

Nanotechnology



NCI **Alliance** for
Nanotechnology
in Cancer

Nanotechnology for cancer therapy: benefits, concerns and effects on the immune system

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Outline

Presentation outline



- Nanotechnology Definitions
- Nanoparticles in Daily Life
- Nanoparticles in Medical Applications
- Nanoparticles for Cancer Diagnosis and Therapy
 - Benefits of nanotechnology
 - Toxicity concerns
- Nanomaterials and the Immune System

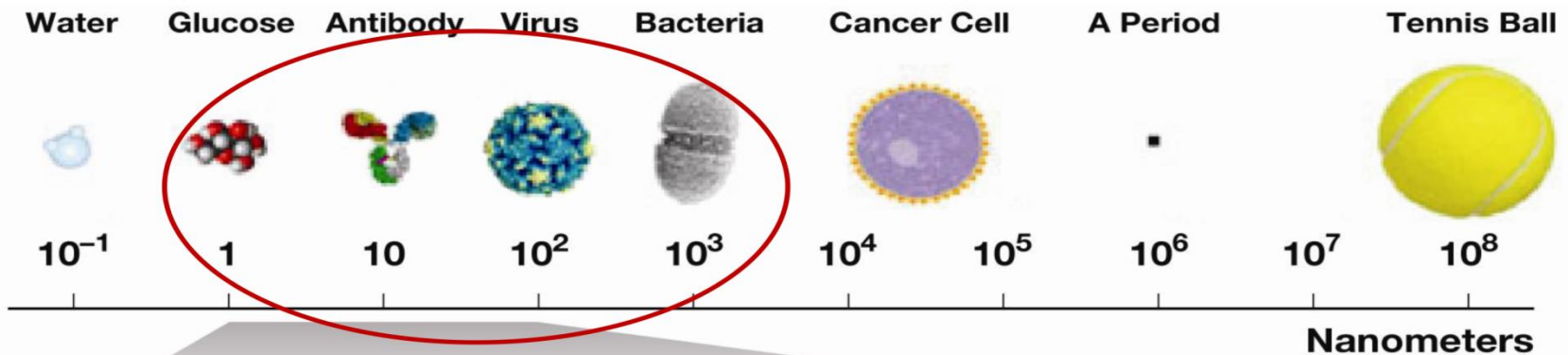
What is nano?

What is Nano?

Nanotechnology:

“Research and technology development at the atomic, molecular or macromolecular scale leading to the controlled creation and use of structures, devices and systems with a length scale of approximately **1 – 100 nanometers** (nm).” (Source: National Nanotech Initiative)

“Whether a material or end product is engineered to exhibit properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, **up to one micrometer (1,000 nm)**” (US FDA)



Nanoparticles

Nanoparticles in Daily Life

■ Consumer products

- 800+ “manufacturer identified” products from 400+ companies in 20+ countries
- Clothing, wound dressings, washing machine liners
- Sunglasses (lens coatings)
- Sporting equipment



Turtle Wax™ makes a nanotech car wax



The Adidas Lone Star™ track shoe includes a lightweight spike-plate made of carbon nanotubes



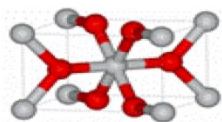
Nanosilver is in supplements and used to treat clothing



Speedo LZR™ Racer Swimsuit is treated in a nanotech cold-plasma process that reduces water absorption.

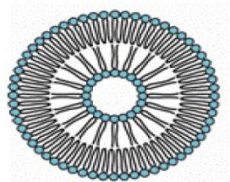
Nanoparticles in daily life

Nanoparticles in Daily Life

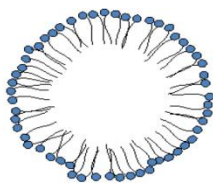


TiO₂

- Nearly all translucent sunscreens contain nanoscale TiO₂ or ZnO₂

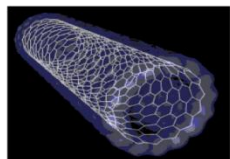


Liposomes



Nanoemulsions

- Liposomes and emulsions are commonly used in cosmetics (L'Oreal holds more than 60 patents related to nanotechnology)

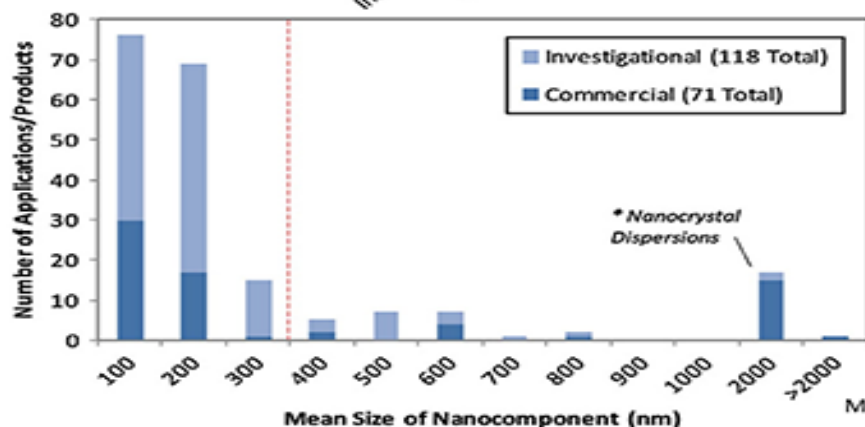
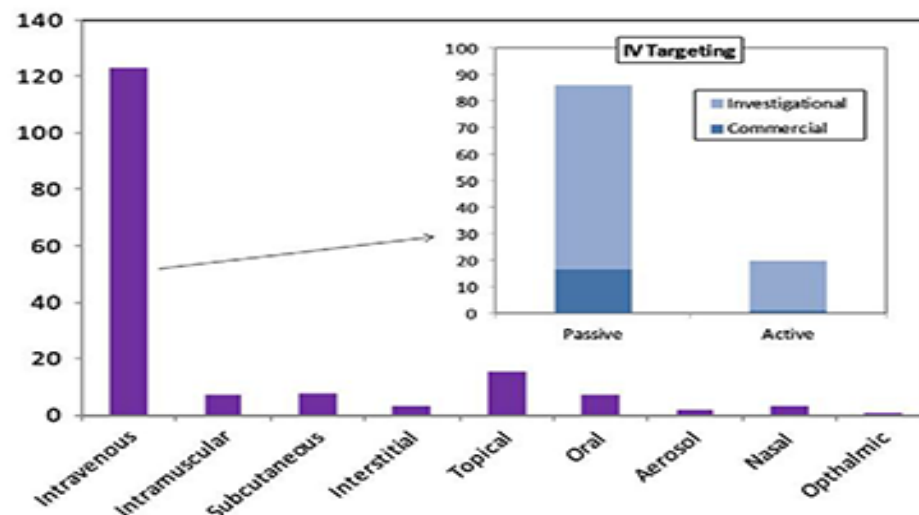
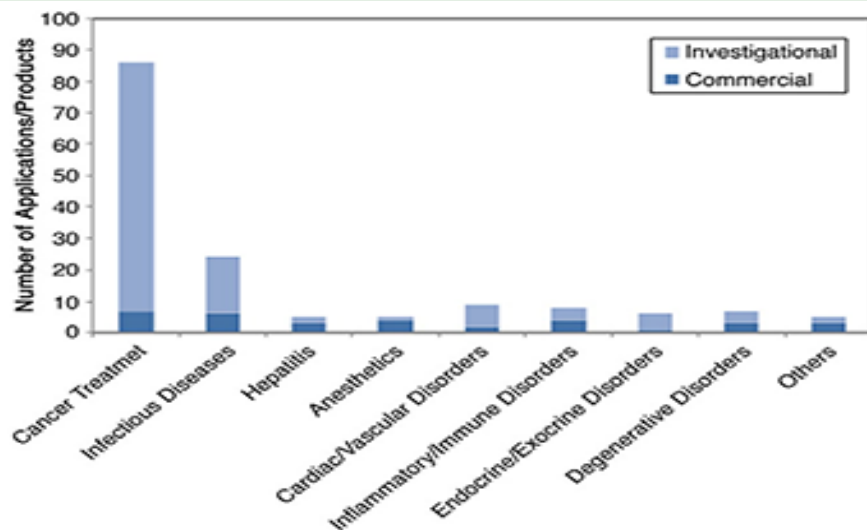


Carbon nanotube

- Carbon nanotubes are used as structural materials

Nanoparticles in medical applications

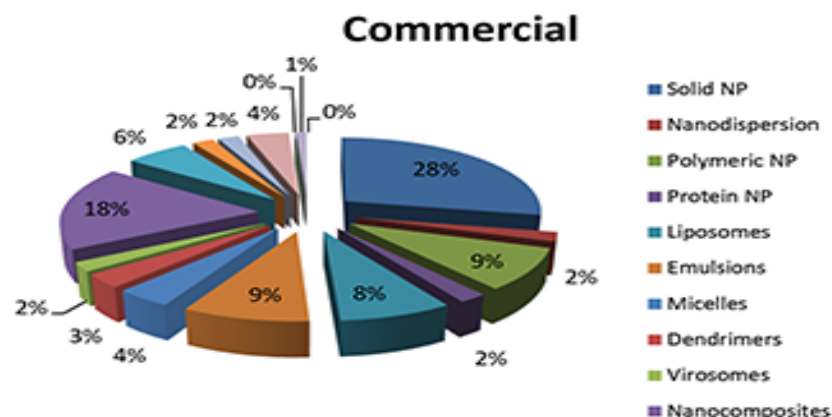
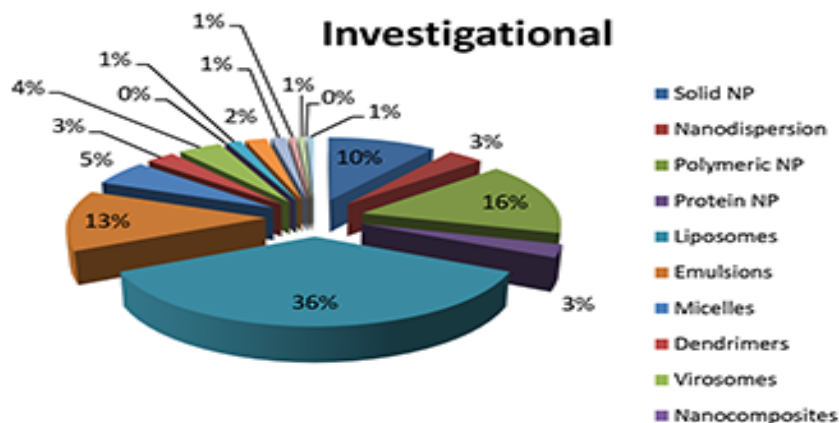
Nanoparticles in Medical Applications



- Common features of Nanomedicines:**
- Primary market is cancer therapy
 - Intravenous administration
 - <350 nm in size
 - Neutral, hydrophilic surfaces
 - Spherical

Nanoparticle type

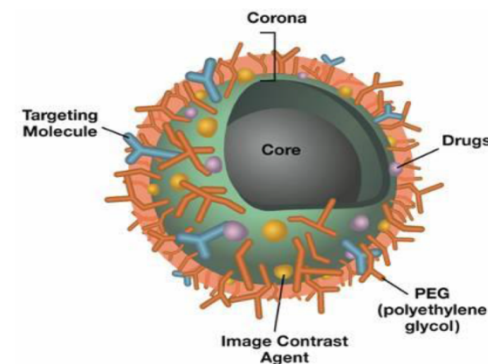
Nanomedicine by Nanoparticle Type



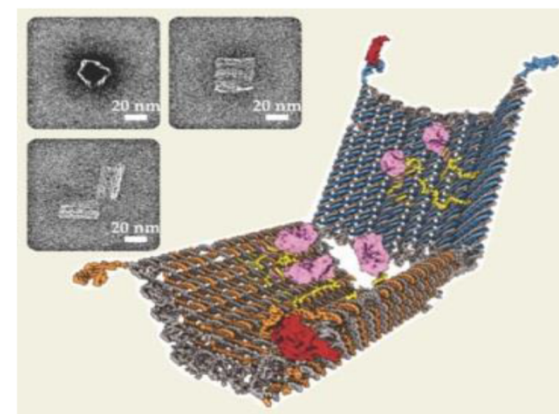
- Dominant (> 10% of total) Investigational Nanomedicines: Liposomes > Polymeric NP > Emulsions > Solid NP
 - Dominant Commercial Nanomedicines: Solid NP > Nanocomposites

Cancer Nanotechnology

- Improve solubility; act as a carrier for hydrophobic drugs.
- Multifunctional capability
- Tumor targeting (reduced toxicity)
- Robotic tasks such as sensing, computation, and actuation; triggered responses.



McNeil, (2005), J. Leuk. Biol., 78:585-594



Douglas et al., (2012) Science, 335 831-834.

