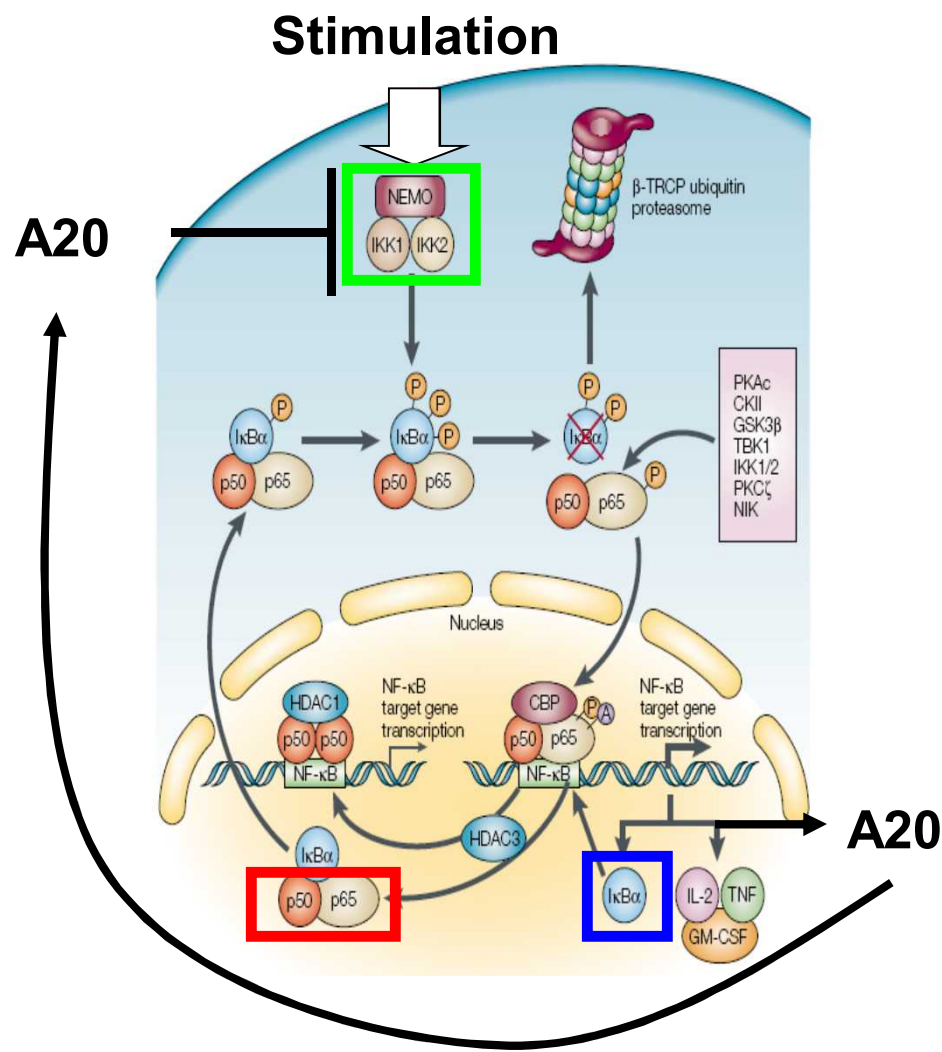


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Why should we expect RelA to oscillate in single cells?



NF-κB (RelA/p65:p50)
Inhibitor κB (IκBα, IκBβ, IκBε)
IκB kinase (IKK)
A20

RelA can oscillate by the following potential mechanisms:

- 1) (-) feedback loops by **IκB** & **A20**
- 2) Extrinsic noise
- 3) Intrinsic noise
- 4) Time-delay



Oscillations are prevalent in biology

Biological Rhythms

Period

Neuronal rhythms	0.0001s to 10s
Cardiac rhythms	1s
Calcium oscillations	sec to min
Biochemical oscillations	30s to 20 min
Mitotic oscillations	10 min to 24 h
Hormonal rhythms	10 min to 3-5 h
Circadian rhythms	24h
P53-MDM2 oscillations	2-3h
Hes1 oscillations	2-3h
NFkB nucleo-cytoplasmic oscillations(?)	2-3h

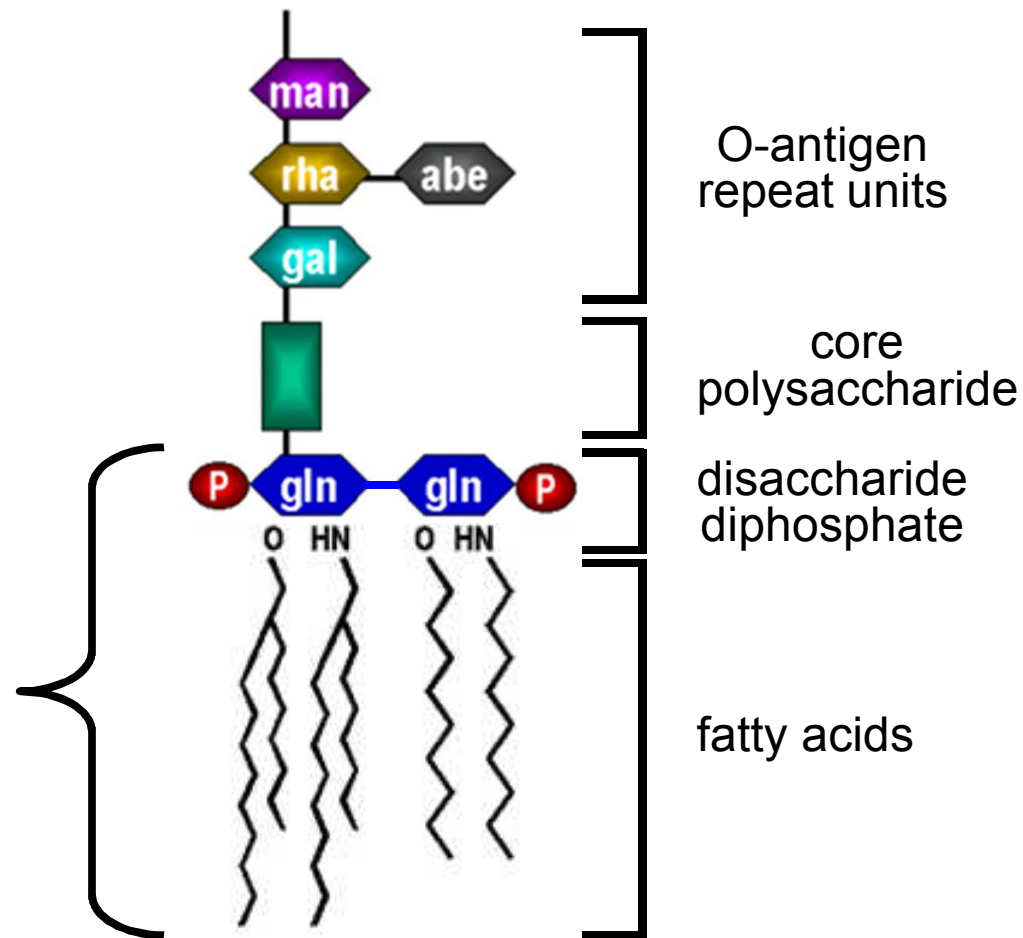


Hypothesis:

