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# Omic Approaches in Nanosafety Research: Prediction of Toxicity and Susceptibility Pathways

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# Potential Health Impacts of Nanomaterials- What endpoints should we focus on?

*Editorial*

## **Air Pollution and Pneumonia**

### **The “Old Man” Has a New “Friend”**

Hospitalization for community-acquired pneumonia (CAP) appears to be occurring more frequently (1, 2). Why is this? To answer this question we need to know why individuals develop this condition. Many predisposing factors to CAP have been described, such as the increased risk associated with extremes of age and comorbid diseases (3, 4).

*A. Zanobetti, Harvard School of Public Health*

*M. Woodhead, Manchester Royal Infirmary*

*Am J Respir Crit Care Med* Vol 181, pp 47-53, 2010

Originally Published in Press as DOI: 10.1164/rccm.200901-0160oc on October 1, 2009

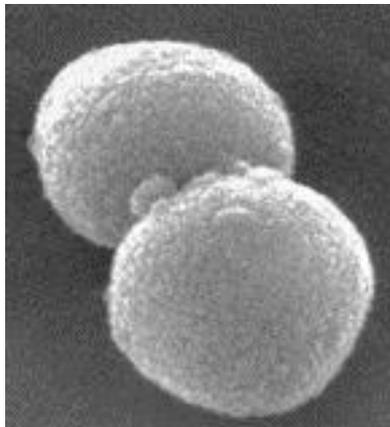
Internet address: [www.atsjournals.org](http://www.atsjournals.org)



## **Long-Term Exposure to Ambient Air Pollution and Risk of Hospitalization with Community-acquired Pneumonia in Older Adults**

Binod Neupane<sup>1</sup>, Michael Jerrett<sup>2</sup>, Richard T. Burnett<sup>3</sup>, Thomas Marrie<sup>4</sup>, Altaf Arain<sup>5</sup>, and Mark Loeb<sup>6</sup>

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*Streptococcus pneumoniae*

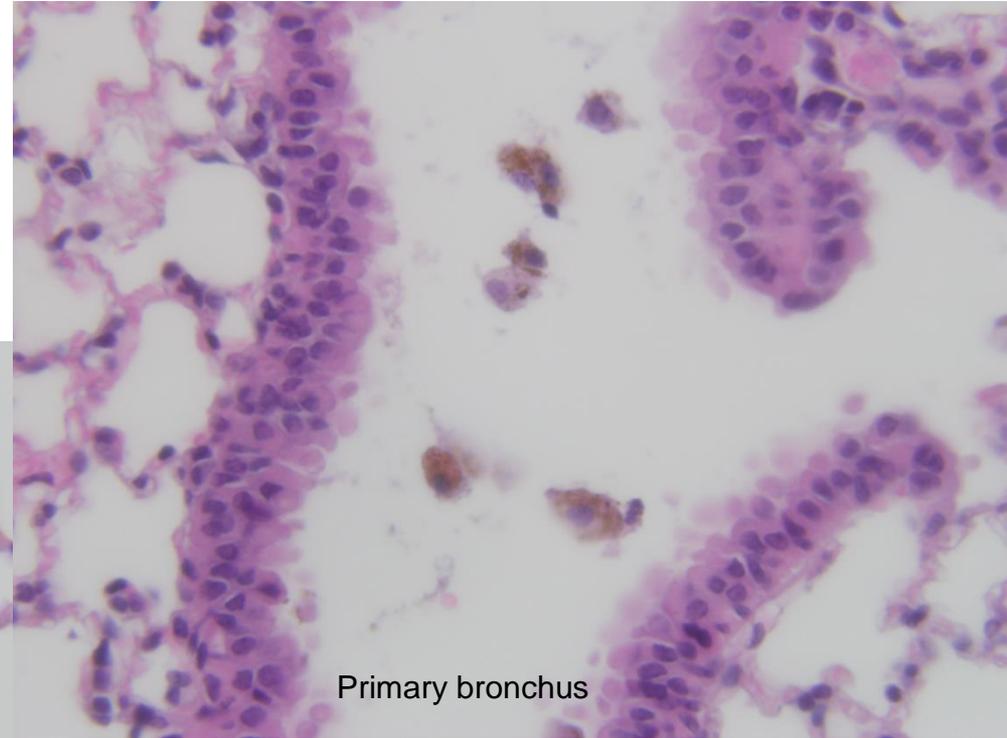
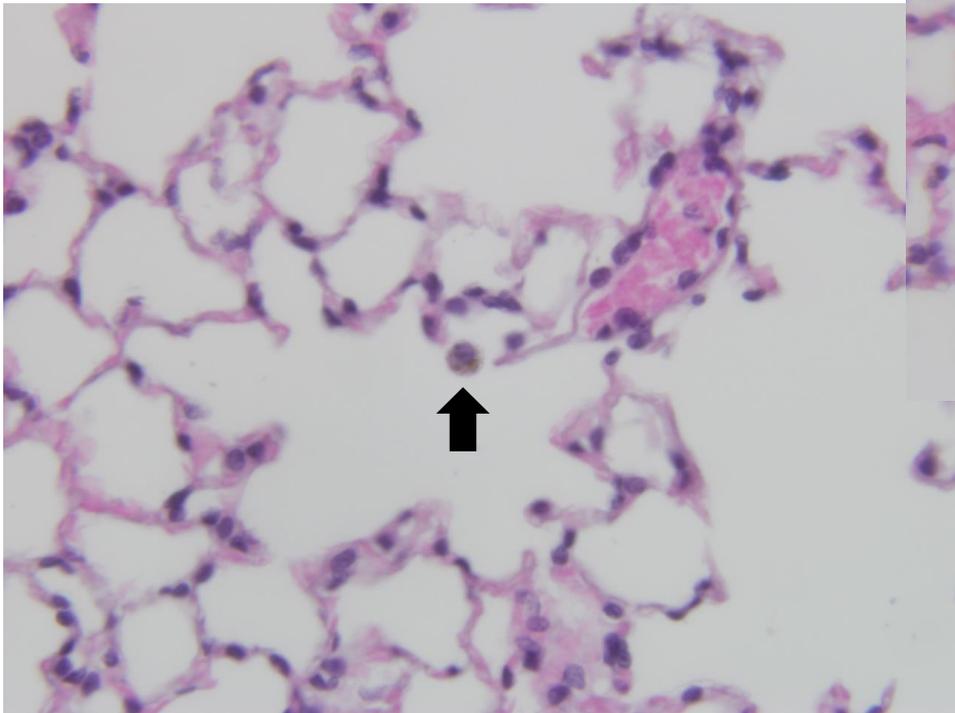


