Nanotechnology

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

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NATIONAL CANCER INSTITUTE

Frederick National Laboratory for Cancer Research

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

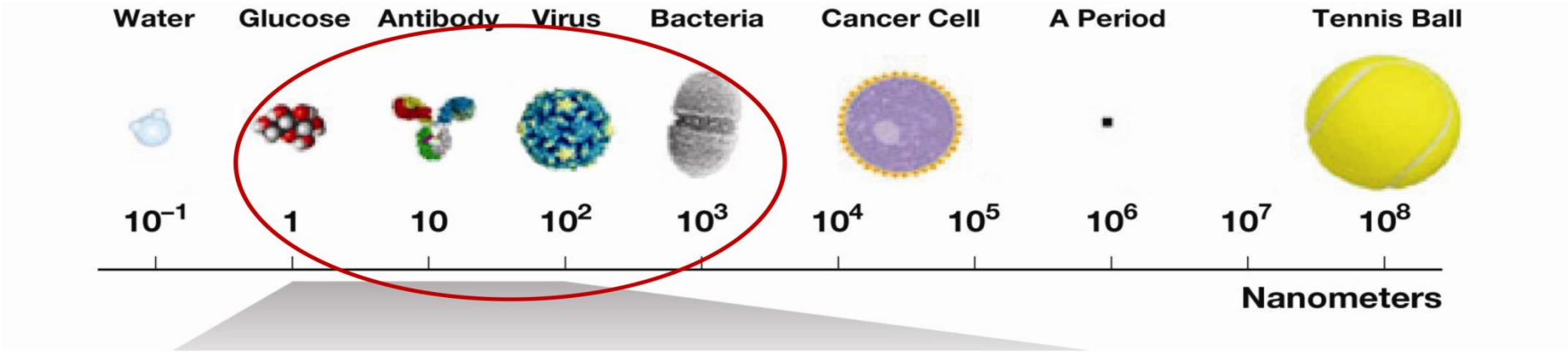
What is nano?

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

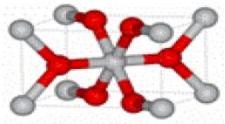
Nanosilver is in supplements and used to treat clothing

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

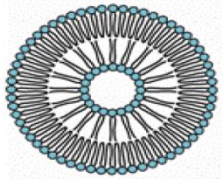
The Adidas Lone Star™ track shoe includes a lightweight spike-plate made of carbon nanotubes

Nanoparticles in daily life

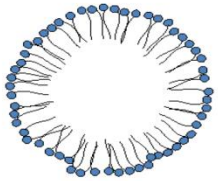
Nanoparticles in Daily Life



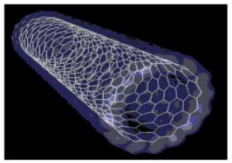
TiO_2



Liposomes



Nanoemulsions

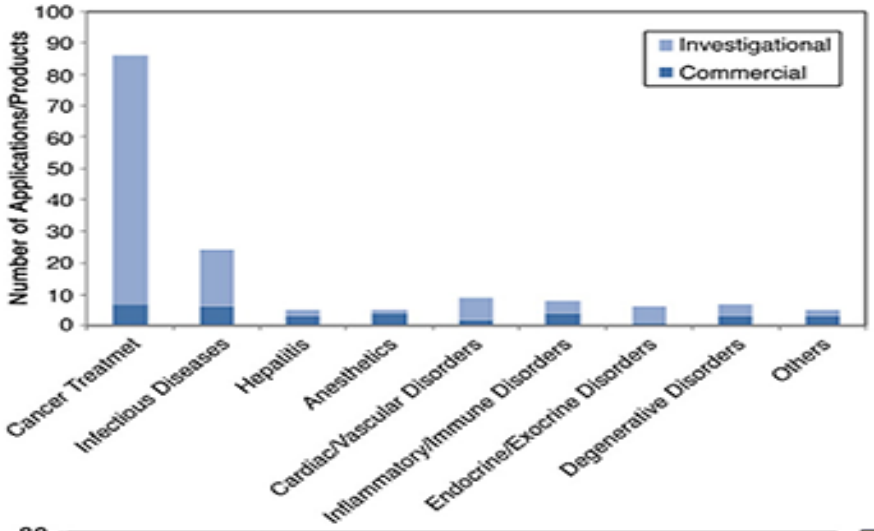


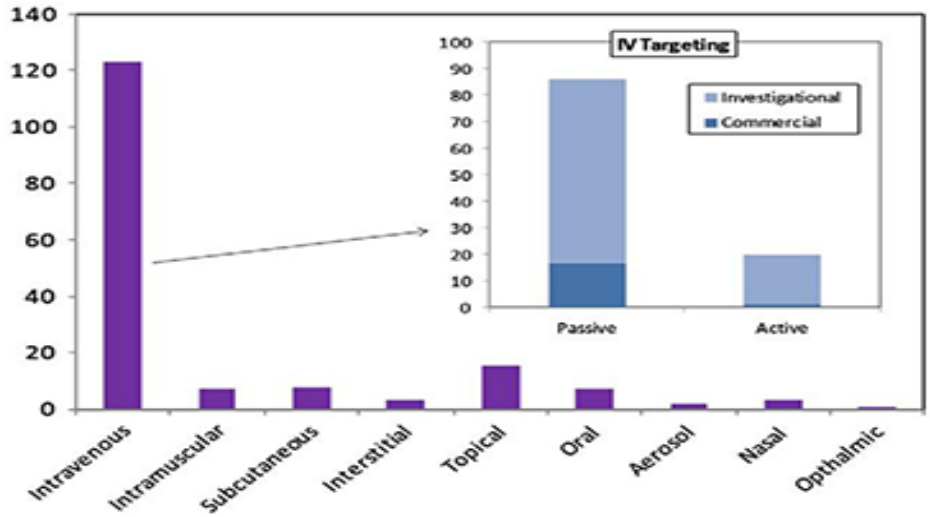
Carbon nanotube

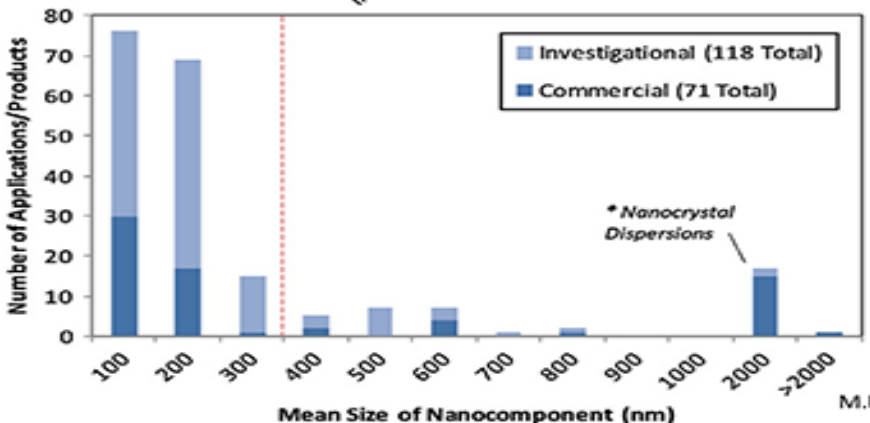
- Nearly all translucent sunscreens contain nanoscale TiO_2 or ZnO_2
- Liposomes and emulsions are commonly used in cosmetics (L'Oreal holds more than 60 patents related to nanotechnology)
- Carbon nanotubes are used as structural materials

Nanoparticles in medical applications

Nanoparticles in Medical Applications





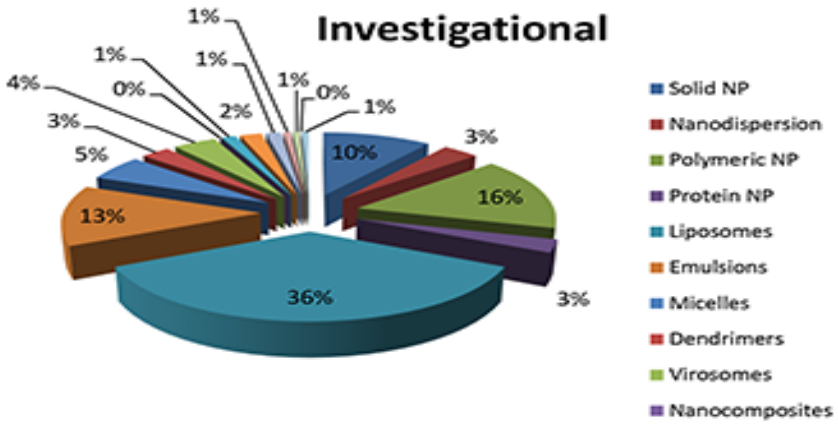


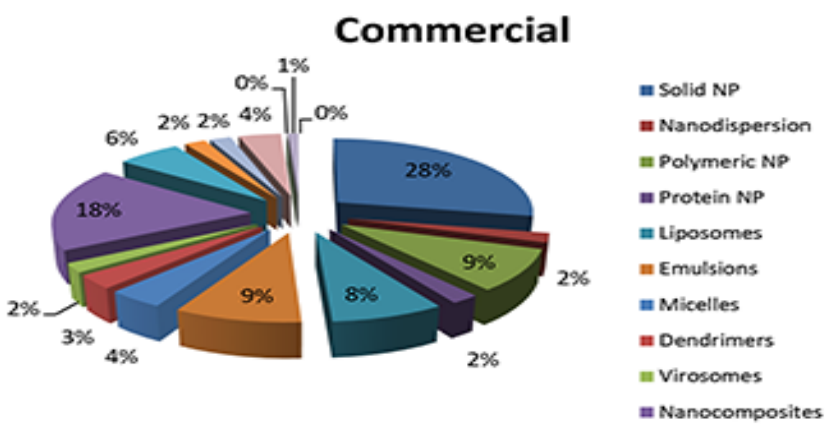
Common features of Nanomedicines:
- Primary market is cancer therapy
- Intravenous administration
- <350 nm in size
- Neutral, hydrophilic surfaces
- Spherical

M.L. Etheridge et al / Nanomedicine: Nanotechnology, Biology, and Medicine 9 (2013) 1–14

Nanoparticle type

Nanomedicine by Nanoparticle Type





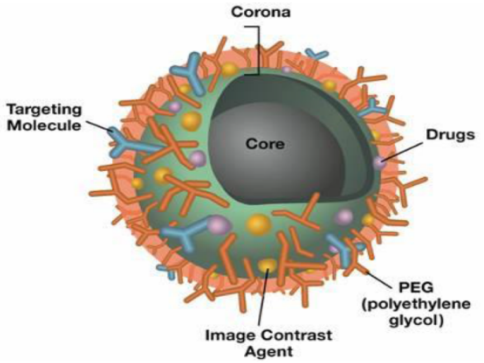
- Dominant (> 10% of total) Investigational Nanomedicines: Liposomes > Polymeric NP > Emulsions > Solid NP
- Dominant Commercial Nanomedicines: Solid NP > Nanocomposites

Plotted based on M.L. Etheridge et al / Nanomedicine: Nanotechnology, Biology, and Medicine 9 (2013) 1–14

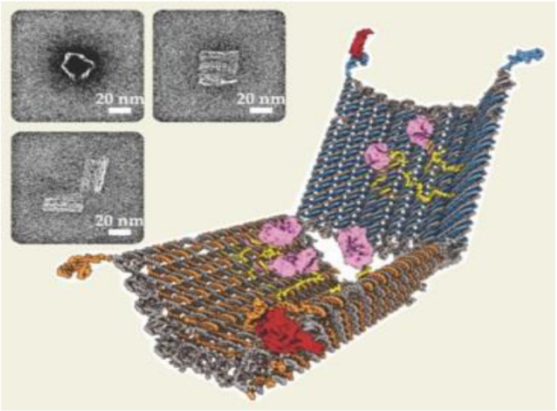
Nanotechnology

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

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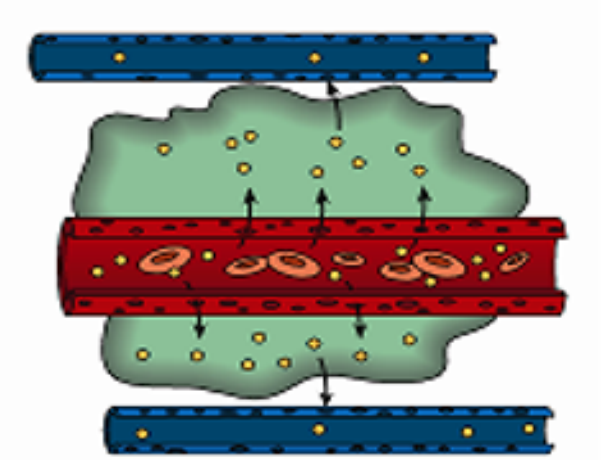
EPR Effect = Passive Targeting

- 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. TNF-α

Reduced toxicity

Benefits: Reduced Toxicity

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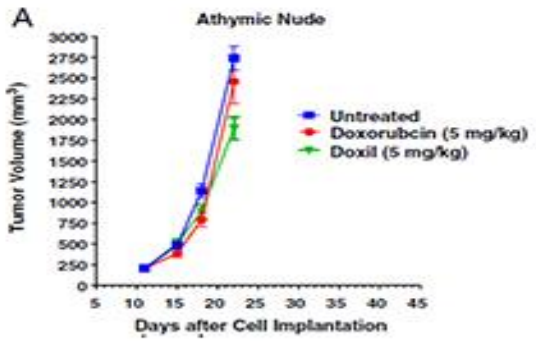
Traditional	Nano
Small Molecules	
Therapeutic Proteins	

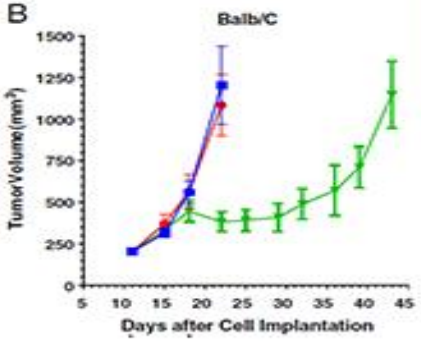
Incorporation of conventional pharmaceuticals into nanotechnology-derived platforms helps decrease their immunotoxicity.