Different [LPS] = Different NF- κ B Oscillations

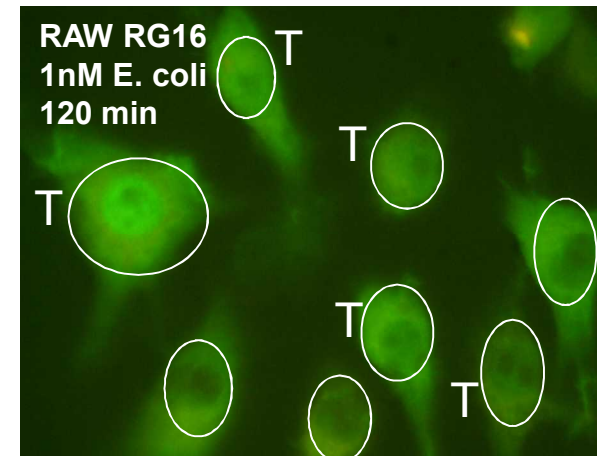
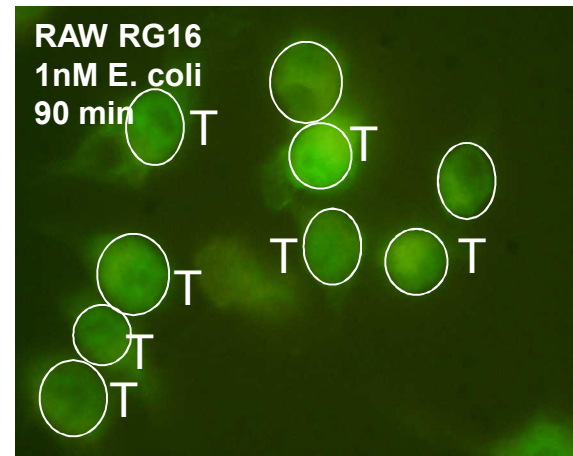
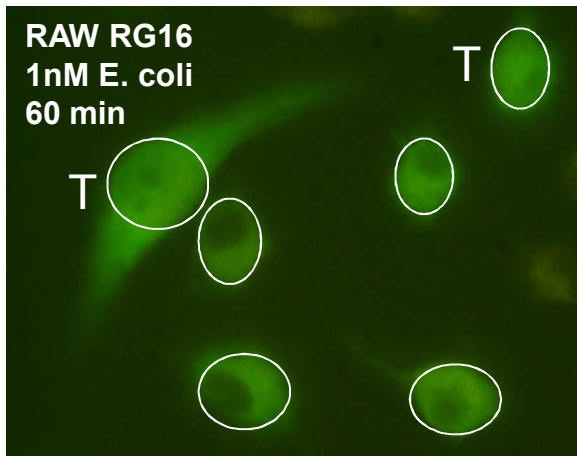
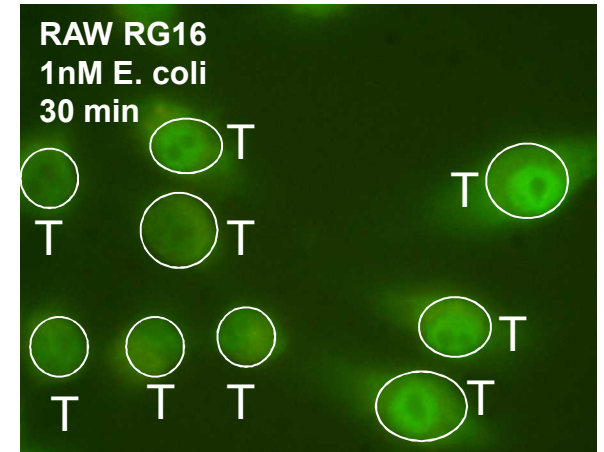
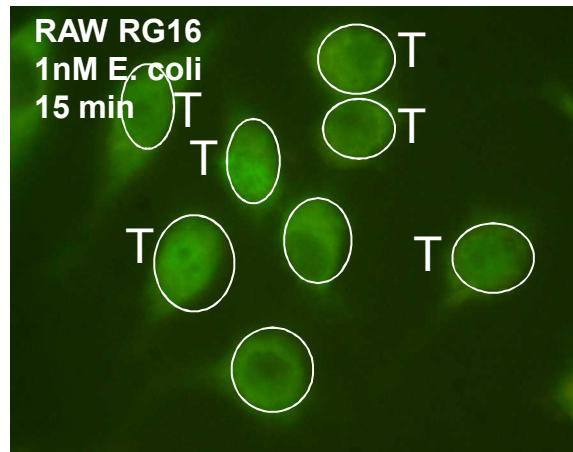
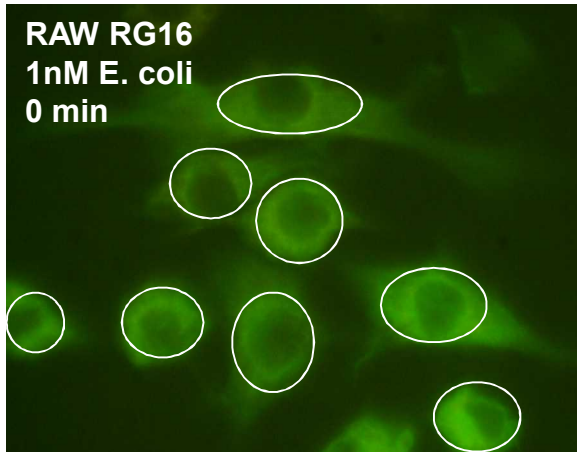
- Gram-negative bacteria produce many types of LPS
- *Y. pestis*
 - Hexaacyl LipidA in vector (21°C) – normal immune response
 - Tetraacyl LipidA in host (37°C) - fails to induce normal immune response
- *E. coli*:
 - Hexaacyl LipidA – normal immune response (control)

lipid A



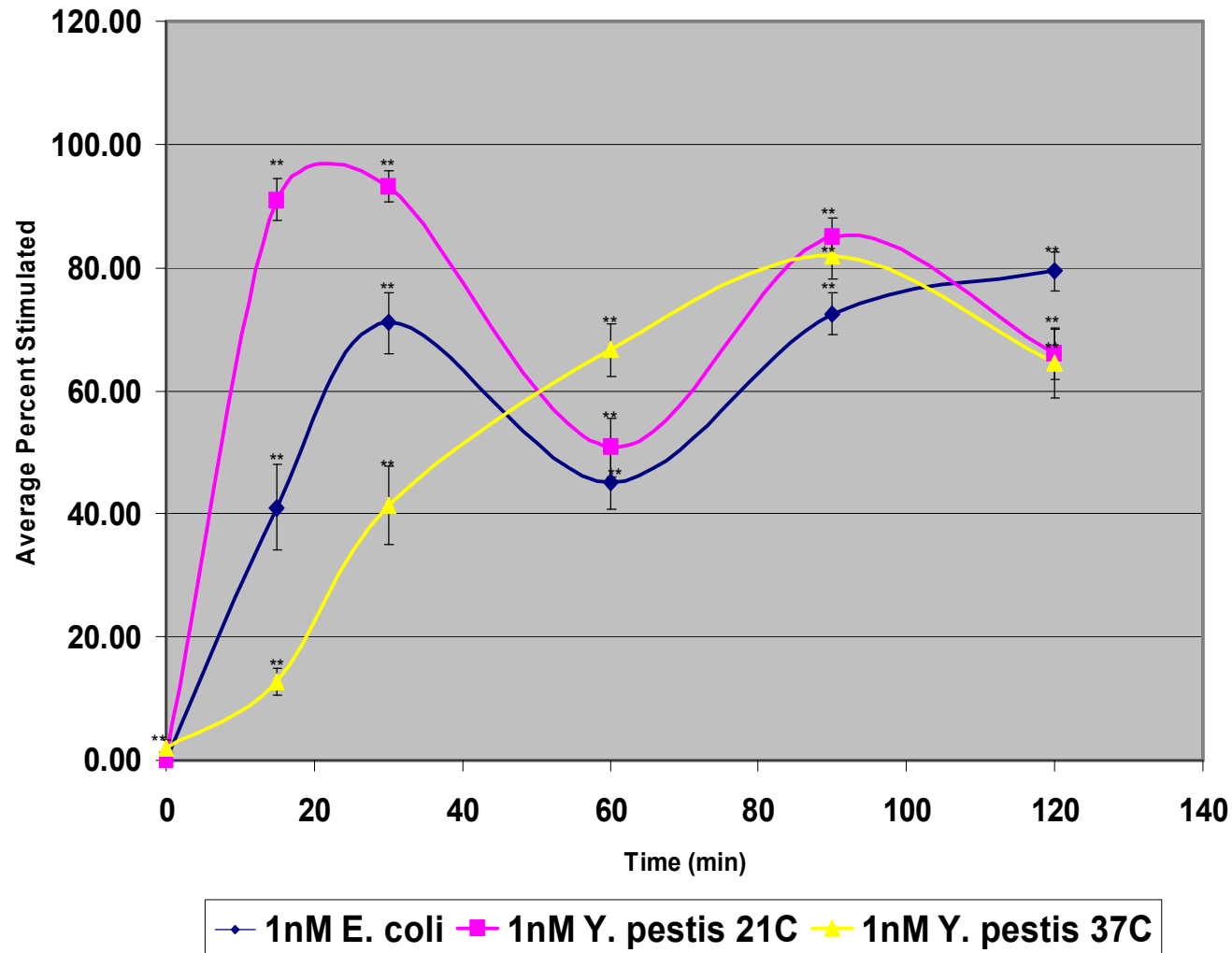


GFP-RelA Oscillation in Macrophages after 1nM *E. coli* LPS Stimulation (fixed cells)



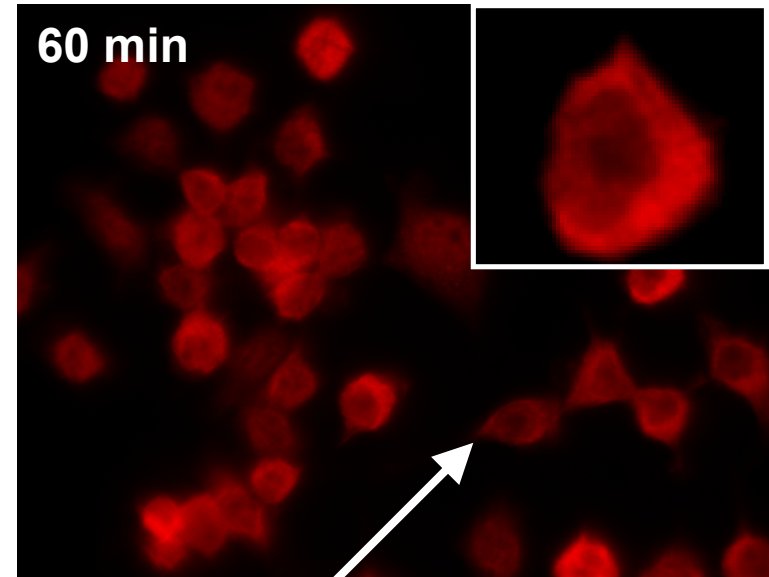
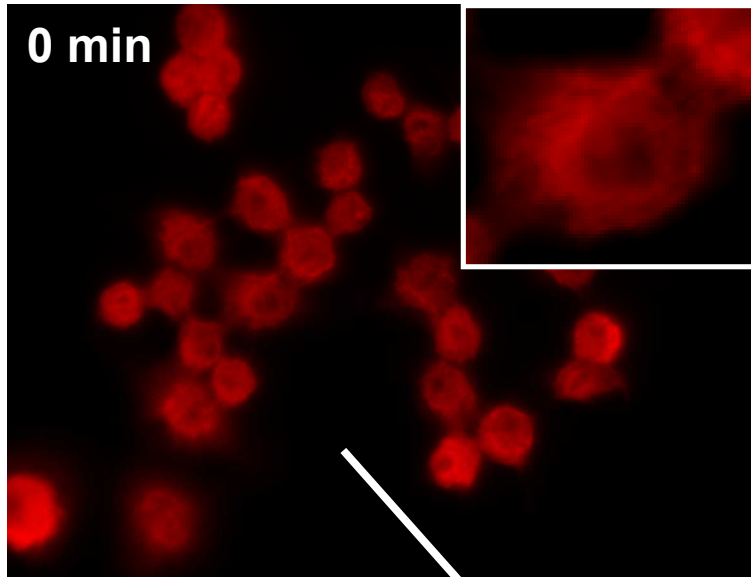