Benefits

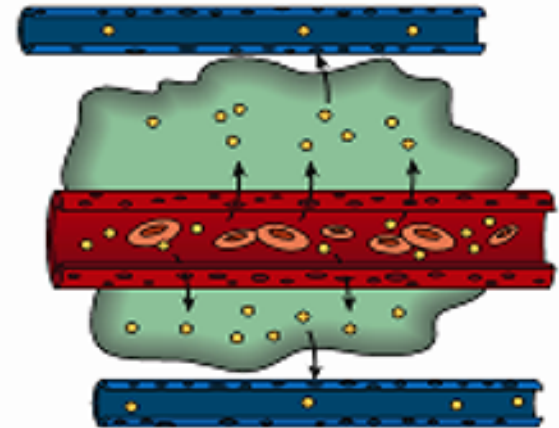
Benefits: Drug Delivery and Targeting



NCI Alliance for
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in Cancer

EPR Effect = Passive Targeting

- Leaky neovasculature
- Particles cross endothelial barrier
- Particles are retained in the tumor



Active Targeting

- Surface chemistry allows functionalization w/ targeting molecules
 - Antibodies, e.g. Herceptin
 - Small molecules, e.g. folic acid
 - Cytokines, e.g. $\text{TNF-}\alpha$

Reduced toxicity

Benefits: Reduced Toxicity



Traditional

Nano

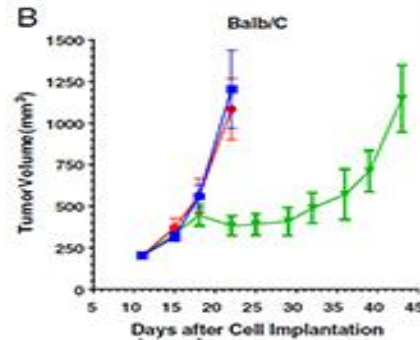
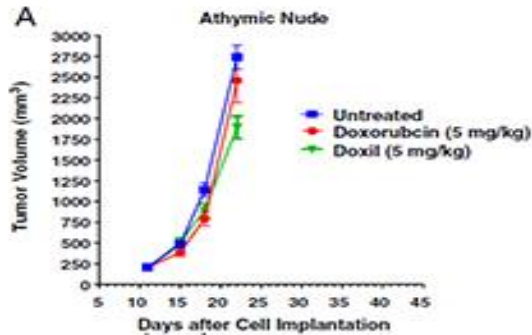
Small Molecules

Therapeutic Proteins

Incorporation of conventional pharmaceuticals into nanotechnology-derived platforms helps decrease their immunotoxicity.

Immunotherapy

Benefits: Immunotherapy



Doxil Synergizes with Cancer Immunotherapies to Enhance Antitumor Responses in Syngeneic Mouse Models

Jonathan Rio-Doria, Nicholas Durham, Leslie Wirtzel, Raymond Rothstein, Jon Chessbrough, Nicholas Hollowocky, Wei Zhao, Ching Ching Leow and Robert Hollingsworth
 MedImmune, Cambridge, MD

■ Untreated
 ■ Doxorubicin (5 mg/kg)
 ■ Doxil (5 mg/kg)

Doxil improves efficacy of cancer immunotherapeutics in CT26 mouse model of colorectal cancer

The Immunotherapy Opdivo & Abraxane for Recurrent HER2-Negative Metastatic Breast Cancer

A Phase 1, Open-Label, Multicenter, Safety Study of Nivolumab (BMS-936558) in Combination With Nab-Paclitaxel Plus or Minus Gemcitabine in Pancreatic Cancer, Nab-Paclitaxel / Carboplatin in Stage IIIB/IV Non-Small Cell Lung Cancer or Nab-Paclitaxel in Recurrent Metastatic Breast Cancer (NCT02309177)

Abraxane is investigated in combination with a-PD-1 in clinical trials for metastatic breast cancer

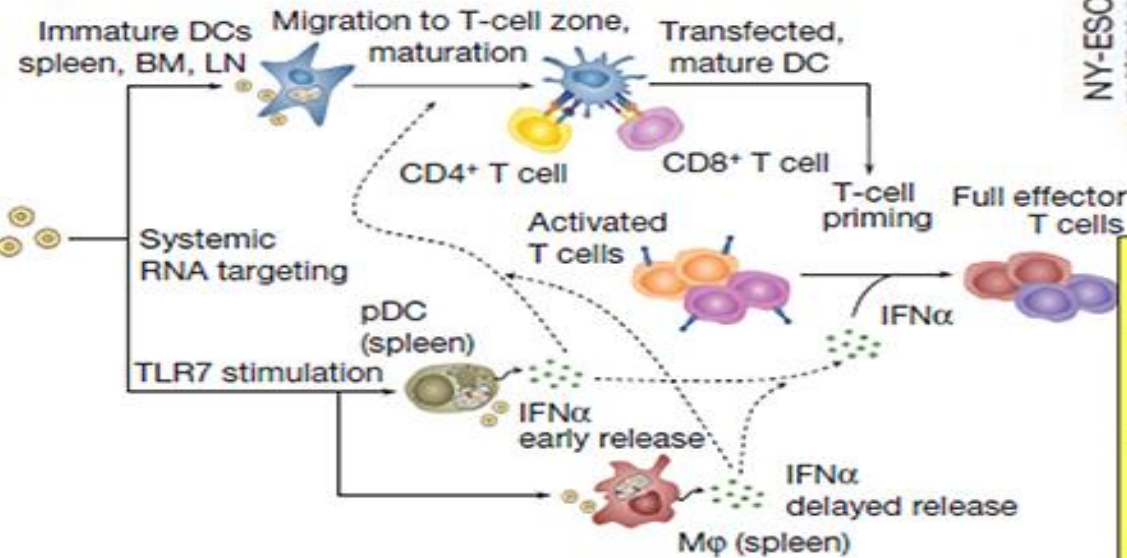
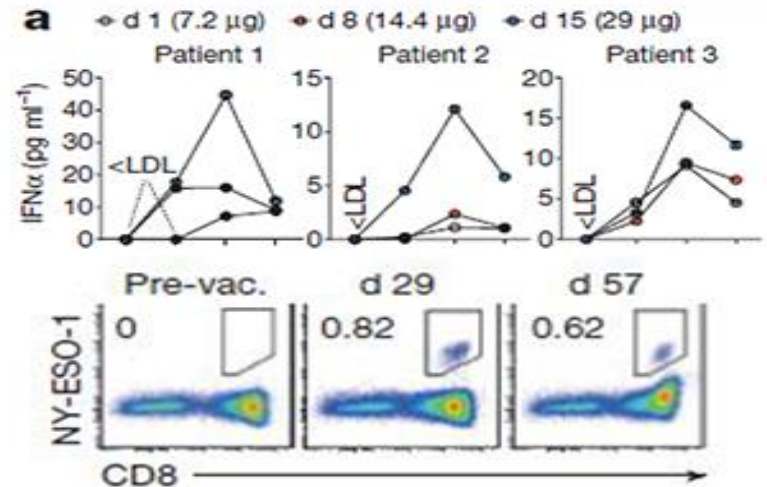
Vaccines

Benefits: Vaccines

LETTER 16 JUNE 2016 | VOL 534 | NATURE | 397

Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy

Leina M. Krause^{1,2*}, Moustafa Elkouss^{1,2*}, Viktorik B. Hain², Sebastian Koster^{1,2}, Carsten Logez², Kerstin C. Rinow¹, Martin Meng², David Fritz², Fabian Vascotto², Howard Hildebrand², Christian Grunewald², Martin Vimmerle^{1,2}, Sven Hübemann¹, Abderrahmane Selmi², Andrius N. Kadis², Janina Buck², Evelyn D. Hoffmann², Richard Kae², Sebastian Artig^{1,2}, Jan Diekmann², Robert A. Tuboi-wicz², Sandra Hirsch², Anika Hesse², Peter Langguth², Stephan Graber², Christoph Huber^{1,2}, Olof Titzel¹ & Ulf G. Kluge^{1,2*}



Systemically administered lipoplex carrying tumor specific RNA:

- LPX protects RNA from degradation, enhances RNA uptake by APC in lymphatic and maturation of DC
- APC express cancer antigens and produce IFN response similar to tat during viral infection
- Induction of strong effector and memory T-cells

Lymphatic delivery

Benefits: lymphatic delivery



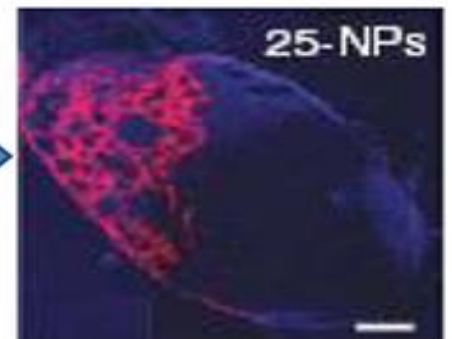
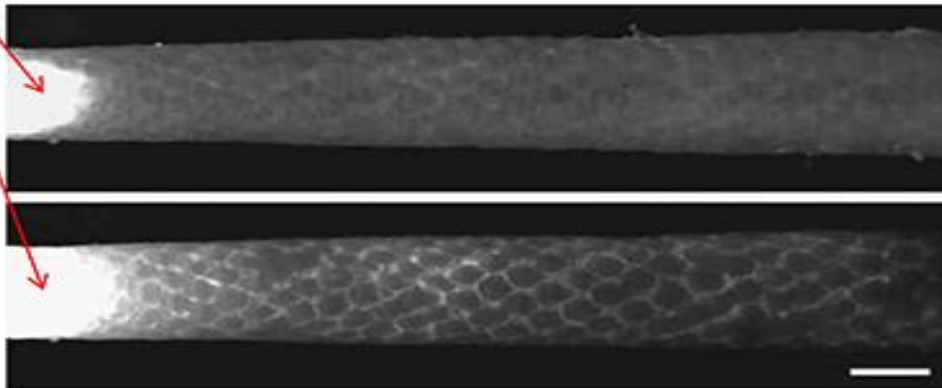
- i.d. injection
- Examine draining lymph nodes

Injection Site

Tail

100nm

25nm



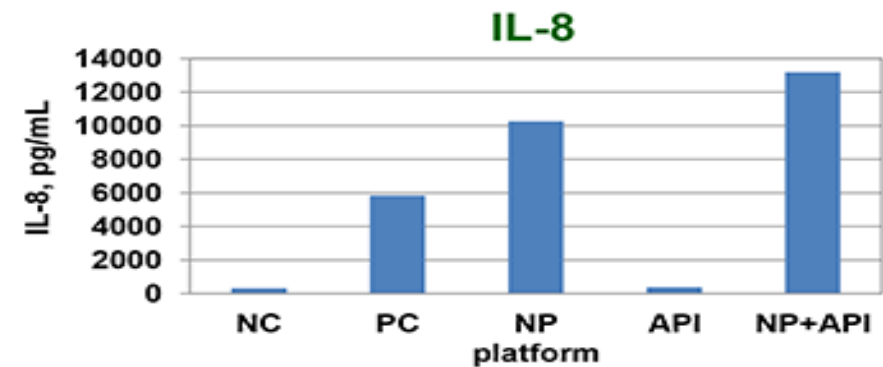
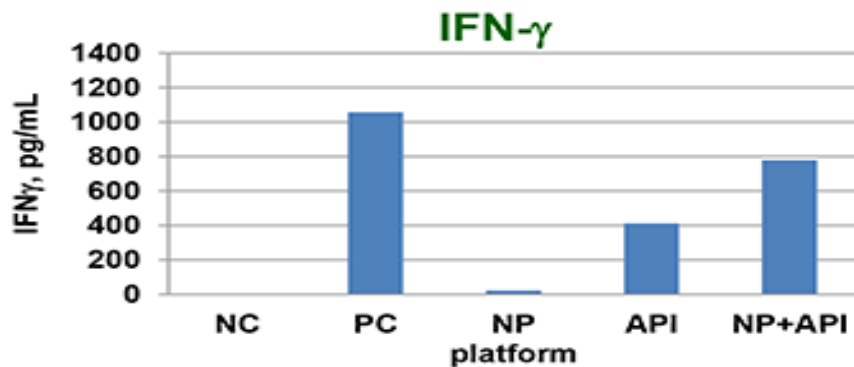
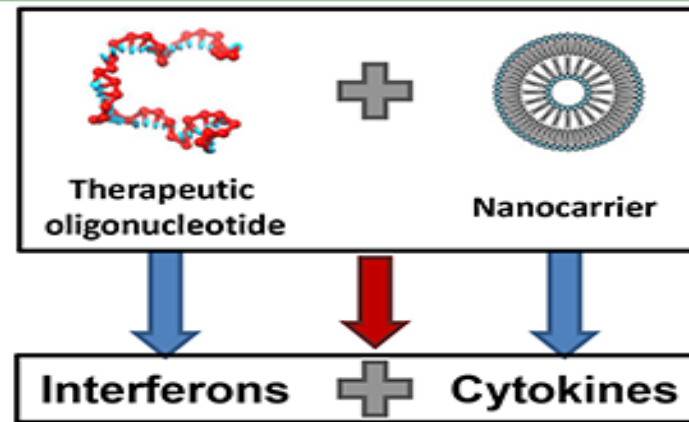
Smaller particles travel through lymphatics. Larger particles do not.

Reddy ST et al, and Hubbell JA. (2007) Nature Biotech., 25 (10):1159-1164

- Particle distribution to lymph nodes after i.d. injection depends on their size
- Lymphatic delivery benefits vaccines, HIV and infectious diseases therapy

Toxicity

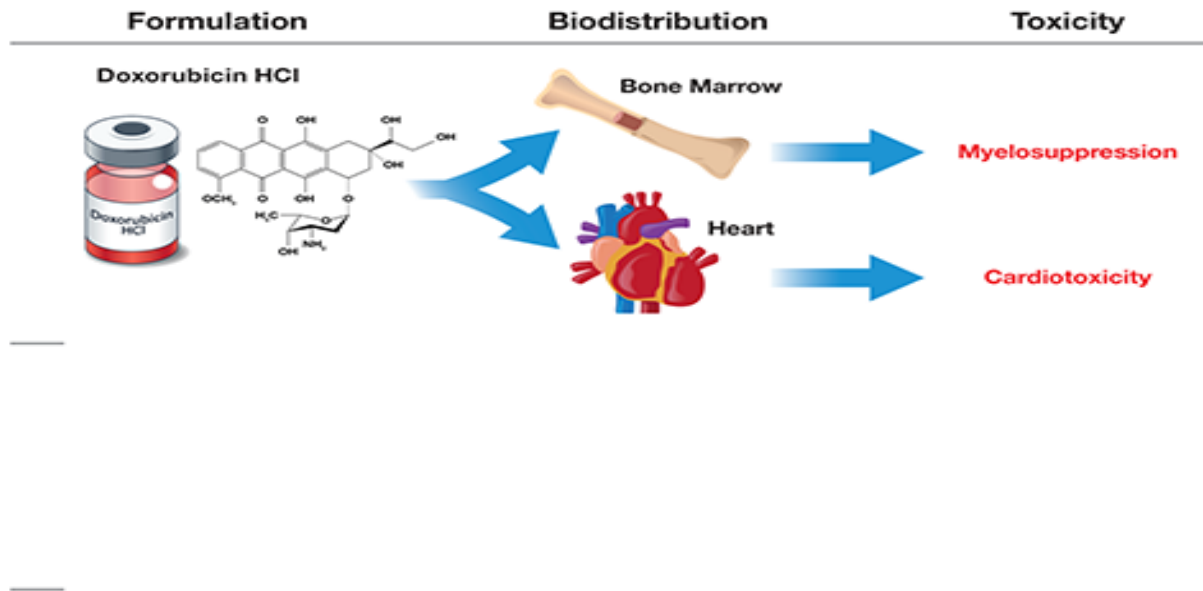
Concerns: Toxicity



- Both nanocarrier and API can be toxic
- Some nanocarriers n a contribute to toxicity of API.

