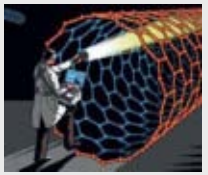
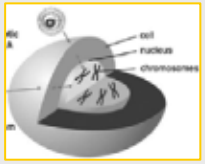
Toxicity of nano-structured biomaterials at the cellular level

Prof. Dr. Ing. Jörg Vienken
BioSciences, Fresenius Medical Care
Bad Homburg, Germany

Toxicity of nano-structured biomaterials at the cellular level

- Biomaterials in medical application
  - Facts and figures
- Biomaterial properties
  - Surface-related observations
  - Effects on the cellular level
- Conclusion





Medical Devices and Nano-Scaled Products

A principle question to be answered

The leaner, the meaner?

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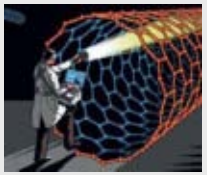
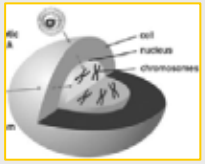




Consensus Conference on Biocompatibility (CCB)
Königswinter, Germany, March 1993
Nephrol Dial Transplant, 9 (Suppl 2): 1-186 (1994)

Biostability

„.....the ability of a substance to remain unchanged in a given biological environment.“

„Blood, a very peculiar liquid“*
…and a perfect medium for extraction!

Plasma
> 900 different compounds

1. Proteins
- **Albumin (60%)**
- **Immunoglobulins (35%)**
- **Fibrinogen (5%)**

2. Water

3 Anorganic salts

4. Transport entities
- **Hormons**
- **Fats**
- **Carbohydrates**
- **Enzyms**

Blood Cells

1. Erythrocytes $4 - 5 \times 10^{12}$ /l

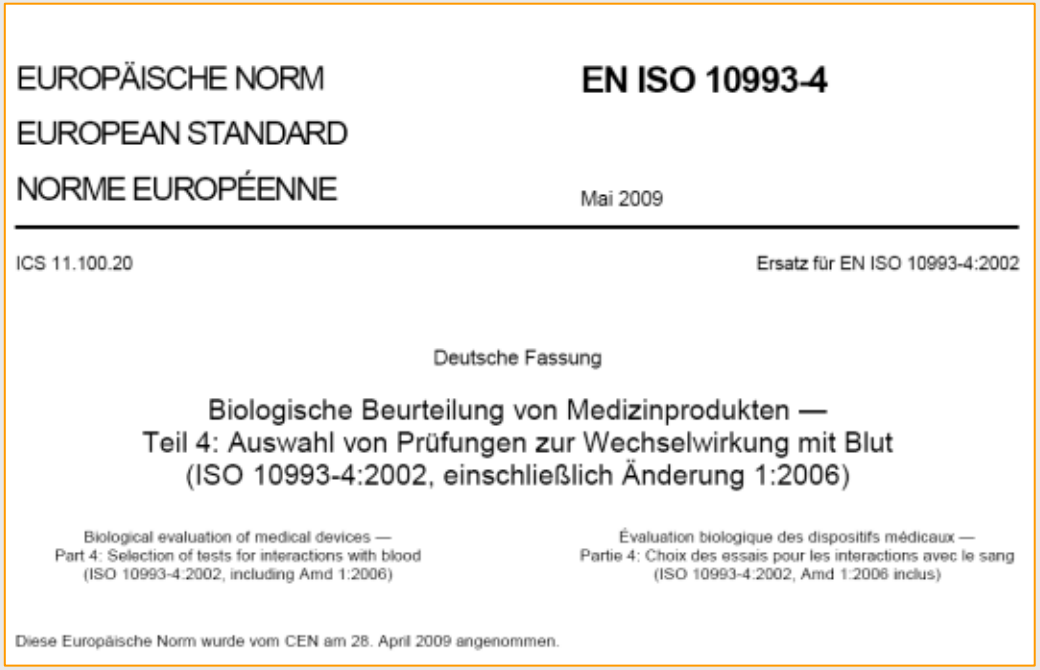
2. Platelets $200\text{-}300 \times 10^{9}$ /l

3. Leukocytes $6\text{-}8 \times 10^{9}$ /l

> 17 different Types
e.g., T_h-, T_s-cells

B-cells
Granulocytes
Monocytes
Killer cells

***Johann Wolfgang von Goethe**
Faust I (1806)



EUROPÄISCHE NORM
EUROPEAN STANDARD
NORME EUROPÉENNE

EN ISO 10993-4

Mai 2009

ICS 11.100.20

Ersatz für EN ISO 10993-4:2002

Deutsche Fassung

Biologische Beurteilung von Medizinprodukten —
Teil 4: Auswahl von Prüfungen zur Wechselwirkung mit Blut
(ISO 10993-4:2002, einschließlich Änderung 1:2006)

Biological evaluation of medical devices —
Part 4: Selection of tests for interactions with blood
(ISO 10993-4:2002, including Amd 1:2006)

Évaluation biologique des dispositifs médicaux —
Partie 4: Choix des essais pour les interactions avec le sang
(ISO 10993-4:2002, Amd 1:2006 inclus)

Diese Europäische Norm wurde vom CEN am 28. April 2009 angenommen.