Comparison of 1 nM LPS Stimulation Results



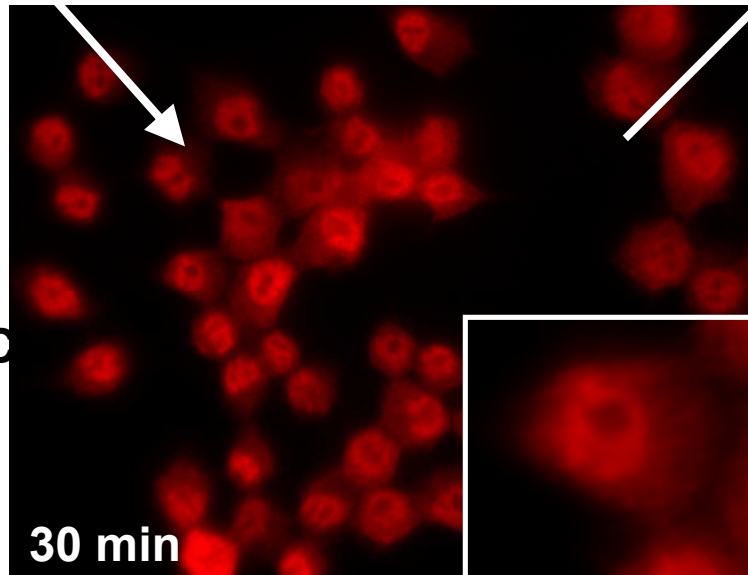


RAW 264.7 Stained with Anti-RelA Antibody



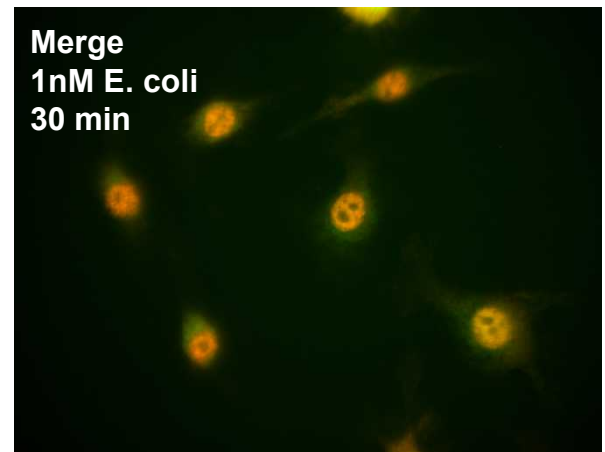
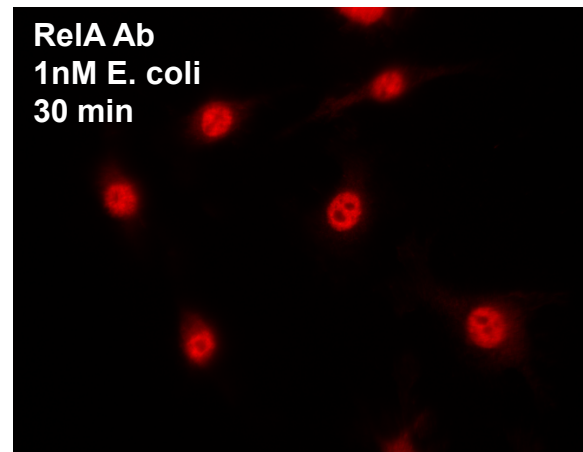
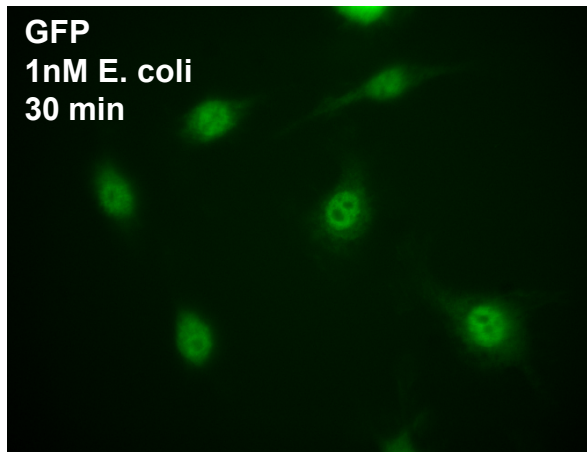
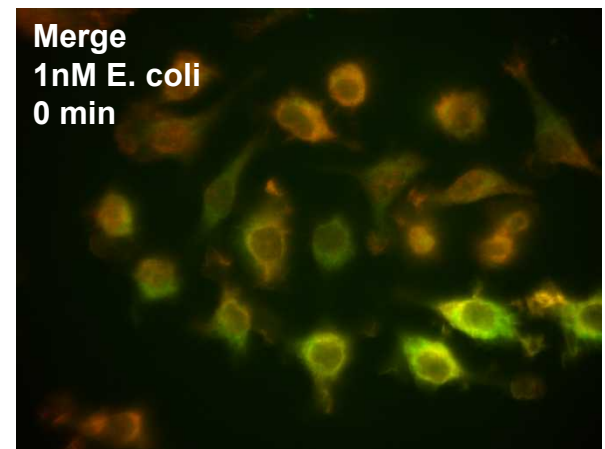
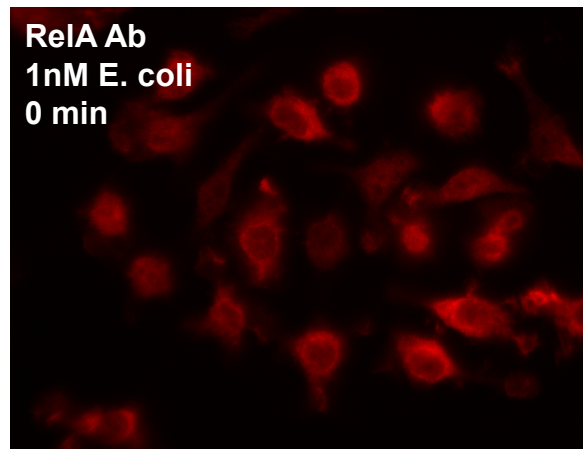
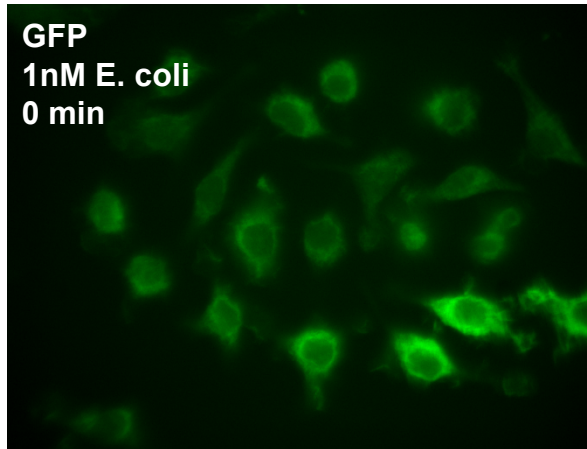
**RAW 264.7 cells
stained with anti-
NF- κ B antibody
(Alexa 568)**