Toxicity

Concerns: Toxicity

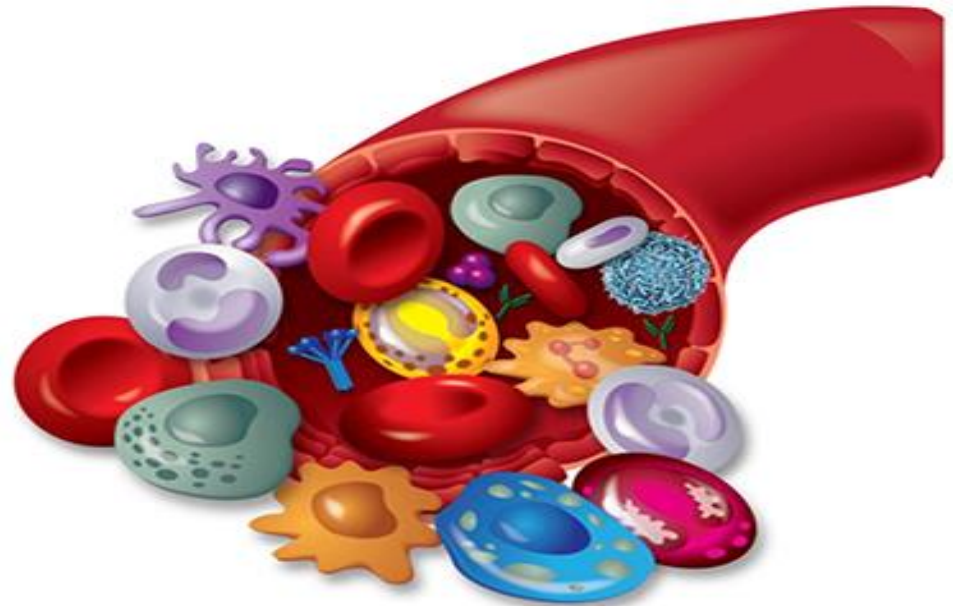


- Both nanocarrier and API can be toxic
- API toxicity can “relocate” depending on the particle biodistribution

Immune system

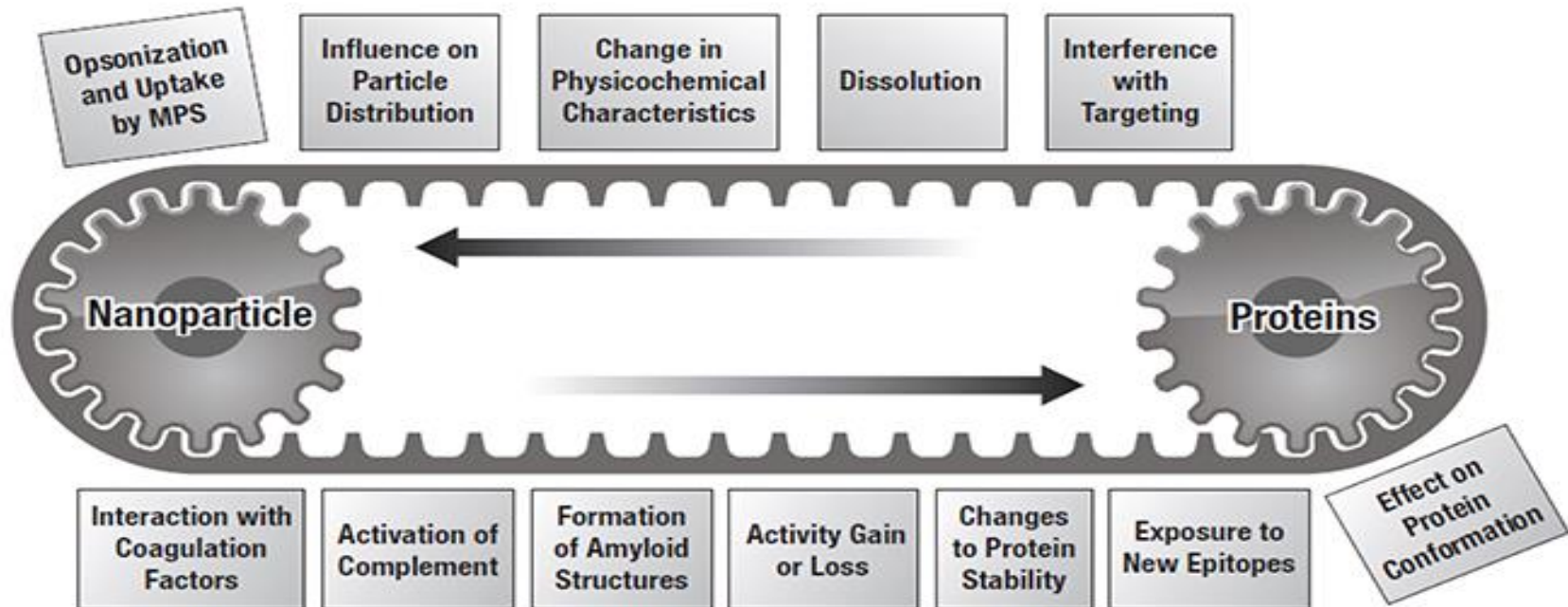
Nanoparticles and the immune system

- Plasma Proteins
 - Biodistribution and MPS uptake
- Effects on erythrocytes
- Blood coagulation system
 - Platelets
 - Leukocytes
 - Endothelial cells
- Allergy
 - Complement activation
 - DTH
- Cytokines
- Immunogenicity



Bidirectional communication

Bidirectional Communication between Nanoparticles and Proteins



**Binding of proteins to nanoparticle surface result in changes in particle properties
Properties and function of some proteins may also change after binding to the nanoparticle**

Particle size

Particle size influences protein binding

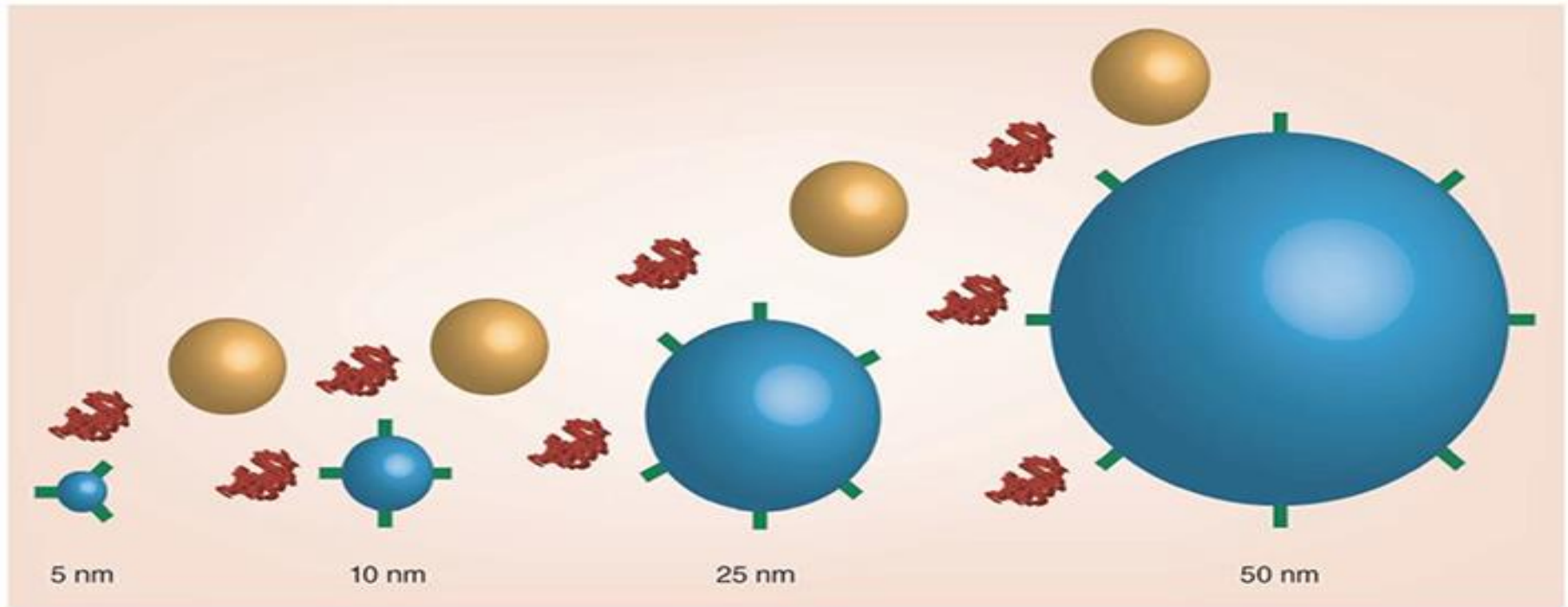


Figure 2. Size of proteins in the corona compared to nanoparticles of varying diameter. Nanoparticles are represented in blue and the diameter is given by the number under each particle in nm. Serum albumin³¹ is shown in red and scaled relative to the nanoparticles. High-density lipoprotein is represented by orange spheres at a size of 12.5 nm diameter.

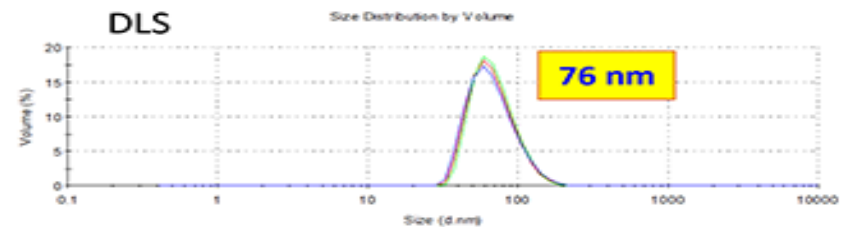
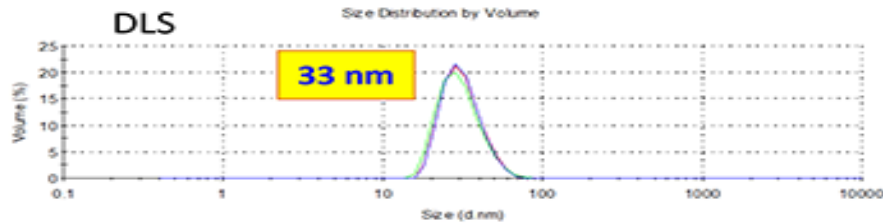
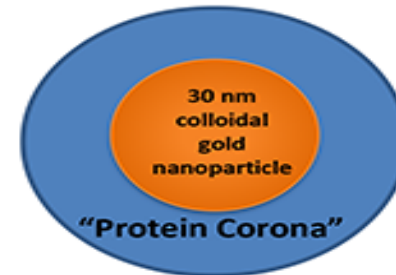
Protein binding

