Sustainable Nanotechnology Organization
Research | Education | Responsibility

UNIVERSITA' DEGLI STUDI DI PARMA

SEVENTH FRAMEWORK PROGRAMME

Titanium dioxide nanoparticles enhance macrophage activation by LPS through a TLR4-dependent intracellular pathway

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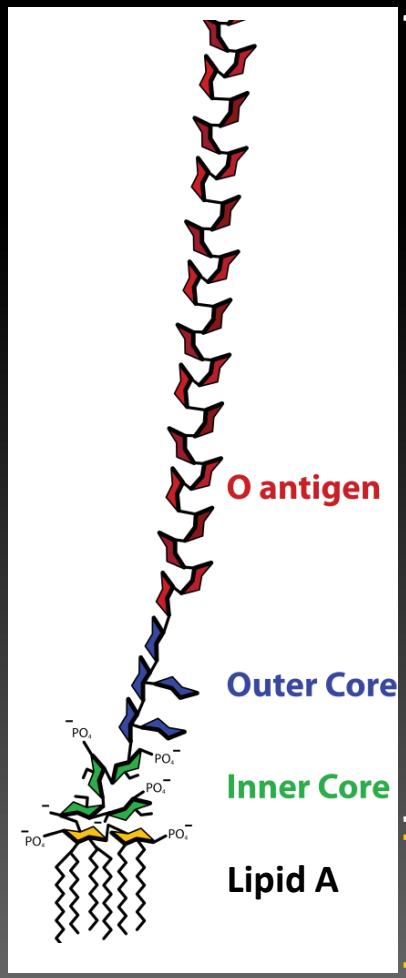
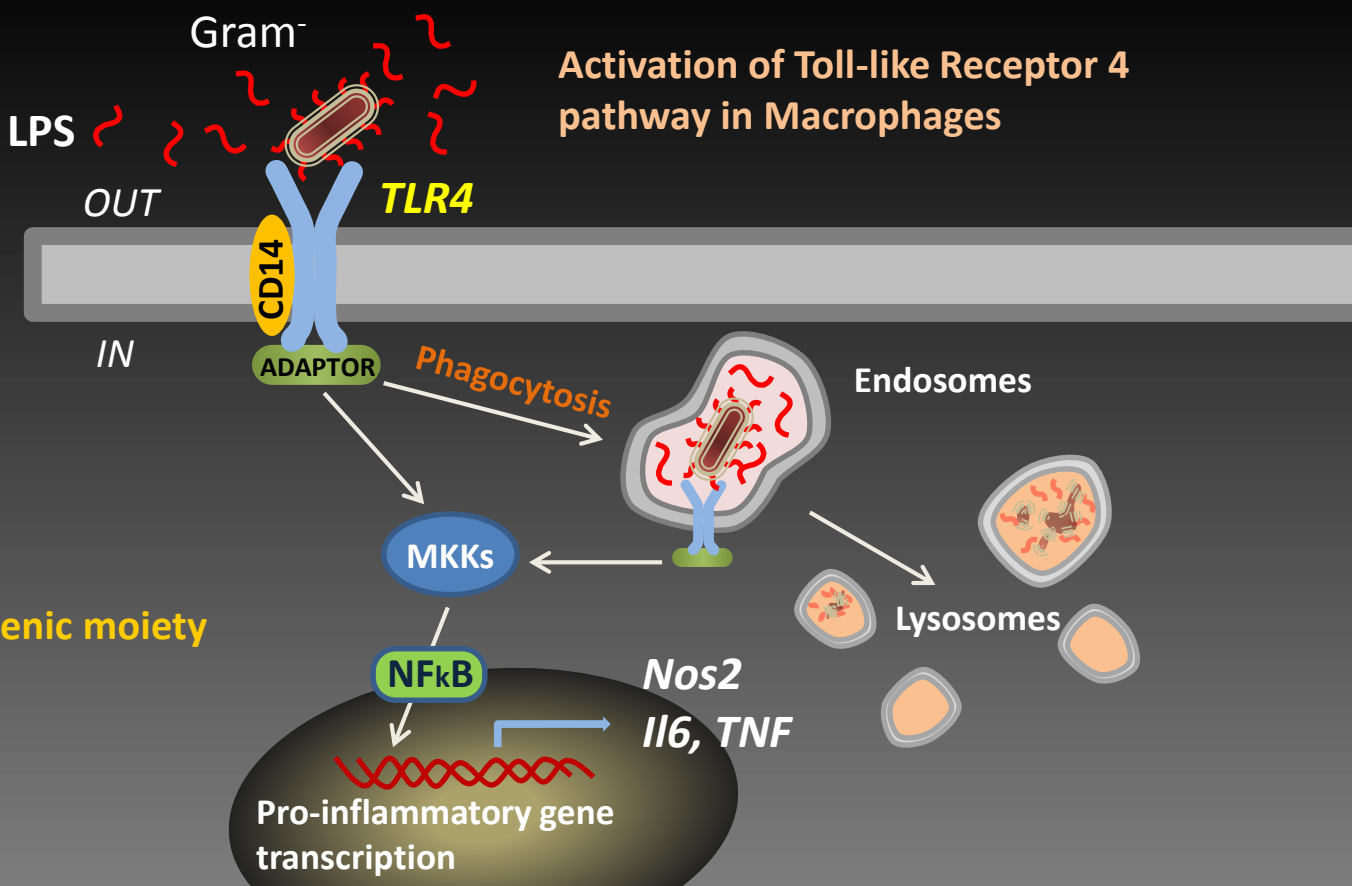
Grant NMP4-SL-2012-280716

BACKGROUND

Bacterial Lipopolysaccharide (LPS or endotoxin)