Extraction: Media and procedures

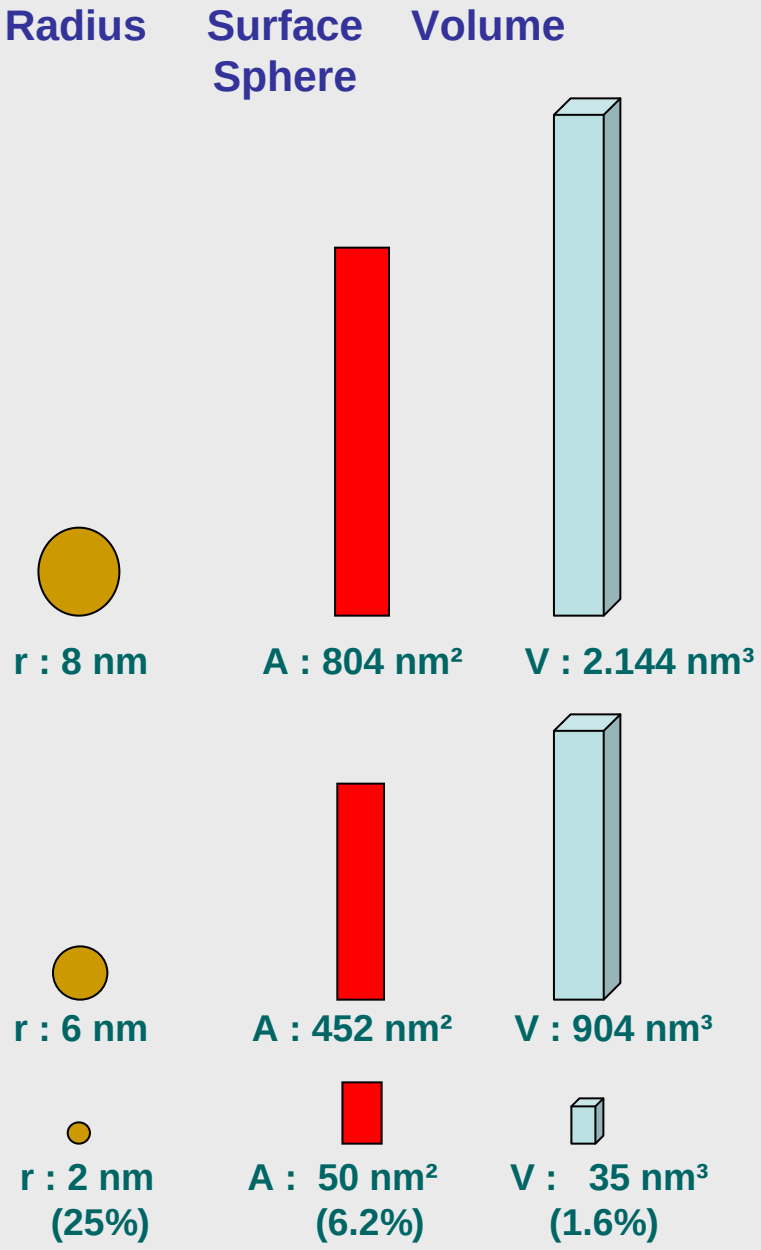
The solvents selected as extractants shall

a) be suitable for use in the specific biological test systems.
b) simulate the extraction which occurs during clinical use of the device and/or
c) maximize the amount of extract

Extraction media:

1. **Polar solutions:** **Water, saline (0.9%) culture media without serum**
2. **Unpolar solutions:** **Vegetable oil (e.g. Sesamoil)**
3. **Additionally:** **Ethanol/water (17,5% w/w), PEG 400, DMSO, culture media & serum**