Protein binding affects particle size

BEFORE



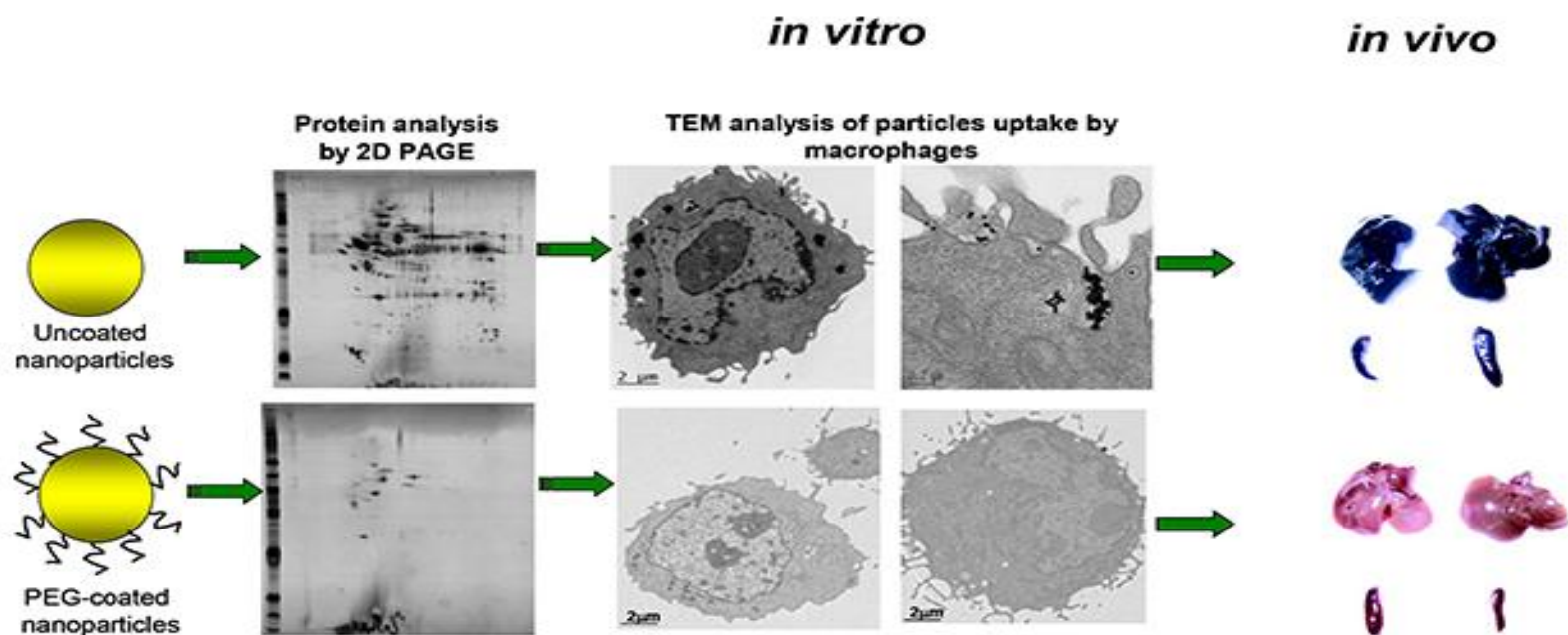
AFTER



Incubation with human plasma increases hydrodynamic size of nanoparticles

Bidistribution

Protein Binding and biodistribution



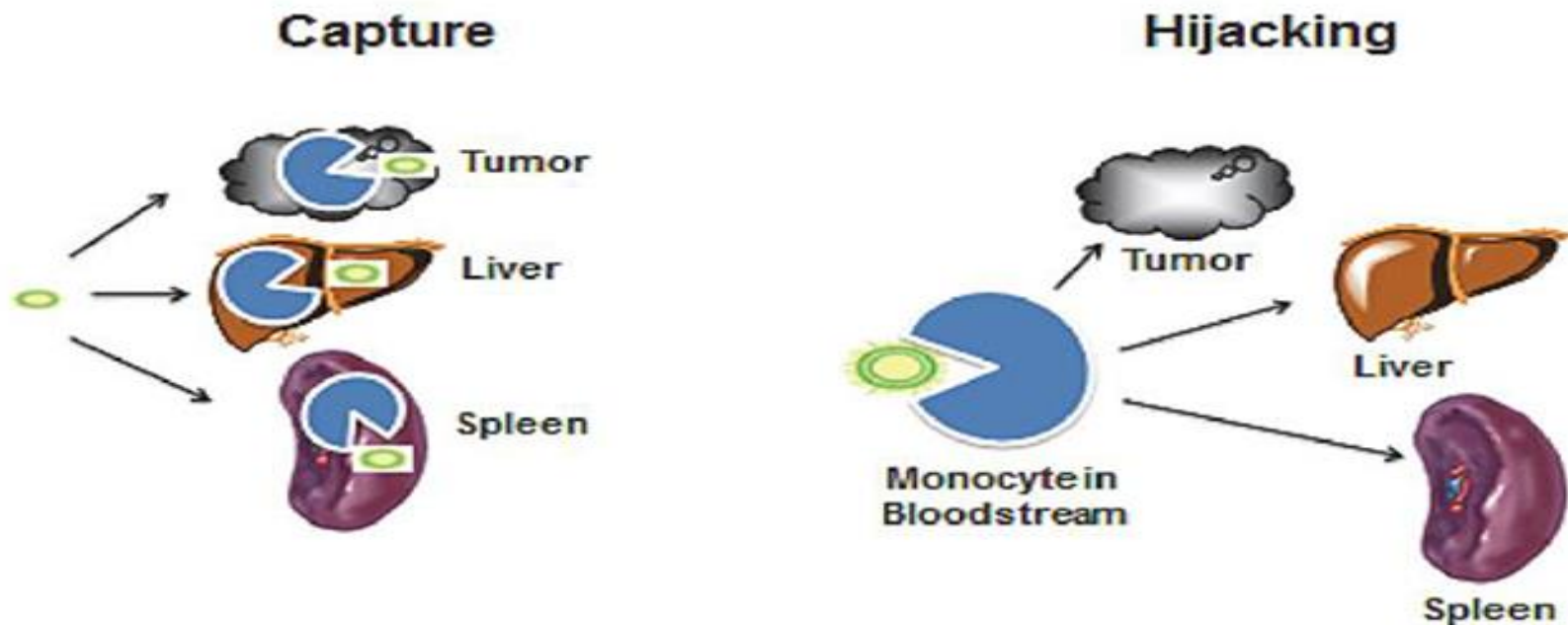
Dobrovol'skaia et al., (2008), *Mol.Pharm.*, 5:487-495.

Paciotti J. et al.,(2004), *Drug Delivery*,11:169-183.

- Particles which bind proteins are eliminated by MPS
- Particle surface protection (e.g with PEG) reduces protein binding and MPS
 - Good correlation between *in vitro* and *in vivo*

MPS uptake

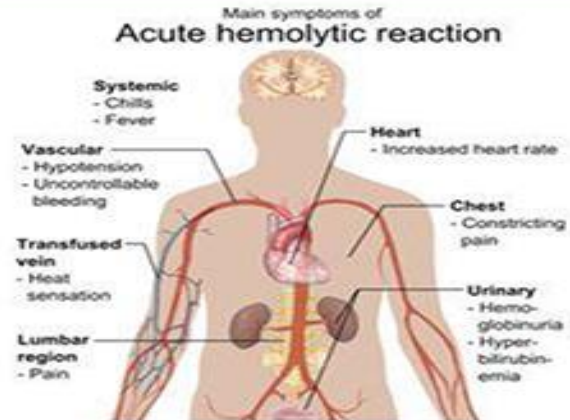
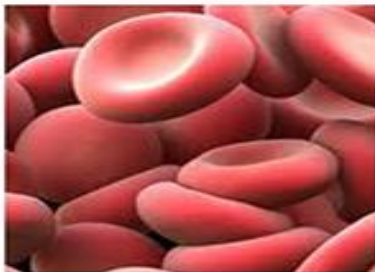
MPS uptake



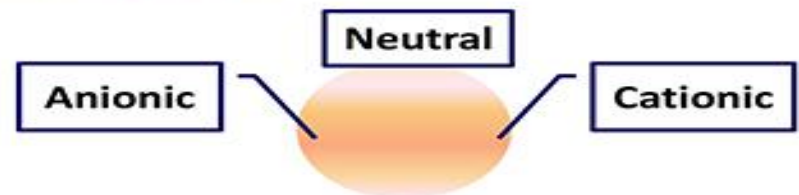
- Two theories about nanoparticle distribution to the MPS
- Capture – uptake by phagocytic cells in the tissue
- Hijacking – uptake by circulating phagocytic cells which then take the particle to tissue

Hemolysis

Hemolysis



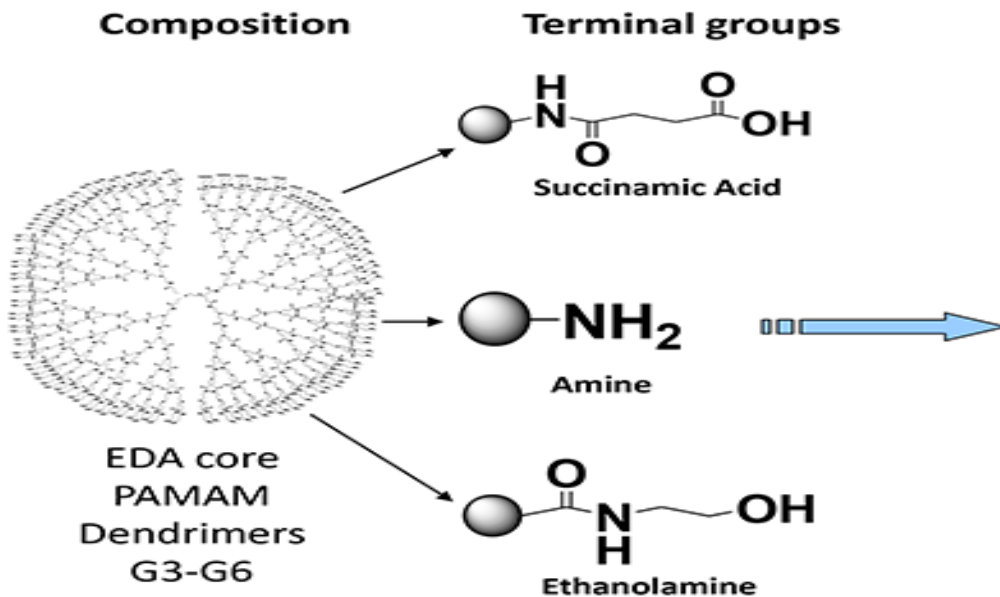
**Role of Nanoparticle
Size**



**Role of Nanoparticle
Surface Charge**

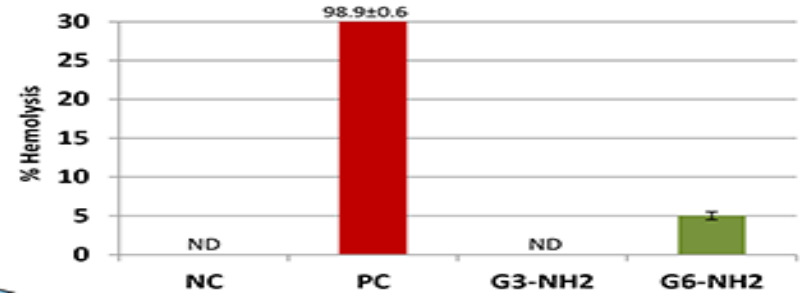
Hemolysis

Hemolysis

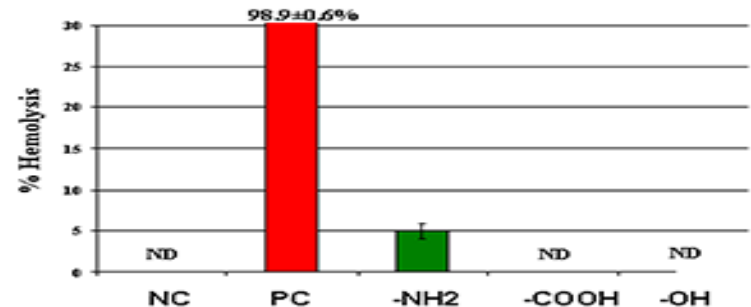


- Cationic dendrimers are more hemolytic than their anionic and neutral counterparts of the same size
- Larger dendrimers are more hemolytic than smaller