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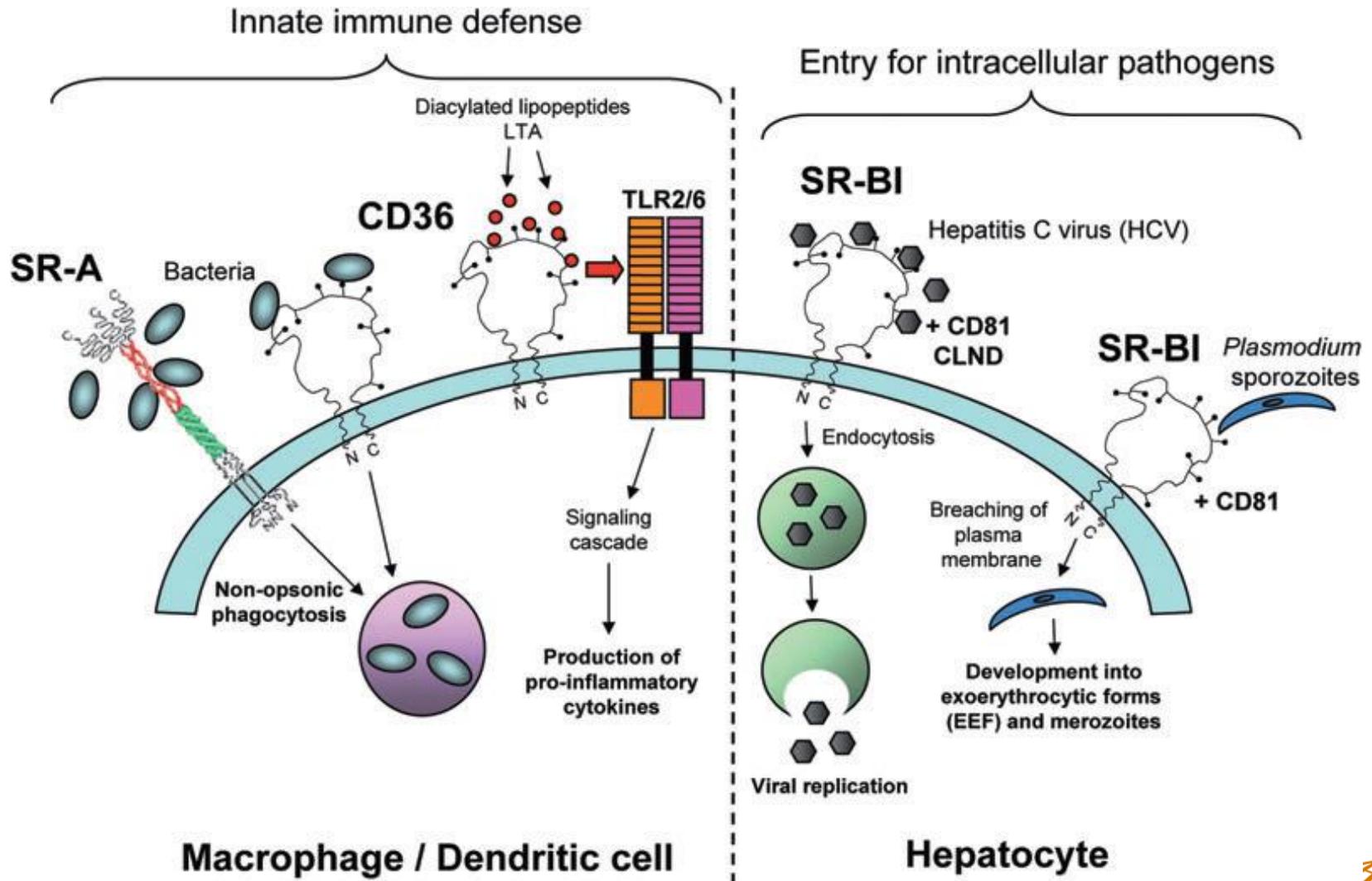
# Macrophages are a Critical Target for Inhaled Nanomaterials

Mouse lung sections-  
48 hrs post inhalation exposure to 13 nm  
superparamagnetic iron oxide (**SPIO**) nanoparticles

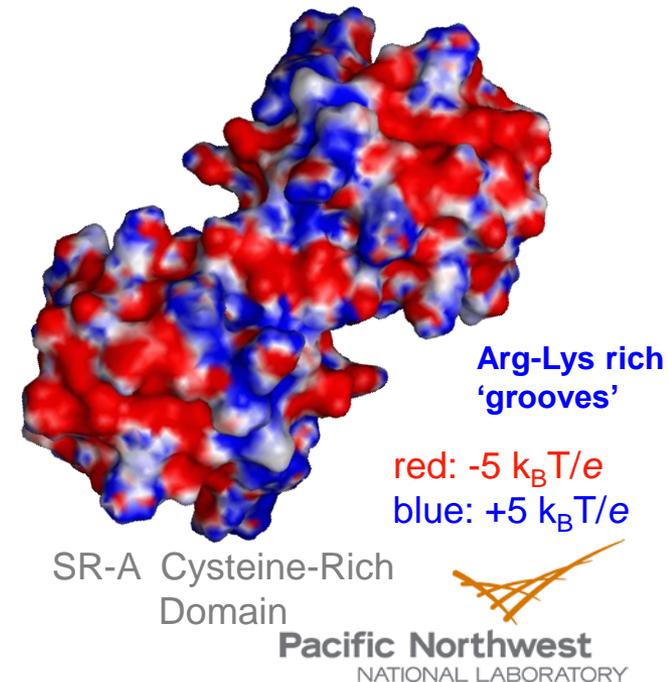
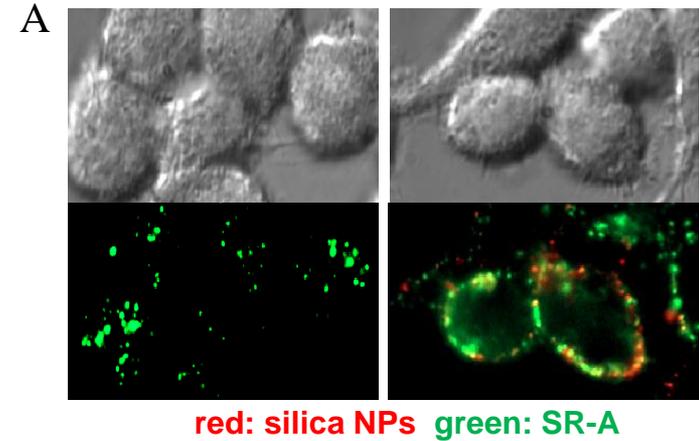
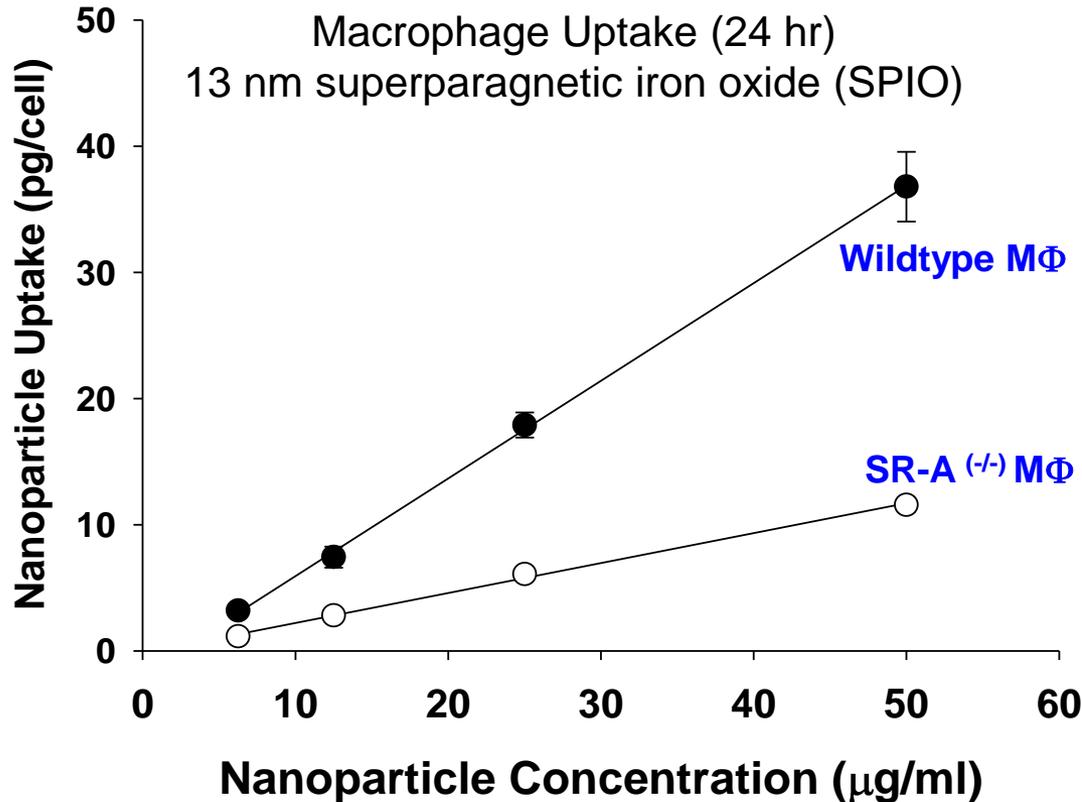
*How is normal macrophage  
function affected by  
nanoparticle exposure?*