Nano-scaled Biomaterials

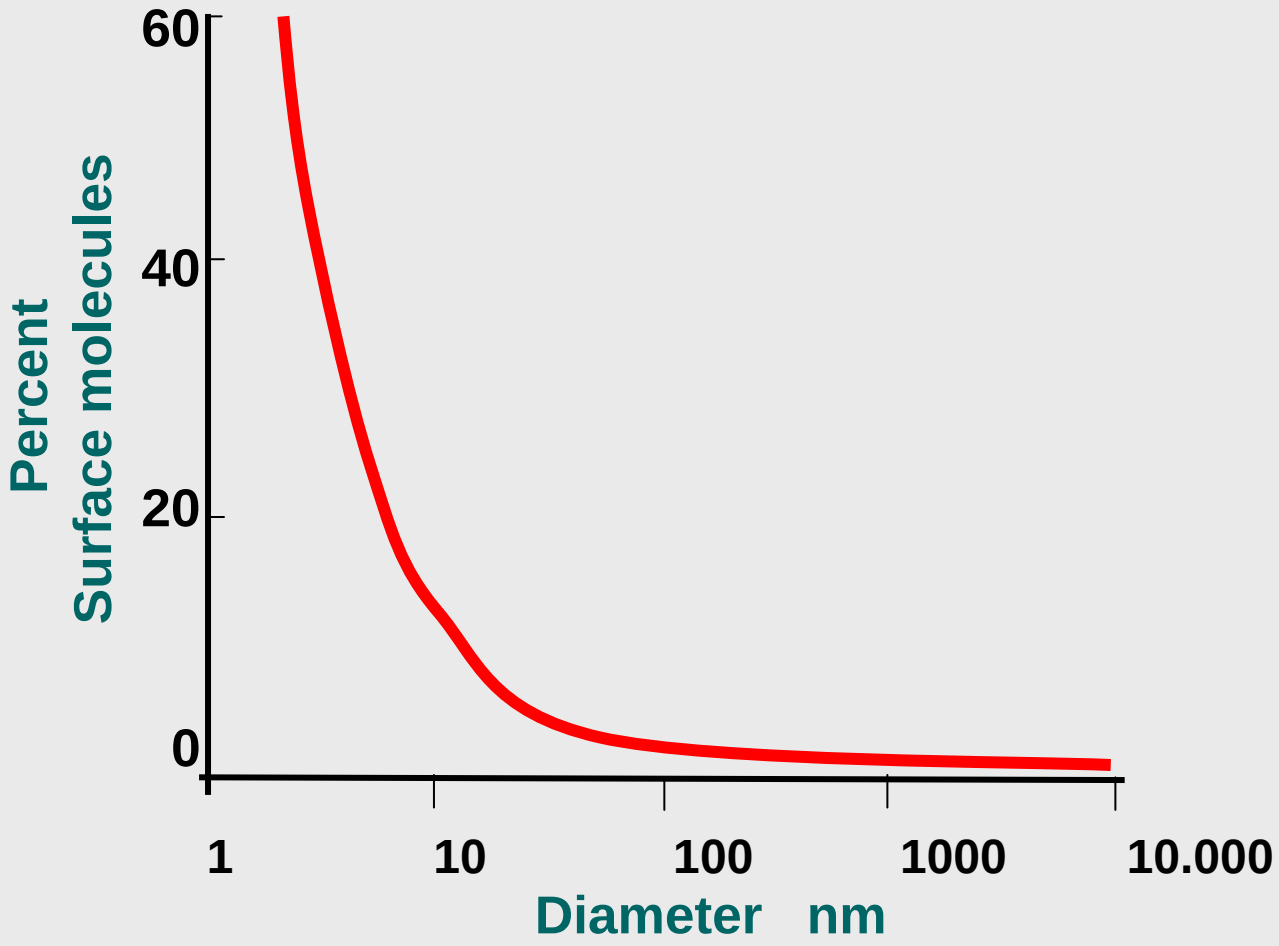


With decreasing radius magnitudes for Volume drop faster than for areas!
Radius dependence:
r^3 *vs* r^2

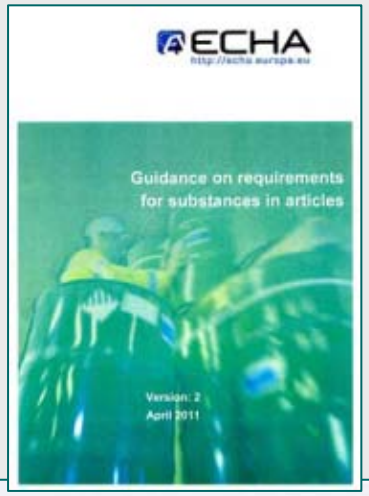
Nanoeffects always Surface effects

Particle Size and Number of Molecules Expressed at Surface

Consequences for leachables



Re: A Nel et al., Science, 311:622 (2006)



ECHA – Model Calculation for Substances of Very High Concern (SVHC)

Requirements for substances in articles

Example 7: Calculation of the average concentration of a SVHC in an article

A chair consists of a wooden part and a plastic part. The weight of the chair is 2.001 kg. The wooden part of the chair contains 10 mg of an SVHC. The weight of the wooden part is 2 kg. The plastic part of the chair contains 1 mg of the same SVHC and weighs 1 g.

The concentration of the SVHC in the chair is calculated using the formula above.

$$Conc_{SVHC\ in\ whole\ article}[\%] = \frac{10 \cdot 10^{-3} g + 1 \cdot 10^{-3} g}{2001 g} \cdot 100 = 0.0005\%$$

Conclusion: The average concentration of the SVHC in the chair does not exceed 0.1% (w/w). Obligations according to Article 7(2) and 33 do not apply.