Effects of Size



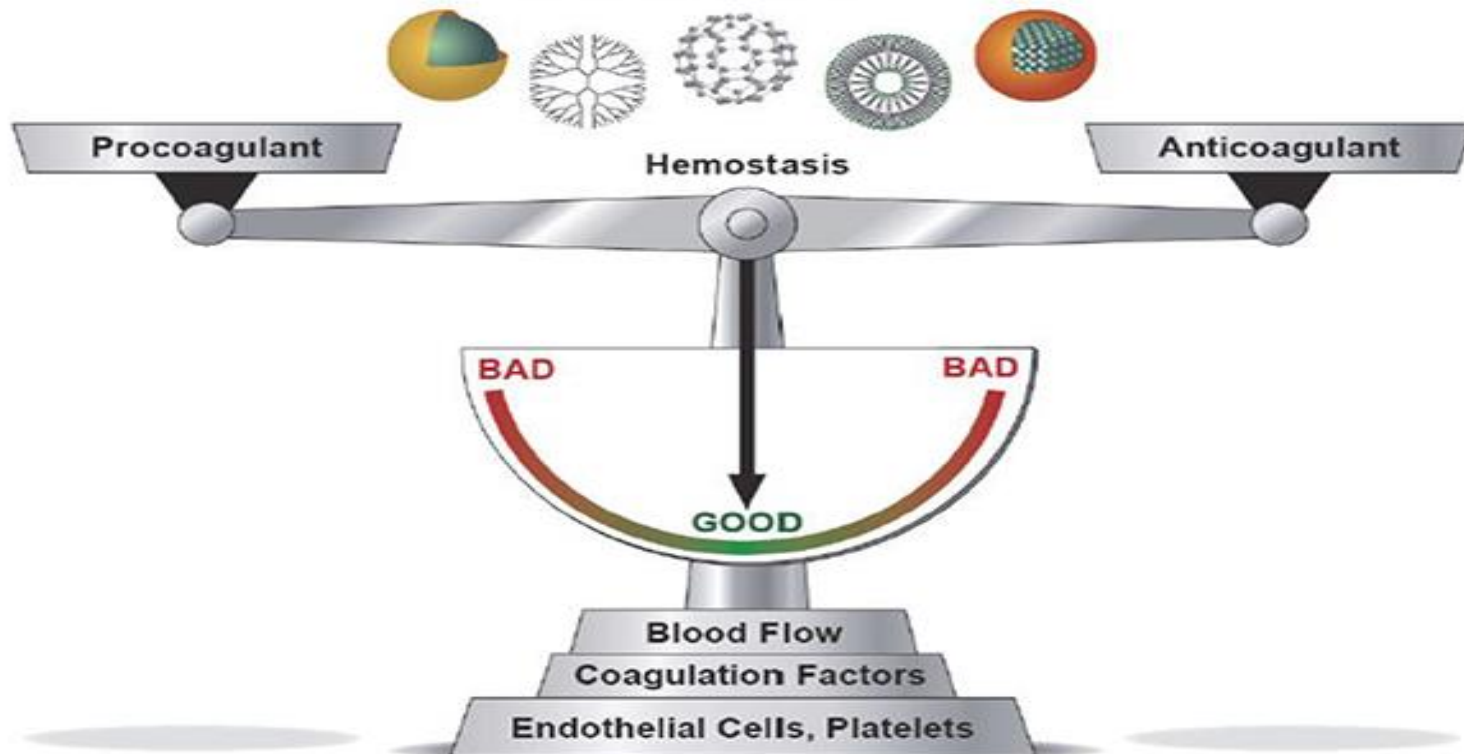
Effects of Surface Charge



Coagulation system

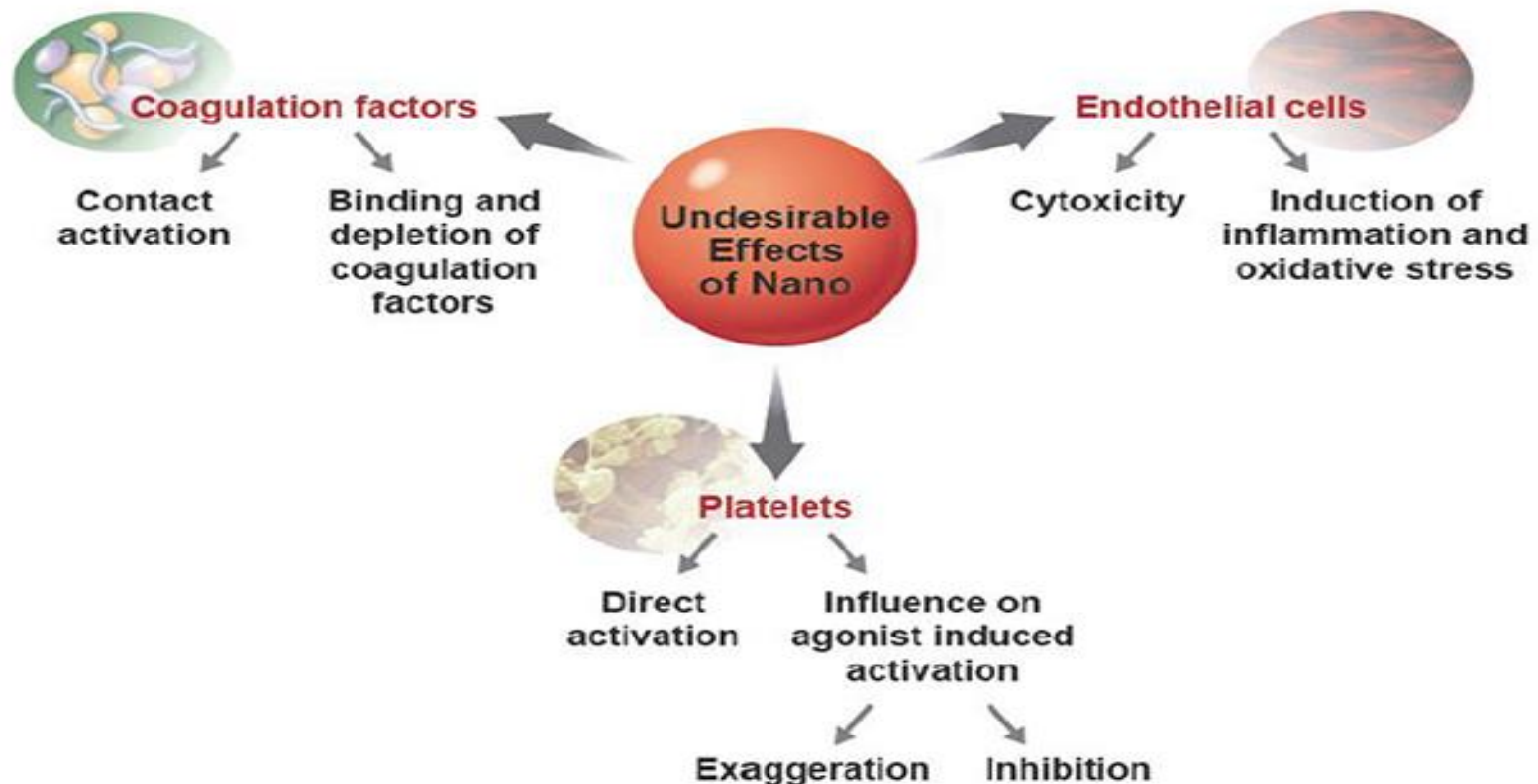
Coagulation system

Nanoparticles can be engineered to avoid or specifically interact with coagulation system.



Undesirable effects

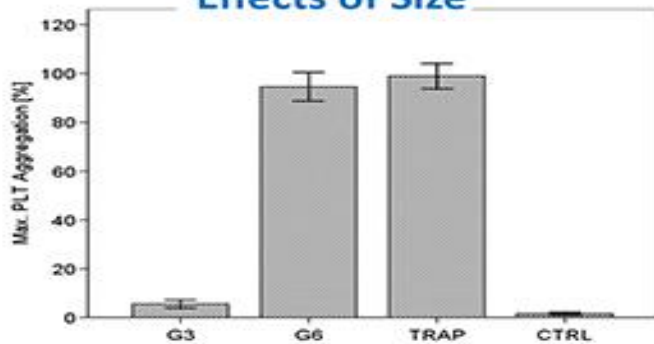
Undesirable effects on coagulation



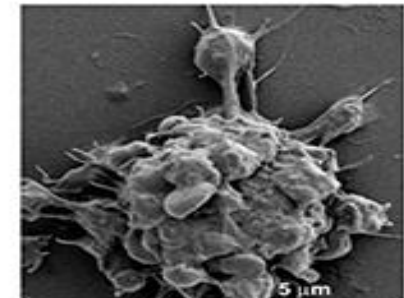
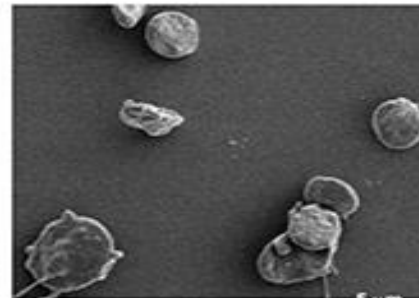
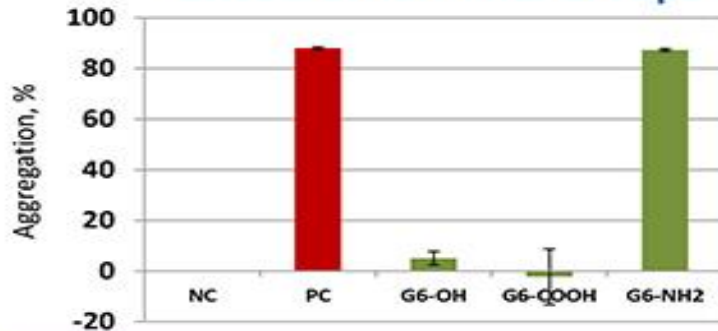
Platelets

Platelets

Effects of Size



Effects of Terminal Groups



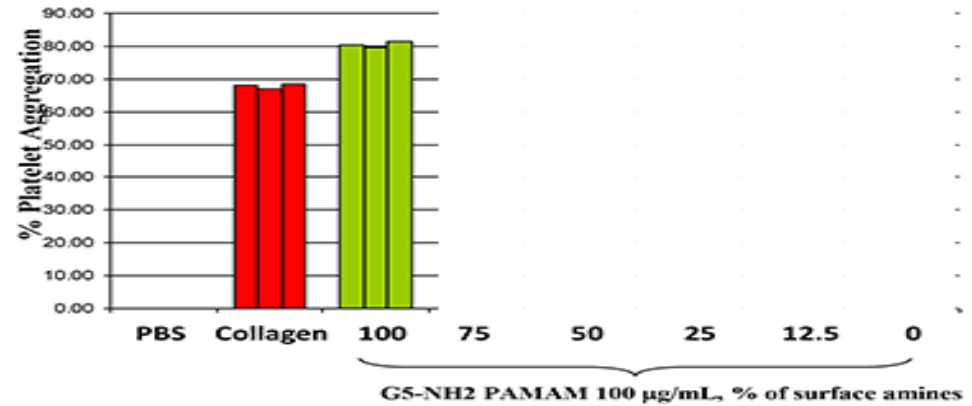
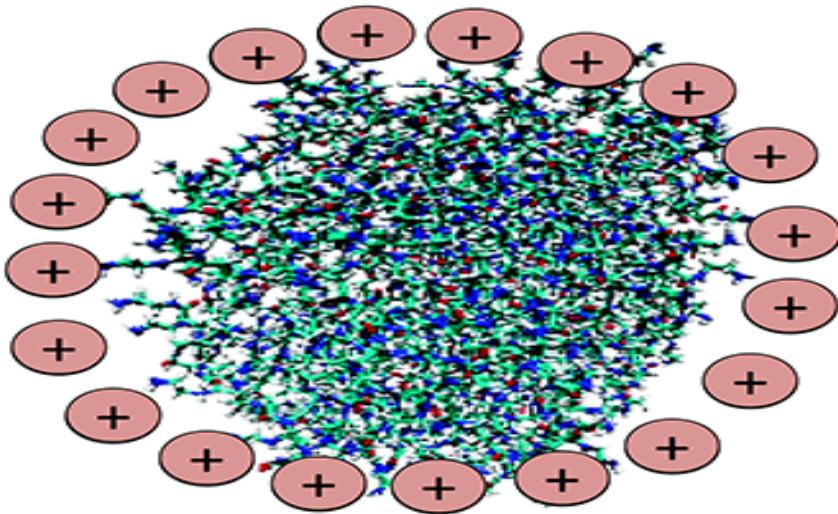
Dobrovolskaia et al., Mol.Pharm, 2011

Platelet aggregation was induced by cationic PAMAM dendrimers, and increased with increasing particle size and greater number of surface amines.

Anionic and neutral dendrimers, irrespective of size and number of surface groups, did not induce platelet aggregation in vitro.

Zeta potential

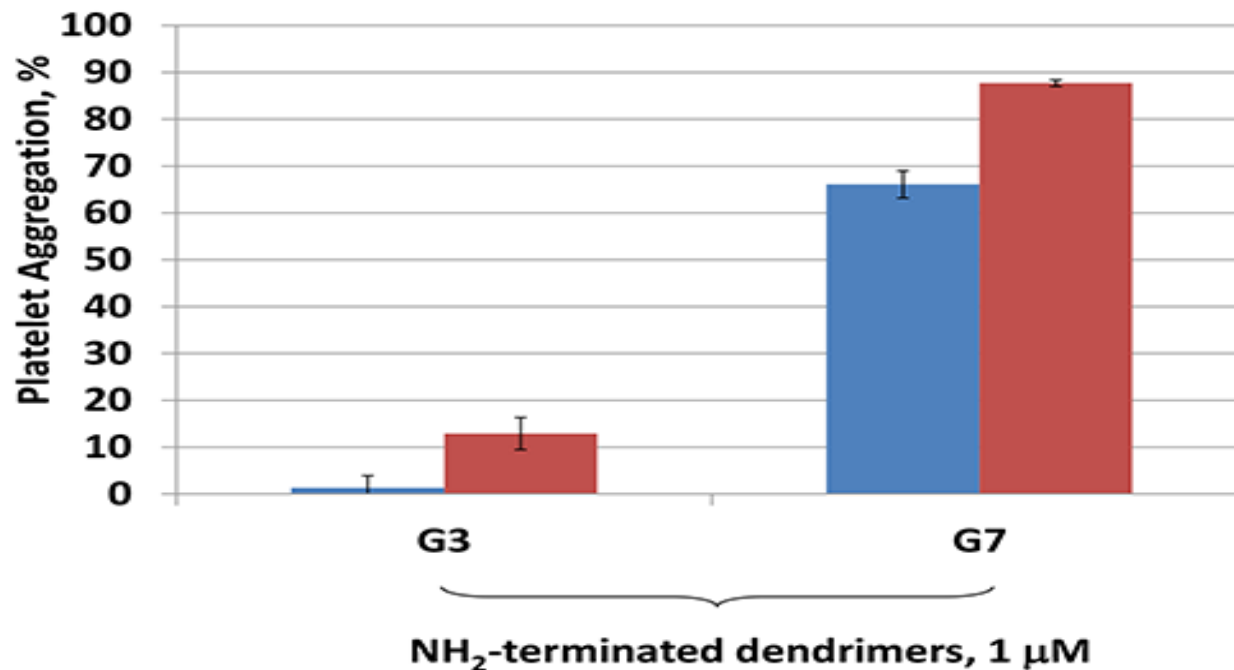
Platelets: role of zeta potential



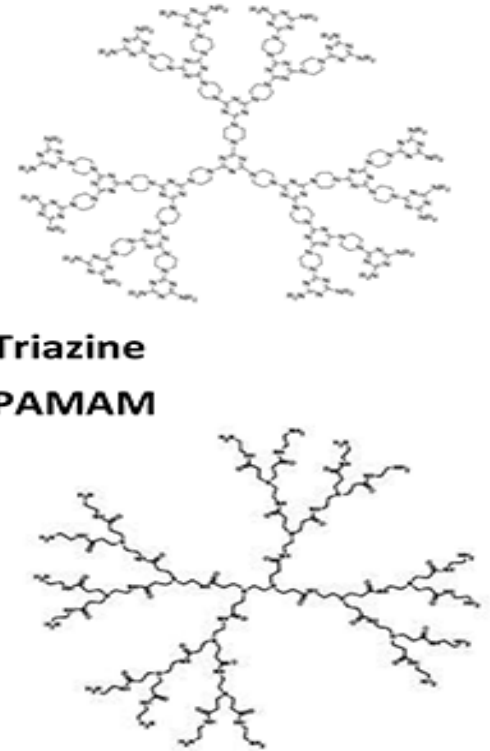
Zeta Potential is important
Less surface amines = less platelet aggregation

Platelets

Platelets: effect of composition



■ Triazine
■ PAMAM



Triazine dendrimers are less potent in inducing platelet aggregation than their PAMAM counterparts

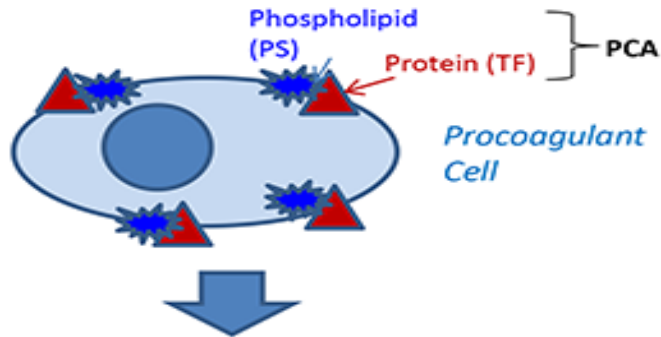
Leukocyte

Leukocyte Procoagulant Activity



Key events
 (occur through multiple mechanisms)