# Scavenger Receptors and Innate Immunity



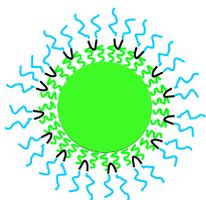
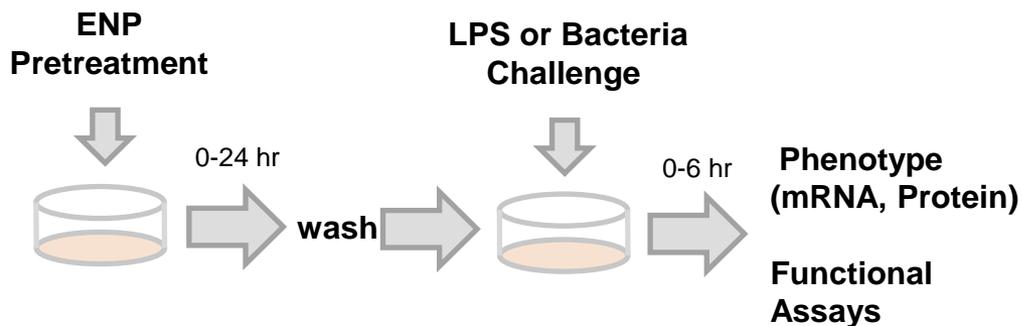
# Scavenger Receptor A (SR-A) Mediates Macrophage Uptake of Engineered Nanoparticles



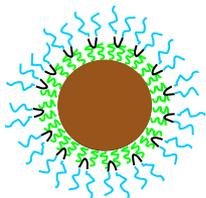
- ❖ Orr *et al. Nanotoxicology*, 2011; 5(3); 296-311.
- ❖ Minard *et al. Biosensor & Bioelectronics*, 2013; 43; 88-93.
- ❖ Mihai *et al.* 2013 (submitted)

# How are Macrophage Activation Pathways Impacted by Nanoparticles?

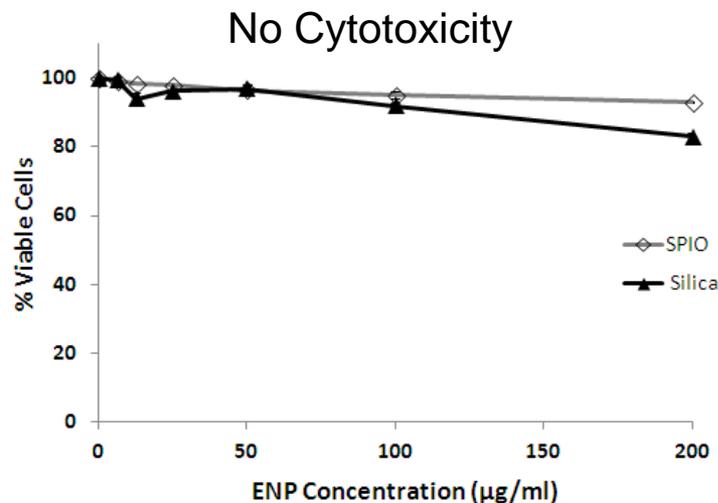
## Study Design:



Amorphous **silica** (fluorescent)  
(50 nm, zeta = -23.5 mV in RPMI)



Superparamagnetic iron oxide (**SPIO**)  
(13 nm, zeta = -21.9 mV in RPMI)



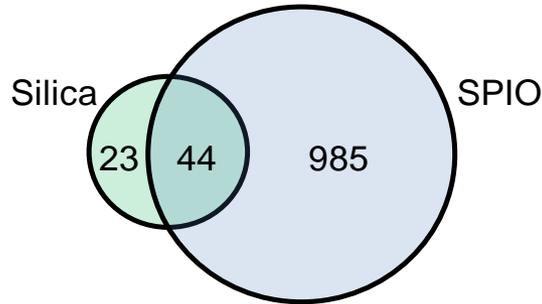
## Expression Profile Results

TABLE 1. Summary of Differentially Expressed Genes in Mouse Bone Marrow-Derived Macrophages Following Exposure to SPIO, Silica, or LPS

pretreatment	challenge	DE genes <sup>a</sup> vs control	DE genes <sup>a</sup> vs LPS (GTA) <sup>b</sup>
none	none		
silica (25 µg/mL)	none	67	
SPIO (25 µg/mL)	none	1029	
none	LPS (10 ng/mL)	5027	
silica	LPS	4954	44 (15)
SPIO	LPS	5483	1044 (499)
	union	6831	1058 (503)

# Gene Regulation Changes Induced by Silica and Iron Oxide Nanoparticles

A.