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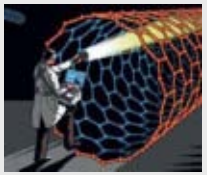
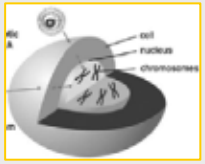
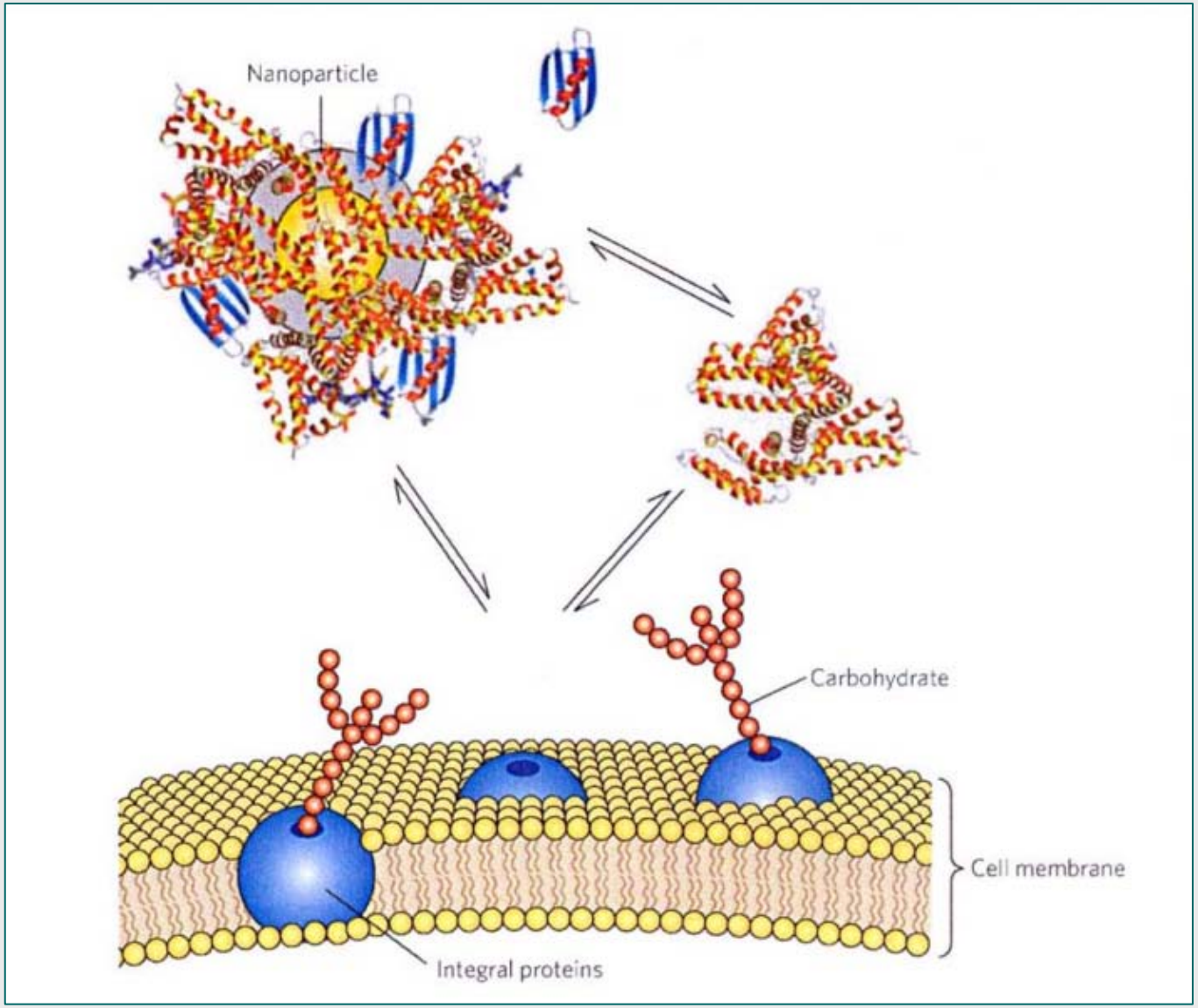




Interaction of Nanoparticles with biological cells -

- determined by protein-coating -



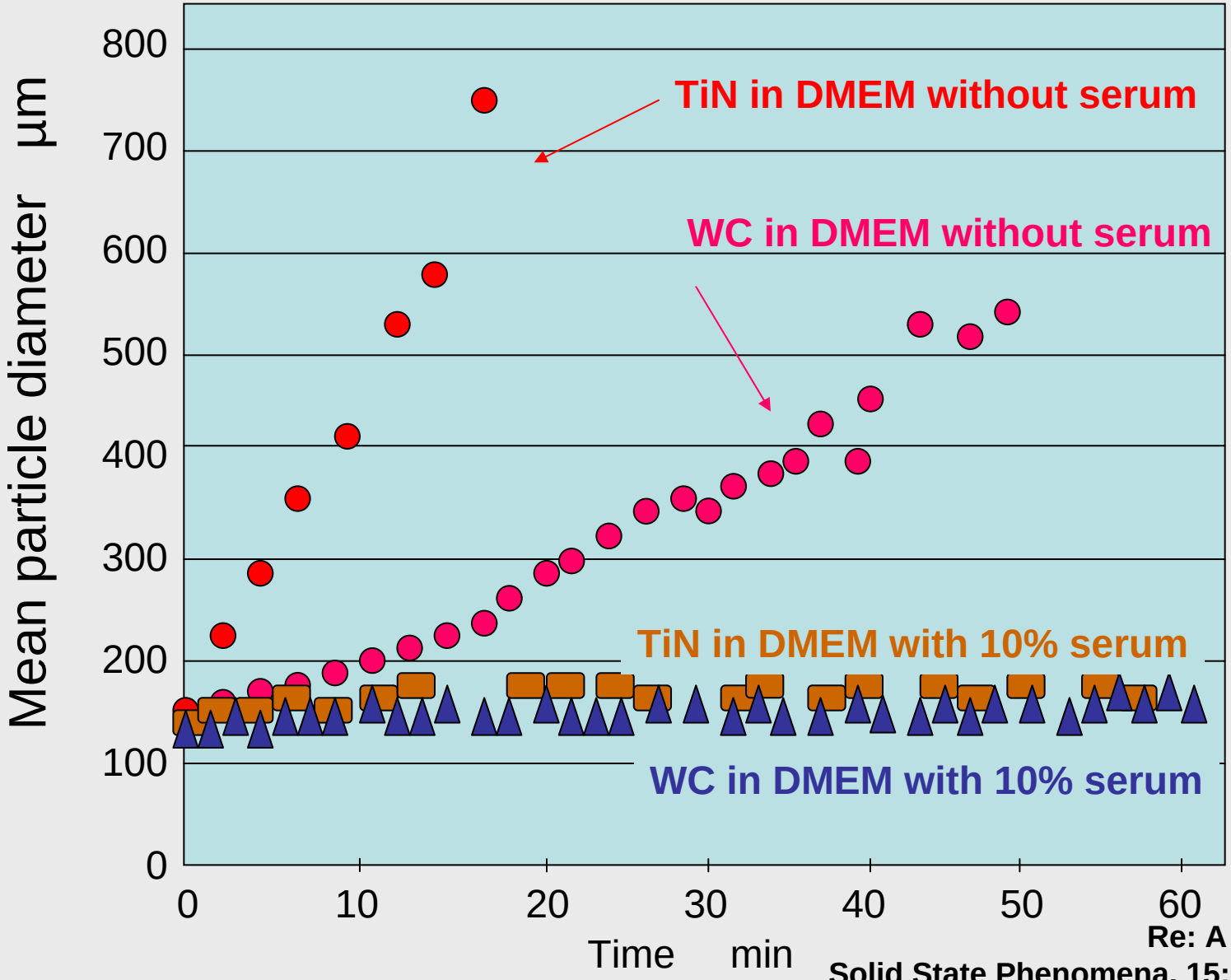
Re: I Lynch
Nature Nanotechn, 4:546-547 (2009)