1. Exposure of phosphatidylserine (PS) on cell surface
2. Expression and/or de-encryption of tissue factor (TF)

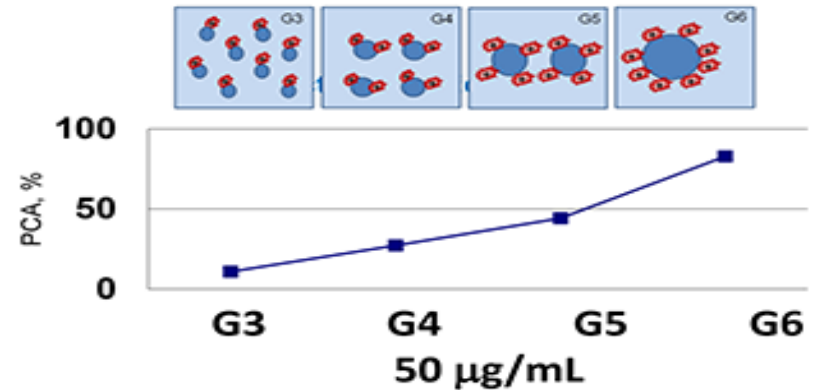


Activation of extrinsic plasma coagulation cascade

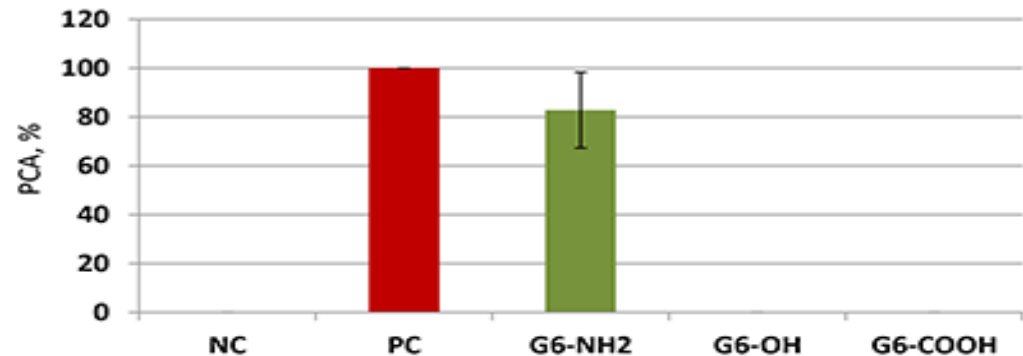
PCA = procoagulant activity

- Cationic, but not anionic or neutral PAMAM dendrimers induced PCA in vitro
- Large particles are more reactive

Effects of Size

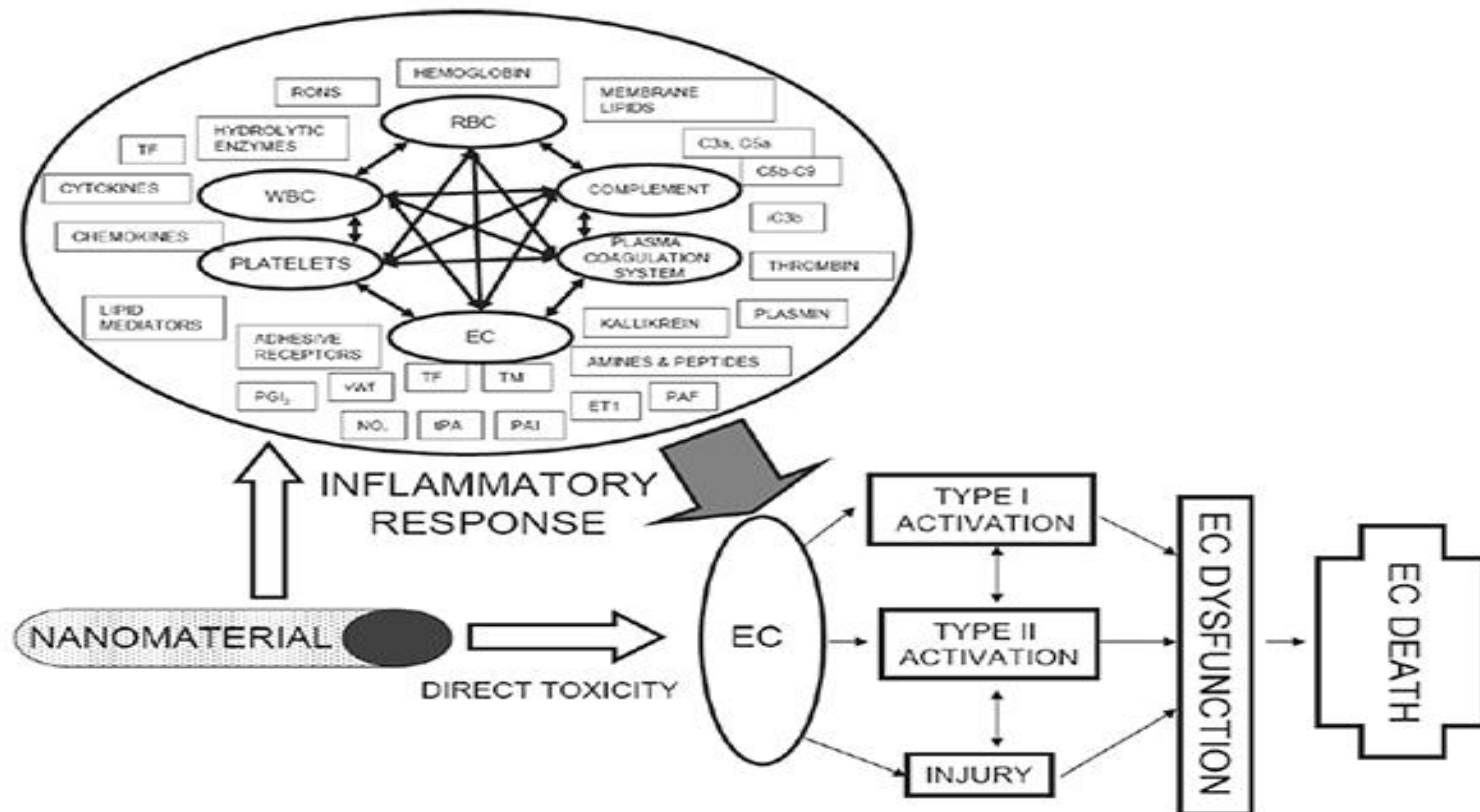


Effects of Surface Charge



Endothelial cells

Effects on endothelial cells



Allergenicity

Allergenicity

Table 2. Main characteristics of the different types of hypersensitivity reactions.

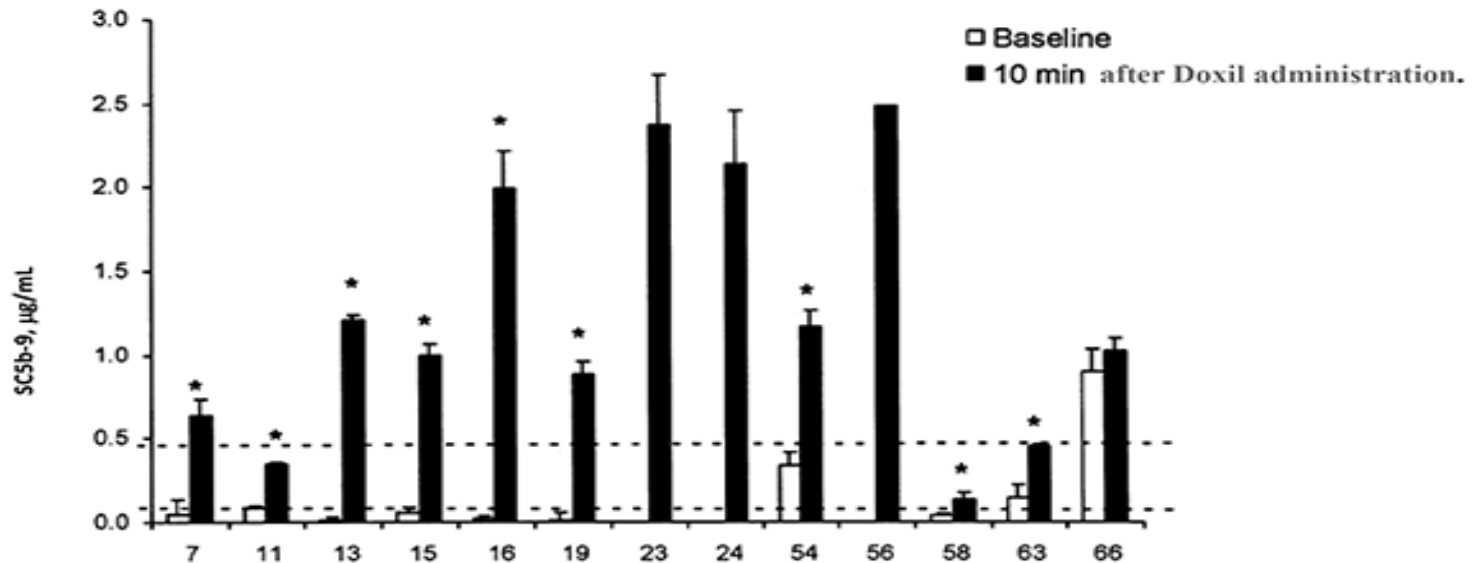
Characteristics	Type I (anaphylactic or immediate hypersensitivity)	Pseudoallergy	Type II (cytotoxic hypersensitivity)	Type III (immune complex mediated hypersensitivity)	Type IV (delayed hypersensitivity)
Main mediators	IgE, mast cells	Complement	Mainly IgG, natural killer cells, complement	IgG, IgM, complement	T helper cells and macrophages
Antigen	Exogenous	Exogenous	Cell surfaces	Soluble antigens	Bacteria, tissues
Time	15–30 min	15–30 min	minutes–hours	3–8 h	48–72 h
Skin reaction	Skin prick positive wheal and flare	Skin prick-negative		Intradermal injection (swelling and redness)	Mantoux-positive (erythema and induration)
Clinical manifestations	Allergic asthma, hay fever, anaphylactic shock	Urticaria, angioedema	Pemphigus, nephritis, autoimmune haemolytic anaemia, Goodpasture's syndrome	Serum sickness, fever, glomerulitis, vasculitis	Tuberculin test, poison ivy, contact dermatitis, maculopapular rashes, granuloma

Gonzalez-Fernandez A et al. Handbook of Immunological properties of Engineered Nanomaterials (2016), Vol 3

- Nanoparticles can be engineered to inhibit allergy (tolerogenic and drug-carrying nanoparticles)
- Some nanoparticle can exaggerate allergy to traditional allergens
- Pseudoallergy is the most common and best studied reaction to nanomaterials
- Rare example of cell-mediated allergy to dendrimers

Allergenicity

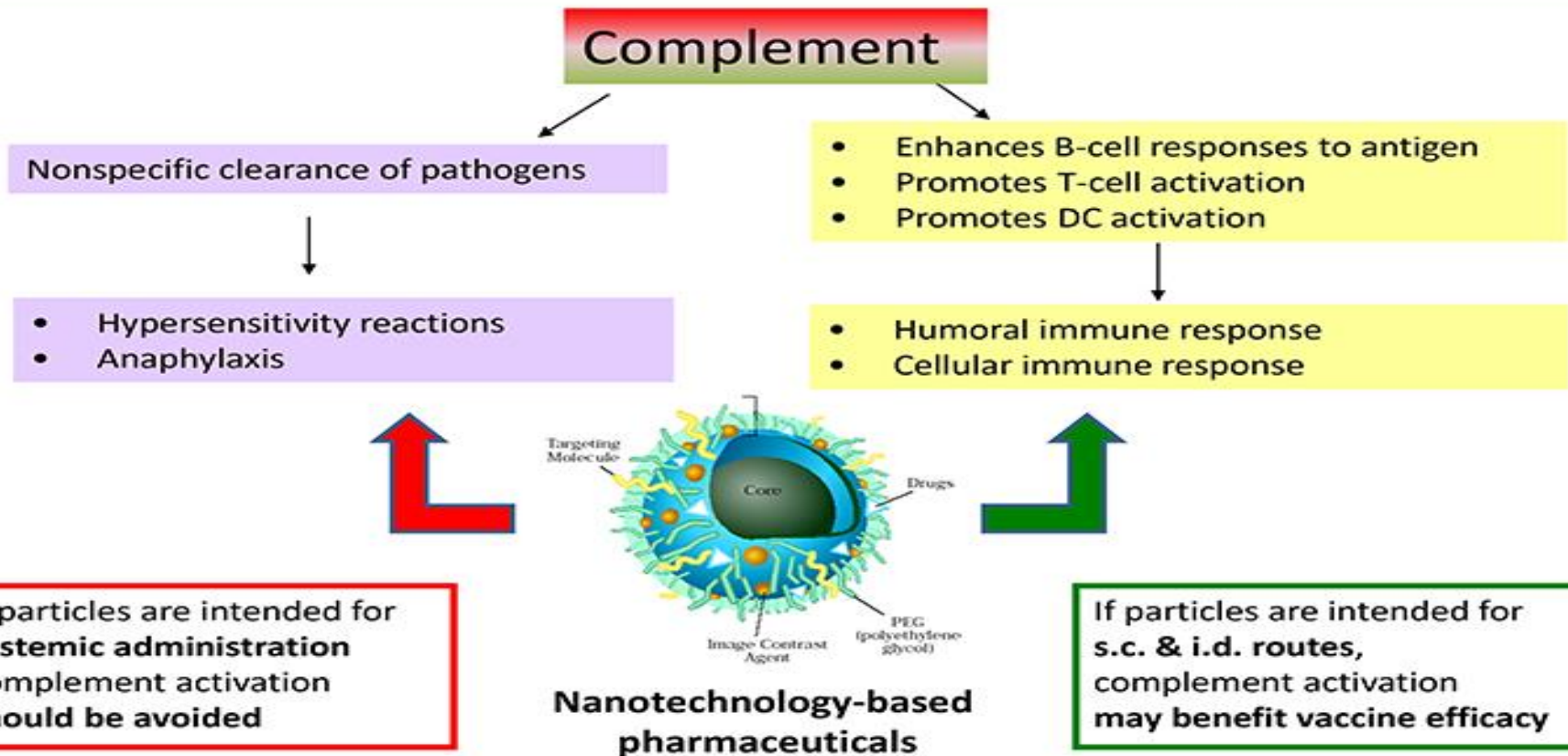
Allergenicity: CARPA to PEG-Liposomes



**Complement activation is dose limiting toxicity
of PEGylated liposomes**

Complement activation

Complement activation: take home message



Allergenicity

Allergenicity: DTH to dendrimers



A case of toxic epidermal necrolysis-like dermatitis evolving from contact dermatitis of the hands associated with exposure to dendrimers

Contact Dermatitis 2008; 59: 122–123

T. Toyama, H. Matsuda, I. Ishida, M. Tani, S. Kitaba, S. Sano and I. Katayama

Department of Dermatology, Course of Integrated Medicine, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

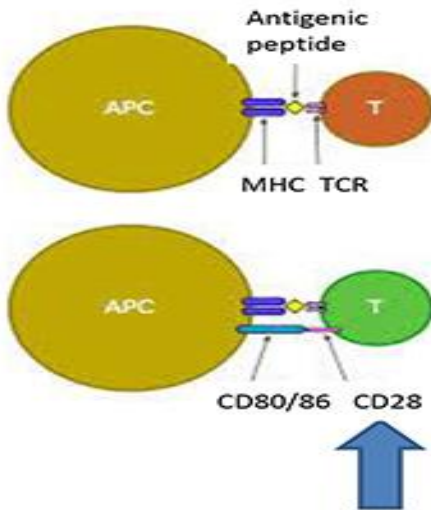
- Only one case of necrotizing dermatitis (type IV reaction) in response to dendrimers is reported in the literature: fever, chills, exudative erythema and fused bullae (Nikolsky's reaction)
- The mechanism is unknown

Cytokine storm

Cytokine storm: Lessons from biotechnology products:



TGN 1412 -
What happened?