DIC = disseminated intravascular coagulation; PCA = procoagulant activity

Immunotherapy

Benefits: Immunotherapy





NEOPLASIA
www.neoplasia.com
Volume 17 Number 8 August 2015 pp. 661–670 661

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

Jonathan Rios-Doria, Nicholas Durham, Leslie Wetzel, Raymond Rothstein, Jon Chesebrough, Nicholas Holowecky, Wei Zhao, Ching Ching Leow and Robert Hollingsworth
MedImmune, Gaithersburg, MD

Untreated
Doxorubcin (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

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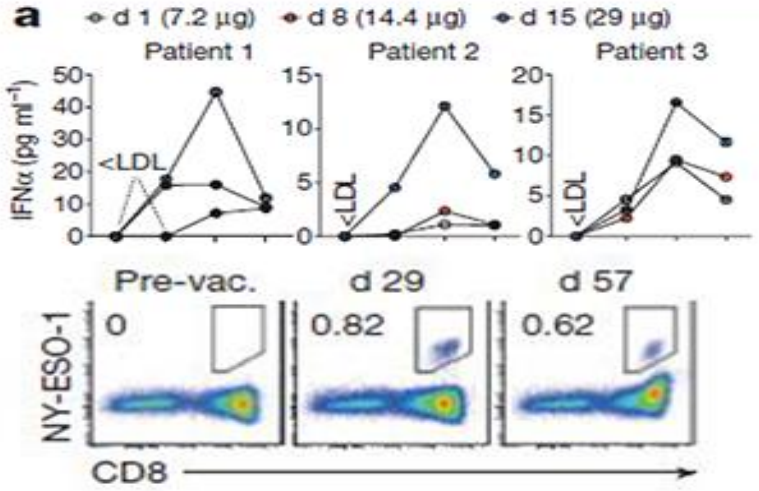
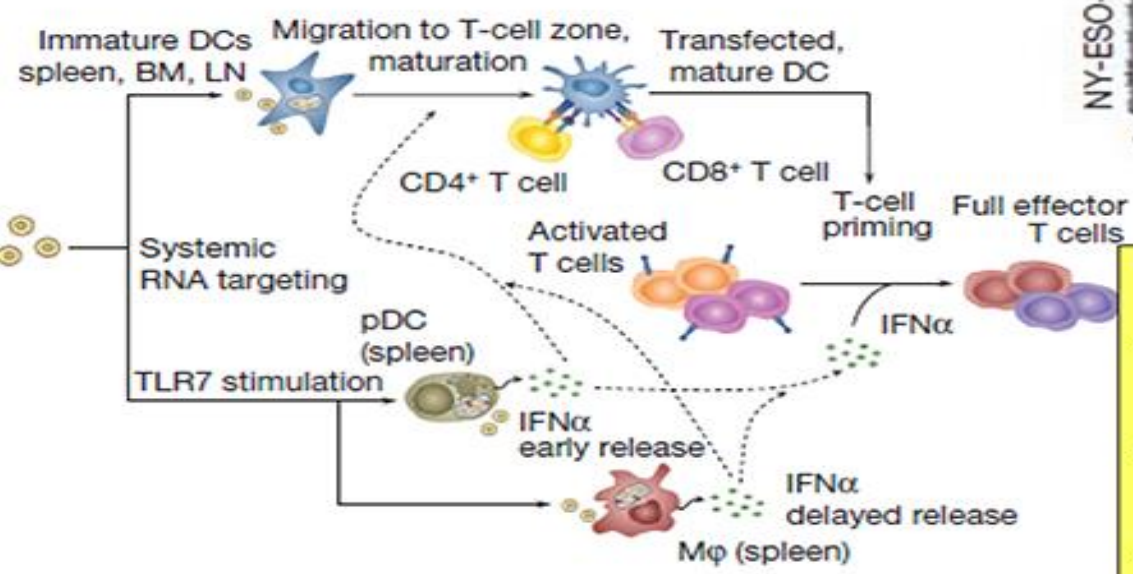
LETTER 16 JUNE 2016 | VOL 534 | NATURE | 397

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

Lena M. Kranz[1,2*], Mustafa Diken[1,2*], Heinrich Haas[3], Sebastian Kreiter[1,2], Carmen Loquai[4], Kerstin C. Reuter[1], Martin Meng[4], Daniel Fritz[1], Fulvia Vascotto[1], Hossam Hefesha[1], Christian Grunwitz[1,2], Mathias Vormehr[1,2], Yves Hüsemann[1], Abderraouf Selmi[1,2], Andreas N. Kuhn[1], Janina Buck[1], Evelyna Derhovanessian[1], Richard Rae[1], Sebastian Attig[1,2], Jan Diekmann[1], Robert A. Jabulowsky[1], Sandra Heesch[1], Jessica Hassel[4], Peter Langguth[5], Stephan Grabbe[4], Christoph Huber[1,2], Özlem Türeci[1] & Ugur Sahin[1,2,3]





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

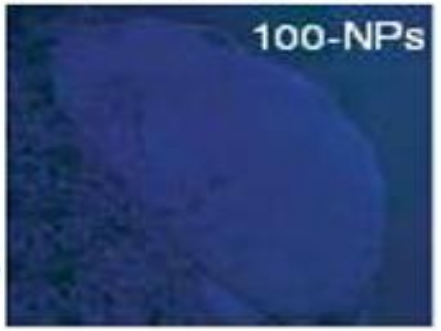
Injection Site

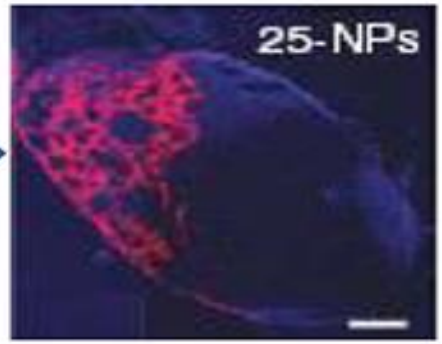
Tail

100nm

25nm

Draining lymph nodes





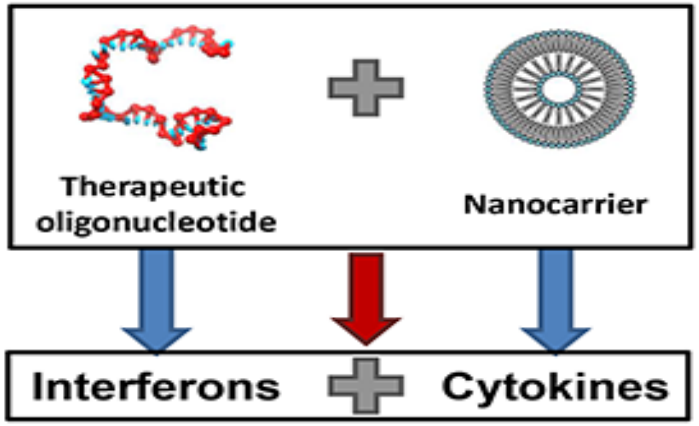
Smaller particles travel through lymphatics. Larger particles do not.

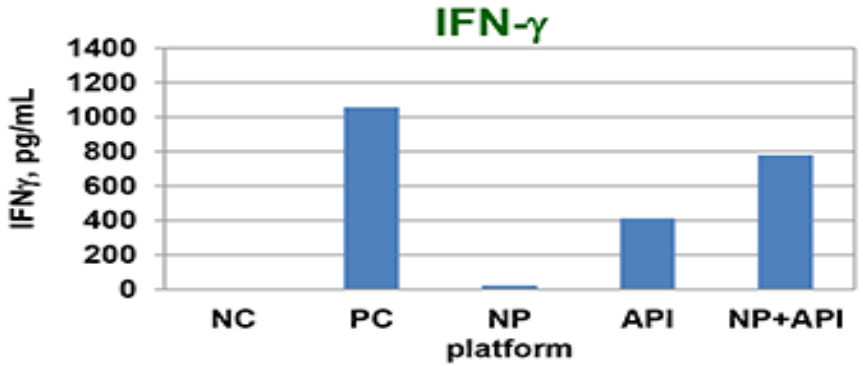
Reddy ST et al, and Hubbell JA. (2007) Nature Biotech., 25 (10):1159-1164

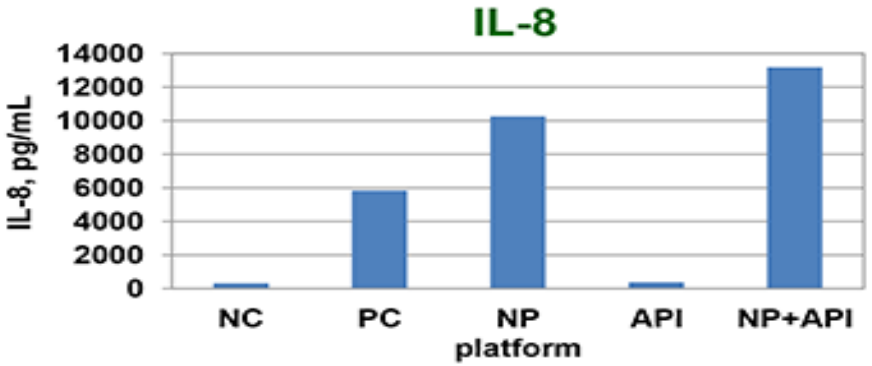
- **Particle distribution to lymph nodes after i.d. injection depends on their size**
- **Lymphatic delivery benefits vaccines, HIV and infectious diseases therapy**

Toxicity

Concerns: Toxicity



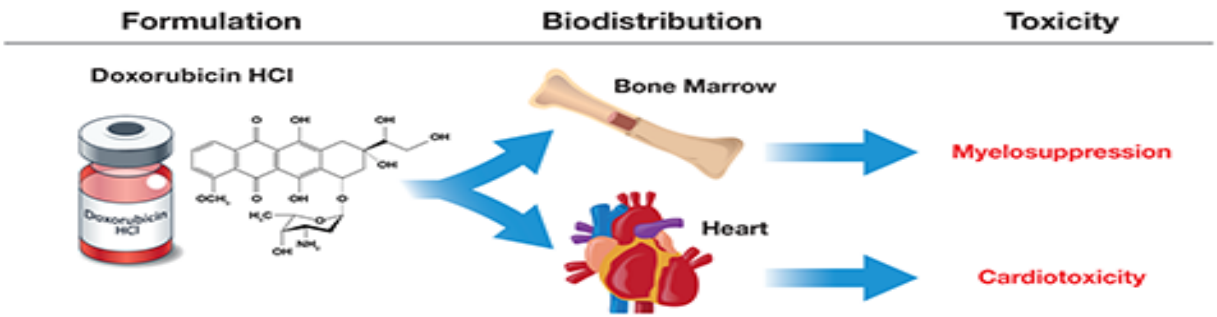




- Both nanocarrier and API can be toxic
- Some nanocarriers n a contribute to toxicity of API.