Protein Deposition on Biomaterials
Sequence under Flow-Conditions

	Depositiontime [s] a=50%	Timefactor for boundary layer formation [s]
Albumin	0,050	9
Fibrinogen	7,4	13
Factor XII	140,0	8
HMWK	68,0	8
Platelets	260,0	18

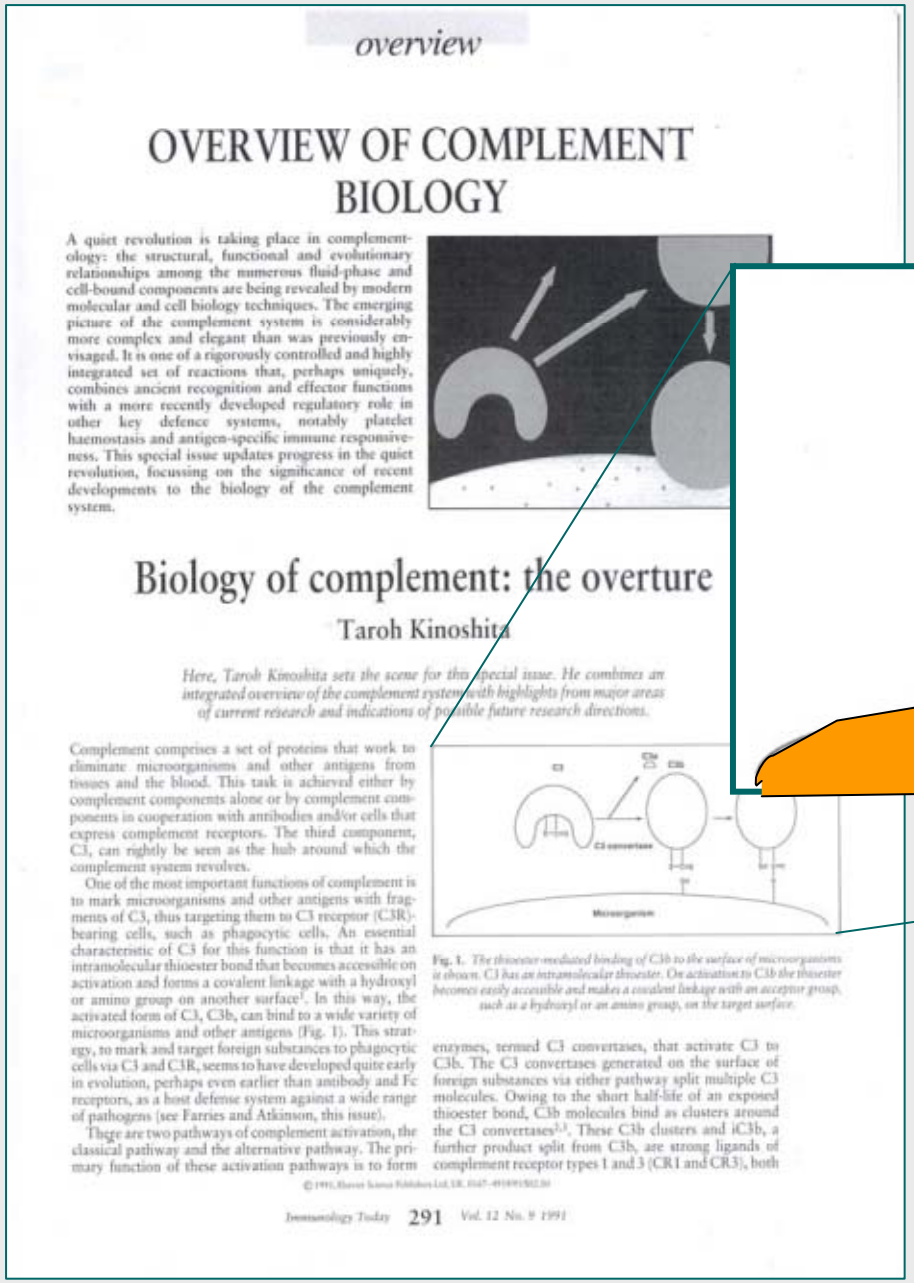
D Basmadjian et al.,
Biomaterials, 18: 1511-1522 (1997)