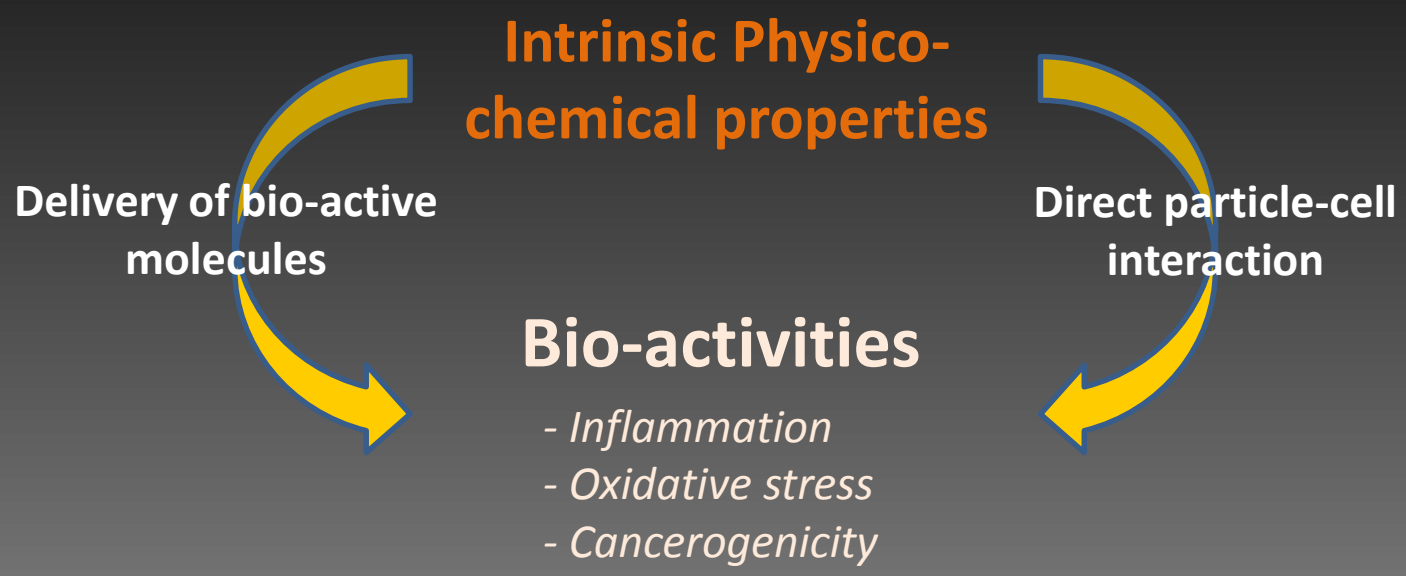
- A common environmental PAMP (macrophage activator)
- Component of the outer membrane of Gram⁻ bacteria
- Elicits strong inflammatory response in competent cells



BACKGROUND

TiO_2 nanoparticles (NPs) at a glance…..

- **One of the most common manufactured metal-based NPs worldwide**
  - *50,400 tons in 2010; expected to increase to 201,500 in 2015*
- **Used in several industrial applications**
  - *Electronics, solar cells, paints, textiles…*
  - *Food, cosmetics, toothpaste……*
  - *Antibacterial and anti-polluting coatings*



BACKGROUND

TiO_2 nanoparticles and the paradigm of "protein corona"

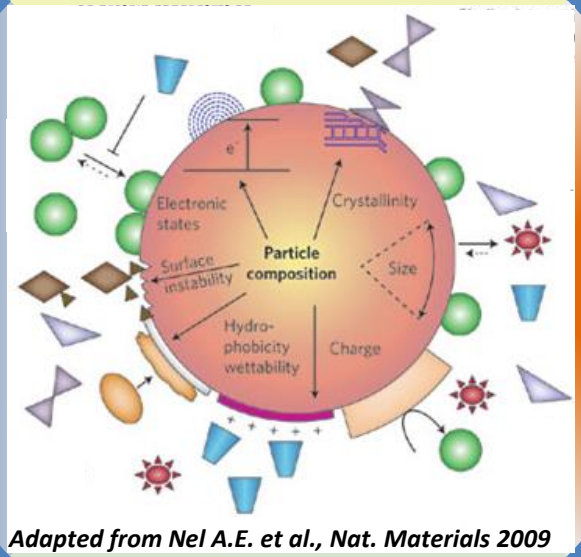
Nanostructured material

Environmental/Biological Matrix

size

surface coatings

shape



Adapted from Nel A.E. et al., Nat. Materials 2009

surface chemistry

Biological behavior

A role for environmental contaminants in TiO_2 NP effects?

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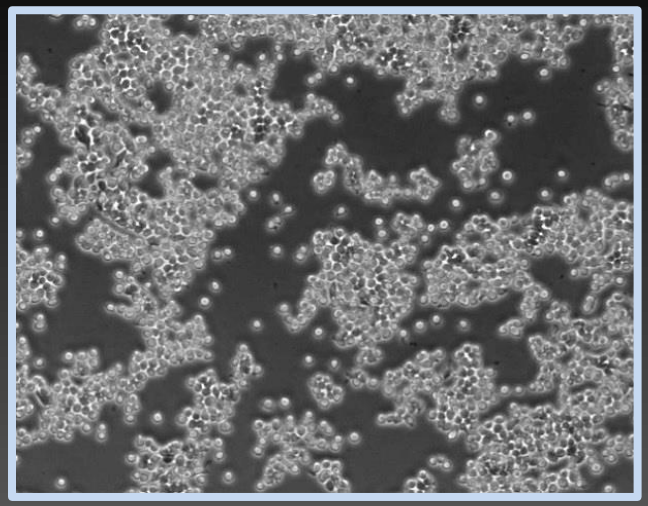
AIM

To asses the effects of TiO_2 NP and LPS on murine macrophage Raw 264.7

Raw 264.7

Immuno-competent cells

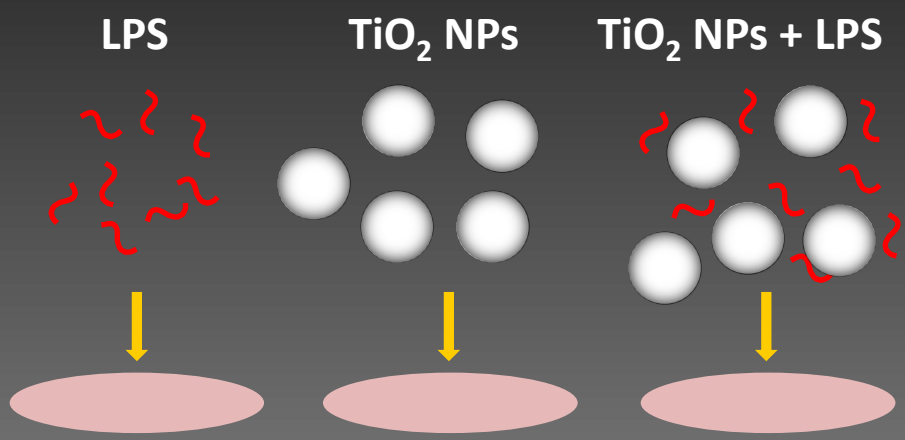
Express TLR4 receptors



Biological parameters evaluated

Cell end points	
Cytotoxicity	**Inflammatory markers**
- Cell viability	- NO production - Pro-inflammatory genes - Cytokine secretion

Experimental design



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BACKGROUND

Physico-chemical properties of NAMA41® and Aeroxide® P25

TiO_2 NP	XRD phase distribution		Density (g/cm^3)	SSA_{BET} (m^2/g)	d_{BET} (nm)
	Anatase (%)	B=Brookite R=Rutile (%)			
NAMA41®	84	16, B	3.98	154	10
Aeroxide® P25	83	17, R	4.10	60	24

Mean size distribution by intensity and ζ potential for 0.125 mg ml−1 of NAMA41® and Aeroxide® P25 dispersed in deionized water and complete culture medium