**Upregulated:**  
lipid metabolism and oxidative stress-response pathways

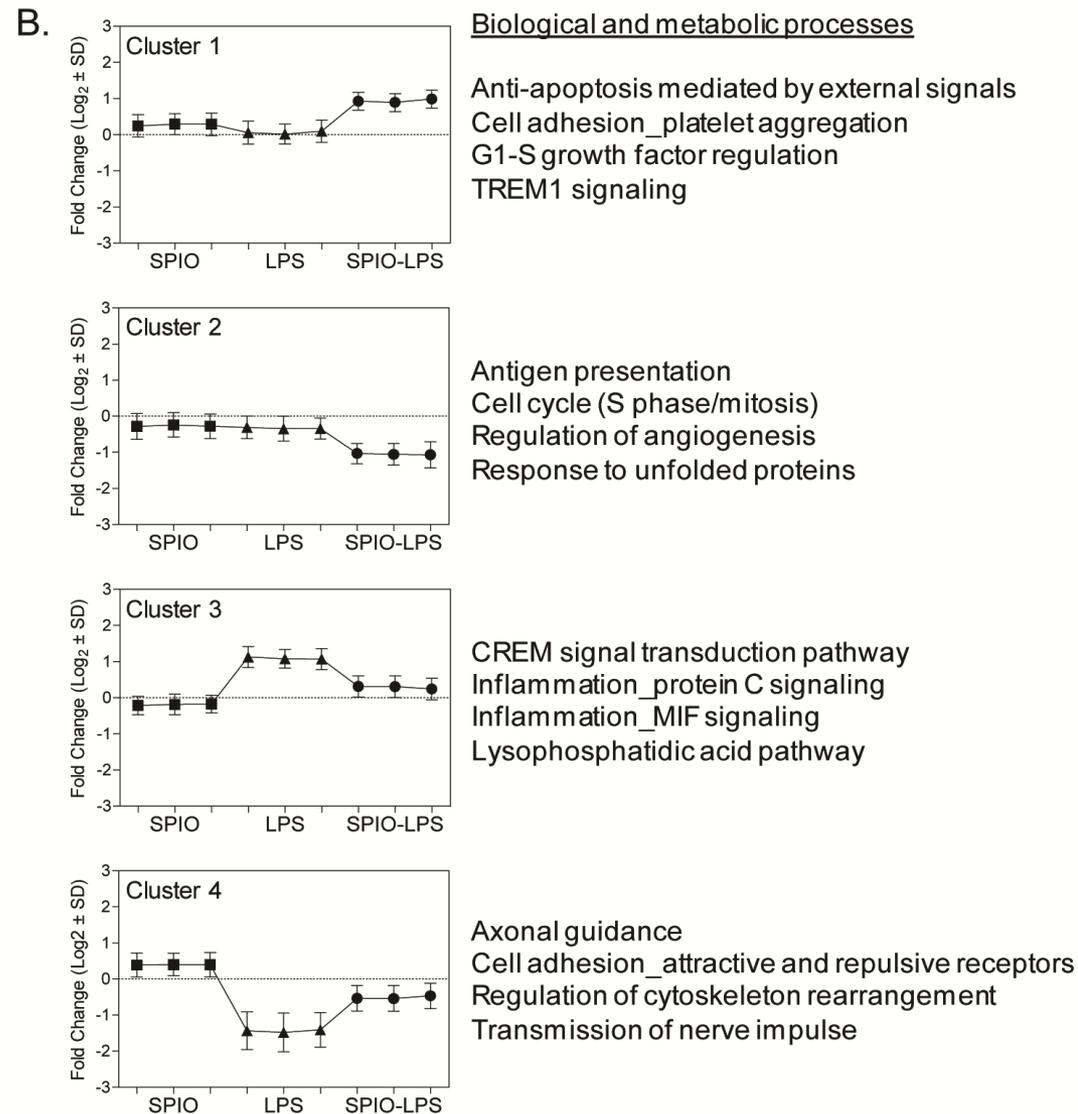
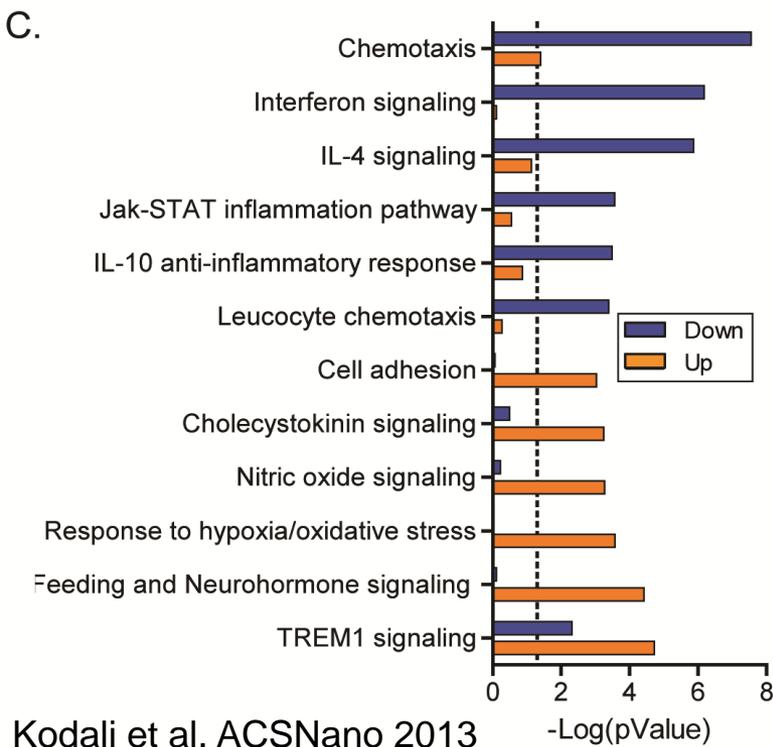
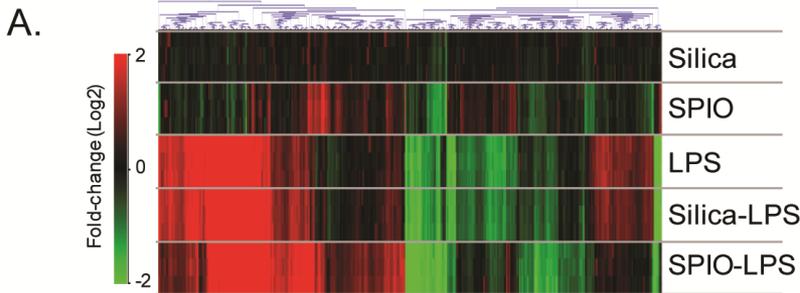
**Suppressed:**  
cell cycle and some immune response pathways

B.

Functional enrichment	SPIO up-regulated genes (p-value)	SPIO down-regulated genes (p-value)
<u>Significant for up-regulated genes</u>		
Ceramide pathway	8.50E-03	6.35E-01
Phosphatidic acid pathway	6.42E-03	1.00E+00
Response to hypoxia and oxidative stress	3.86E-04	9.59E-01
Sphingomyelin pathway	3.54E-03	5.40E-01
Steroid metabolism_Cholesterol biosynthesis	2.42E-07	1.00E+00
<u>Significant for down-regulated genes</u>		
Cell adhesion_Leucocyte chemotaxis	6.70E-01	5.67E-03
Cell adhesion_Platelet-endothelium-leucocyte interactions	2.36E-01	3.31E-05
Cell cycle_Core	9.47E-01	3.95E-07
Cell cycle_G2-M	7.48E-01	1.39E-07
Cell cycle_Mitosis	4.71E-01	1.04E-04
Cell cycle_S phase	6.75E-01	7.09E-03
Chemotaxis	1.89E-01	2.35E-07
Cytoskeleton_Spindle microtubules	7.44E-01	1.05E-05
Development_Blood vessel morphogenesis	2.83E-01	9.04E-13
Regulation of epithelial-to-mesenchymal transition	9.74E-01	1.10E-03
Immune response_Th17-derived cytokines	6.03E-01	8.24E-03
Inflammation_Interferon signaling	9.15E-01	9.40E-04
Inflammation_MIF signaling	7.95E-01	5.18E-04
Reproduction_Progesterone signaling	5.12E-01	3.56E-03
<u>Significant for up- and down-regulated genes</u>		
Blood coagulation	5.00E-03	3.16E-02
Development_Regulation of angiogenesis	1.29E-02	2.53E-05
N-acyl-sphingosine phosphate pathway	2.27E-02	1.69E-04
Transport_Iron transport	1.67E-02	3.06E-03

***Even 'benign' nanoparticles which lack direct cytotoxic or proinflammatory effects can alter the regulation of hundreds of genes.***

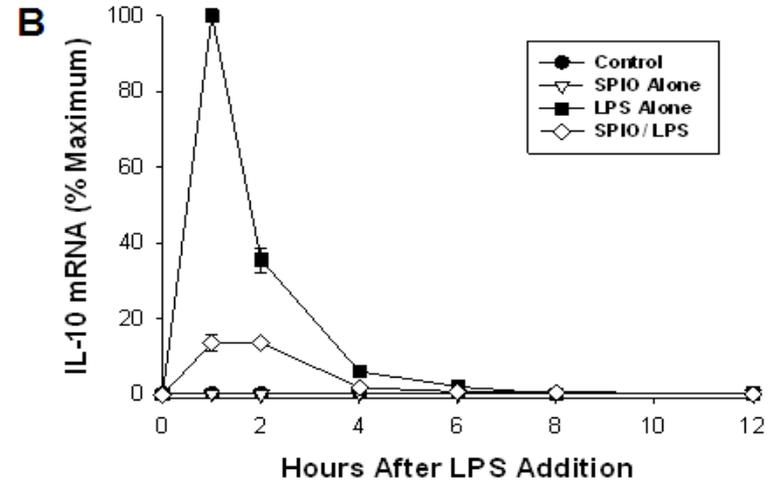
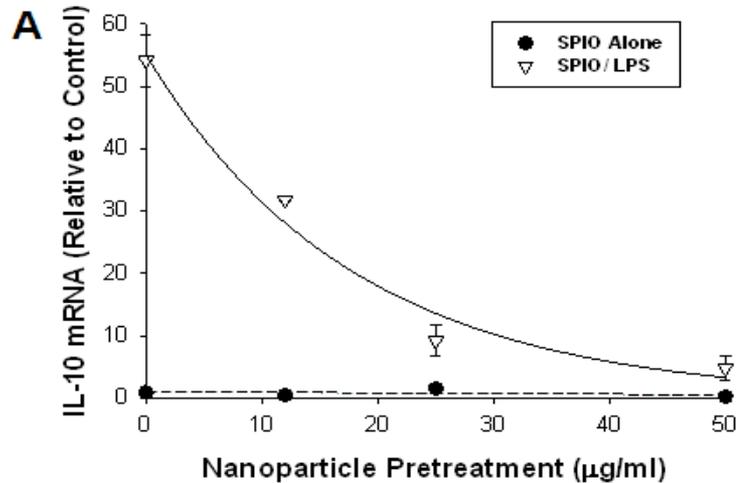
# Nanoparticle Exposure Shifts the LPS Mediated Activation Profile of Macrophages