TGN1412 = CD28 Super-MAB

Preclinical studies in NHP and rodents
did not reveal cytokine storm



Phase I clinical trial: 6 of 6 volunteers
experienced cytokine storm which lead to
multiple organ failure



In vitro experiments using human PBMC
showed high TNF levels in response to
TGN1412

Cytokine storm

Cytokine Storm to nanomaterials can be predicted in vitro

In vivo

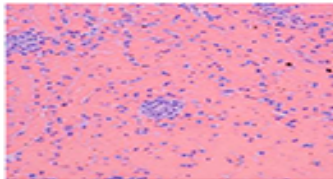
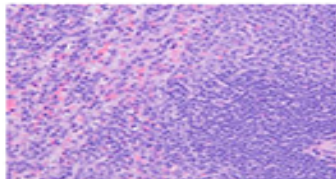


Metal oxide nanoparticles



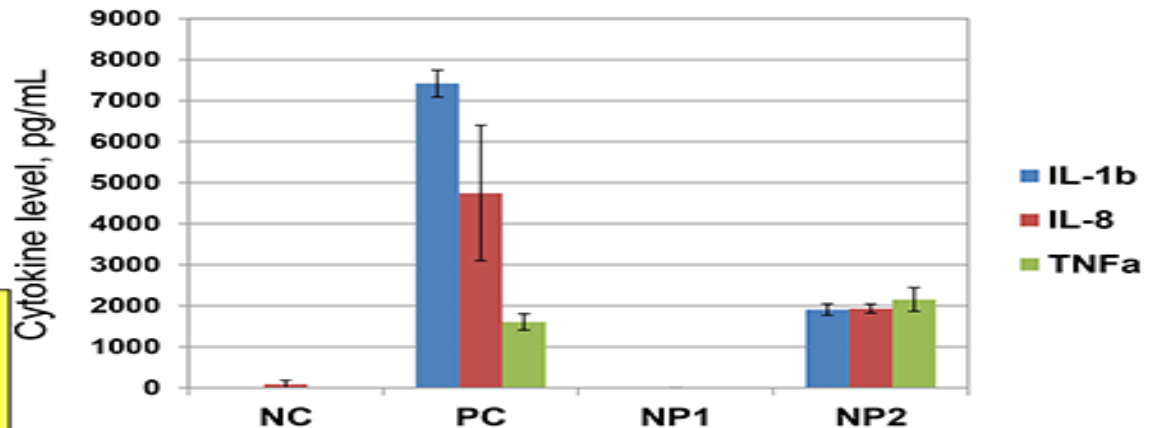
Control: Normal spleen

NP2: congestion in spleen



- Endotoxin is undetectable in NP1 and NP2 by gel-clot LAL
- NP2 but not NP1 is toxic
- Necropsy reveals congestion and multiple organ damage similar to that seen in septic shock
- Analysis of plasma samples reveals elevated cytokines

In vitro (human PBMC)

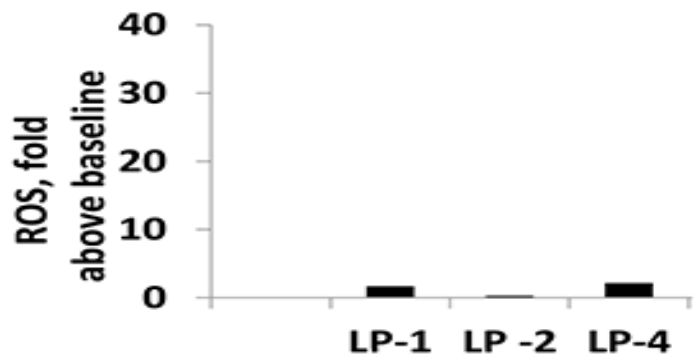
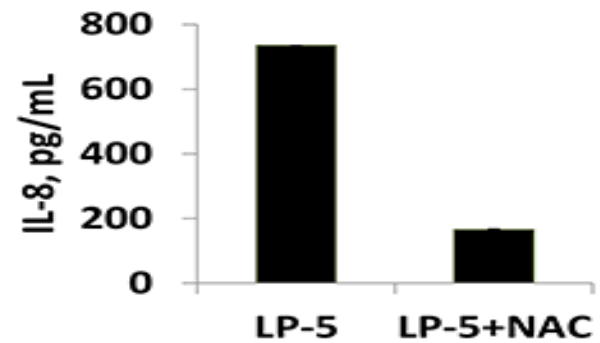
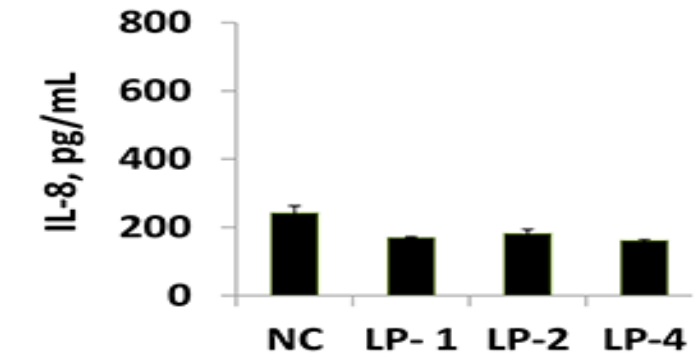


Results are reproduced in vitro using human PBMC

NP = nanoparticle; PBMC = peripheral blood mononuclear cells; IL- interleukin; TNF = tumor necrosis factor; LAL = limulus amoebocyte lysate

IL-8

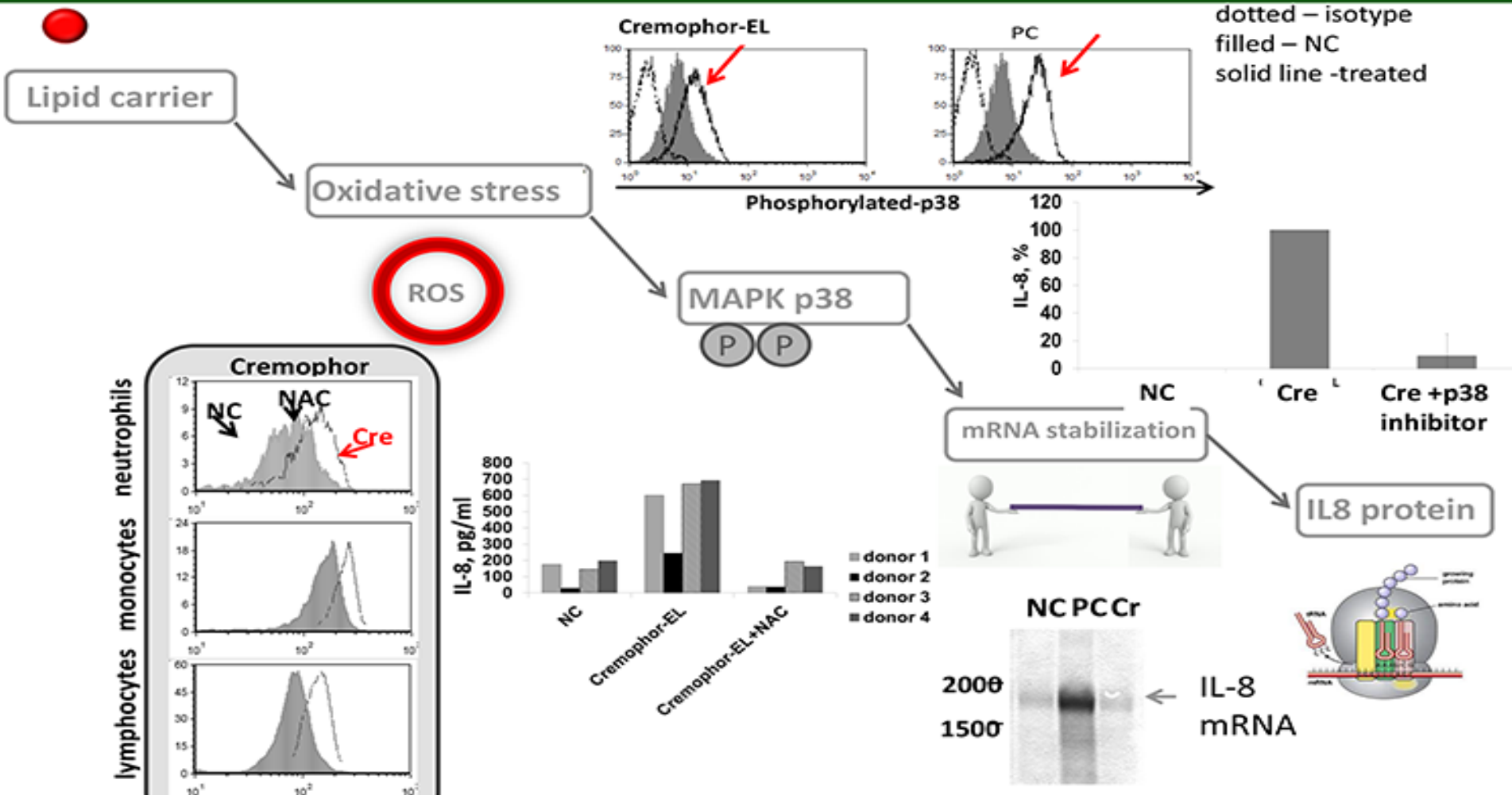
Lipid based particles induce IL-8



- Induction of IL-8 by liposomes follows induction of oxidative stress and can be prevented by antioxidant N-acetyl cysteine

Oxidative stress

Mechanism of IL-8 induction: oxidative stress



IL-1

Fibrous and Cationic Nanoparticles induce IL-1



NCI Alliance for
Nanotechnology
in Cancer

Particle and Fibre Toxicology

BioMed Central

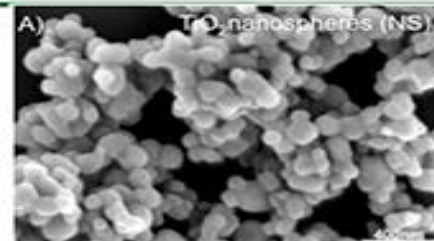
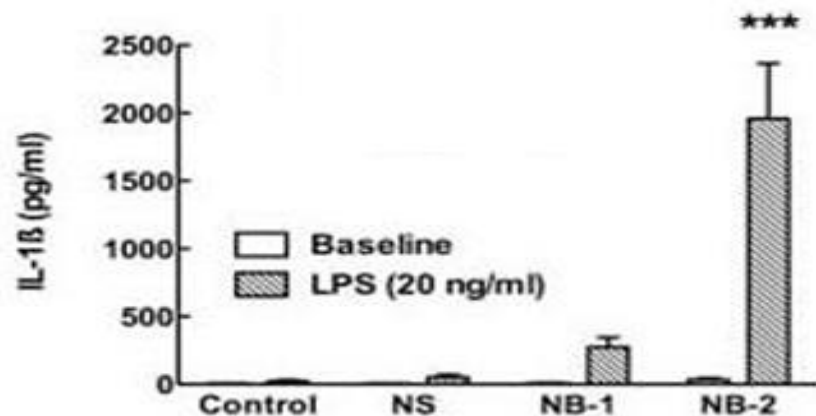
2009, 6:35

Open Access

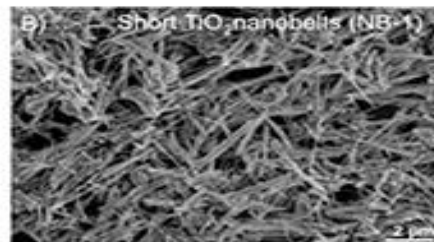
Research

Particle length-dependent titanium dioxide nanomaterials toxicity and bioactivity

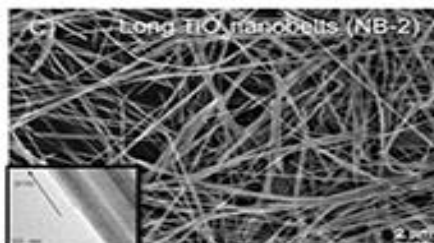
Raymond F Hamilton Jr¹, Nianqiang Wu², Dale Porter³, Mary Buford¹, Michael Wolfarth³ and Andriy Holian^{*1}