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

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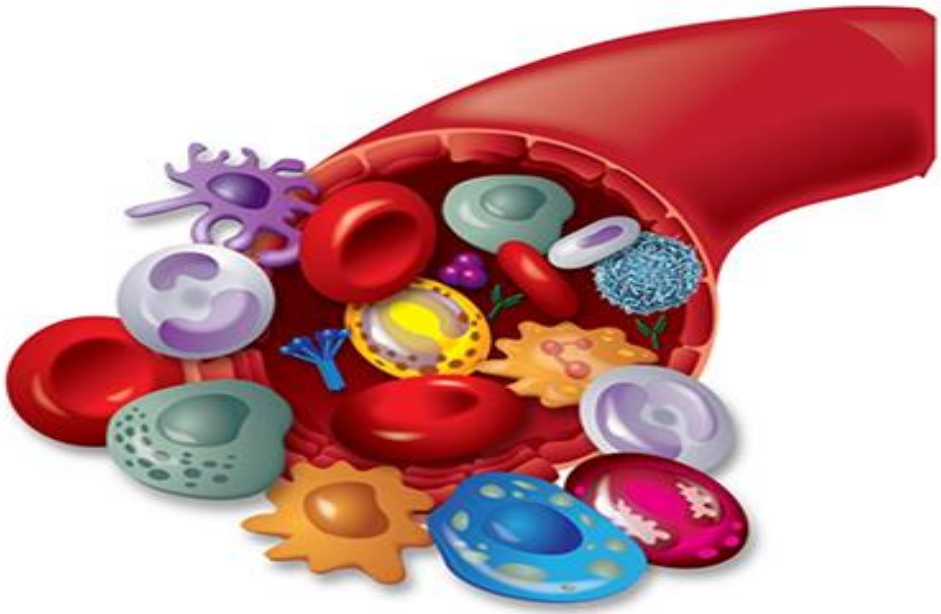
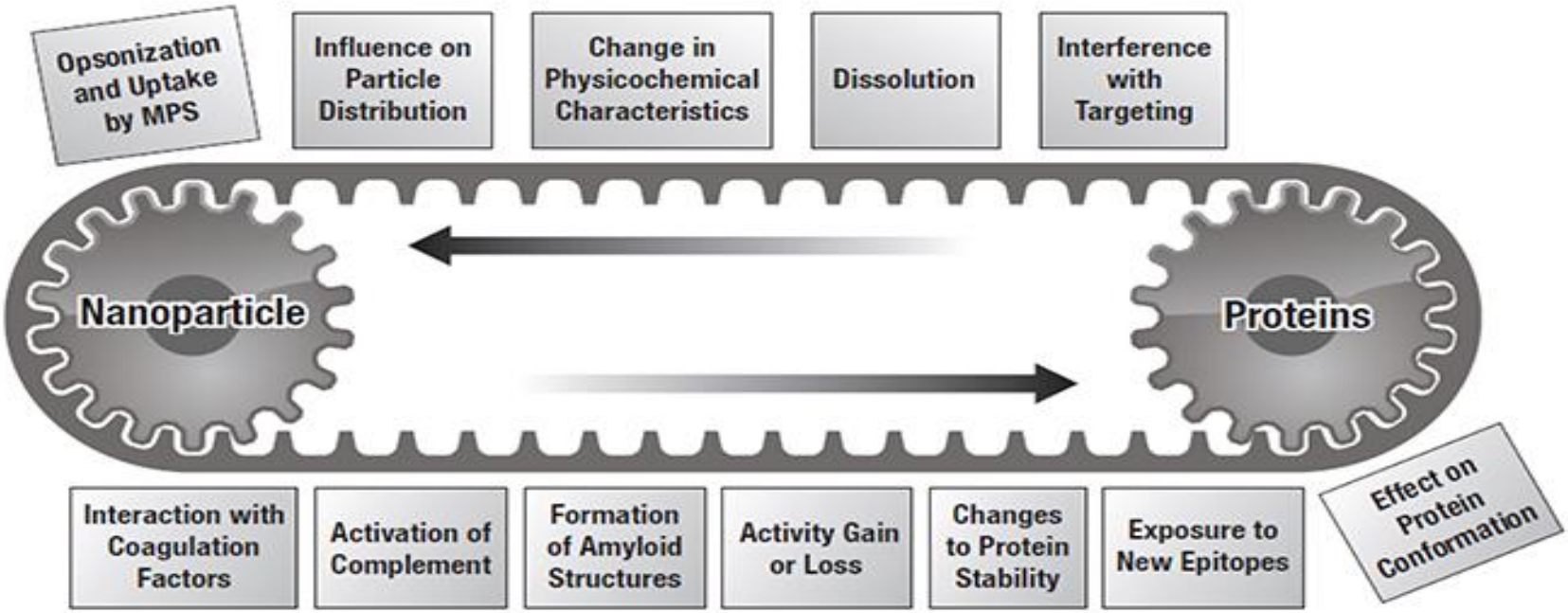
- Plasma Proteins
  - Biodistribution and MPS uptake
- Effects on erythrocytes
- Blood coagulation system
  - Platelets
  - Leukocytes
  - Endothelial cells
- Allergy
  - Complement activation
  - DTH
- Cytokines
- Immunogenicity



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

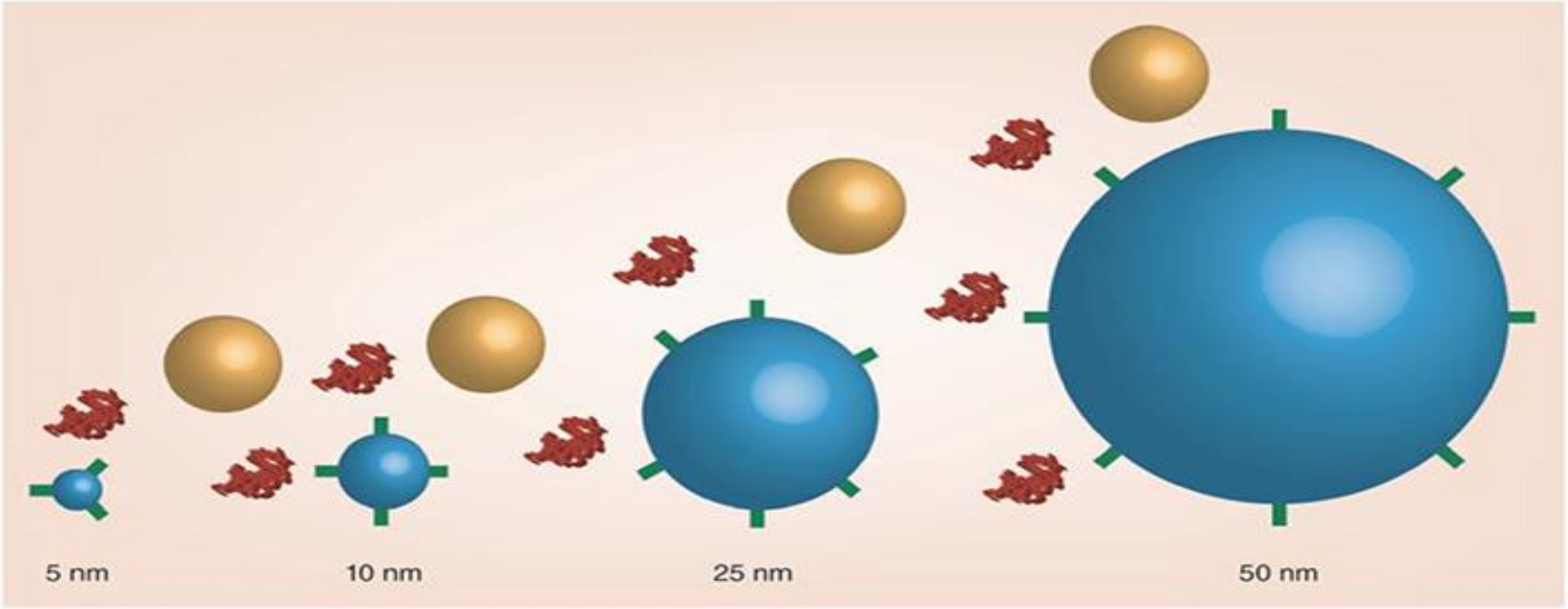


Figure 2. Size of proteins in the corona compared to nanoparticles of varying diameter. Nanoparticles are represented in blue and the diameter is given by the number under each particle in nm. Serum albumin[31] is shown in red and scaled relative to the nanoparticles. High-density lipoprotein is represented by orange spheres at a size of 12.5 nm diameter.

Cedervall Tet al, 2016. Handbook of Immunological properties of engineered nanomaterials

Protein binding

Protein binding affects particle size

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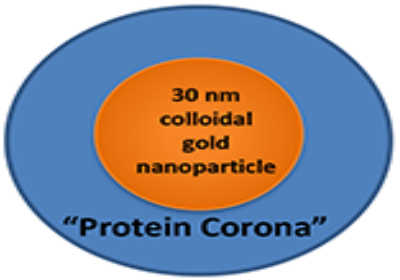
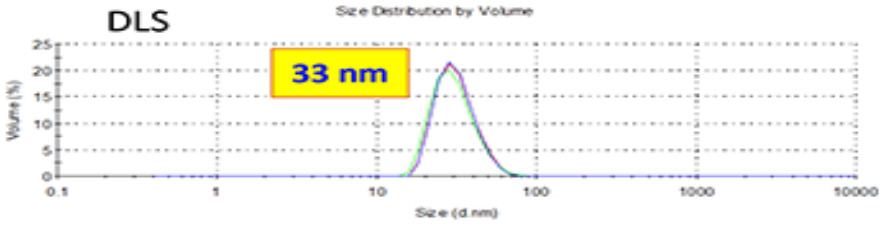
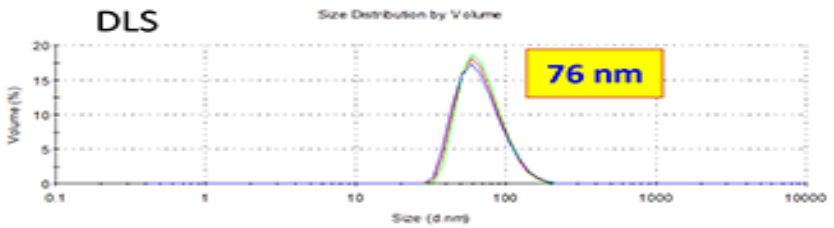
BEFORE

AFTER





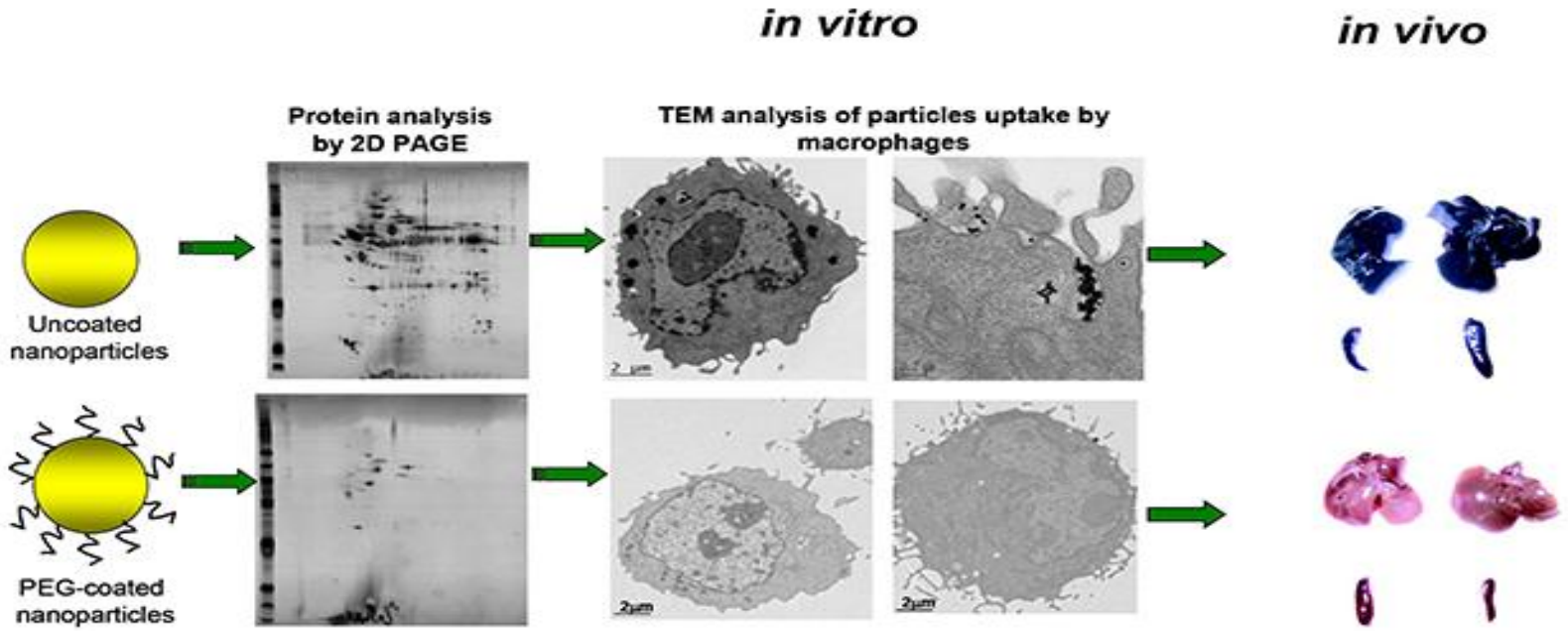


Incubation with human plasma increases hydrodynamic size of nanoparticles

Dobrovolskaia et al, (2009), Nanomed. Nanotechnol. Biol. Med., 5:106-117.