	Deionized water$_{natural\ pH}$				Deionized water$_{medium\ pH}$				Complete culture medium			
TiO_2 NP	pH	Size (d. nm)	PdI	ζ pot. (mV)	pH	Size (d. nm)	PdI	ζ pot. (mV)	pH	Size (d. nm)	PdI	ζ pot. (mV)
NAMA41®	3,9	45	0,48	41,2	7,3	9864	0,76	-15,9	7,3	1962	0,98	-10,9
DEV. ST		1	0,09	0,0		2390	0,30	0,4		147	0,03	0,5
Aeroxide®P25	6,5	286	0,30	37,4	7,7	3425	0,36	-11,0	7,7	532	0,53	-10,8
DEV. ST		4	0,04	0,9		226	0,10	0,1		16	0,11	0,4

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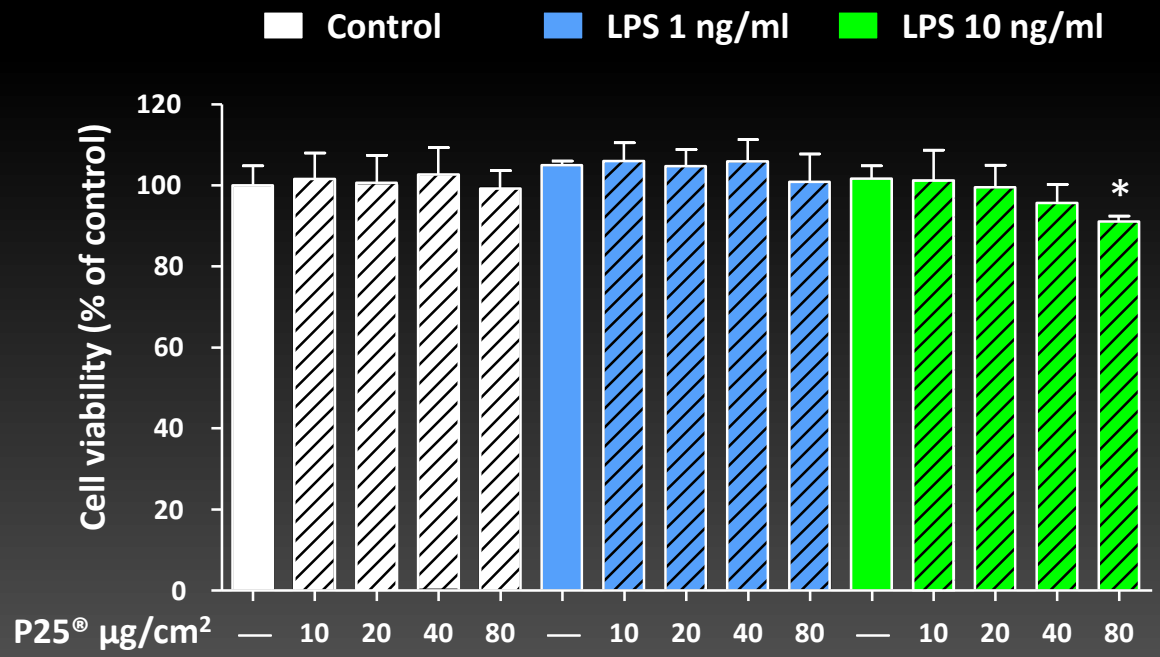
Bianchi et al., Toxicol. Res. 4, 385-398, 2015

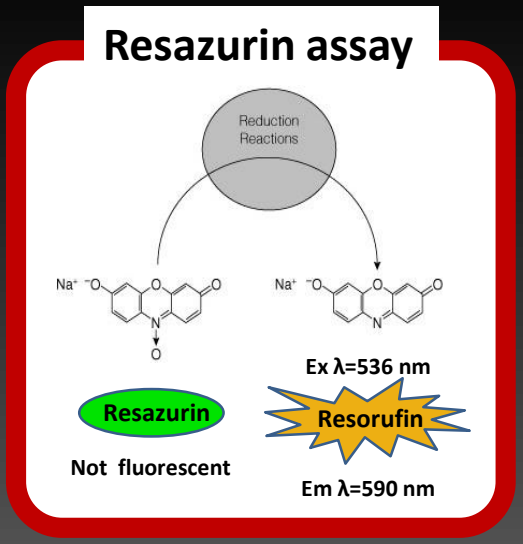
RESULTS

Cytotoxicity

Effects of P25® and LPS on cell viability



*p < 0.05 vs. Untreated cultures



P25® do not markedly affect cell viability up to 48h

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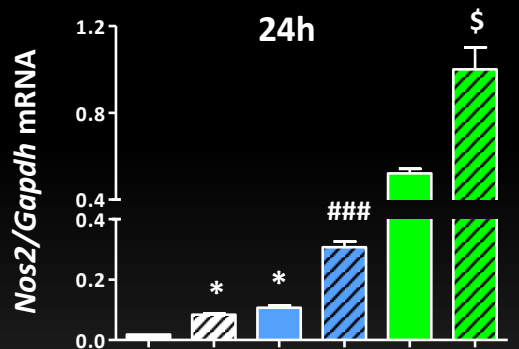
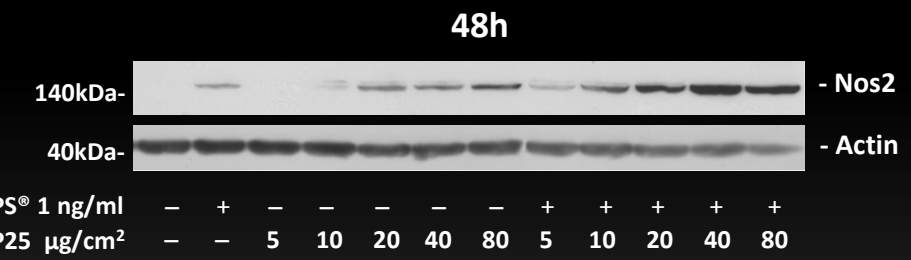
RESULTS

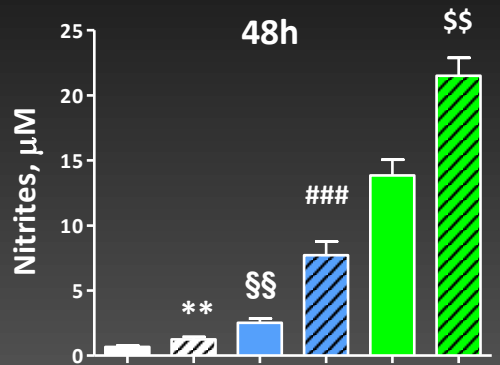
Inflammatory markers

Effects of P25® and LPS on NO production

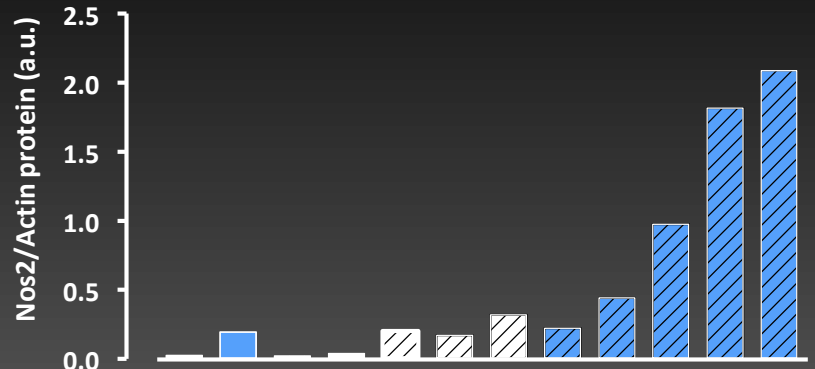


*p < 0.05 vs. Untreated cultures
###p < 0.001 vs. cultures treated with LPS 1 ng/ml alone
$p < 0.05 vs. LPS 10 ng /ml alone