Agglomeration Behaviour of WC and TiN Particles
- at a concentration of 10 µg/ml -



Re: A Potthoff et al.,
Solid State Phenomena, 15:183-189 (2009)

C3b Complement Binding to Microorganisms



overview

OVERVIEW OF COMPLEMENT BIOLOGY

A quiet revolution is taking place in complement-ology: the structural, functional and evolutionary relationships among the numerous fluid-phase and cell-bound components are being revealed by modern molecular and cell biology techniques. The emerging picture of the complement system is considerably more complex and elegant than was previously envisaged. It is one of a rigorously controlled and highly integrated set of reactions that, perhaps uniquely, combines ancient recognition and effector functions with a more recently developed regulatory role in other key defence systems, notably platelet haemostasis and antigen-specific immune responsiveness. This special issue updates progress in the quiet revolution, focussing on the significance of recent developments to the biology of the complement system.

Biology of complement: the overture

Taroh Kinoshita

Here, Taroh Kinoshita sets the scene for this special issue. He combines an integrated overview of the complement system with highlights from major areas of current research and indications of possible future research directions.

Complement comprises a set of proteins that work to eliminate microorganisms and other antigens from tissues and the blood. This task is achieved either by complement components alone or by complement components in cooperation with antibodies and/or cells that express complement receptors. The third component, C3, can rightly be seen as the hub around which the complement system revolves.

One of the most important functions of complement is to mark microorganisms and other antigens with fragments of C3, thus targeting them to C3 receptor (C3R)-bearing cells, such as phagocytic cells. An essential characteristic of C3 for this function is that it has an intramolecular thioester bond that becomes accessible on activation and forms a covalent linkage with a hydroxyl or amino group on another surface[1]. In this way, the activated form of C3, C3b, can bind to a wide variety of microorganisms and other antigens (Fig. 1). This strategy, to mark and target foreign substances to phagocytic cells via C3 and C3R, seems to have developed quite early in evolution, perhaps even earlier than antibody and Fc receptors, as a host defense system against a wide range of pathogens (see Farries and Atkinson, this issue).

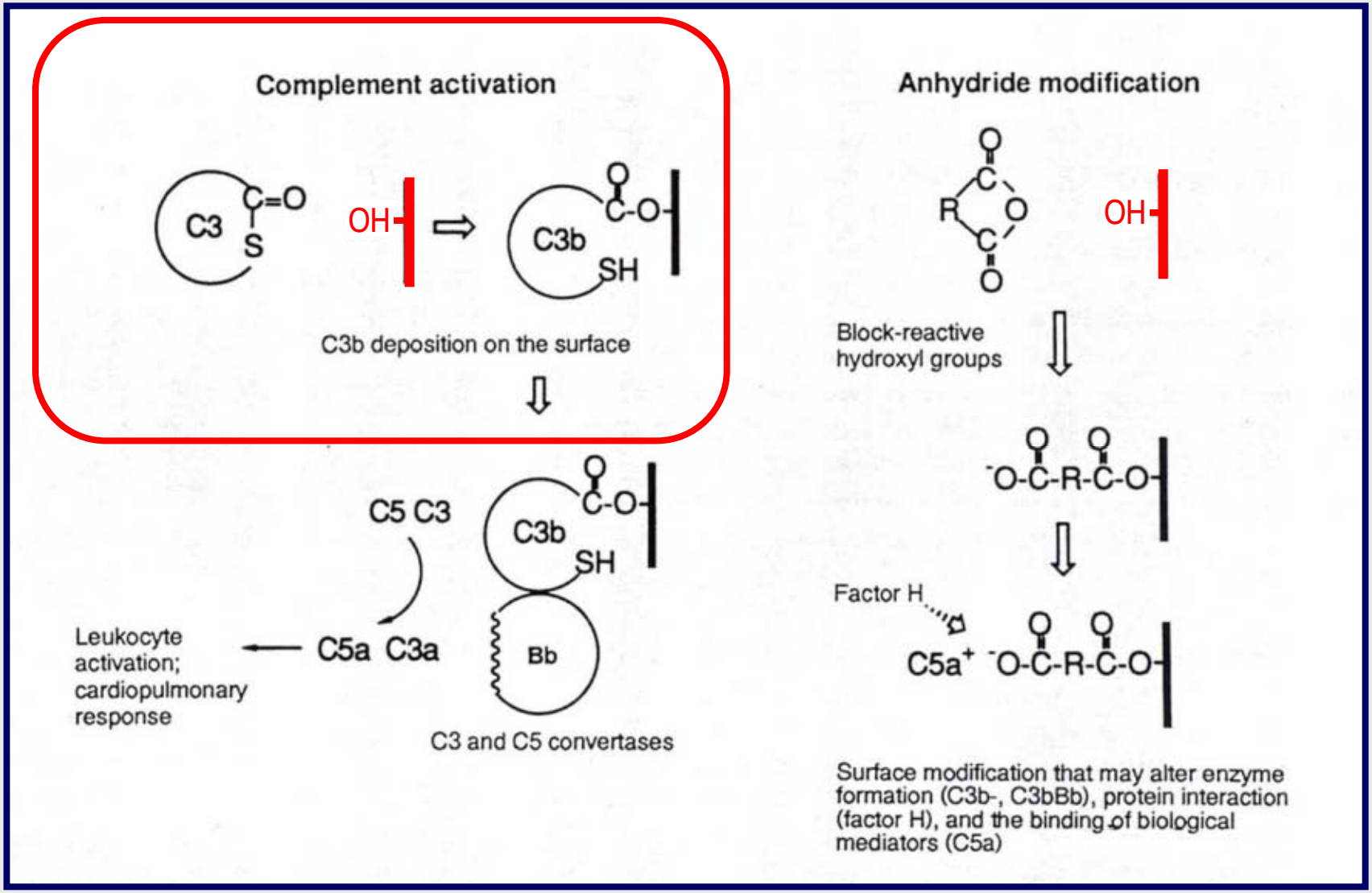
There are two pathways of complement activation, the classical pathway and the alternative pathway. The primary function of these activation pathways is to form enzymes, termed C3 convertases, that activate C3 to C3b. The C3 convertases generated on the surface of foreign substances via either pathway split multiple C3 molecules. Owing to the short half-life of an exposed thioester bond, C3b molecules bind as clusters around the C3 convertases[2,3]. These C3b clusters and iC3b, a further product split from C3b, are strong ligands of complement receptor types 1 and 3 (CR1 and CR3), both