# Pro-Inflammatory and Anti-Inflammatory Cascades are Uncoupled in Nanoparticle Exposed Macrophages

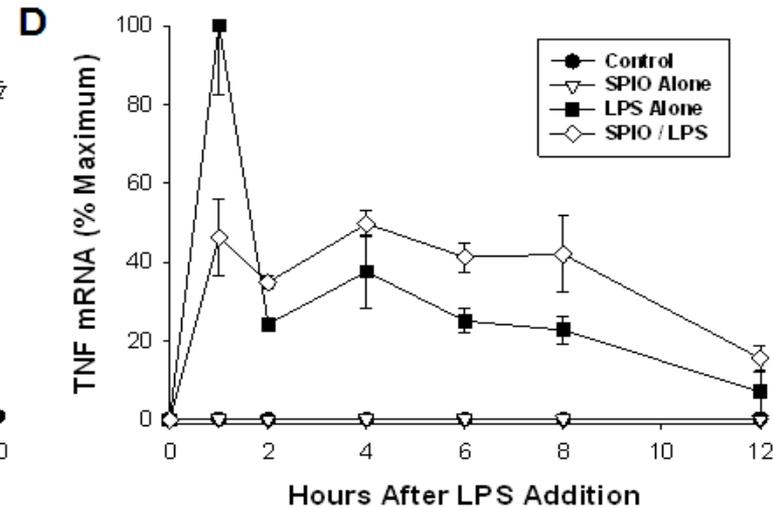
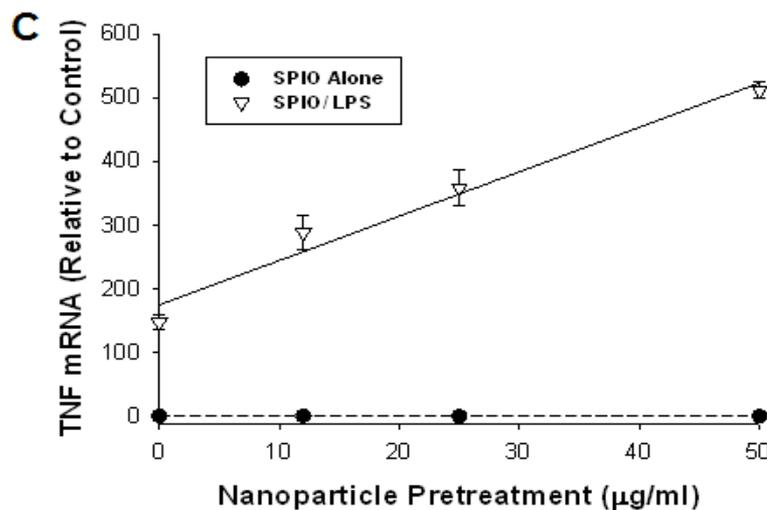
## •IL-10

Anti-inflammatory  
Feedback Pathway  
Suppressed

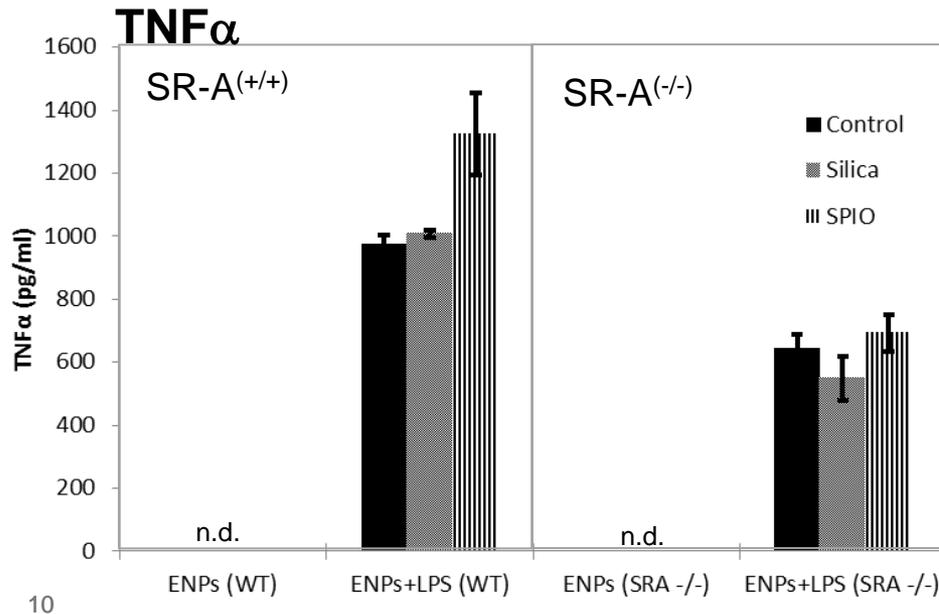
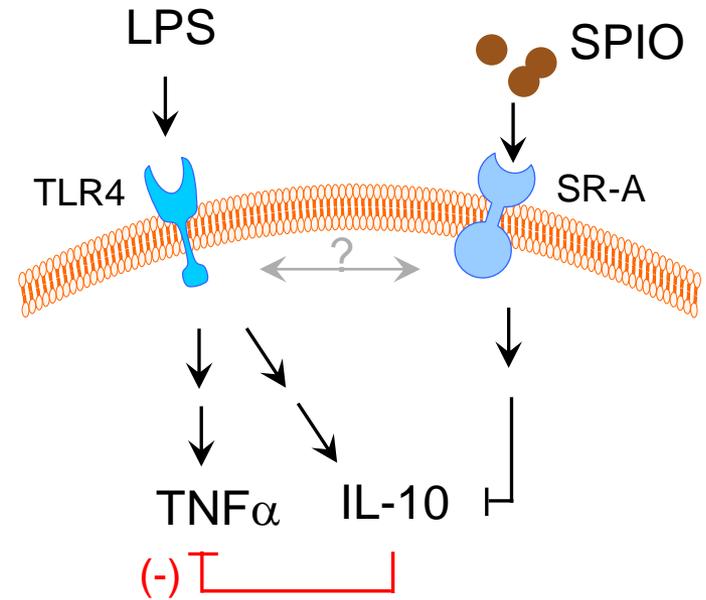
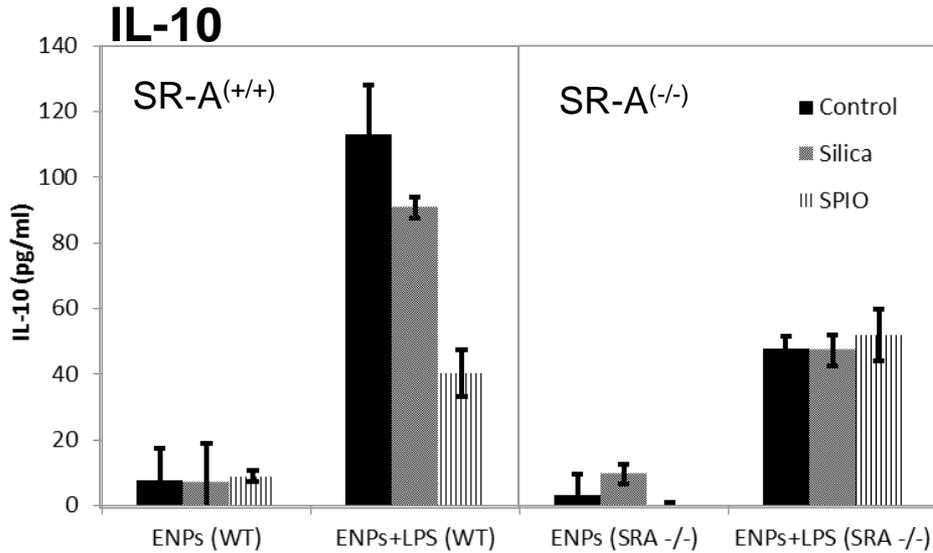