**p < 0.01, ***p < 0.001 vs. untreated cultures
##p < 0.001 vs. cultures treated with LPS 1 ng/ml alone
$$p < 0.01 vs. LPS 10 ng /ml alone.



P25® synergize the LPS-mediated stimulation of *Nos2* gene/protein expression and of NO production

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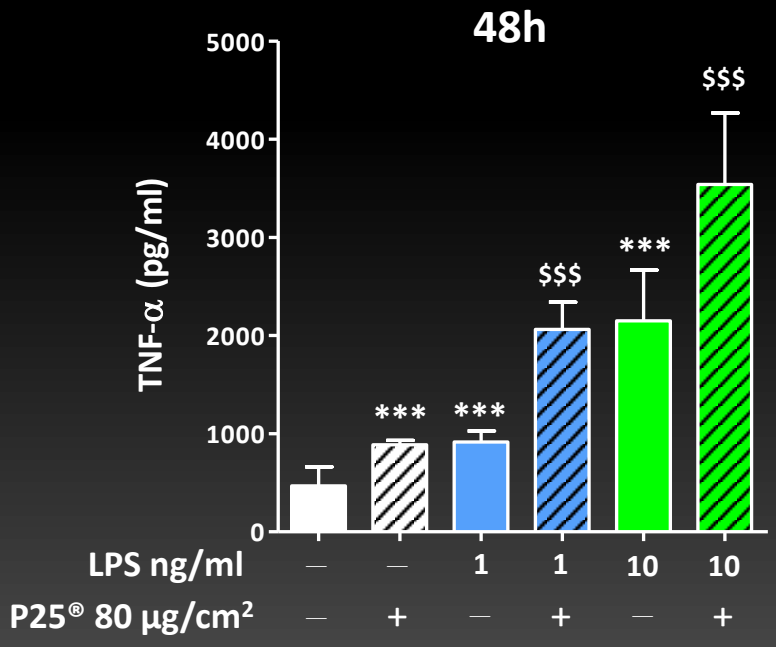
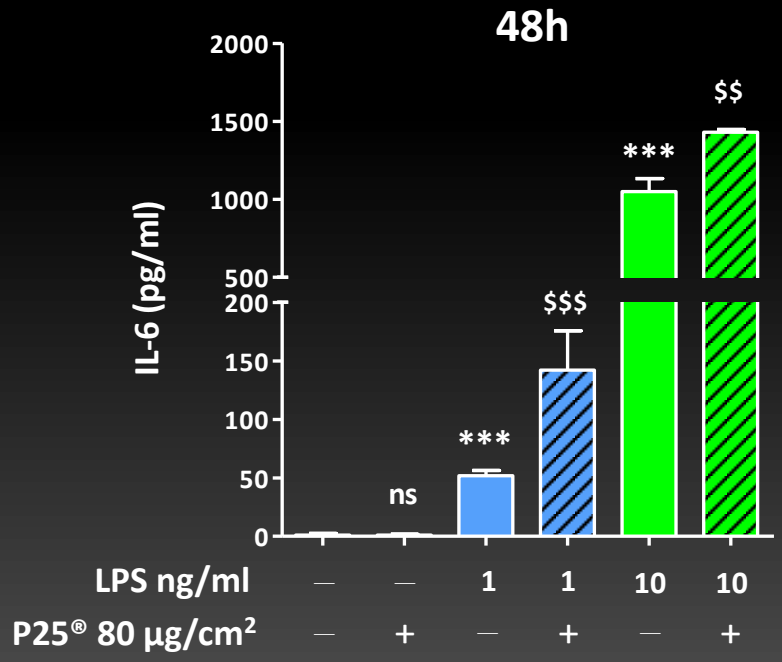
Bianchi et al., Toxicol. Res. 4, 385-398, 2015

RESULTS

Inflammatory markers

Effects of P25® and LPS on cytokine secretion





P25® synergize also the secretion of inflammatory cytokines induced by LPS

*p < 0.05, ***p < 0.001 vs. untreated cultures; ##p < 0.01, ###p < 0.001 vs. cultures treated with LPS 1 ng/ml alone; $p < 0.05, $$$p < 0.001 vs. LPS 10 ng/ml alone