Bidistribution

Protein Binding and biodistribution



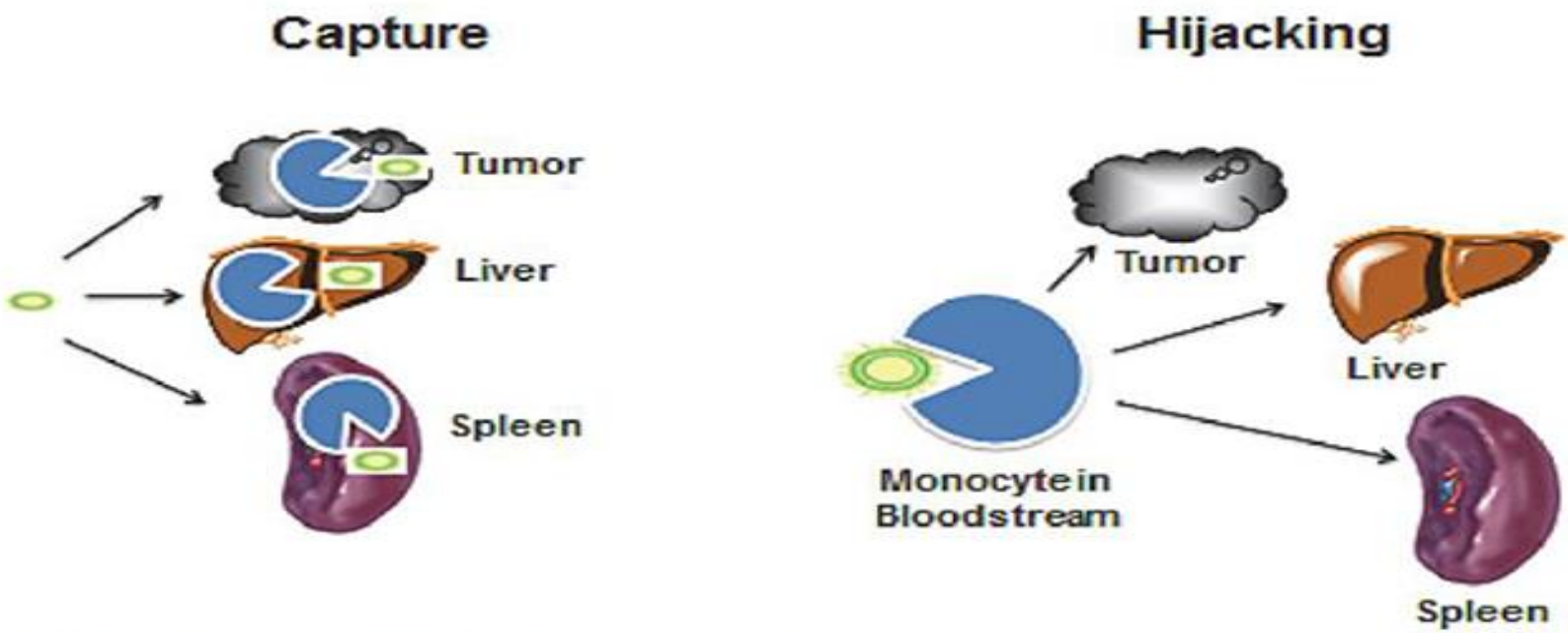
Dobrovolskaia et al., (2008), Mol.Pharm., 5:487-495.

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

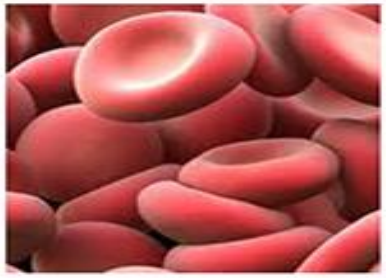


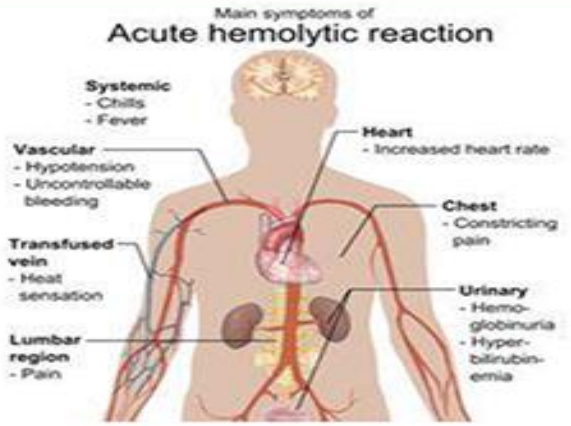
- Two theories about nanoparticle distribution to the MPS
- Capture – uptake by phagocytic cells in the tissue
- Hijacking – uptake by circulating phagocytic cells which then take the particle to tissue

Zamboni et al, Handbook of Immunological properties of engineered nanomaterials(2016). V3

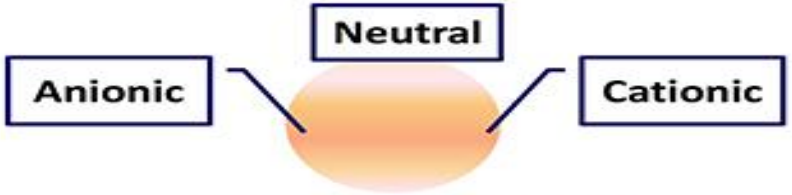
Hemolysis

Hemolysis





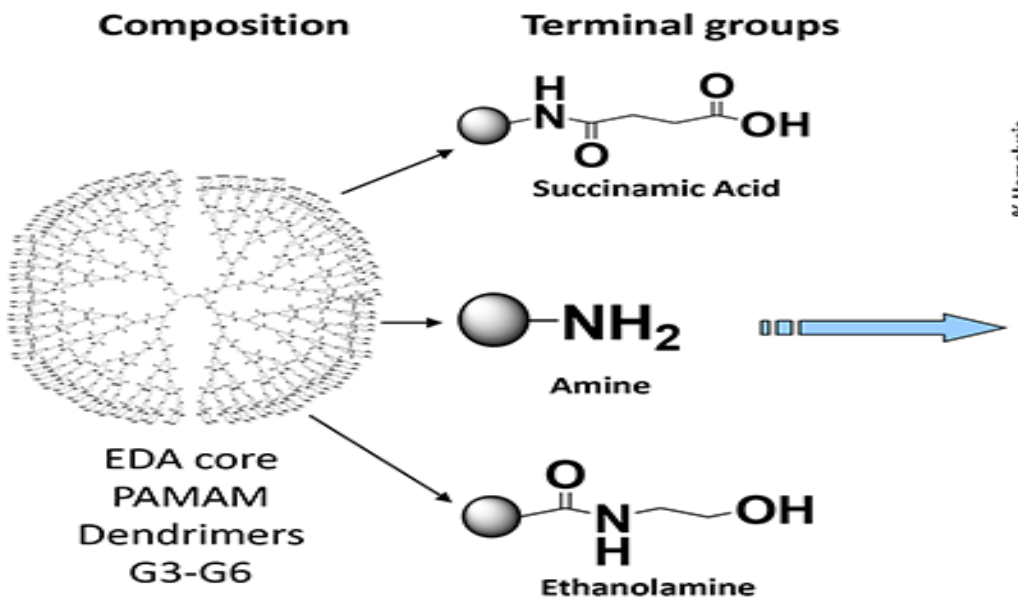
Role of Nanoparticle Size

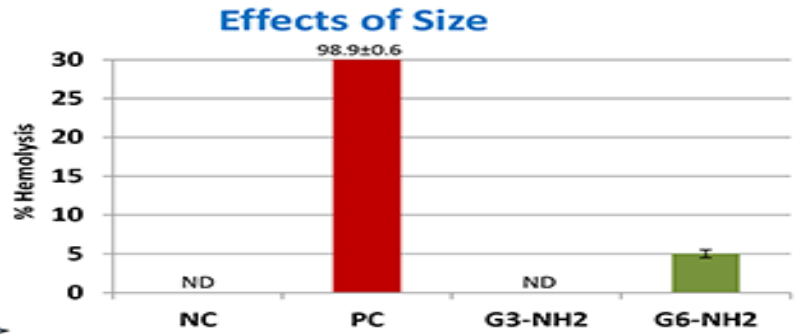


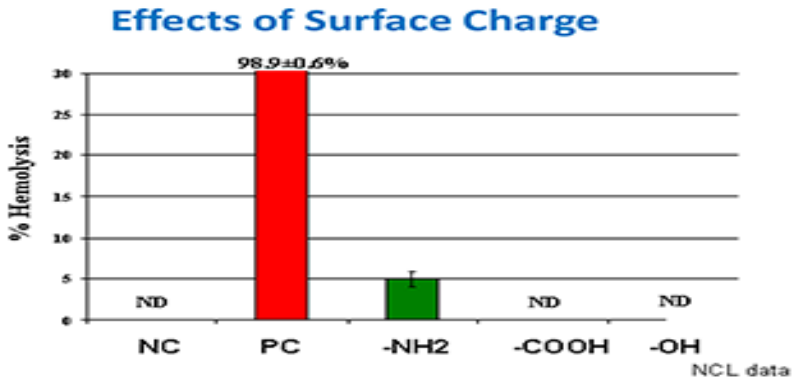
Role of Nanoparticle Surface Charge

Hemolysis

Hemolysis







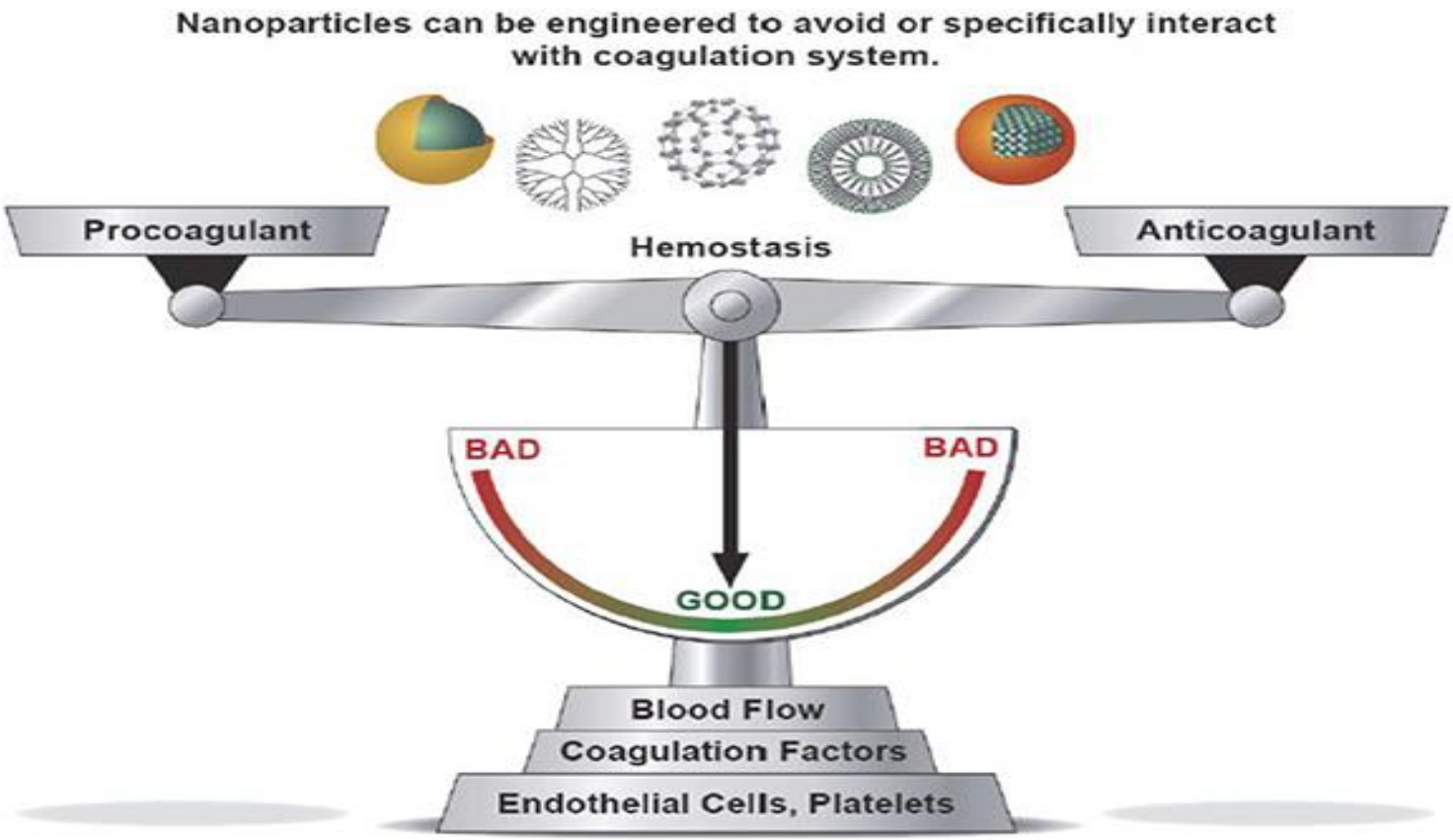
- Cationic dendrimers are more hemolytic than their anionic and neutral counterparts of the same size
- Larger dendrimers are more hemolytic than smaller

NC = negative control;; PC = positive control; EDA = ethylenediamine; PAMAM = poly(amidoamine)