**Cells challenged
with *Y. pestis* 21°C
LPS**





Imaging of GFP-RelA Macrophages Stained with Anti-RelA Antibody





Computer Model Predicts that NF κ B Oscillatory Behavior Varies with LPS Dosage

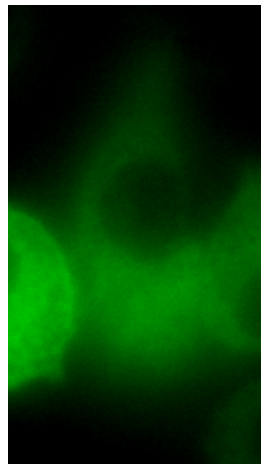
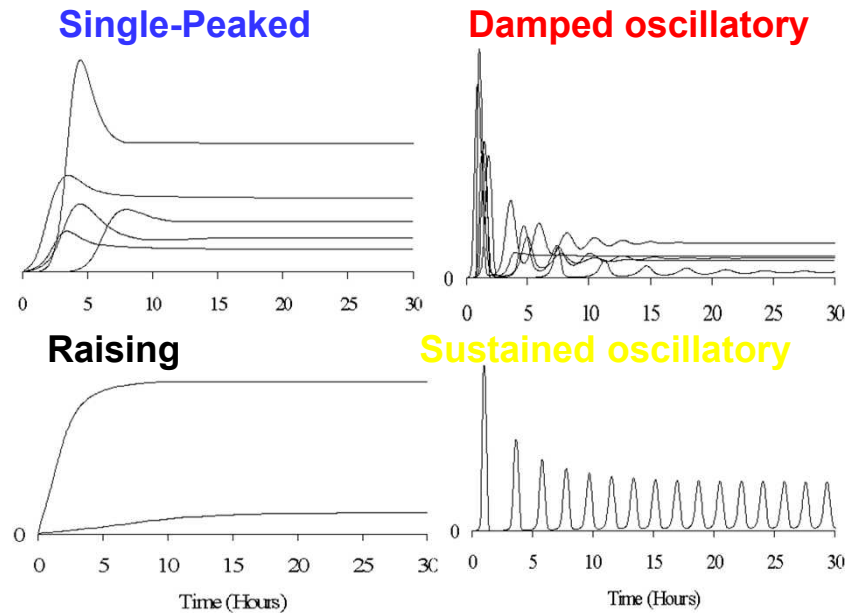
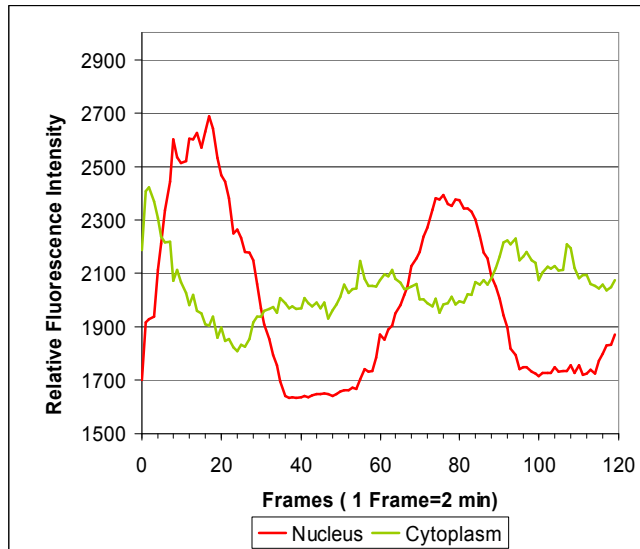
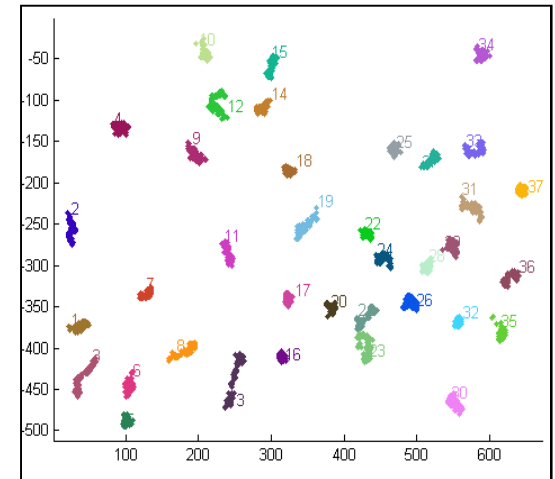
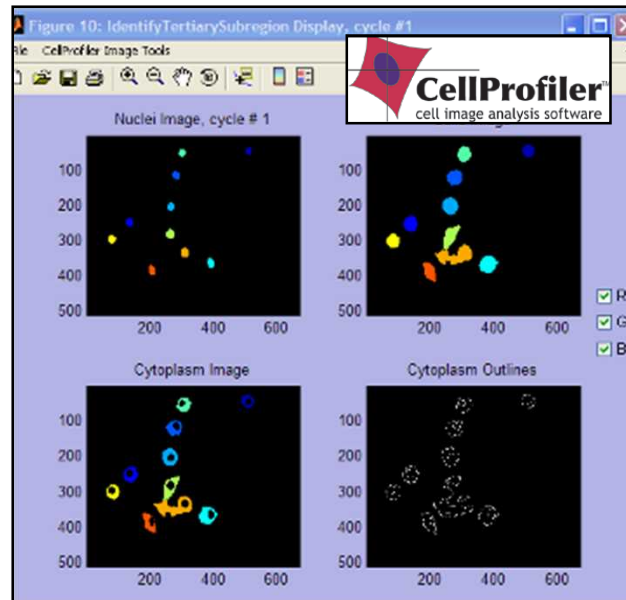
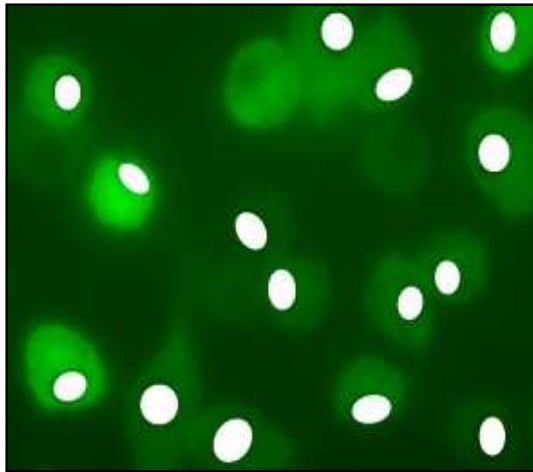
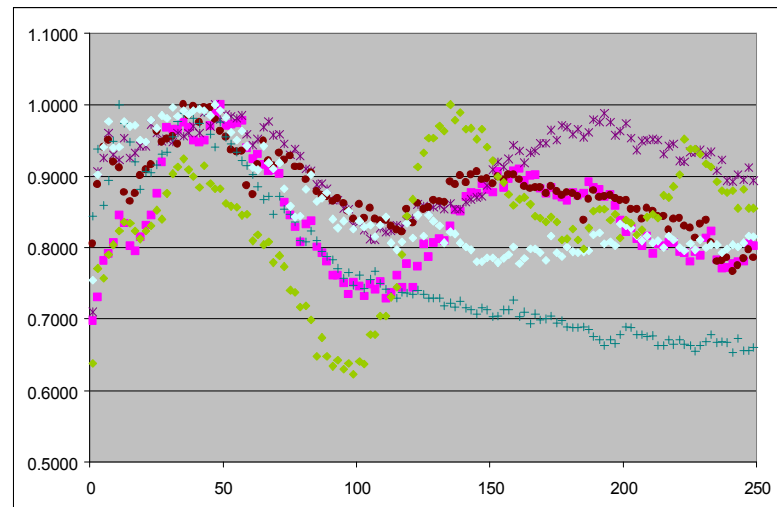




Image Analysis

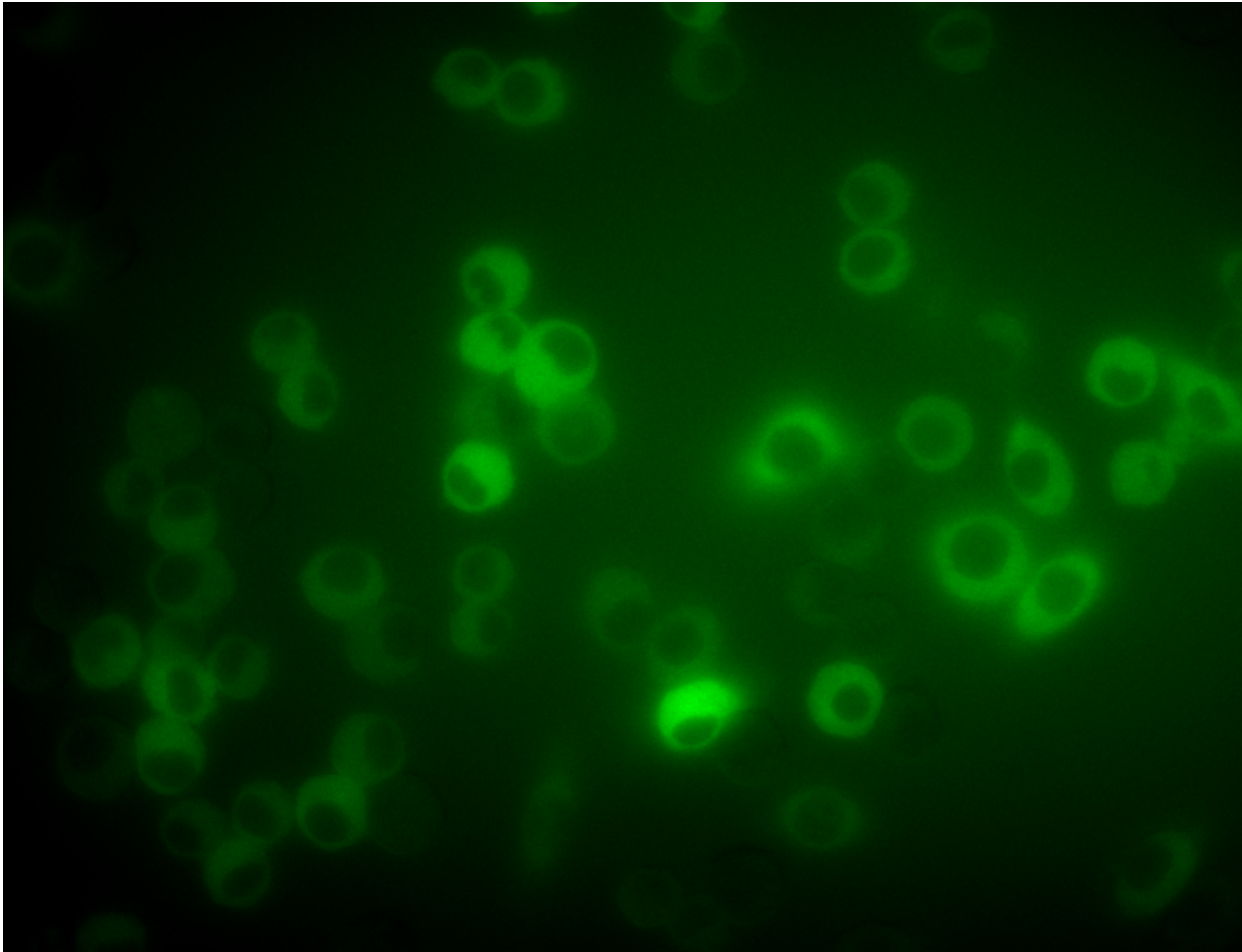


- Nuclei are manually selected
- Intensities of nuclei are extracted and normalized using CellProfiler™
- Cell positions are tracked using MatLab®
- Intensity vs. Time curves exported with Excel®