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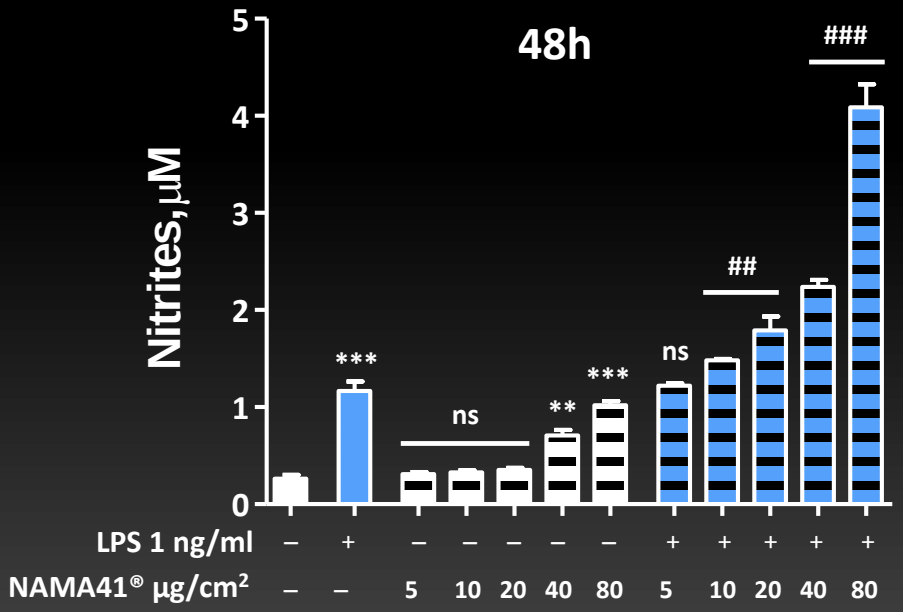
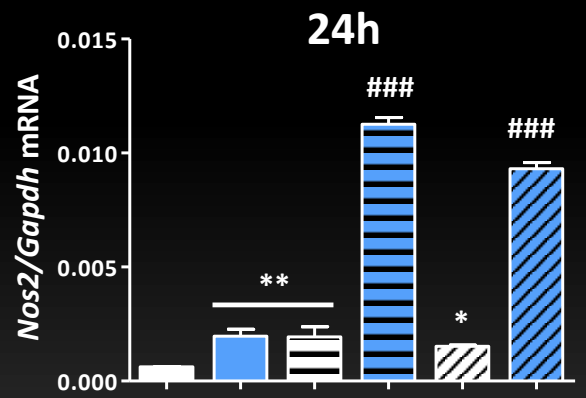
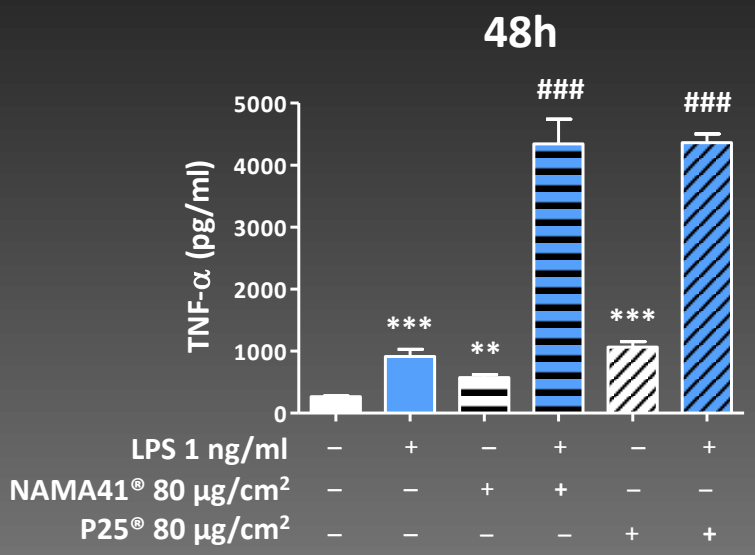
RESULTS

Inflammatory markers

A comparison between the effects mediated by P25® and NAMA41®







The synergistic effect of P25® on LPS-dependent macrophage activation is shared by NAMA41® (another industrial preparation of TiO_2 NPs)

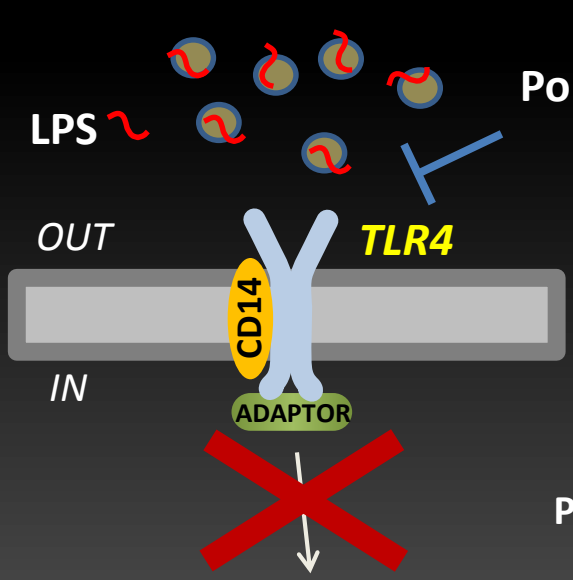
**p < 0.01, ***p < 0.001 vs. untreated cultures;
##p < 0.01, ###p < 0.001 vs. cultures treated with LPS 1 ng/ml alone.

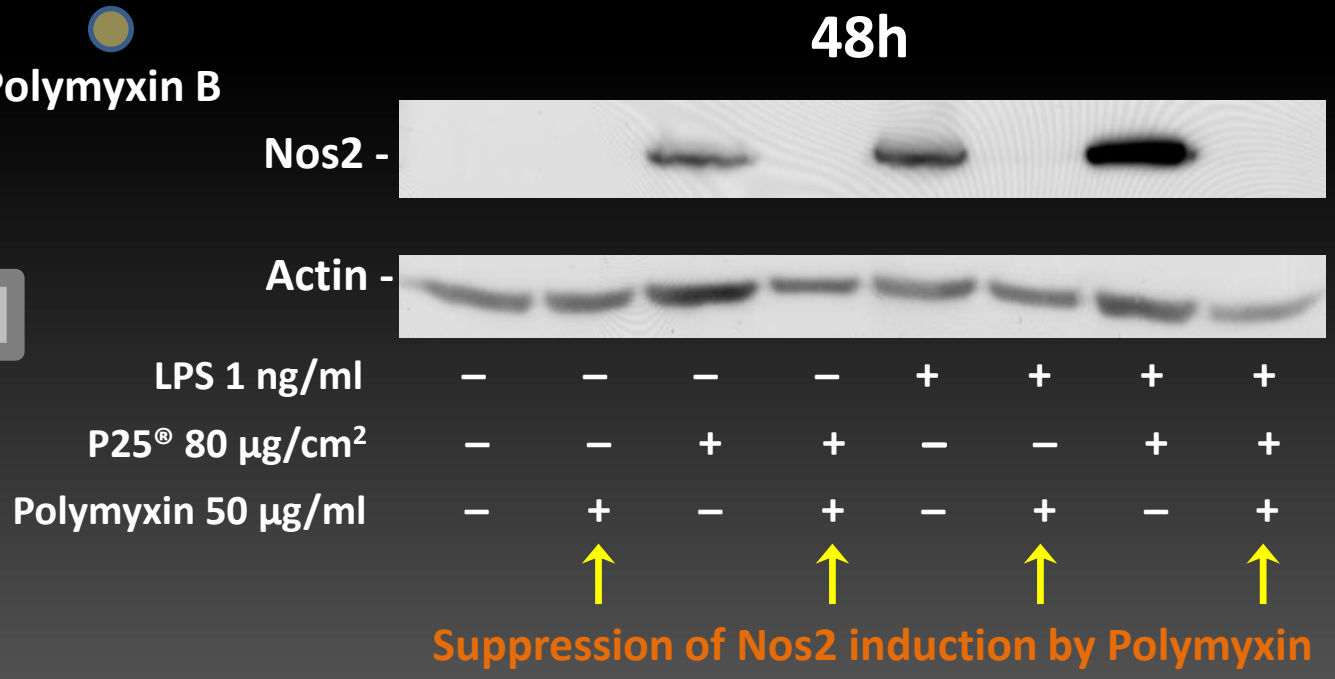
Bianchi et al., Toxicol. Res. 4, 385-398, 2015

RESULTS

Mechanism characterization

Role of TLR4 on the P25®-mediated synergistic induction of Nos2: effect of polymyxin B