TiO₂
Nanospheres
(NS)



TiO₂
Short Nanobelts
(NB-1)



TiO₂
Long Nanobelts
(NB-2)

- Long fibrous TiO₂ nanoparticles enhanced endotoxin-mediated IL-1
- Cationic dendrimers have similar property
- Enhancement of endotoxin-mediated inflammation is a serious safety concern due to common contamination of nanomaterials with bacterial LPS

IL-1

Mechanism of IL-1 induction: Proton sponge effect

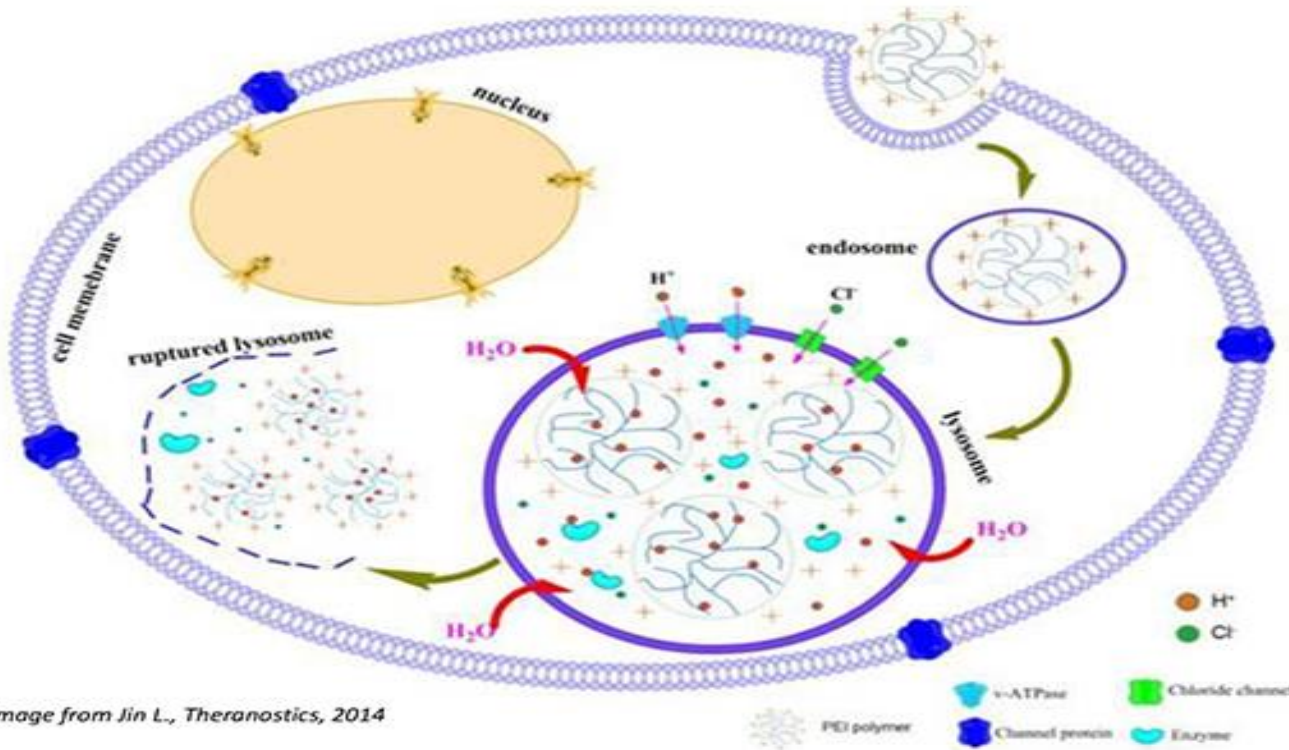


Image from Jin L., *Theranostics*, 2014

Cationic particles induce IL-1 β through activation of NLRP3 inflammasome triggered by a proton-sponge mechanism

Immunogenicity

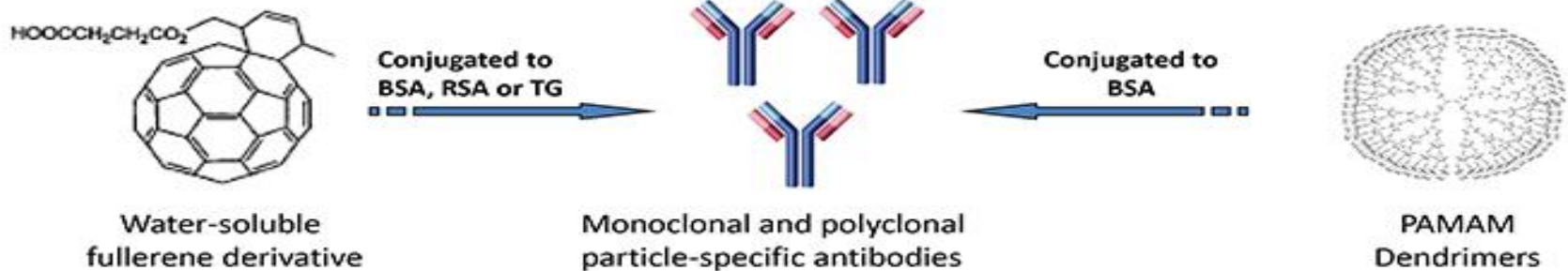
Immunogenicity

Not immunogenic even in the presence of strong adjuvant

- Other C60-derivatives
- Gold colloids
- other PAMAM dendrimers



Immunogenic after conjugation to a protein carrier

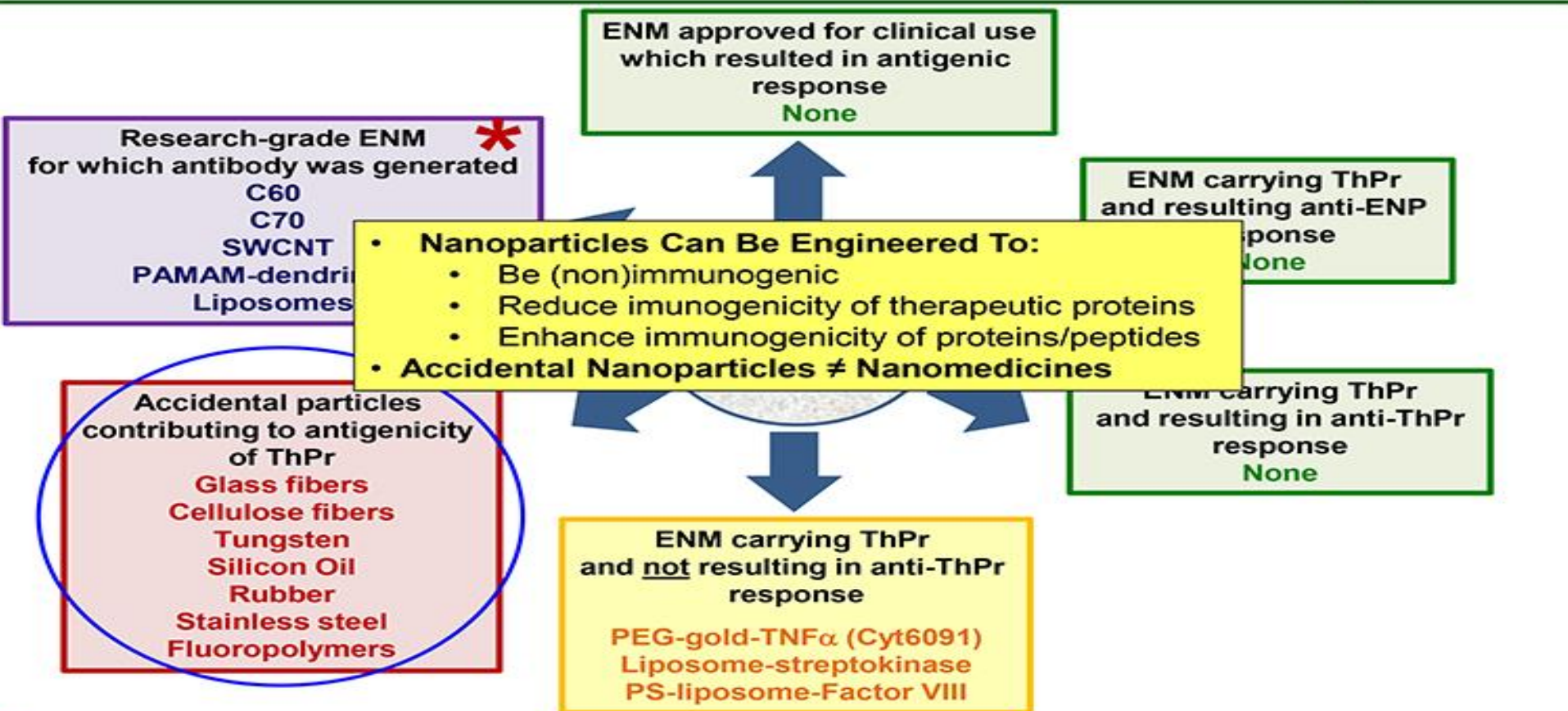


Chen et al. (1998) PNAS, 95:10809-10813
Braden et al. (2000) PNAS 97:12193-12197

Lee SC et al. (2004) BioMed Microdevices 6:191-202

Immunogenicity

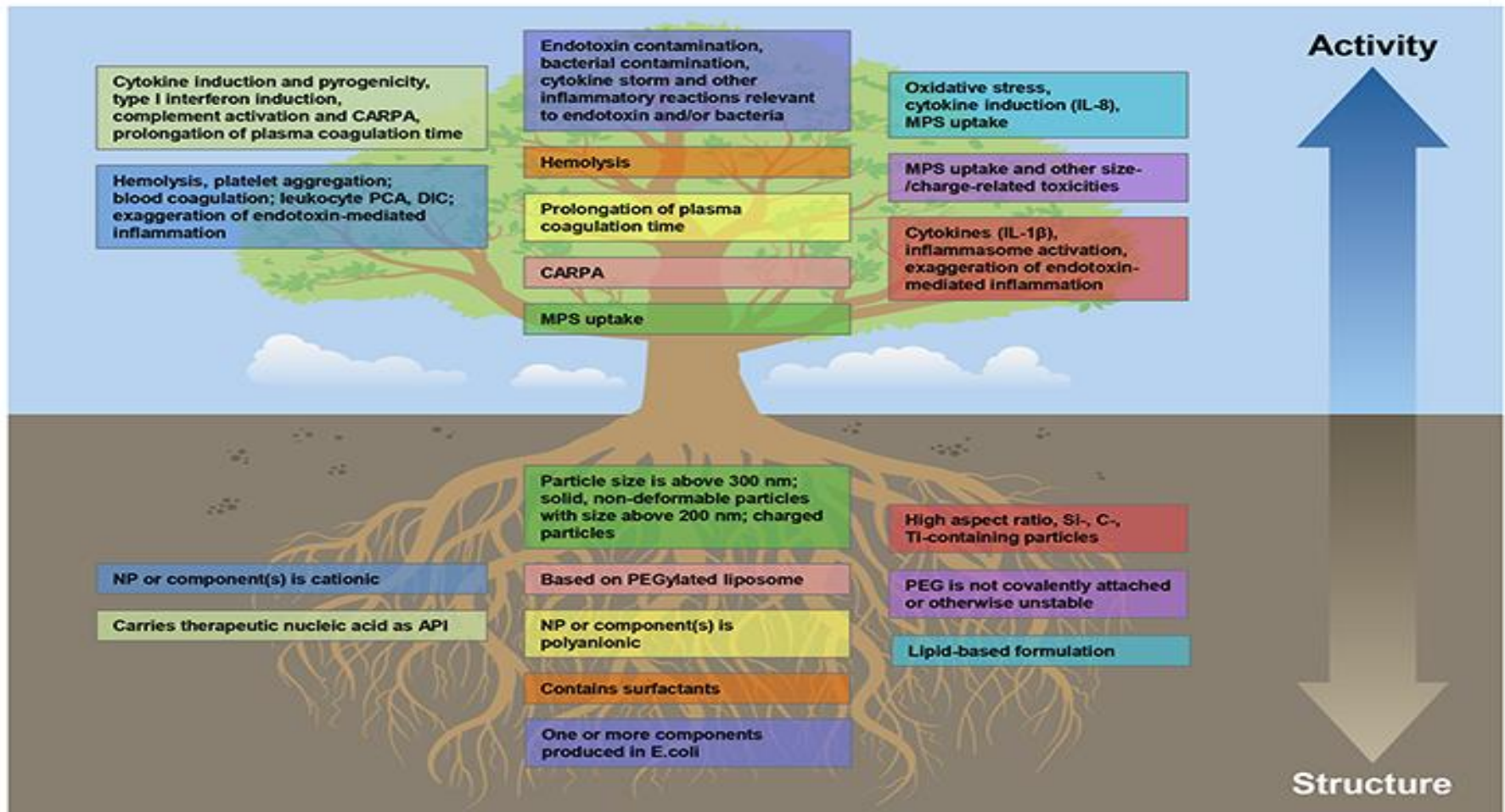
Immunogenicity



* - antibodies were generated ONLY after conjugation to protein carrier and injection in the presence of strong adjuvants
 ENM = engineered nanomaterials; ThPr = therapeutic protein; SWCNT = single wall carbon nanotubes; PAMAM = polyamidoamine; TNF = tumor necrosis factor
 Dobrovolskaia & McNeil. *Handbook of Immunological properties of engineered nanomaterials*. WSP, 2013, ISBN 978-981-4390-25-5.

Immune system

Overall summary of effects on the immune system



Take home message

Take home messages



- Nanotechnology can benefit cancer therapy by improving formulation of traditional drugs (SM, biotechnology products and immunotherapeutics)
- Nanoparticles physicochemical properties determine particle toxicity
- Nanoparticles can be engineered to either specifically interact with or avoid the immune system
- Nanoparticle interaction with the components of the immune system can be desirable or undesirable
- Desirable interactions can benefit therapies of many disorders including cancer