## •TNF

Pro-Inflammatory  
Th1 Response  
Enhanced



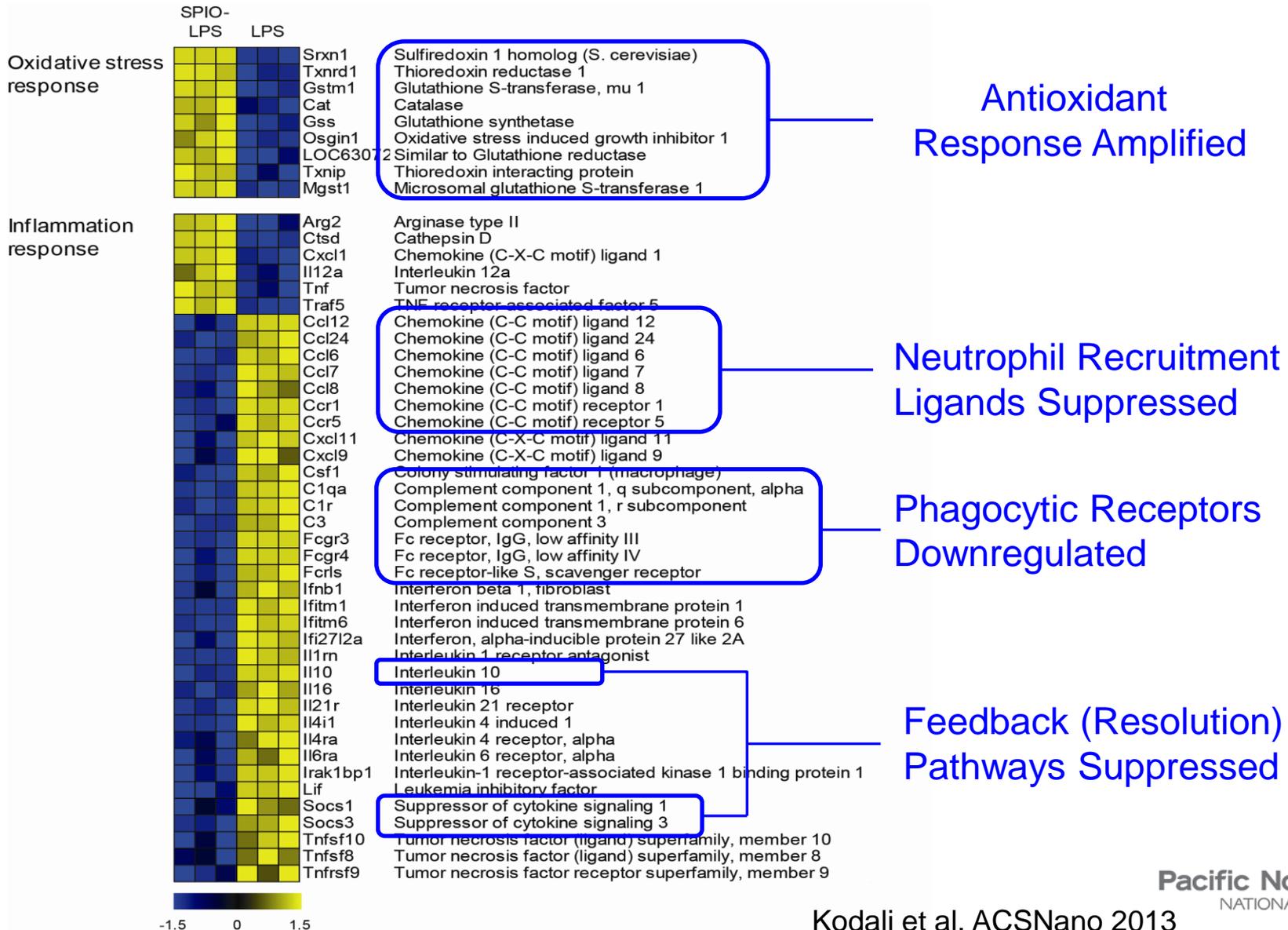
# SPIO Disrupts the IL-10 Anti-inflammatory Feedback Pathway



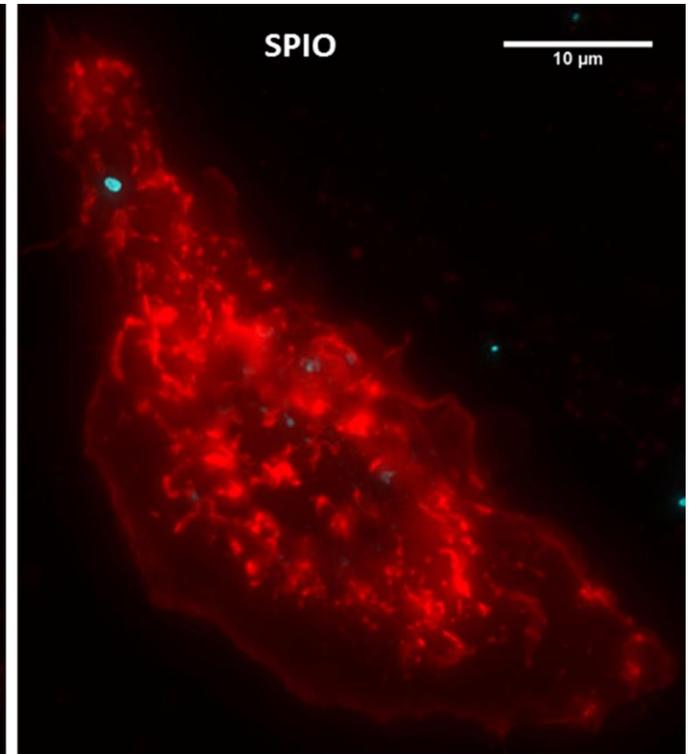
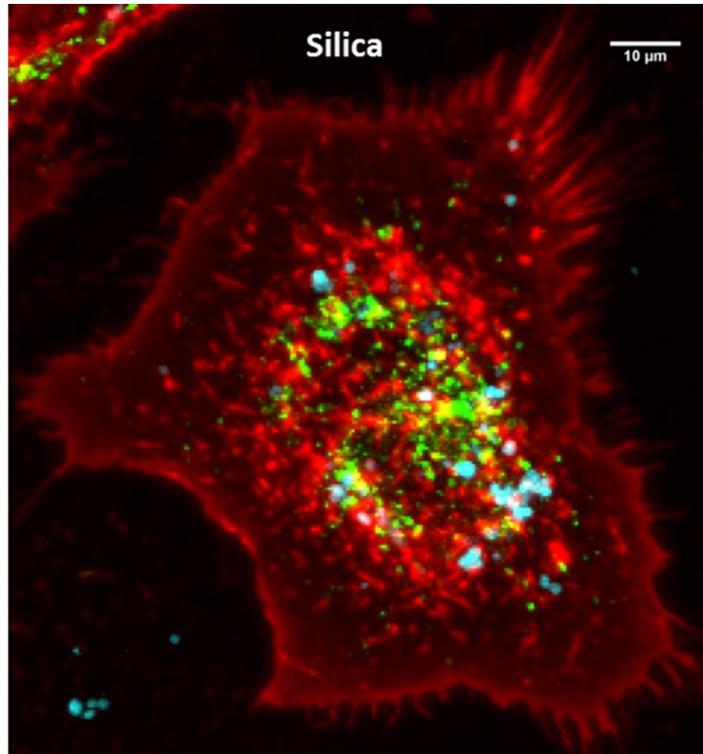
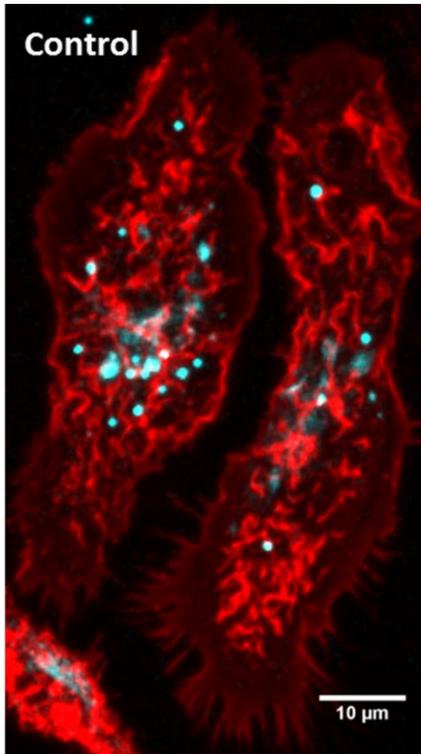
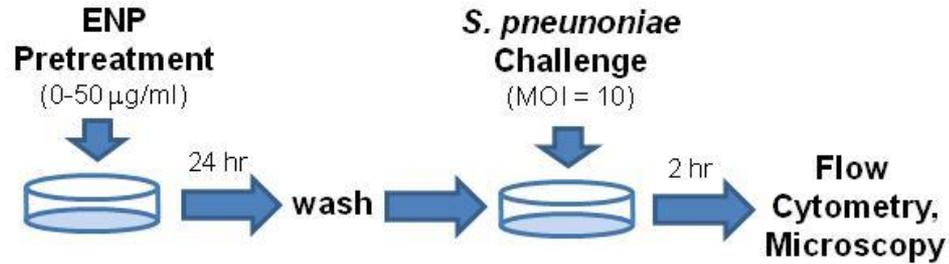
***IL-10 needed for resolution of inflammation.***