Fig. 1. The thioester-mediated binding of C3b to the surface of microorganisms is shown. C3 has an intramolecular thioester. On activation to C3b the thioester becomes easily accessible and makes a covalent linkage with an acceptor group, such as a hydroxyl or an amino group, on the target surface.

Alternative complement pathway is part of innate immunity!

Re: T Kinoshita, Immunol Today, 12:291-294 (1991)

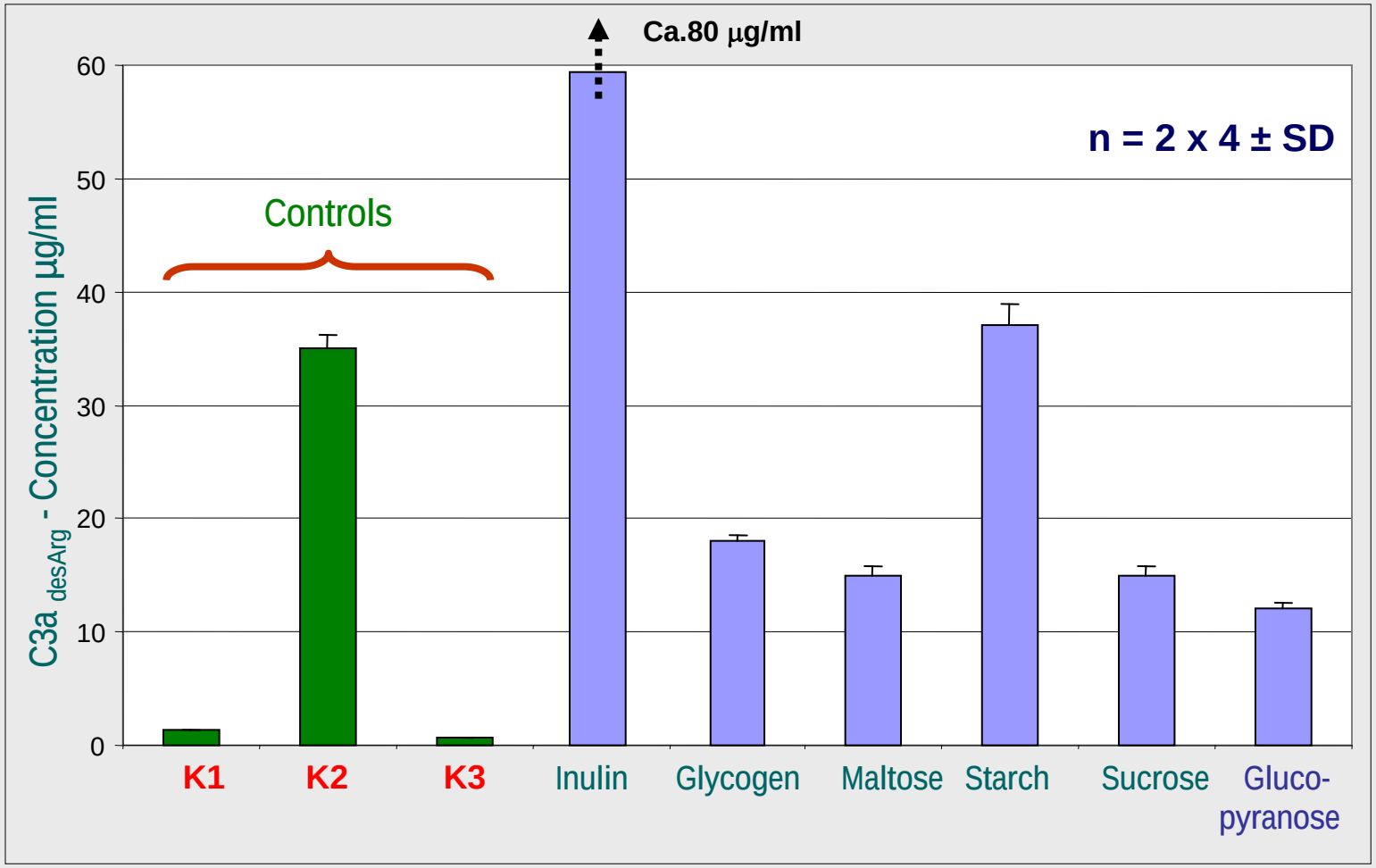
C3b Complement Binding to Biomaterials with OH-Groups