NCL data

Coagulation system

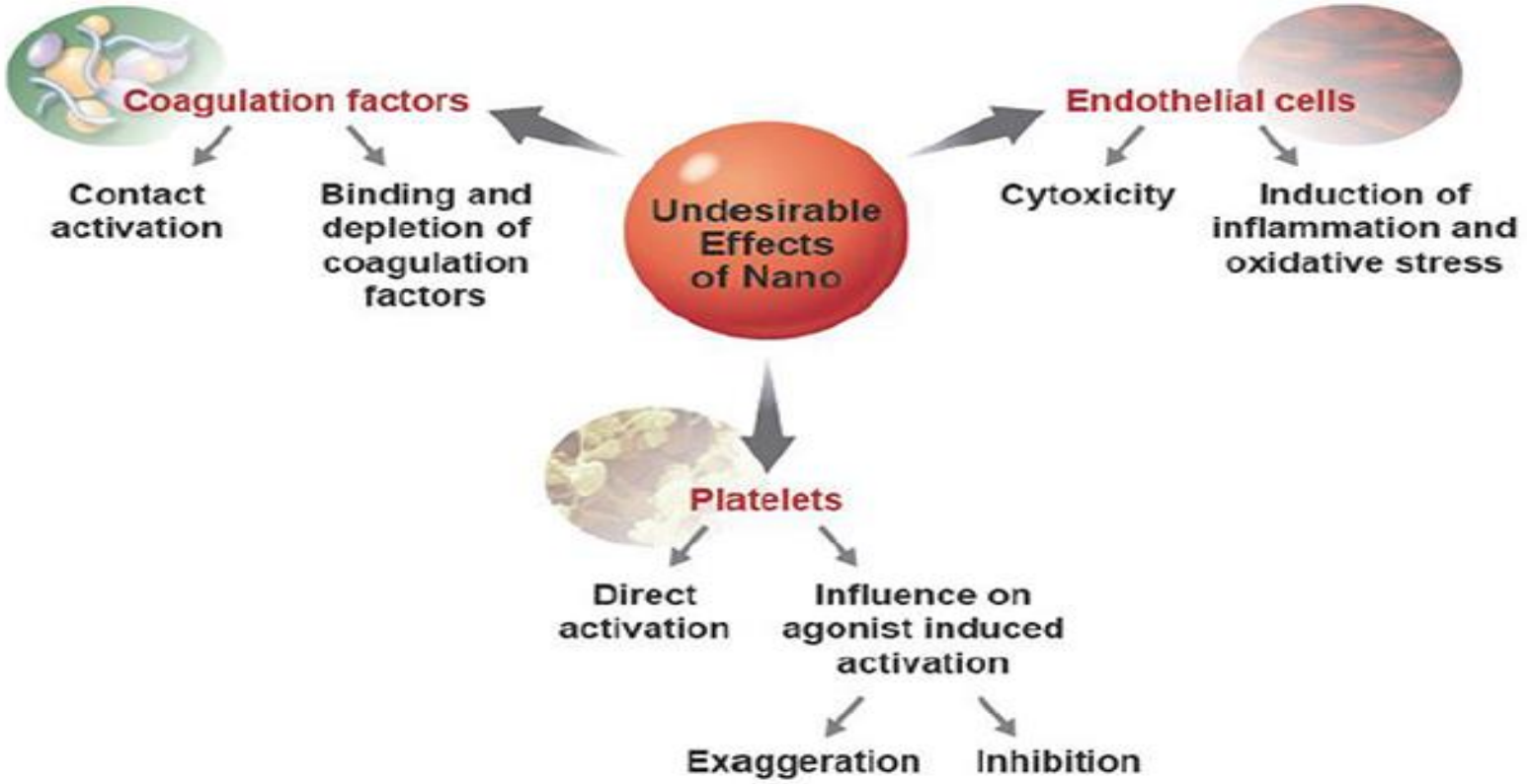
Coagulation system



Ilinskaya A& Dobrovolskaia MA. Handbook of Immunological properties of Engineered Nanomaterials (2016), Vol 2

Undesirable effects

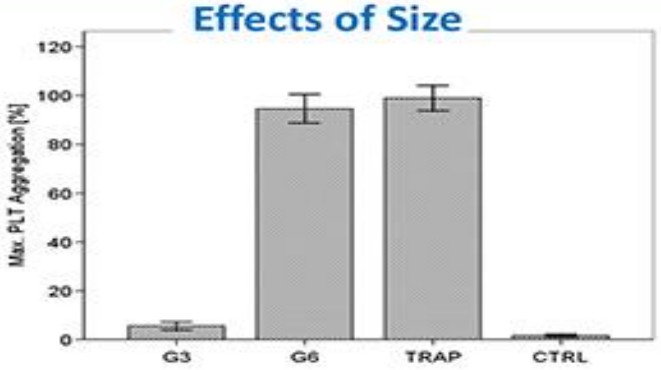
Undesirbale effects on coagulation

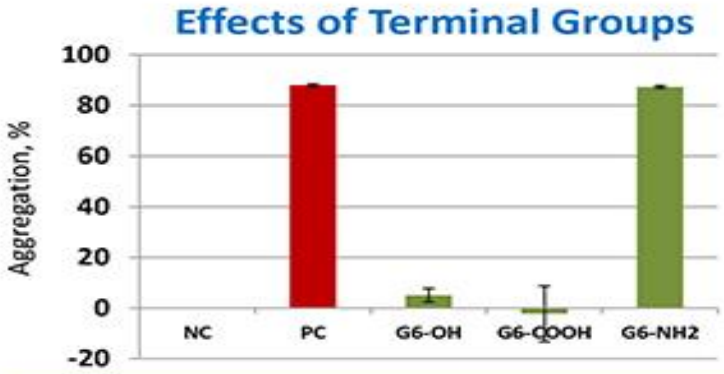


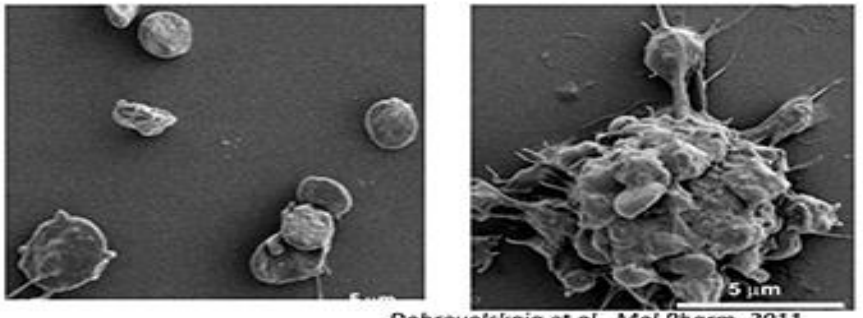
Ilinskaya A& Dobrovolskaia MA. Handbook of Immunological properties of Engineered Nanomaterials (2016), Vol 2

Platelets

Platelets





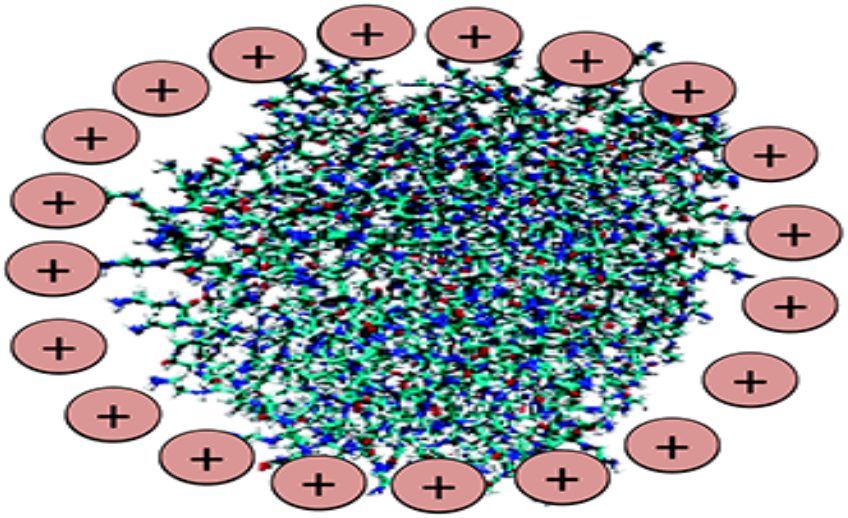


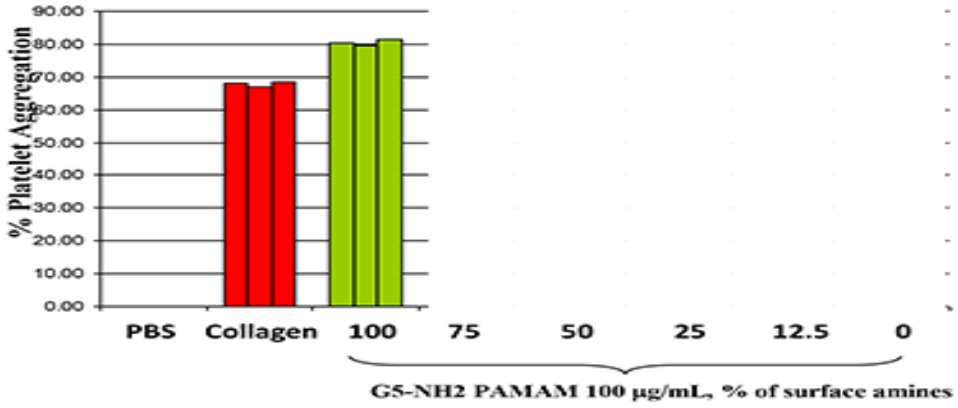
Dobrovolskaia et al., Mol.Pharm, 2011

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

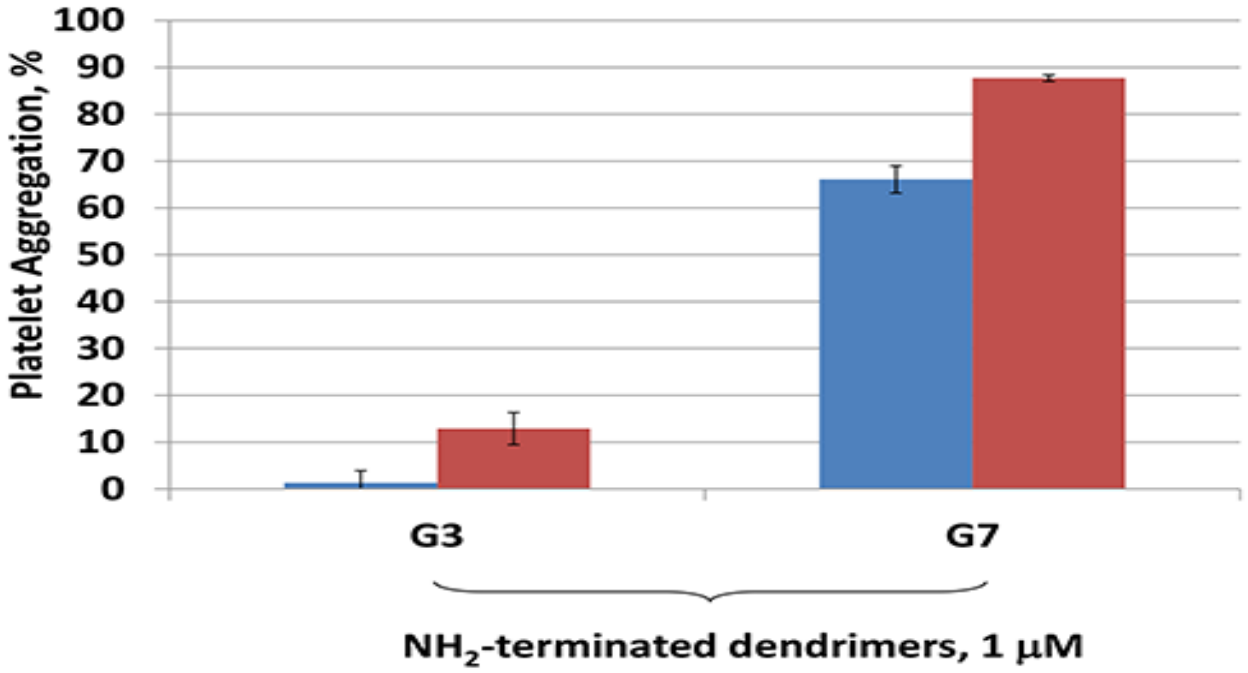


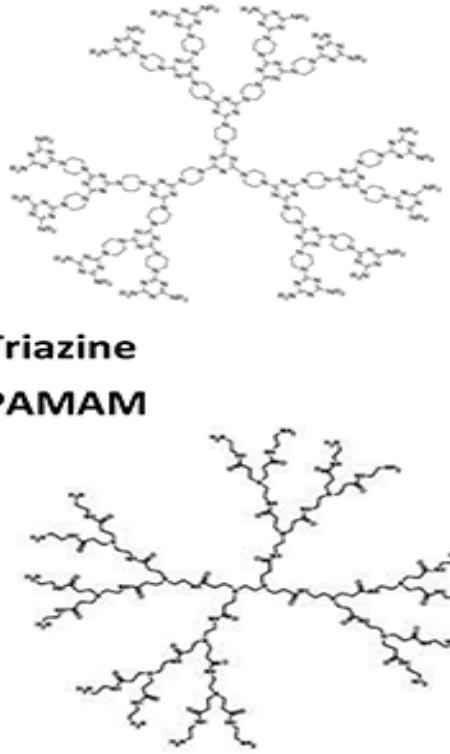


Zeta Potential is important
Less surface amines = less platelet aggregation

Platelets

Platelets: effect of composition



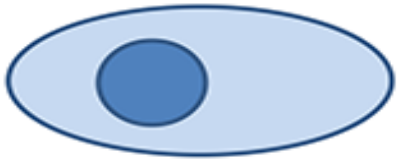


Triazine dendrimers are less potent in inducing platelet aggregation than their PAMAM counterparts

Triazine dendrimers were kindly provided by Dr.Eric Simanek, Texas Christian University