***Regulation of IL-10 depends on SR-A.***

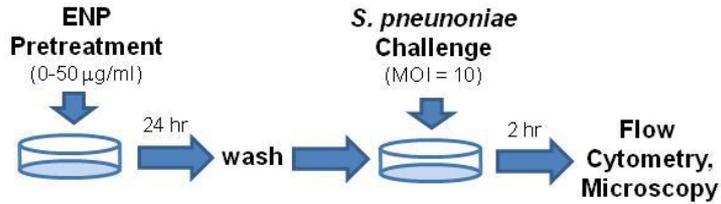
# SPIO Nanoparticle Treatment Reprograms Macrophage Toll 4 Activation



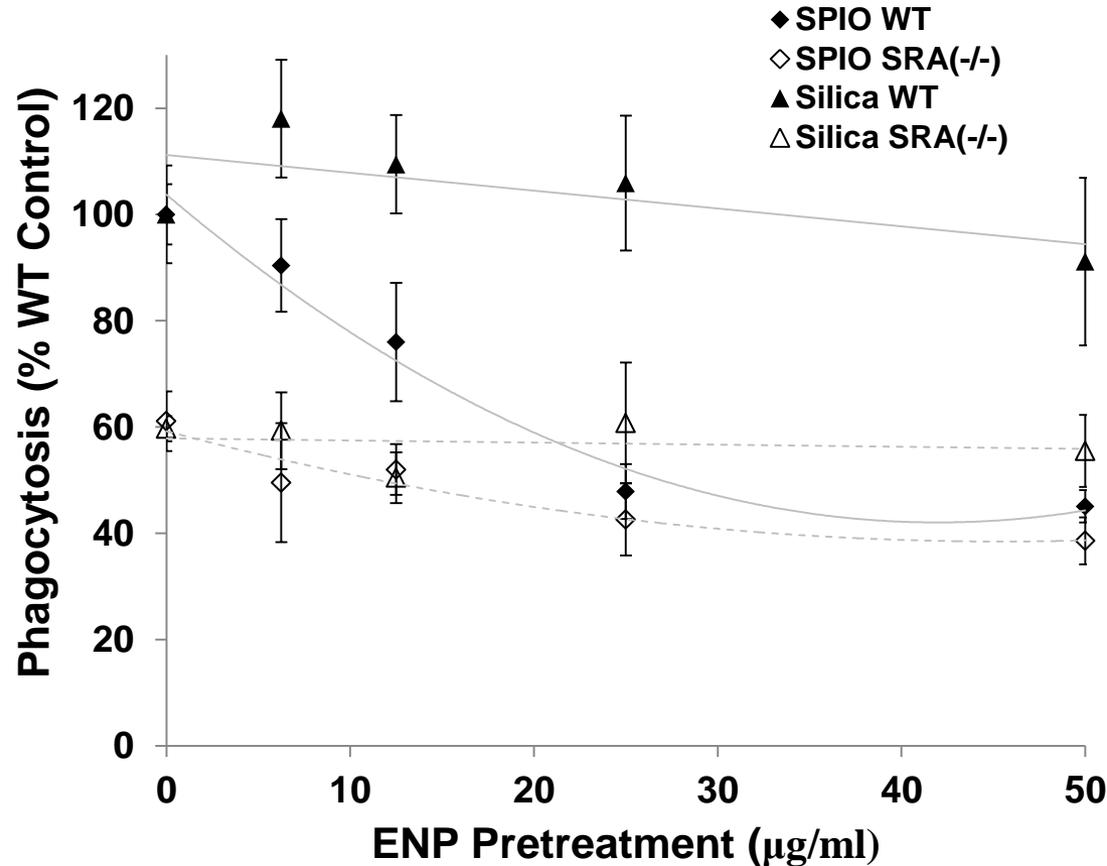
# Impact of Engineered Nanoparticles on Pathogen Phagocytosis



# Impact of ENPs on Pathogen Phagocytosis

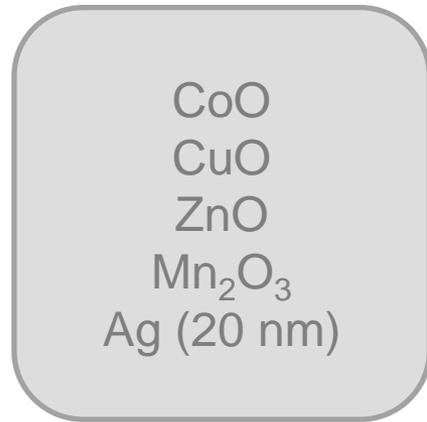


- Phagocytosis inhibited in a dose-dependent manner by SPIO
- Results confirm gene expression predictions- particle specific effect
- SR-A required for recognition of *S. pneumoniae*