P25® enhance macrophage activation by LPS via a TLR4-dependent mechanism

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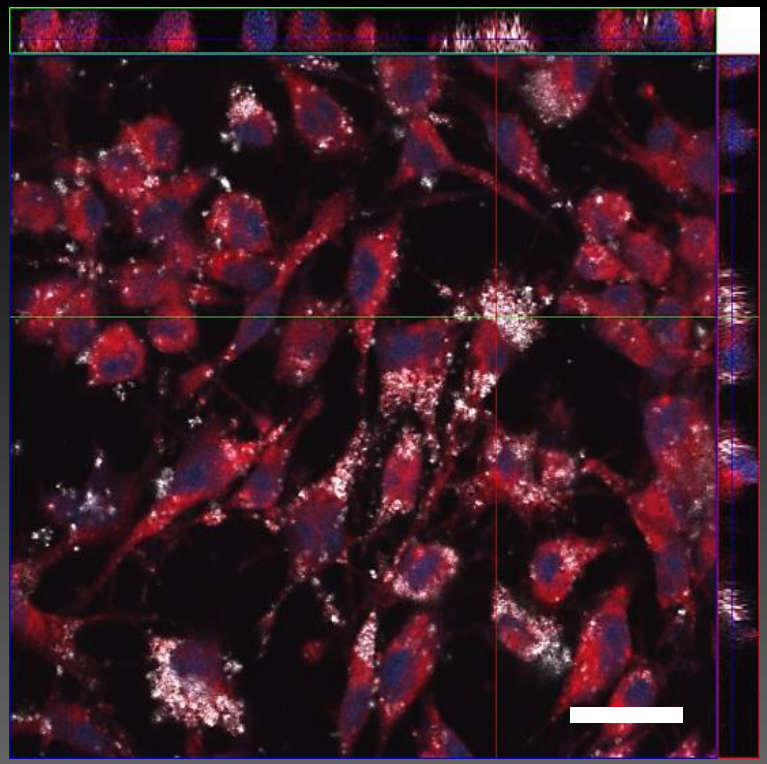
RESULTS

Mechanism characterization

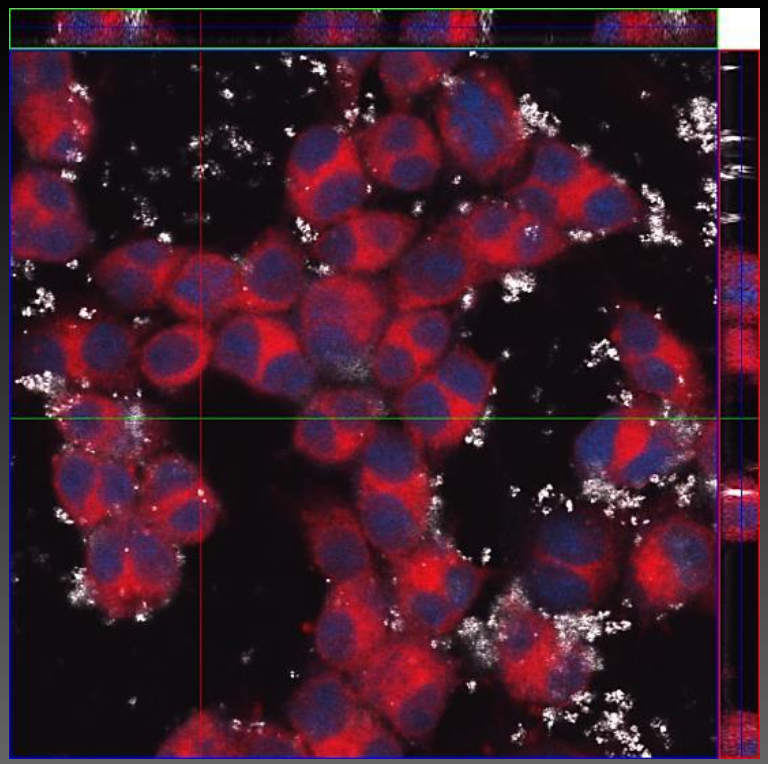
Effect of cytoskeletal disorganization on NO production and P25® internalization

P25® 10 µg/cm² + LPS 1 ng/ml

w/o inhibitor



Cytochalasin 5µg/ml



Cytochalasin blokes the endocytosis of P25®

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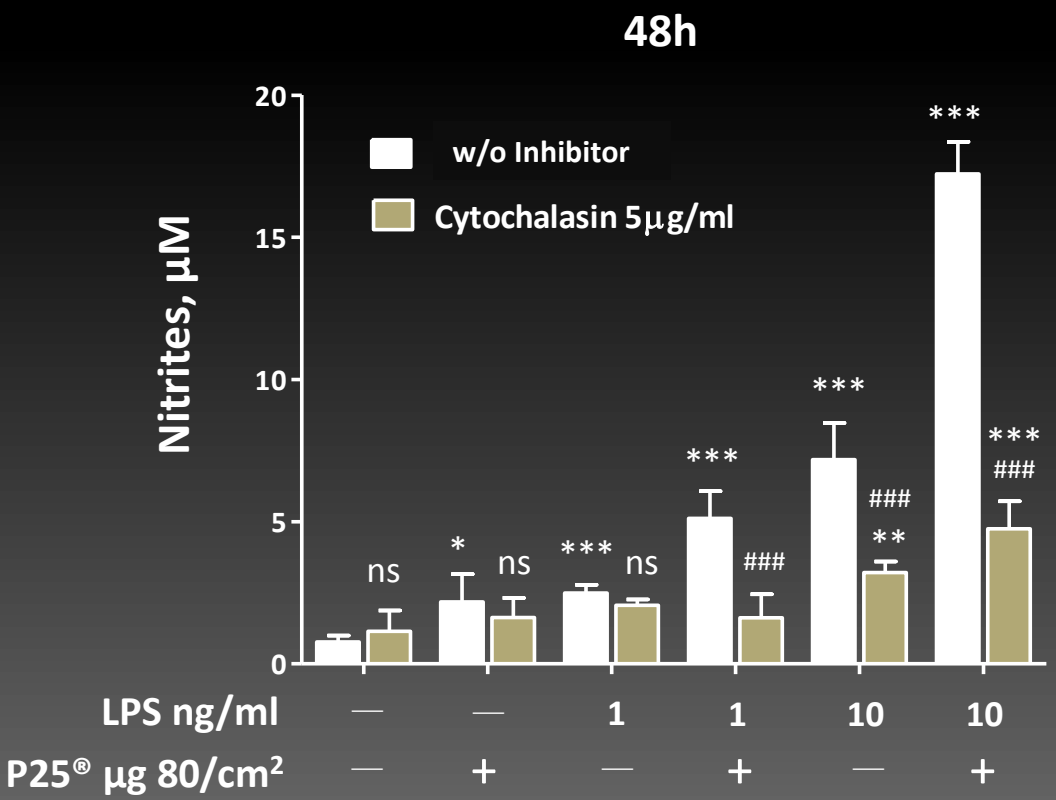
RESULTS