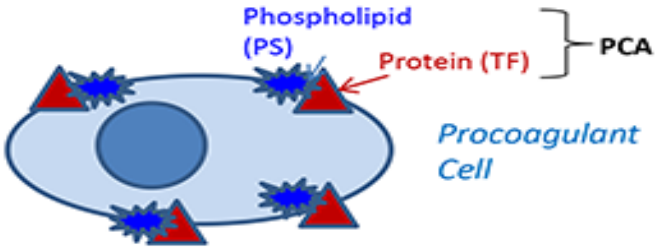
Leukocyte

Leukocyte Procoagulant Activity

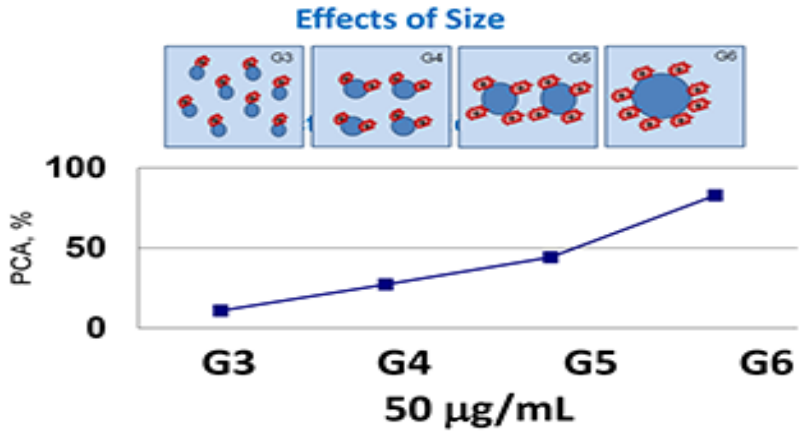


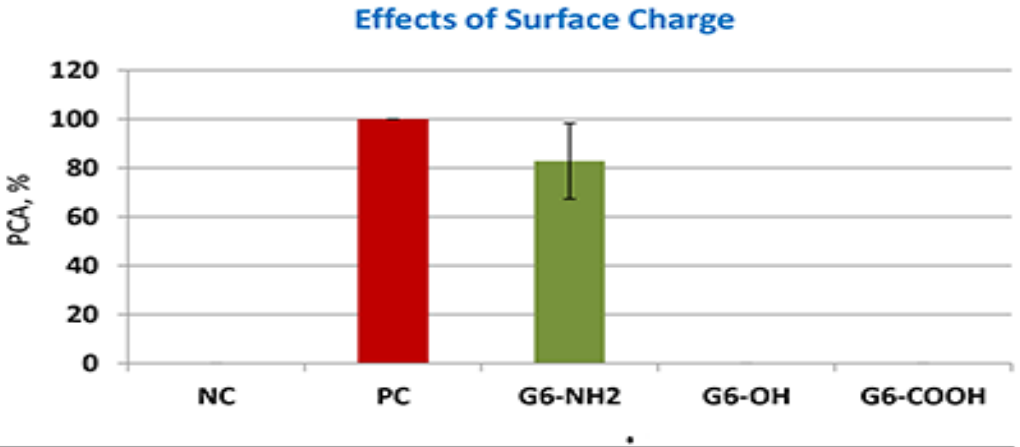
Key events
(occur through multiple mechanisms)

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



Activation of extrinsic plasma coagulation cascade



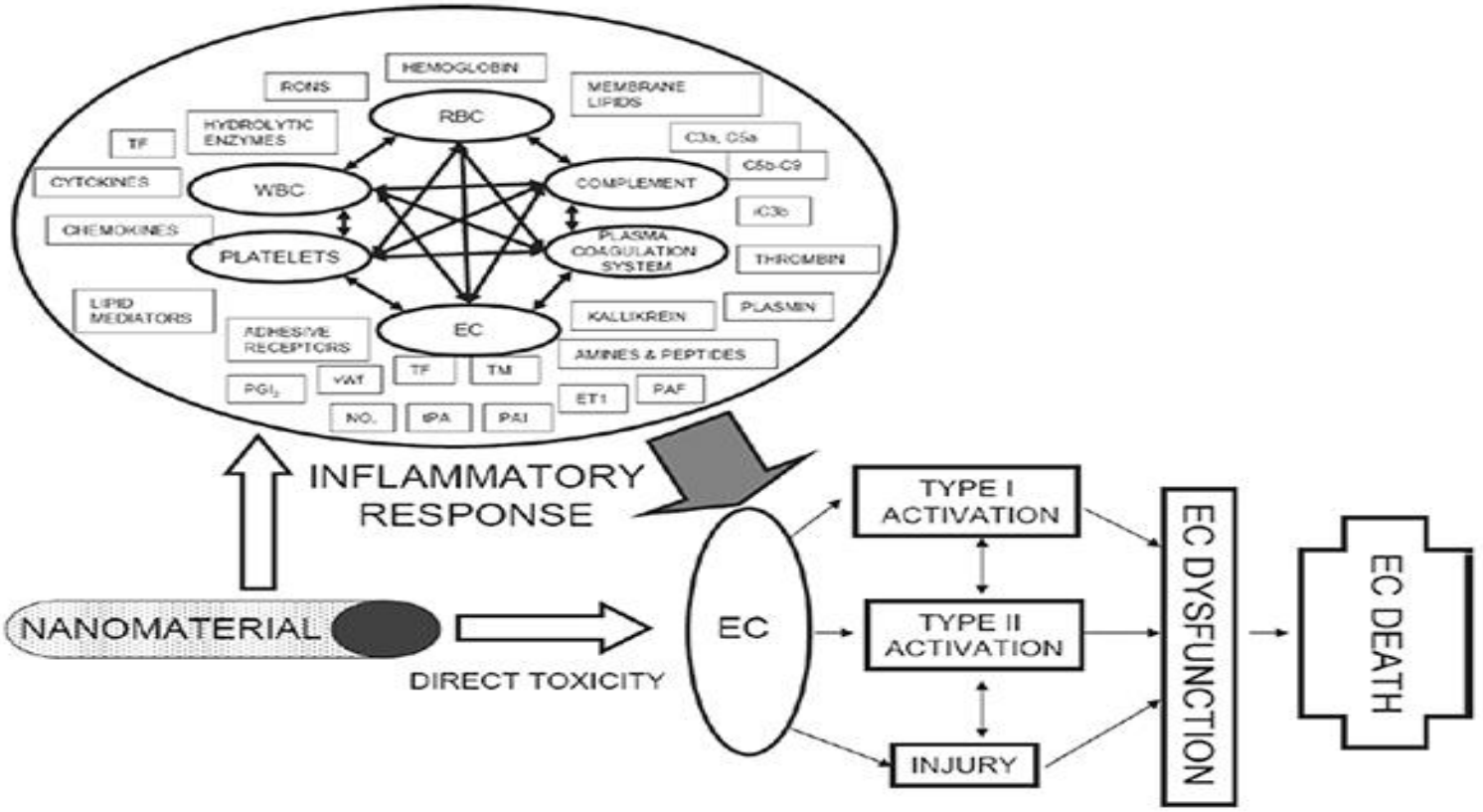


- Cationic, but not anionic or neutral PAMAM dendrimers induced PCA in vitro
- Large particles are more reactive

PCA = procoagulant activity

Endothelial cells

Effects on endothelial cells



Simak J. Handbook of Immunological properties of Engineered Nanomaterials (2016), Vol 2

Allergenicity

Allergenicity

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

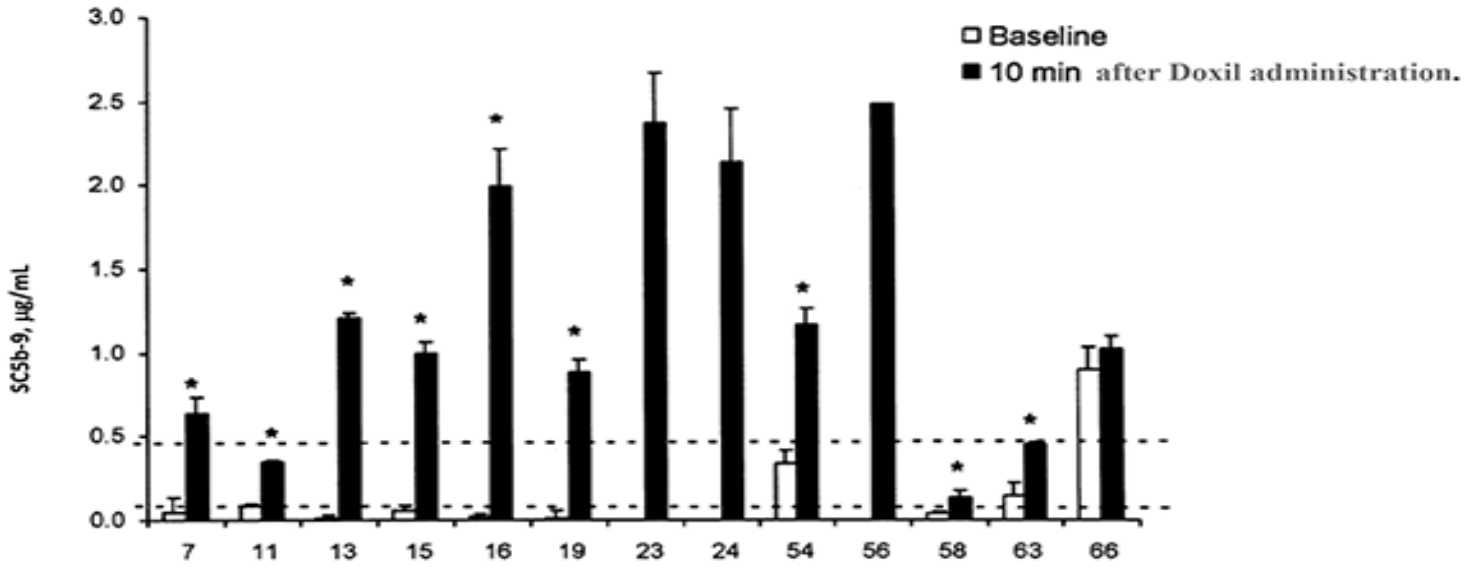
Characteristics	Type I (anaphylactic or immediate hypersensitivity)	Pseudoallergy	Type II (cytotoxic hypersensitivity)	Type III (immune complex mediated hypersensitivity)	Type IV (delayed hypersensitivity)
Main mediators	IgE, mast cells	Complement	Mainly IgG, natural killer cells, complement	IgG, IgM, 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**

Allerginicity

Allerginicity: CARPA to PEG-Liposomes

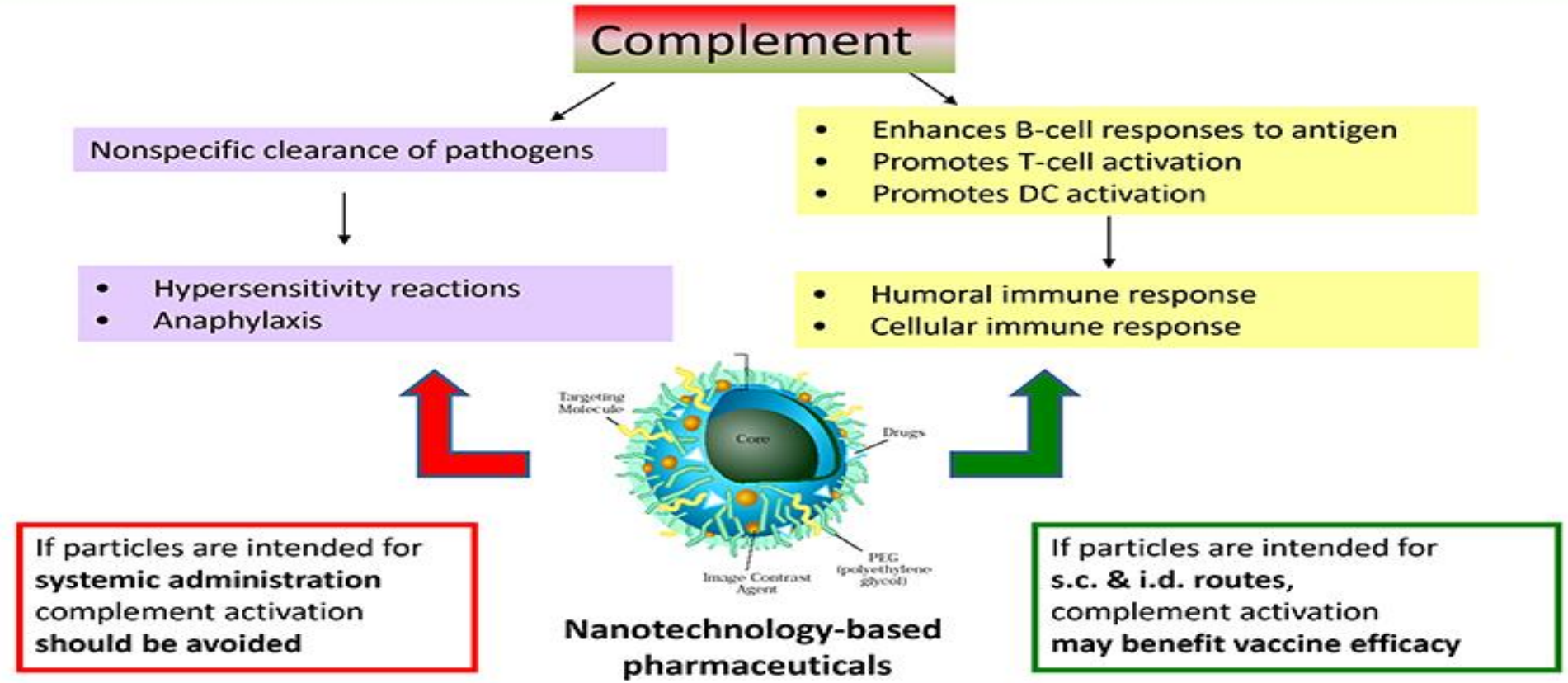


Complement activation is dose limiting toxicity of PEGylated liposomes

Chanan-Khan et al., (2003) Ann. Oncol., 14:1430-1437

Complement activation

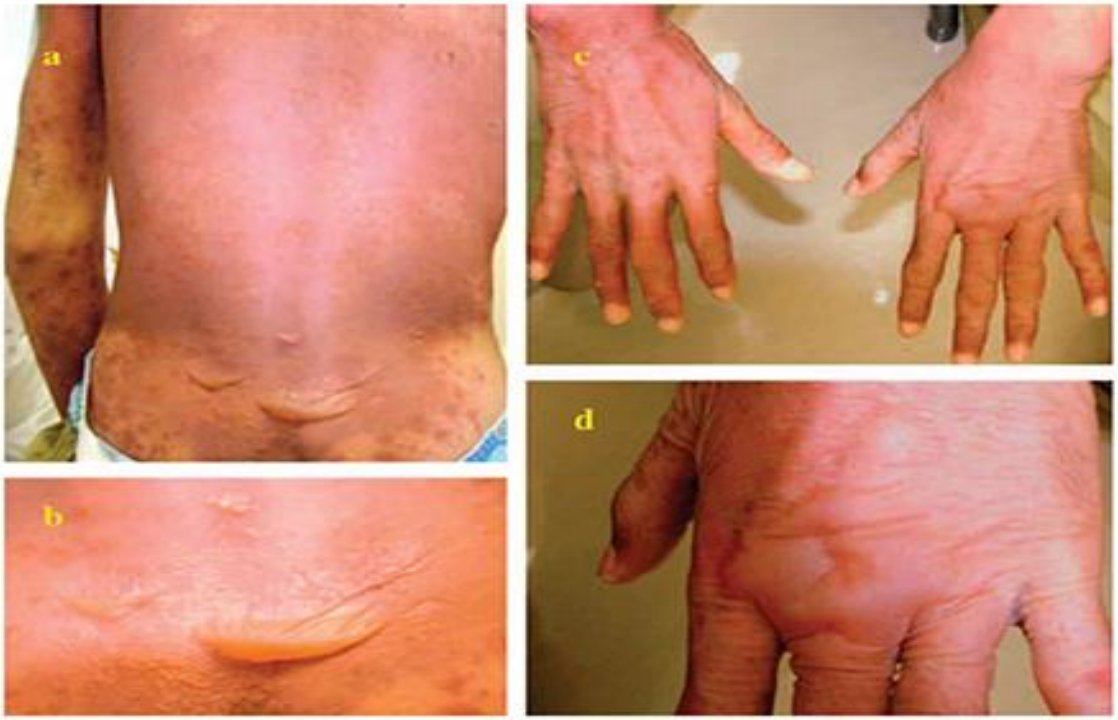
Complement activation: take home message