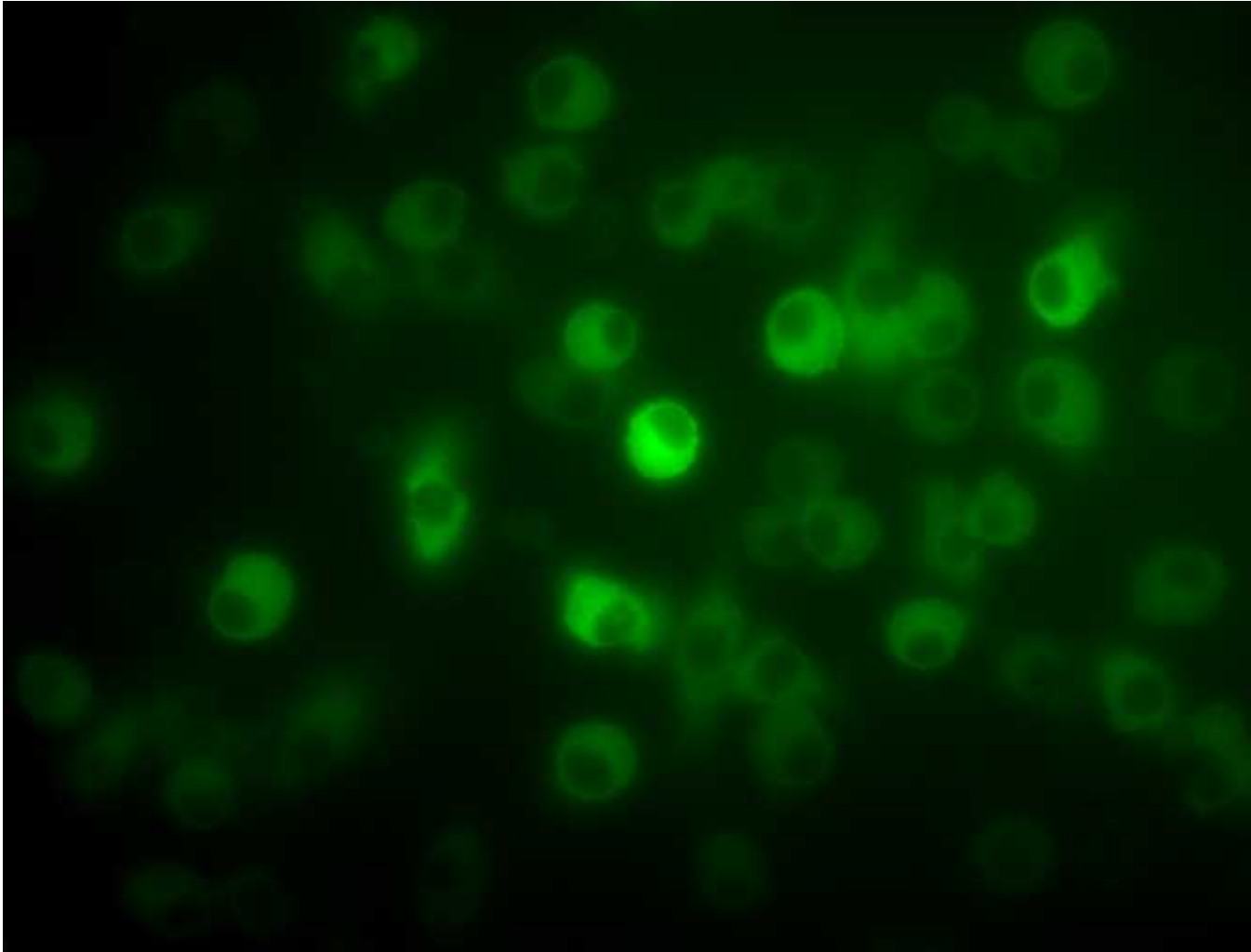
Timelapse of Oscillation 1 nM *E. coli* LPS





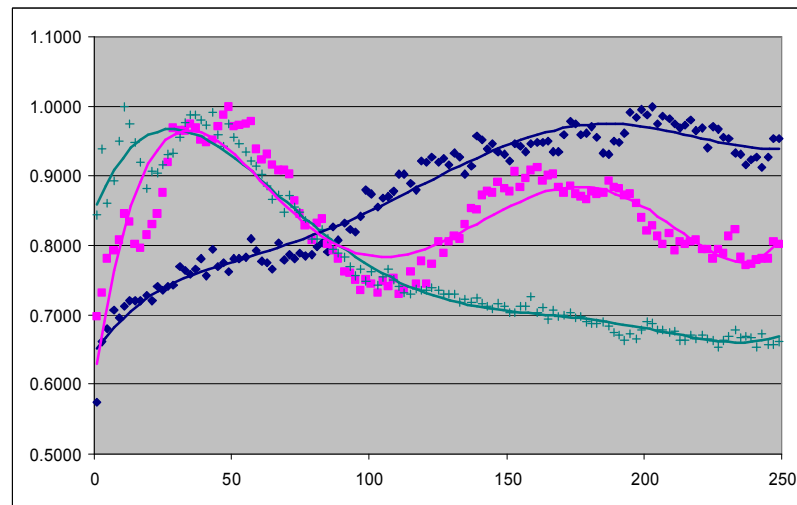
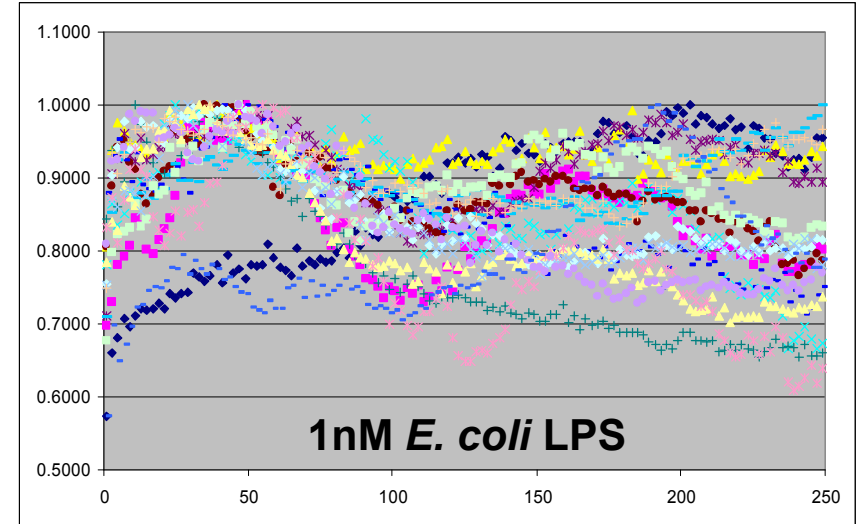
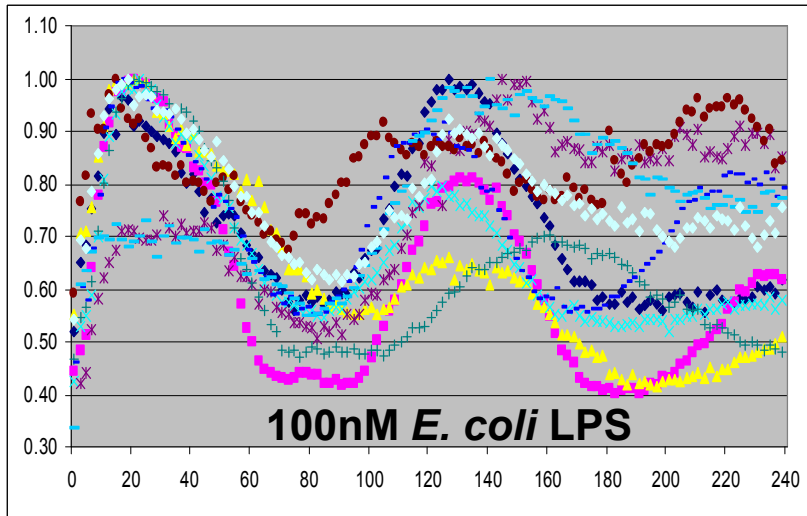
Timelapse of Oscillation