Mechanism characterization

Effect of cytoskeletal disorganization on NO production and P25® internalization



*p < 0.05, **p < 0.01,***p < 0.001 vs. untreated cultures;
###p < 0.001 vs. cultures treated under the same conditions without the inhibitor

Endocytosis blockade inhibits the synergistic effect of P25® on LPS-dependent NO production

Involvement of an intracellular site

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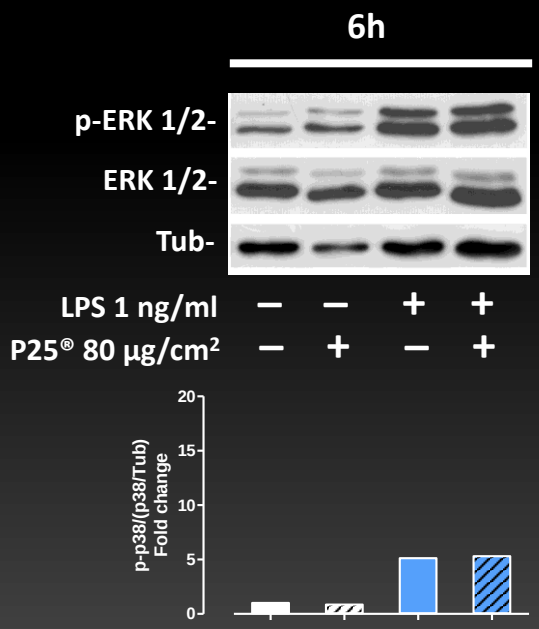
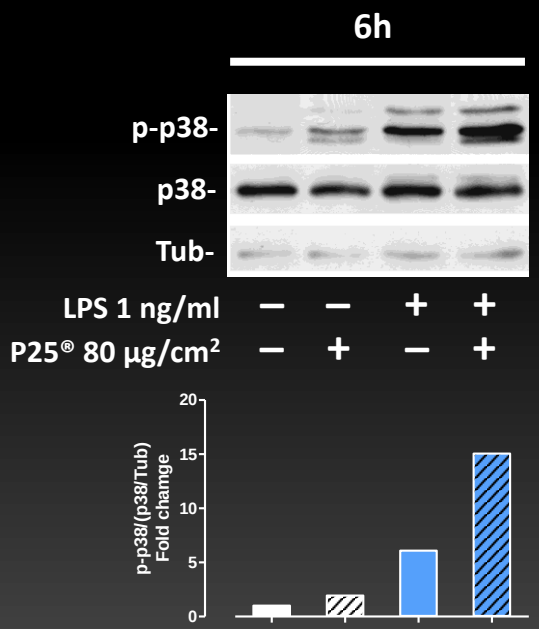
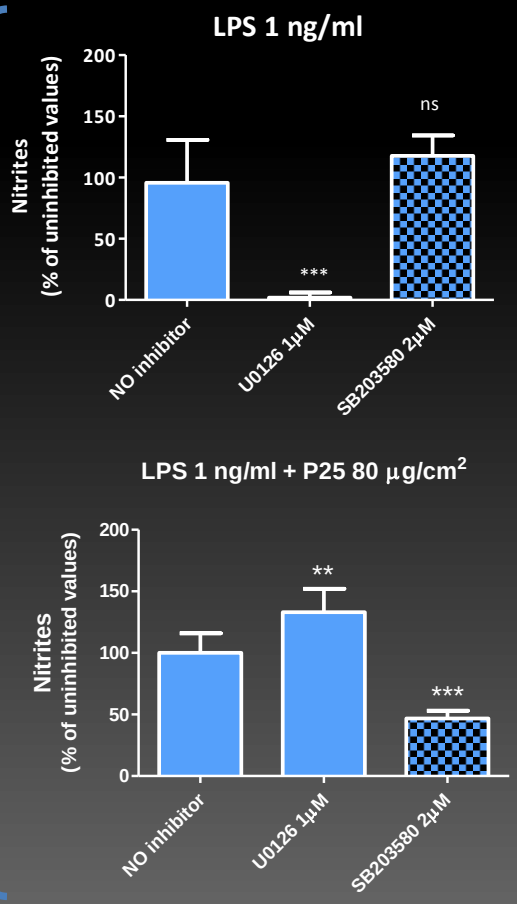
RESULTS

Mechanism characterization

Role of MKKs in the LPS and P25® effects







LPS-P25® synergy involves p38 but not ERK 1/2 MAPK

**p < 0.01 and ***p < 0.001 vs. cultures incubated with the same doses of LPS and TiO2 NPs in the absence of inhibitors

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SUMMING UP

- ***TiO_2 NP synergize LPS inflammogenic activity***

  - Enhanced NO production, pro-inflammatory gene expression, cytokine secretion

- ***The effect requires TLR4 signalling, phagocytosis and the phosphorylation of p38 MAPK***

  - TLR4 inhibitors, blockade of TiO_2 NP internalization and Inhibition of p38 phosphorylation prevent macrophage activation

Hypothesis

↓

Do TiO_2 NP bind LPS ?