DC = dendritic cell; s.c. = subcutaneous; i.d. = intradermal

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

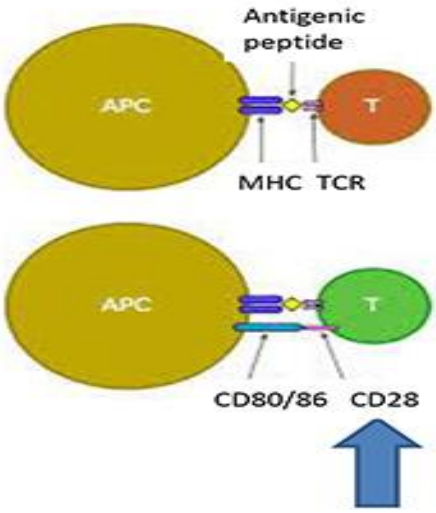
- **Only one case of necrotizing dermatitis (type IV reaction) in response to dendrimers is reported in the literature: fever, chills, exudative erythema and fused bullae (Nikolsky's reaction)**
- **The mechanism is unknown**

Cytokine storm

Cytokine storm: Lessons from biotechnology products:

TeGenero

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

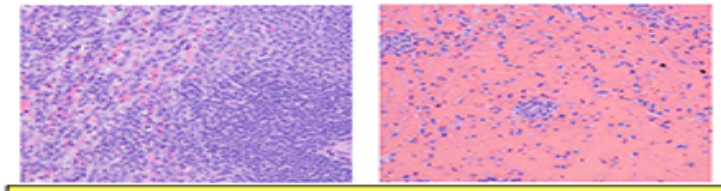
Cytokine Storm to nanomaterials can be predicted in vitro

Metal oxide nanoparticles

In vivo

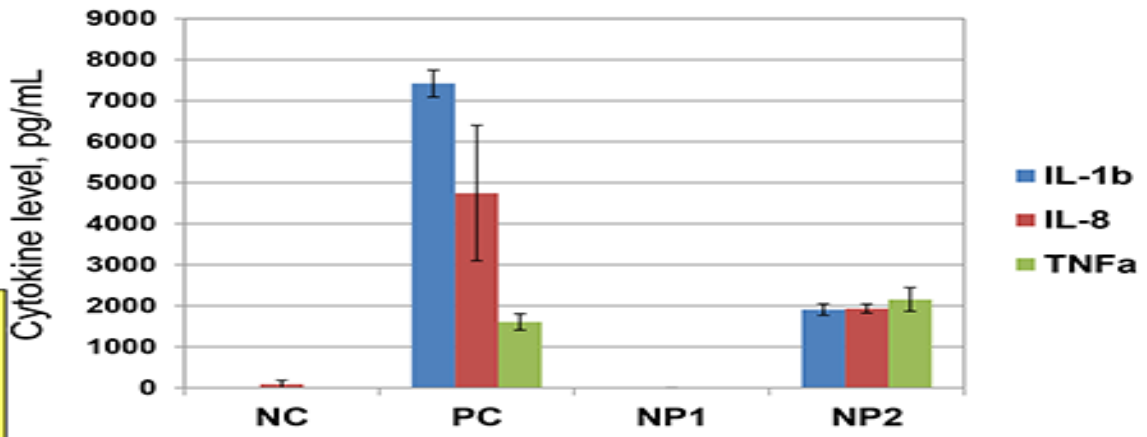
Control: Normal spleen

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)**

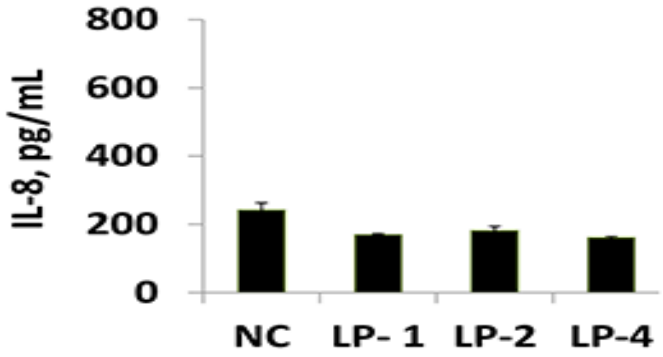


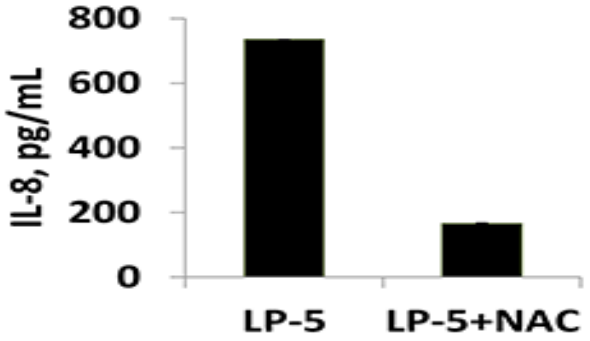
Results are reproduced in vitro using human PBMC

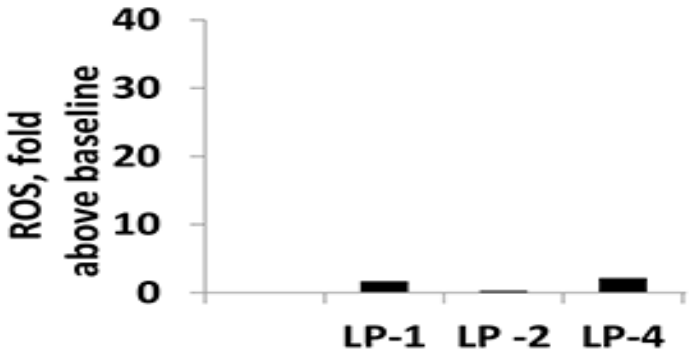
NP = nanoparticle; PBMC = peripheral blood mononuclear cells; IL- interleukin; TNF = tumor necrosis factor; LAL = limulus amebocyte lysate

IL-8

Lipid based particles induce IL-8

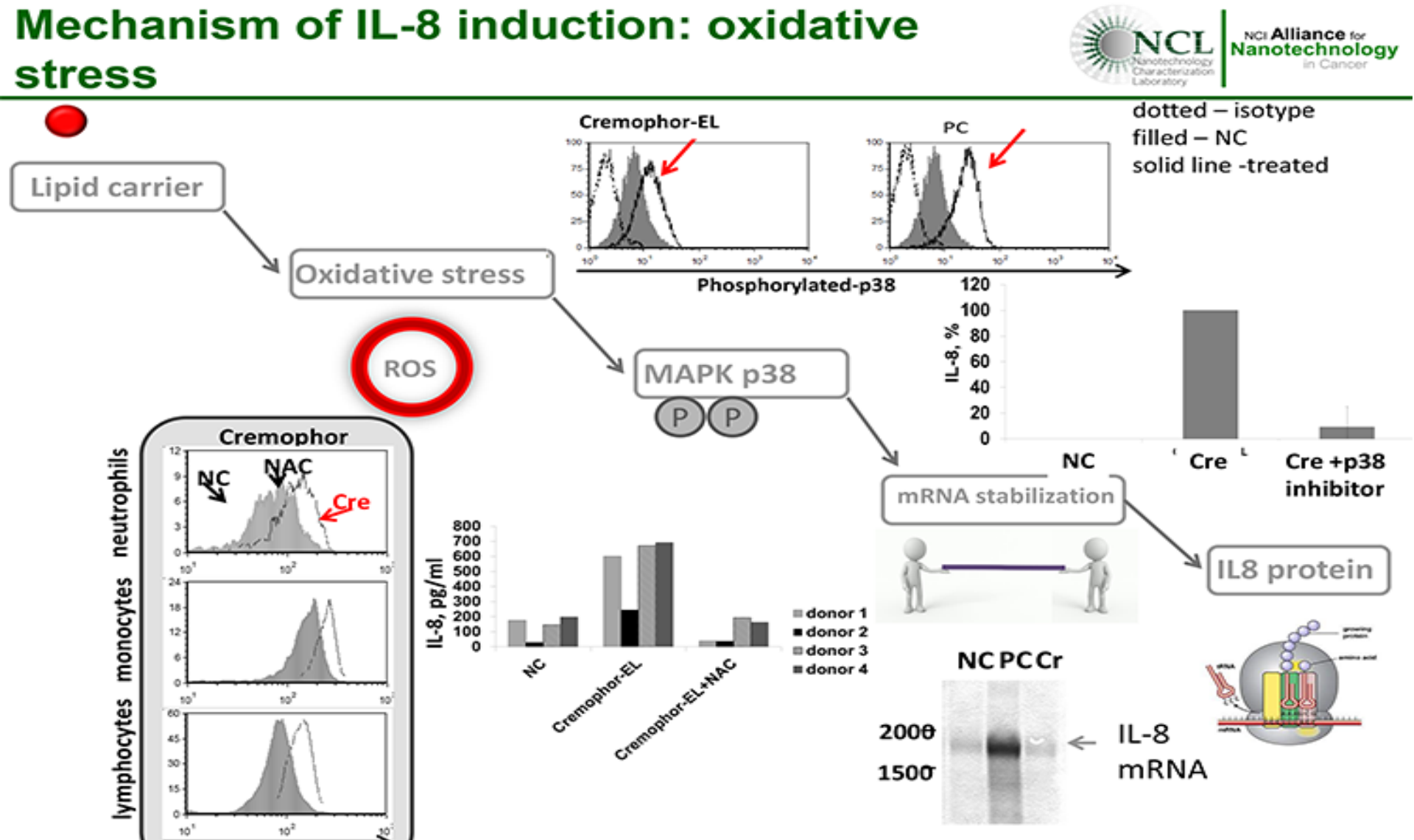






- **Induction of IL-8 by liposomes follows induction of oxidative stress and can be prevented by antioxidant N-acetyl cysteine**

Oxidative stress



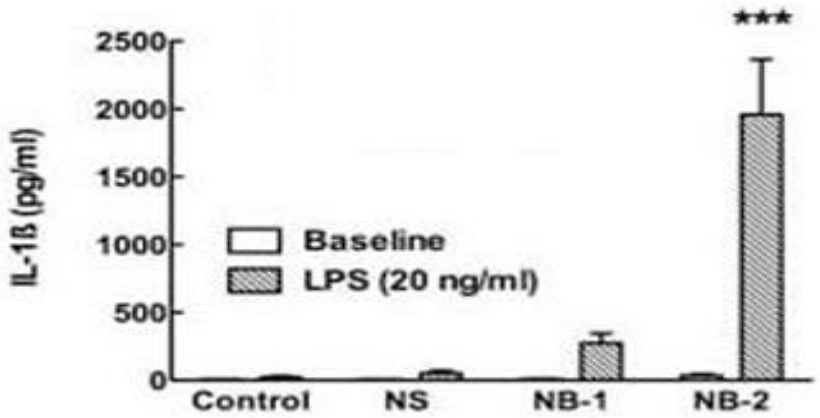
IL-1

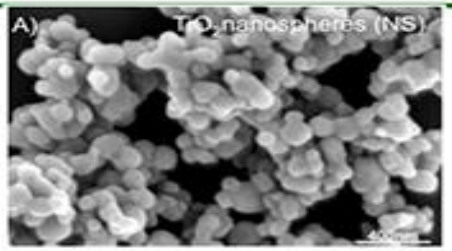
Fibrous and Cationic Nanoparticles induce IL-1