100 nM *E. coli* LPS





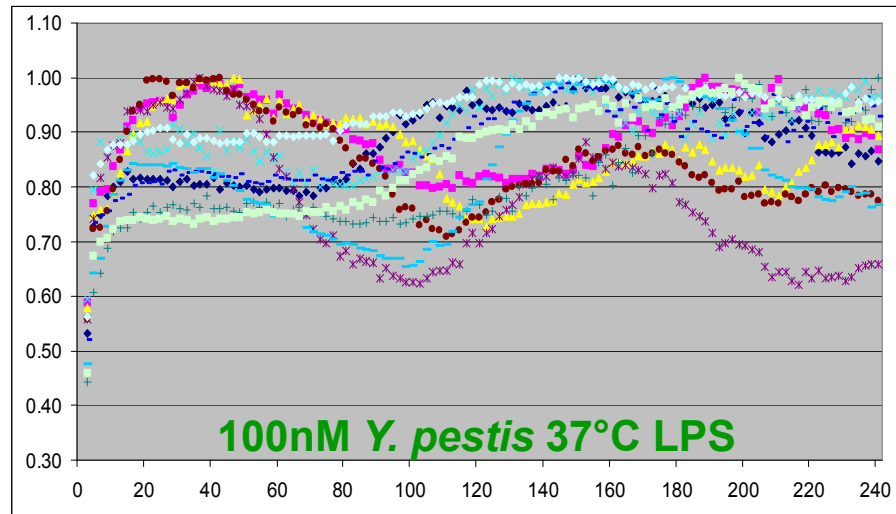
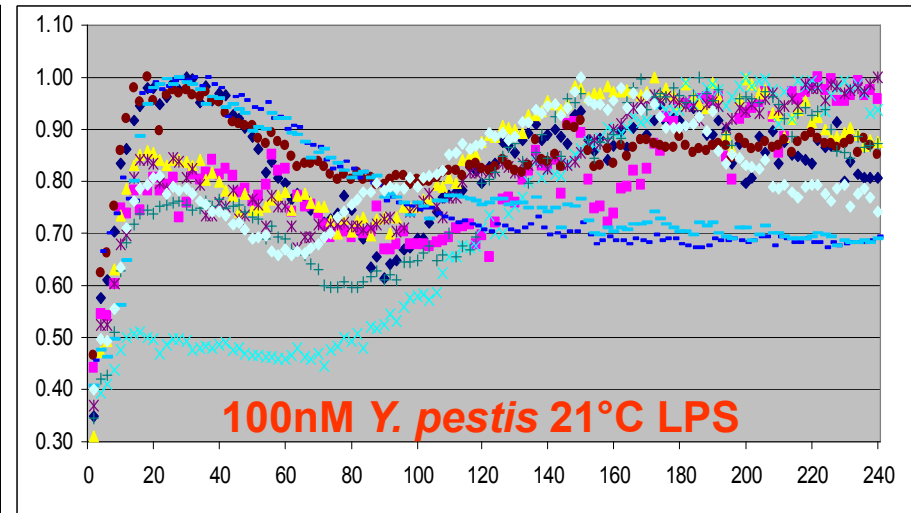
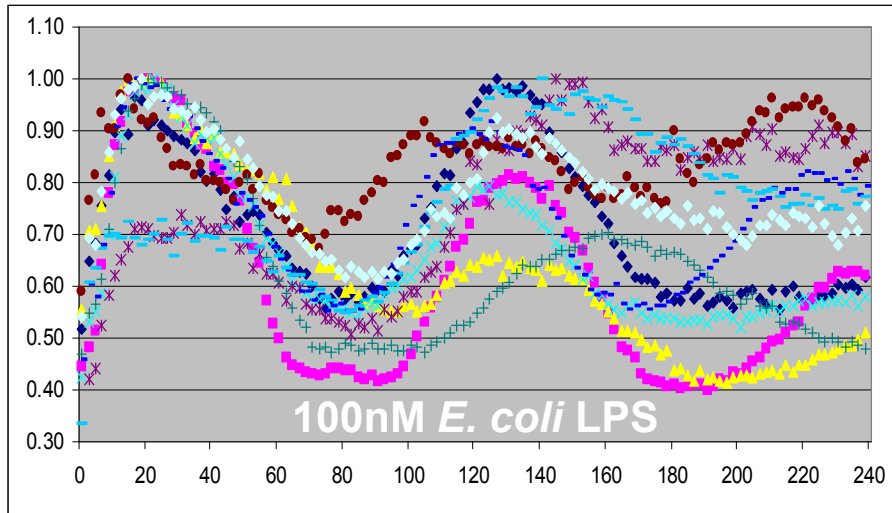
LPS concentration affects RelA-GFP Oscillation Patterns



Rising pattern
Damped-oscillation
Single peak oscillation



LPS Chemotypes Change GFP-RelA Oscillation Patterns





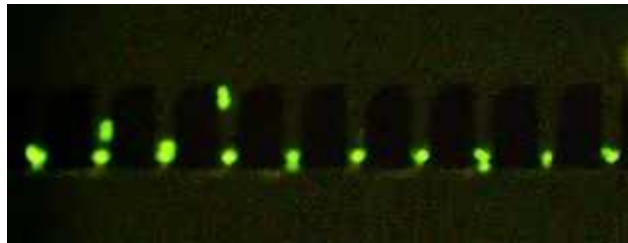
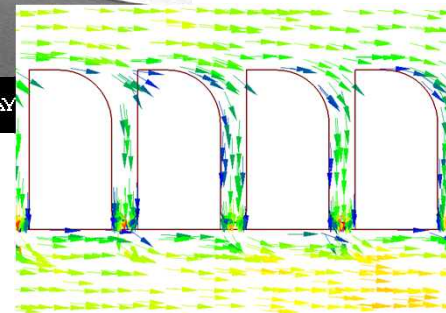
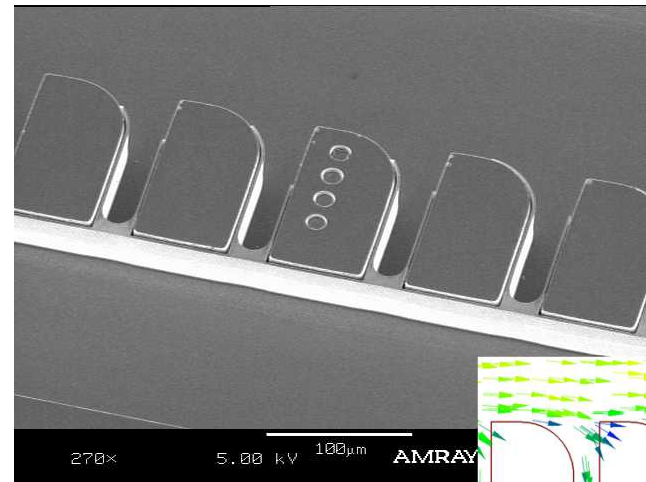
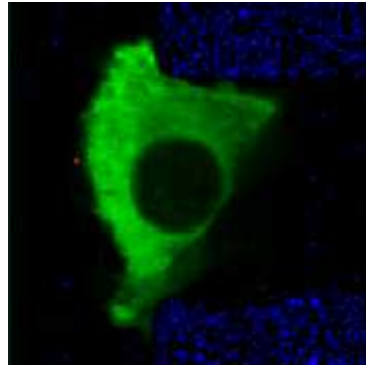
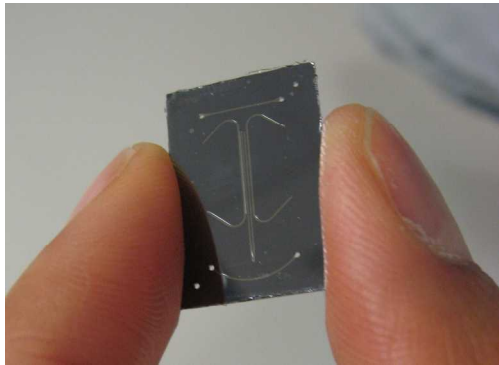
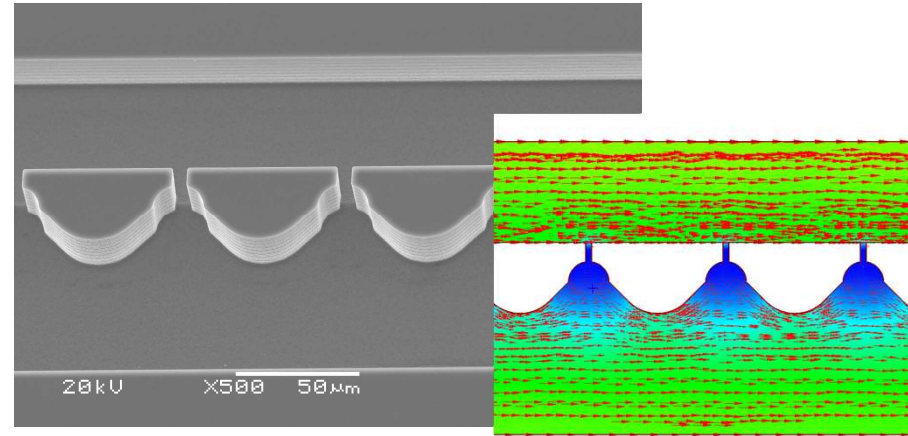
Oscillatory Patterns for Different LPS Concentrations and Chemotypes

	<i>E. coli</i> (1nM)	<i>E. coli</i> (100nM)	<i>Y. pestis</i> 21°C (100nM)	<i>Y. pestis</i> 37°C (100nM)
Cells counted	19	69	25	33
Average First Translocation Time (min)	40 ± 9	20 ± 5	25 ± 6	29 ± 10
% Cells with First Translocation	90%	100%	100%	85%
Average 2nd Translocation Time (min)	167 ± 19	137 ± 13	185 ± 21	164 ± 30
% Cells with 2nd Translocation	53%	94%	32%	61%