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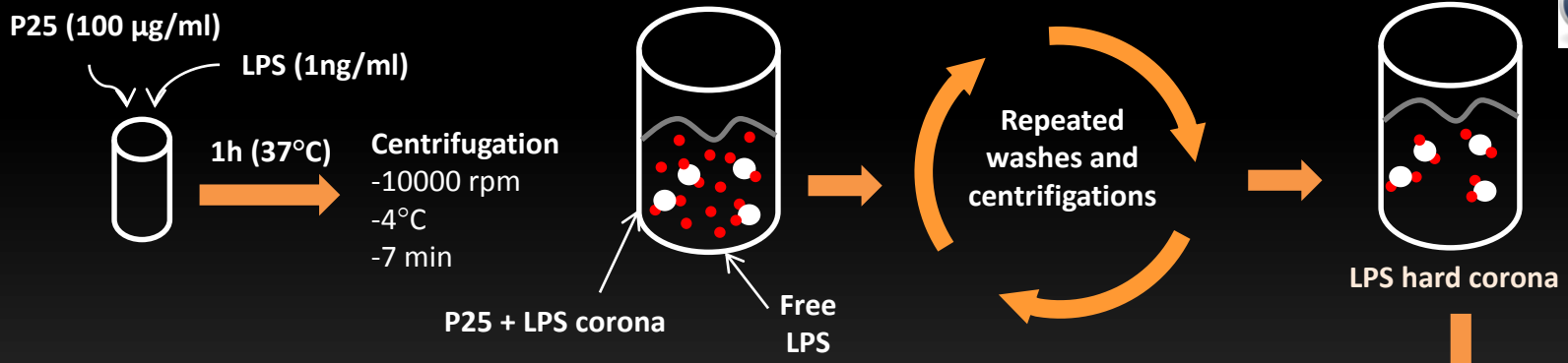
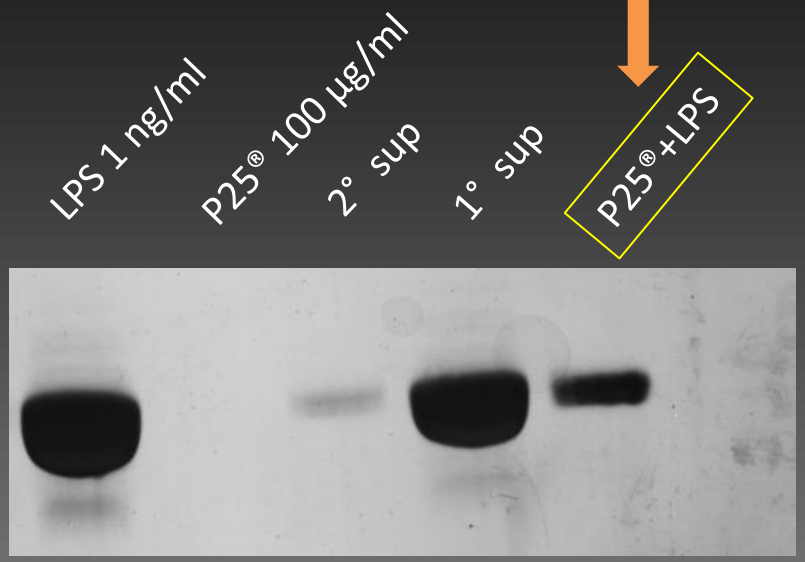
PRELIMINARY RESULTS

P25®-LPS binding

Assessment of LPS corona on P25® by SDS-PAGE and Silver Staining





TiO_2 NP bind LPS and are likely responsible for LPS intracellular delivery

SUMMING UP

- ***TiO_2 NP synergize LPS inflammogenic activity***

  - Enhanced NO production, pro-inflammatory gene expression, cytokine secretion

- ***The effect requires TLR4 signalling, phagocytosis and the phosphorylation of p38 MAPK***

  - TLR4 inhibitors, blockade of TiO_2 NP internalization and Inhibition of p38 phosphorylation prevent macrophage activation

- ***TiO_2 NP are able to deliver high amounts of LPS in to the cell***

  - LPS corona formation on TiO_2 has been demonstrated

TiO_2 NP as "TROJAN HORSES"

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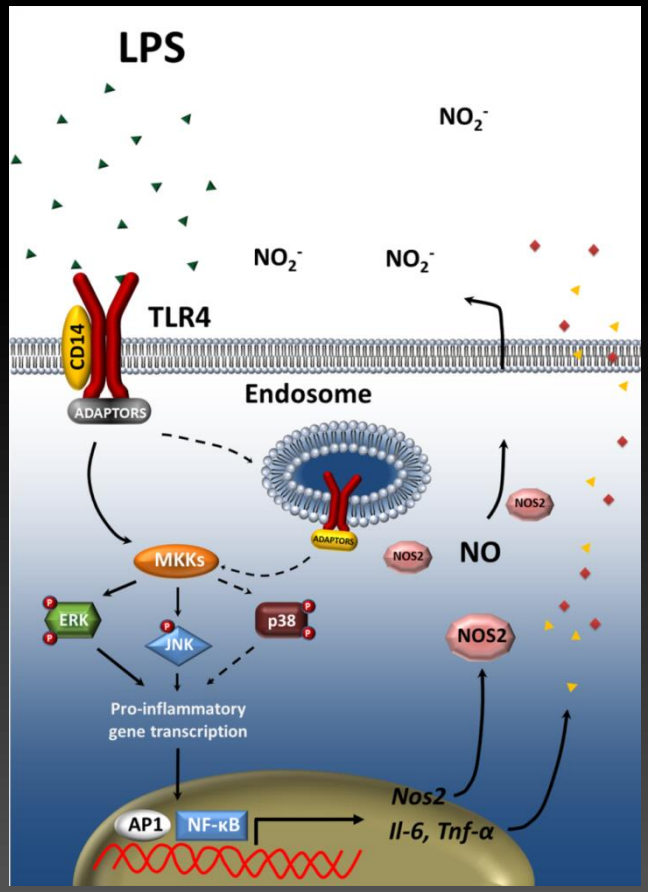
CONCLUSIONS

A working model….



Nanomaterials change the biopersistence and/or bioavailability of PAMPs

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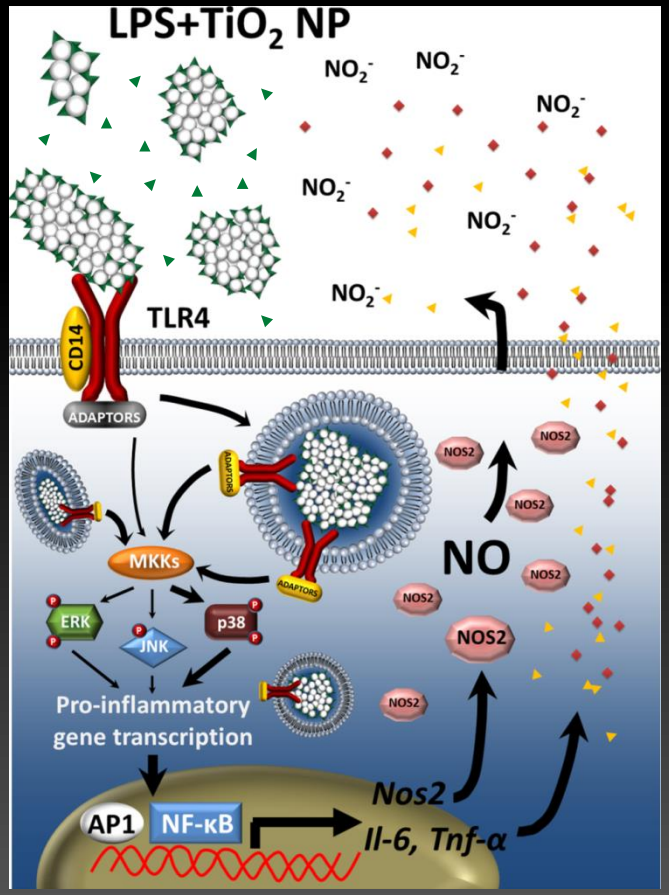
Biological effects depend (also) from the bioactive molecules present in the tissue (contaminants, PAMP, etc.)

Exploitable for modulating inflammatory responses ?



Free LPS

"Out-door" activation of TLR4 receptors on plasma membrane

Free LPS + TiO_2@LPS

"Out-door"+ "In-door" activation of TLR4 receptors in endosomal compartments

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Bianchi et al., Toxicol. Res. 4, 385-398, 2015

Thank you all……!!