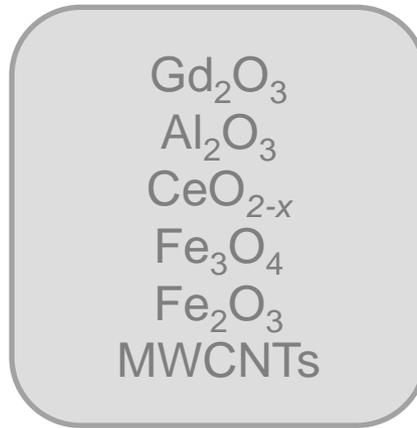


# Hazard Analysis Based on Macrophage Function

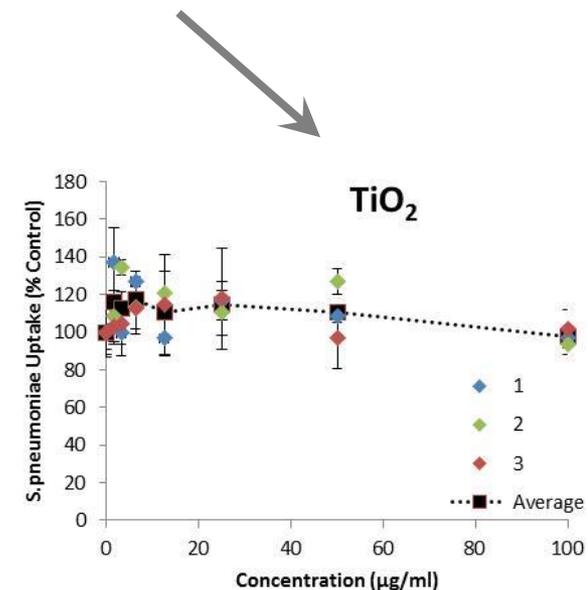
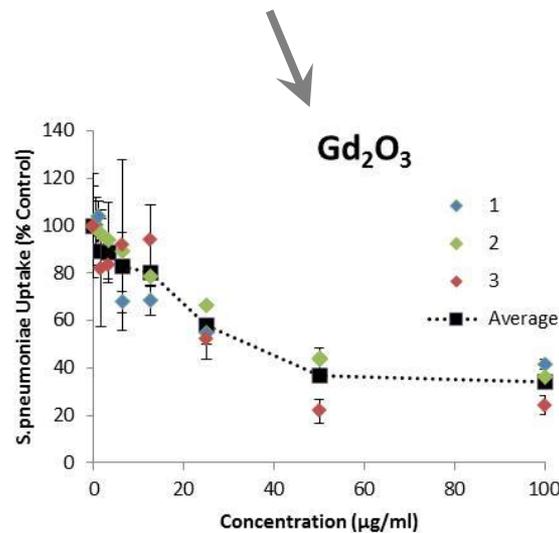
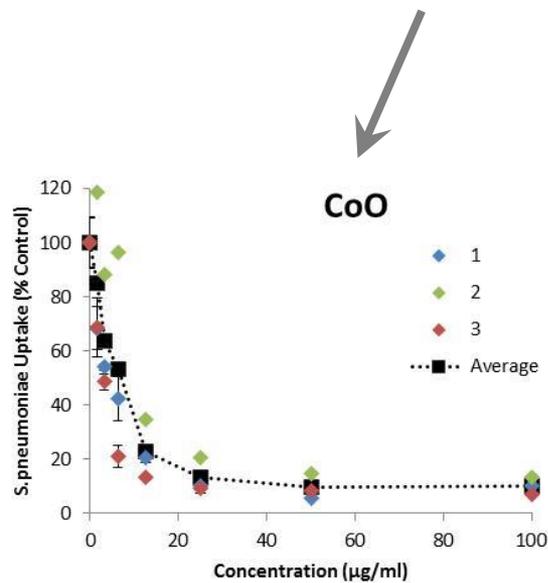
**Strong Phagocytic Inhibitors**  
Cytotoxic, Strong ROS



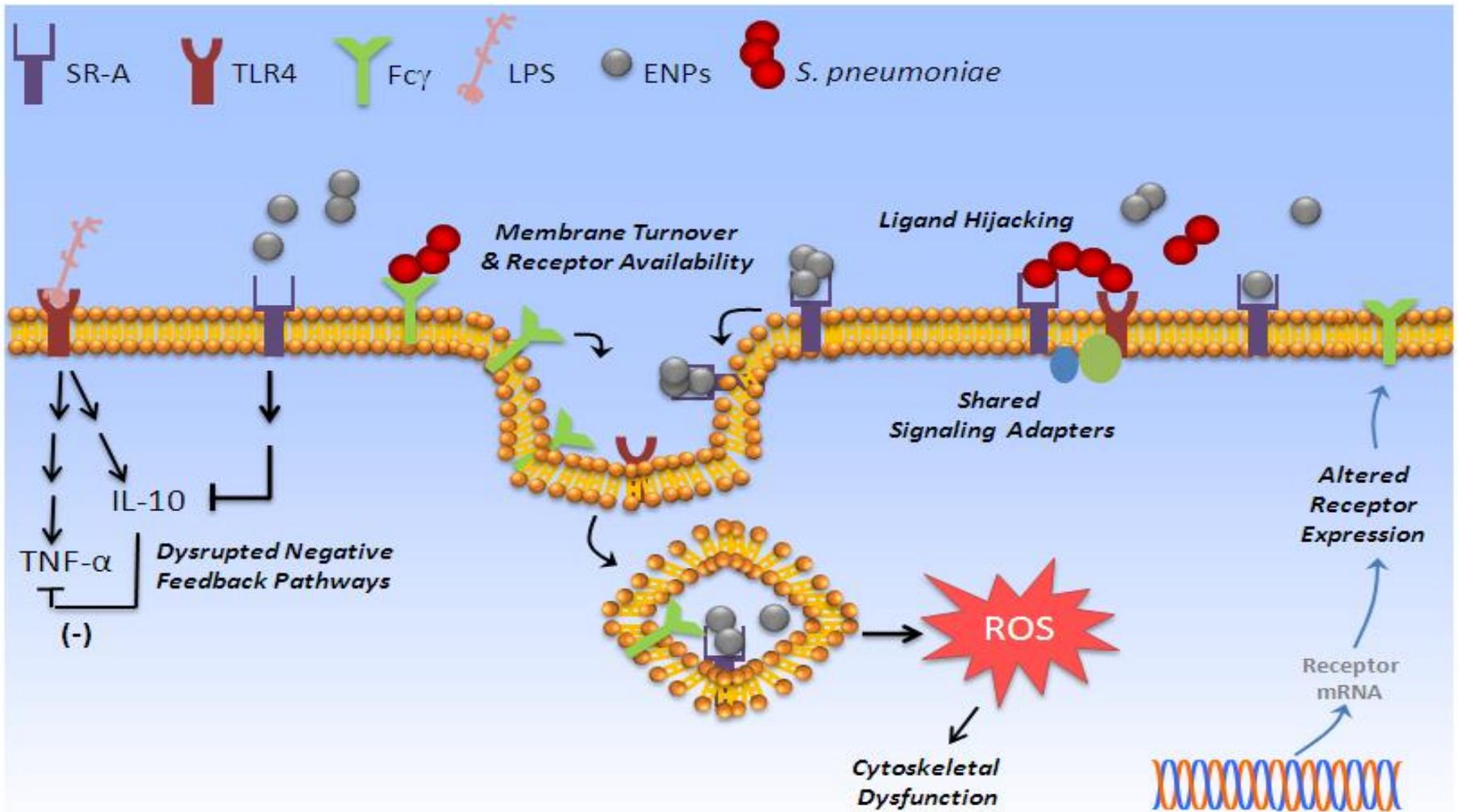
**Phagocytic Inhibitors**  
Noncytotoxic/Mod. Cytotox



**Normal Phagocytic Activity**  
Noncytotoxic



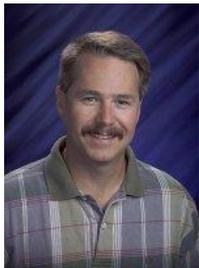
# Shared Receptor Uptake Pathways For Nanomaterials and Pathogens - Potential Mechanisms for Interactions



# Summary

- ▶ Macrophages use common receptor based mechanisms for clearance of bacteria and nanoparticles- creates potential mechanisms for dysregulating innate immune functions
- ▶ Even non-cytotoxic nanoparticles can alter the regulation of hundreds of genes.
- ▶ Phenotypic effects of ‘transcriptional reprogramming’ of cells by nanoparticles are not always readily apparent until cells are challenged
- ▶ Altered susceptibility to pathogens or other co-exposures may be more important health outcome than “direct toxicities” of nanoparticles

# Multidisciplinary Team



Justin Teeguarden  
Dosimetry & Kinetic Modeling

Bobbie-Jo Webb-Robertson  
Statistics

Susan Tilton  
Bioinformatics

Katrina Waters  
Bioinformatics

Rick Zangar  
Disease Biomarkers



Matt Littke



Vamsi Kodali



Kasey Hostetler



Gaurav Sharma



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Joel Pounds  
Toxicology Biomarkers

Galya Orr  
Cell Microscopy

Brian Thrall  
Cell&Molecular Biology

Charles Frevert  
U. Washington Vet Pathology



Hadley Jolley



Matt Gaffrey



Jordan Smith  
Toxicology, PK Modeling

Liang Shi  
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Kevin Minard  
Biophysics MR Imaging

Tao Liu  
Mass Spectrometry



R01 ES016212  
U19 ES019544