Particle and Fibre Toxicology
BioMed Central
2009, 6:35
Research
Open Access

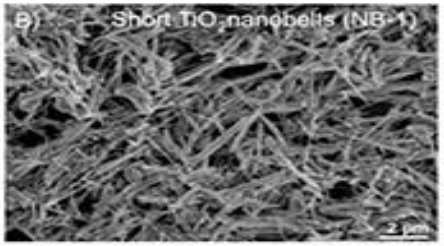
Particle length-dependent titanium dioxide nanomaterials toxicity and bioactivity

Raymond F Hamilton Jr1, Nianqiang Wu2, Dale Porter3, Mary Buford1, Michael Wolfarth3 and Andrij Holian*1

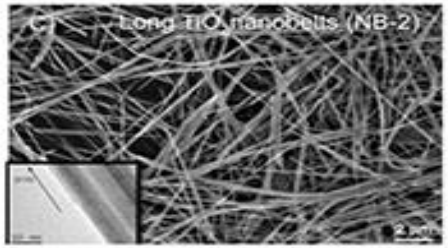




TiO_2 Nanospheres (NS)



TiO_2 Short Nanobelts (NB-1)



TiO_2 Long Nanobelts (NB-2)

- **Long fibrous TiO_2 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

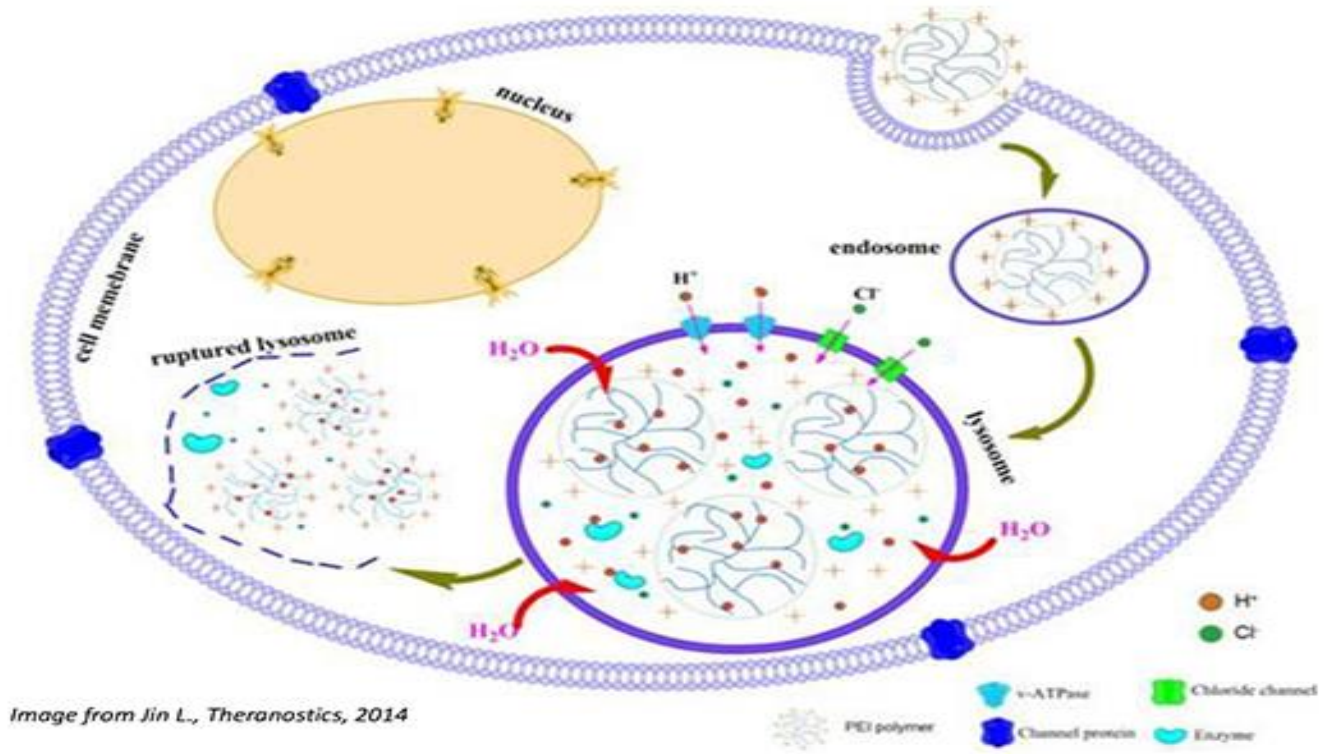


Image from Jin L., Theranostics, 2014

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

Immunogenicity

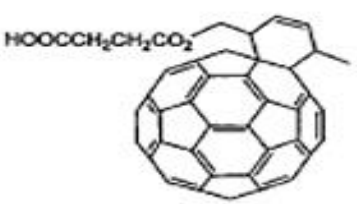
Immunogenicity

NCL Nanotechnology Characterization Laboratory | NCI Alliance for Nanotechnology in Cancer

Not immunogenic even in the presence of strong adjuvant

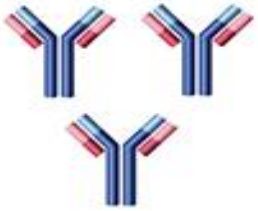
- Other C60-derivatives
- Gold colloids
- other PAMAM dendrimers

Immunogenic after conjugation to a protein carrier



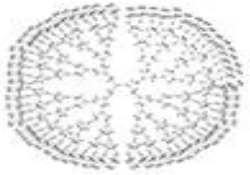
Water-soluble fullerene derivative

Conjugated to BSA, RSA or TG



Monoclonal and polyclonal particle-specific antibodies

Conjugated to BSA



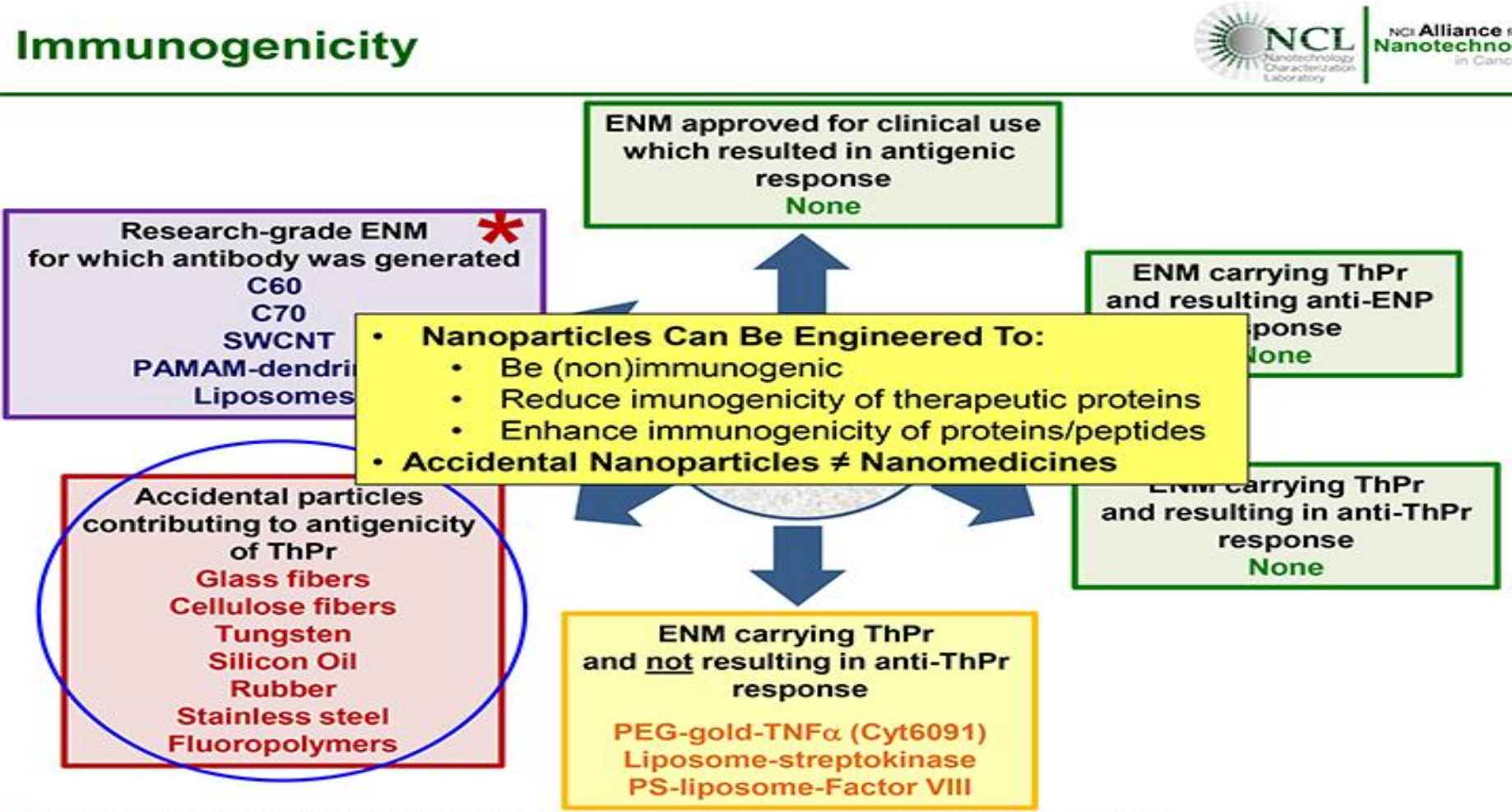
PAMAM Dendrimers

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

BSA = bovine serum albumin; RSA = rabbit serum albumin; TG = thyroglobulin

Immunogenicity



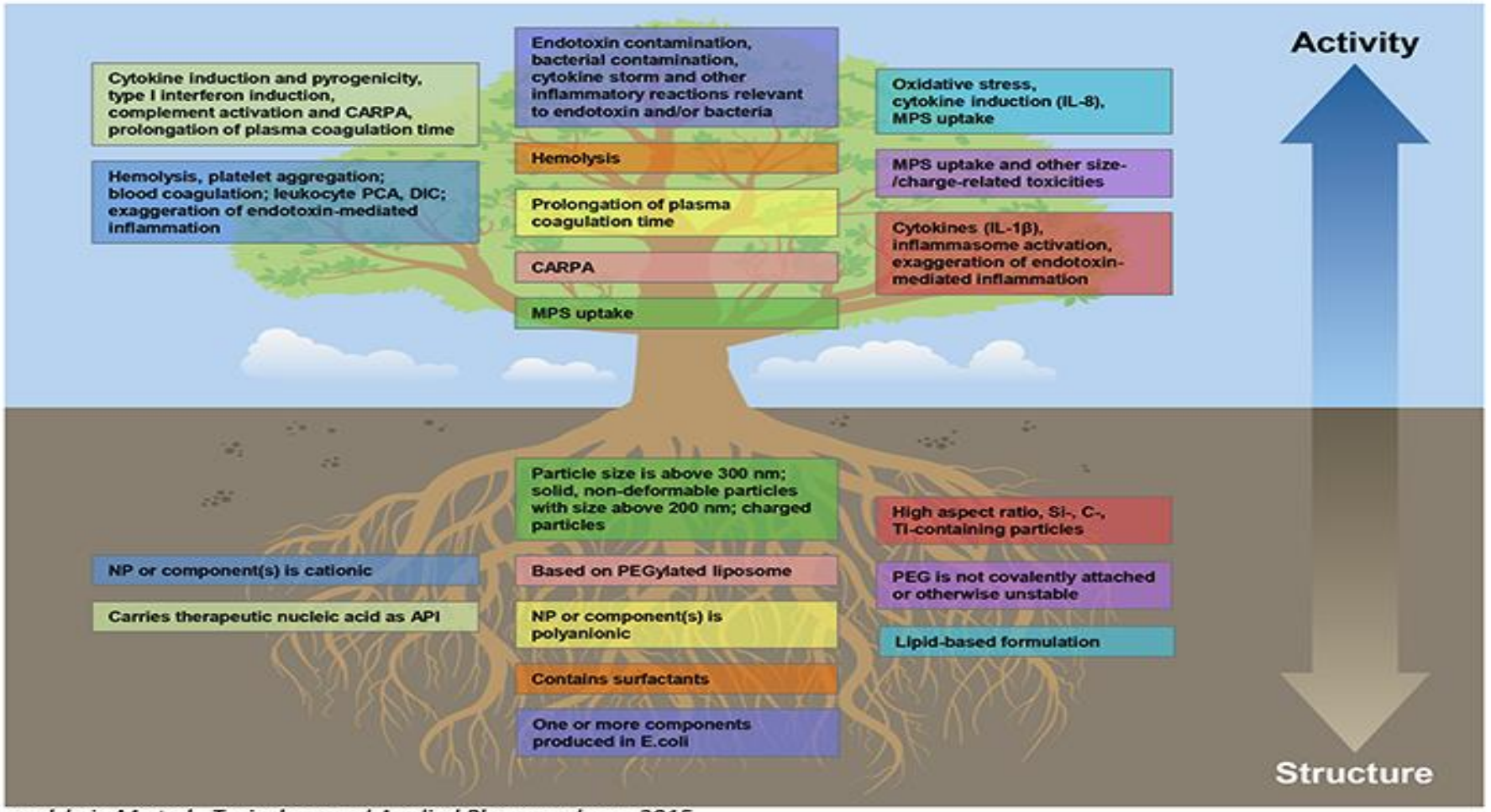
* - antibodies were generated ONLY after conjugation to protein carrier and injection in the presence of strong adjuvants

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



Dobrovolskaia M et al., Toxicology and Applied Pharmacology, 2015.

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