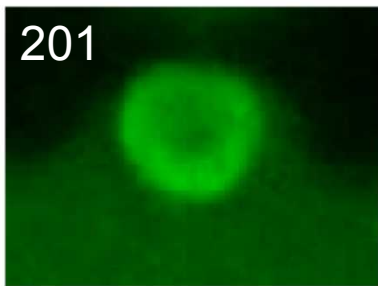
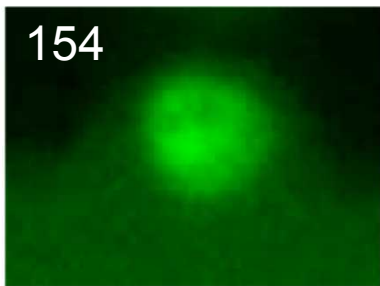
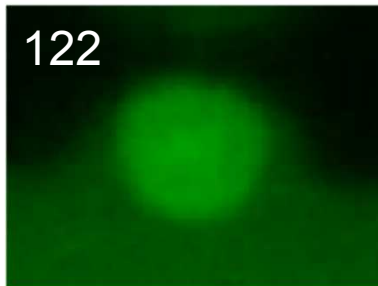
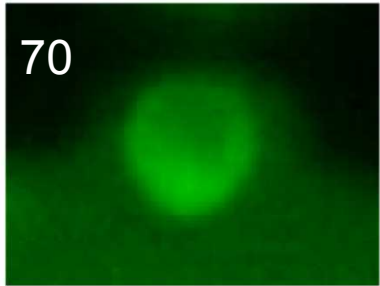
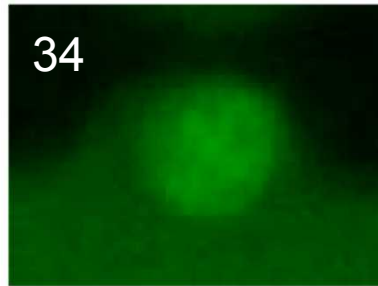
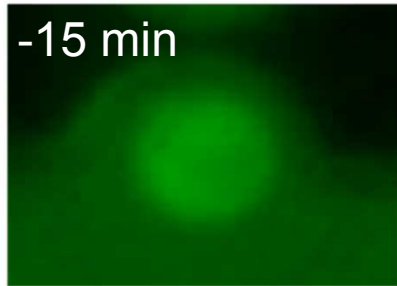
SCA Chip Configuration

- Flow constrictions for mechanical capture of single cells in pre-defined locations
- High-resolution imaging interface
- Fluidic isolation of single cells
- Multiple chambers for experiments



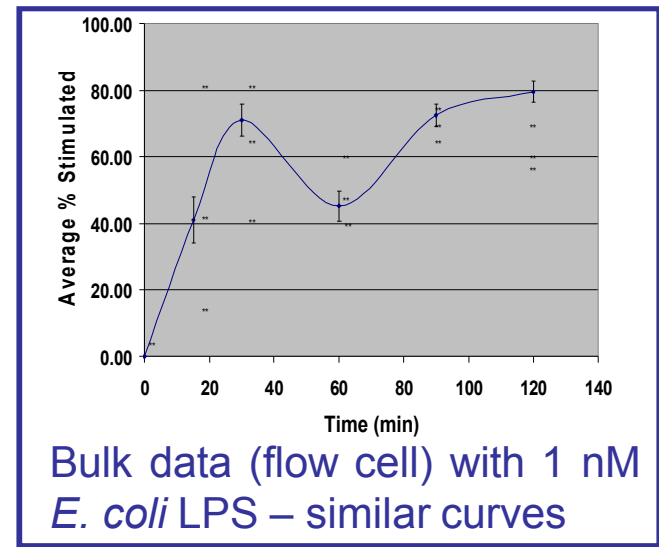
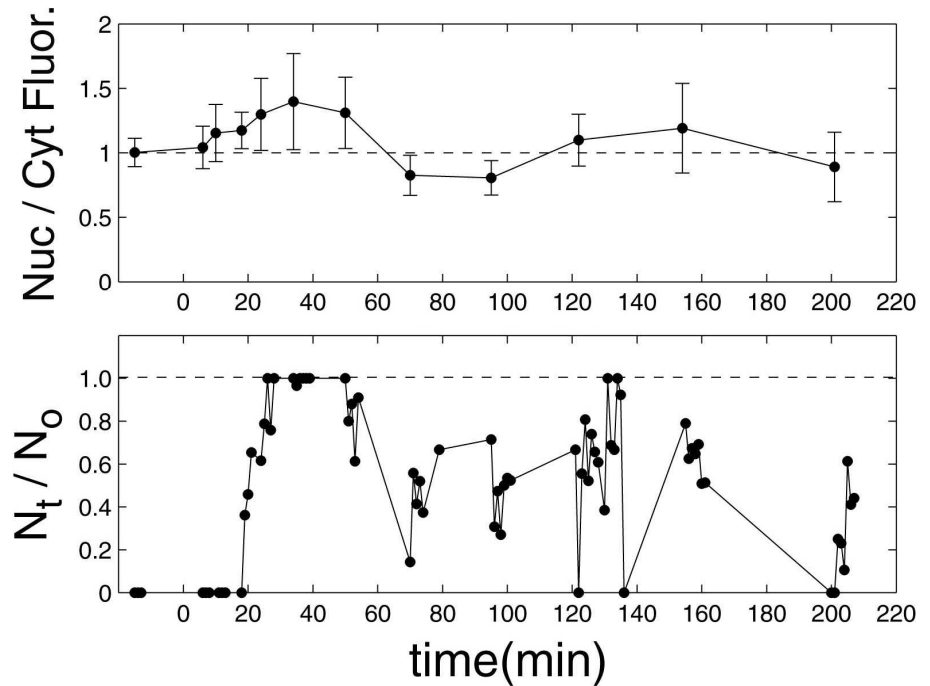


GFP-RelA Oscillations in Single Cells



1 nM *E. coli* LPS at $t = 0$ min
Oscillation Period ~ 110 min

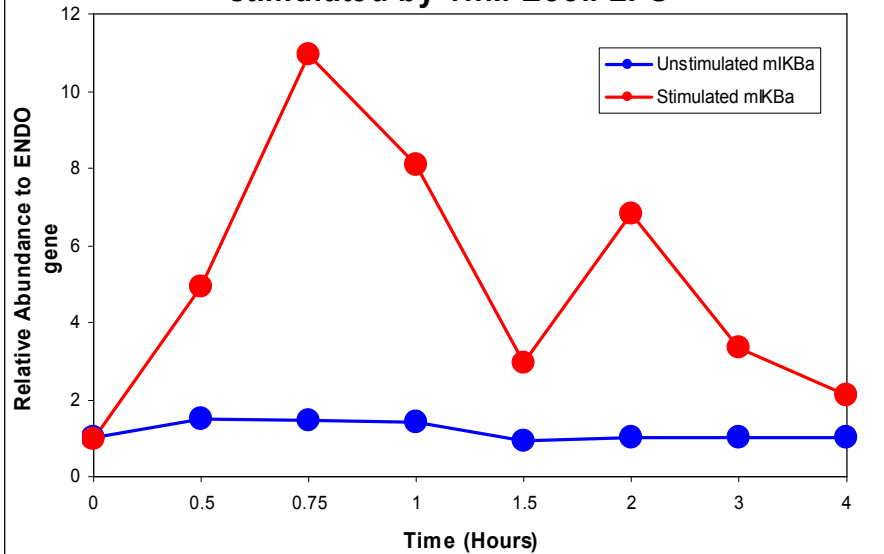
Conrad James, PhD, SNL



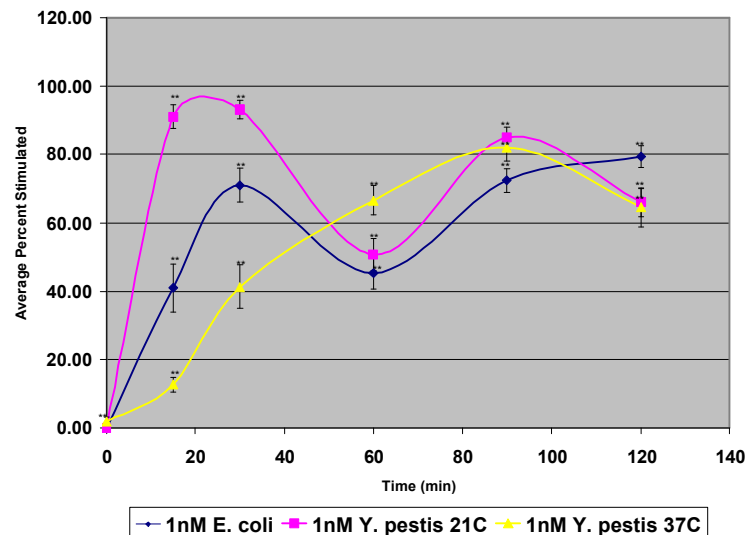
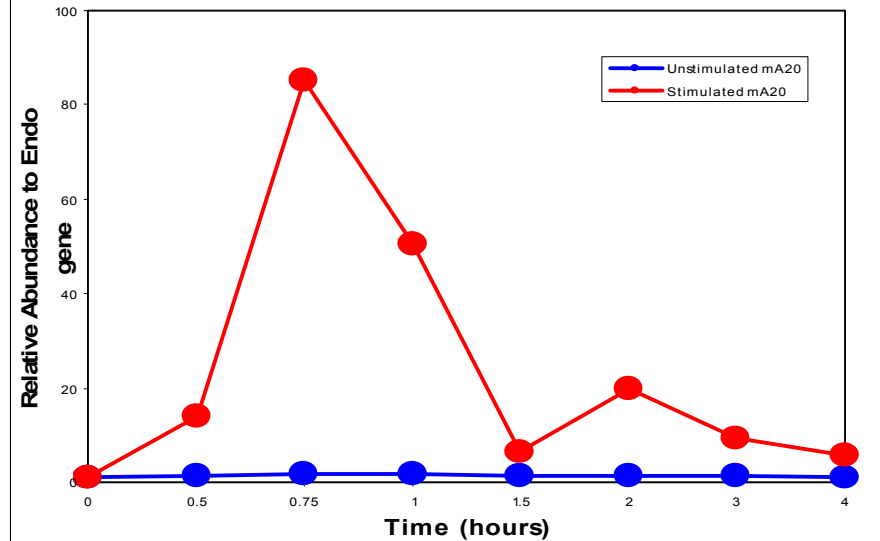


RelA Target Genes Oscillate

mRNA IKBa profiles: transfected RAW stimulated by 1nM Ecoli LPS

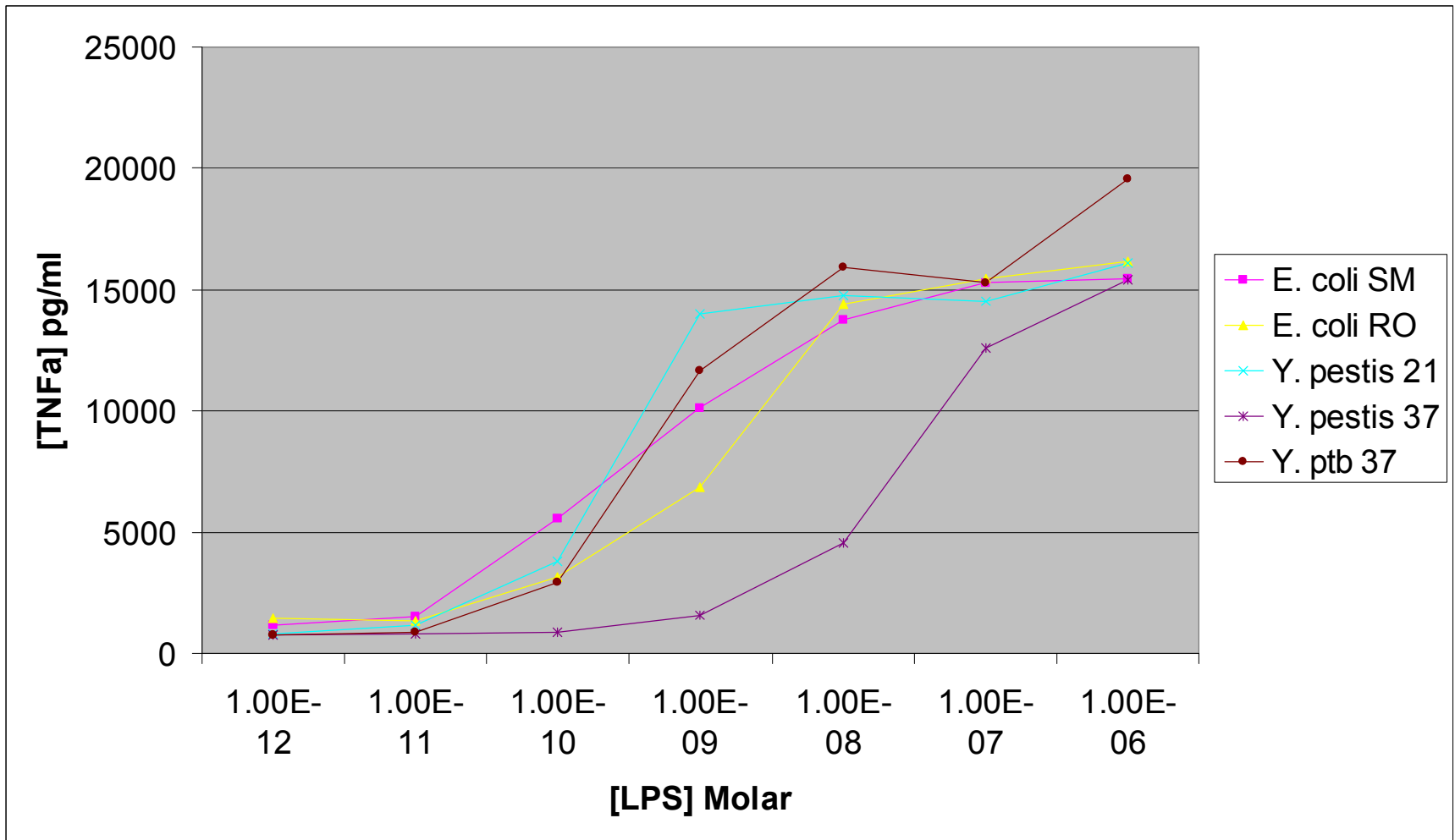


mRNA A20 profiles: Transfected RAW stimulated by 1nM E.Coli LPS





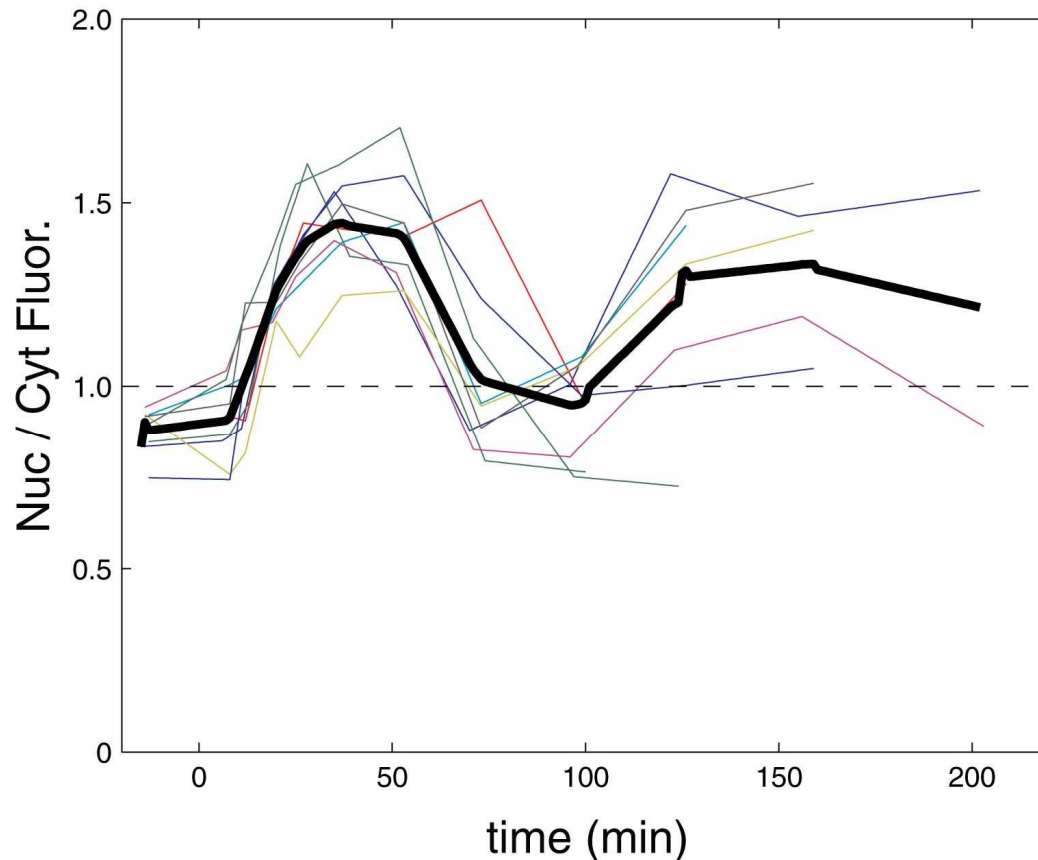
TNF α ELISA in Response to Different LPS Chemotypes





GFP-RelA Oscillations in the SCA

E. coli LPS 1 nM, n=10 cells



Relevant metrics:

Nuc/Cyt (t_0) = 0.9

$t_{max1} = 40 \pm 4$ min

$t_{period} = 116 \pm 22$ min

Bulk Data	<i>E. coli</i> (1nM)
# Cells	19
Average First Translocation (min)	40 $\pm$ 9
% Cells with First Translocation	17 (90%)
Period between 1 st and 2 nd Translocation (min)	127 $\pm$ 21
% Cells with 2nd Translocation	10 (53%)

Next: Continue to track relevant metrics for model refinement, TNF α challenges, etc.



Summary & Future Work

- **Computer model predicted RelA oscillations**
- **RelA oscillates in response to LPS**
 - Oscillation is dependent on LPS concentration and LPS type.
 - Three distinct oscillatory patterns have been observed and predicted by modelling
- **Biocore:** Generated TNF- α transcriptional reporter that will be transfected to measure TNF- α in oscillating vs. non-oscillating cells.
- **Biocore:** TNF- α staining of single cells on grid.
- **Platform:** TNF α experiments – examine strength of response in comparison to LPS – Comp. Model
- **Platform:** Optimize fluidic isolation of single cells in the SCA for comparison with computational model predictions



A Multi-Disciplinary Team

Biology Core Team:

Cathy Branda
Bryan Carson
Jens Poschet
Roberto Rebeil
Elizabeth Carles
Jacquelyn Murton

Platform and Detection Systems Core Team:

Conrad James
Ron Manginell
Matt Moorman

Computational Biology Core Team:

Jean-Loup Faulon
Jaewook Joo

Funding: Sandia LDRD Program