Re: R Johnson et al., Blood Purif, 8:318-328 (1990)

Complement Activation by Polysaccharides (PS)

- PS linked to microtiter plates (solid phase) -

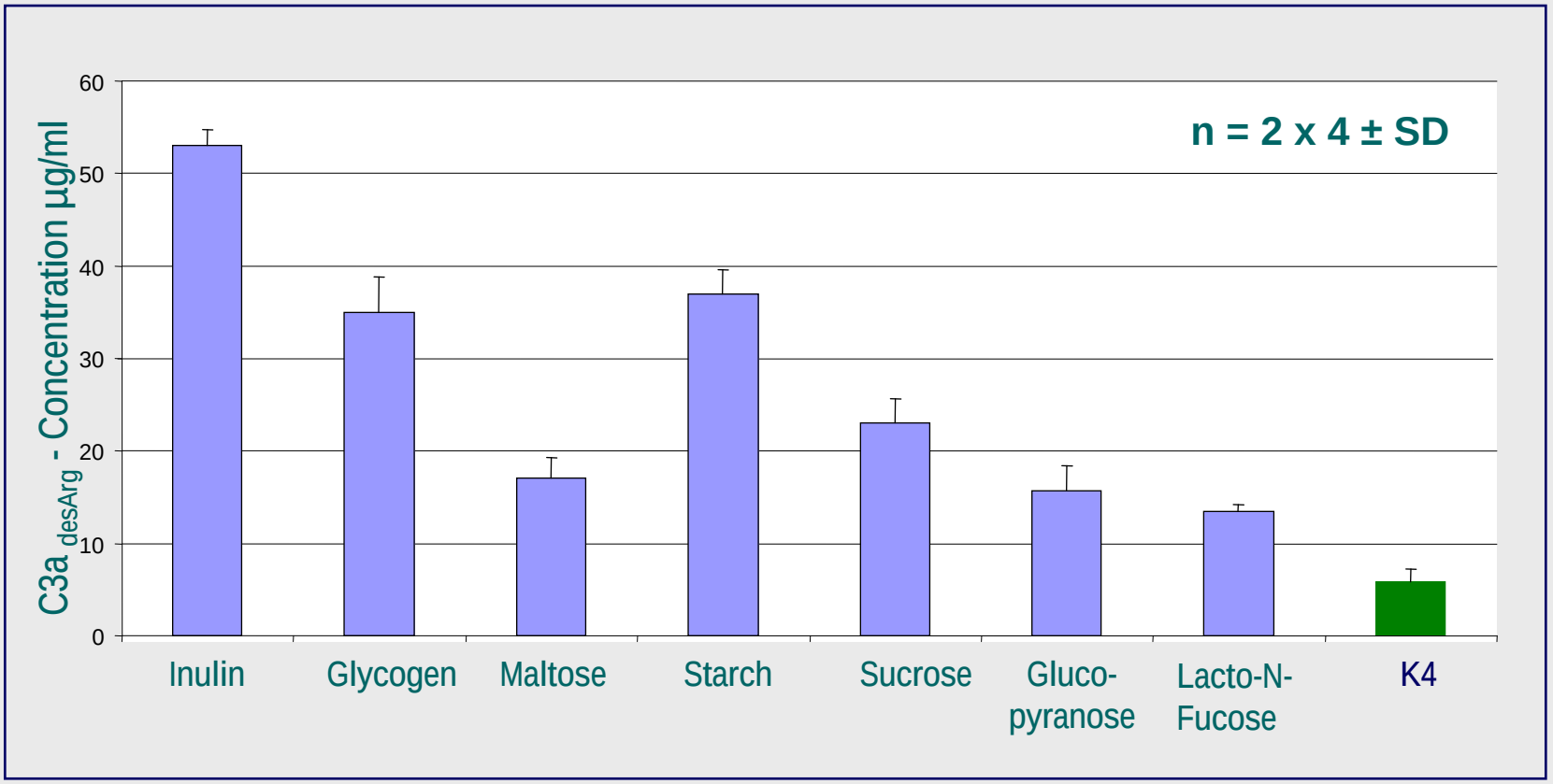


PS linked to microtiter plates precoated with activated human serum albumin (mHSA).

K1: no poly-saccharide (PS)
K2: no PS, serum with complement and activator
K3: blank (no PS, no serum)

Complement Activation by Polysaccharides

- PS exposed to serum in solution -

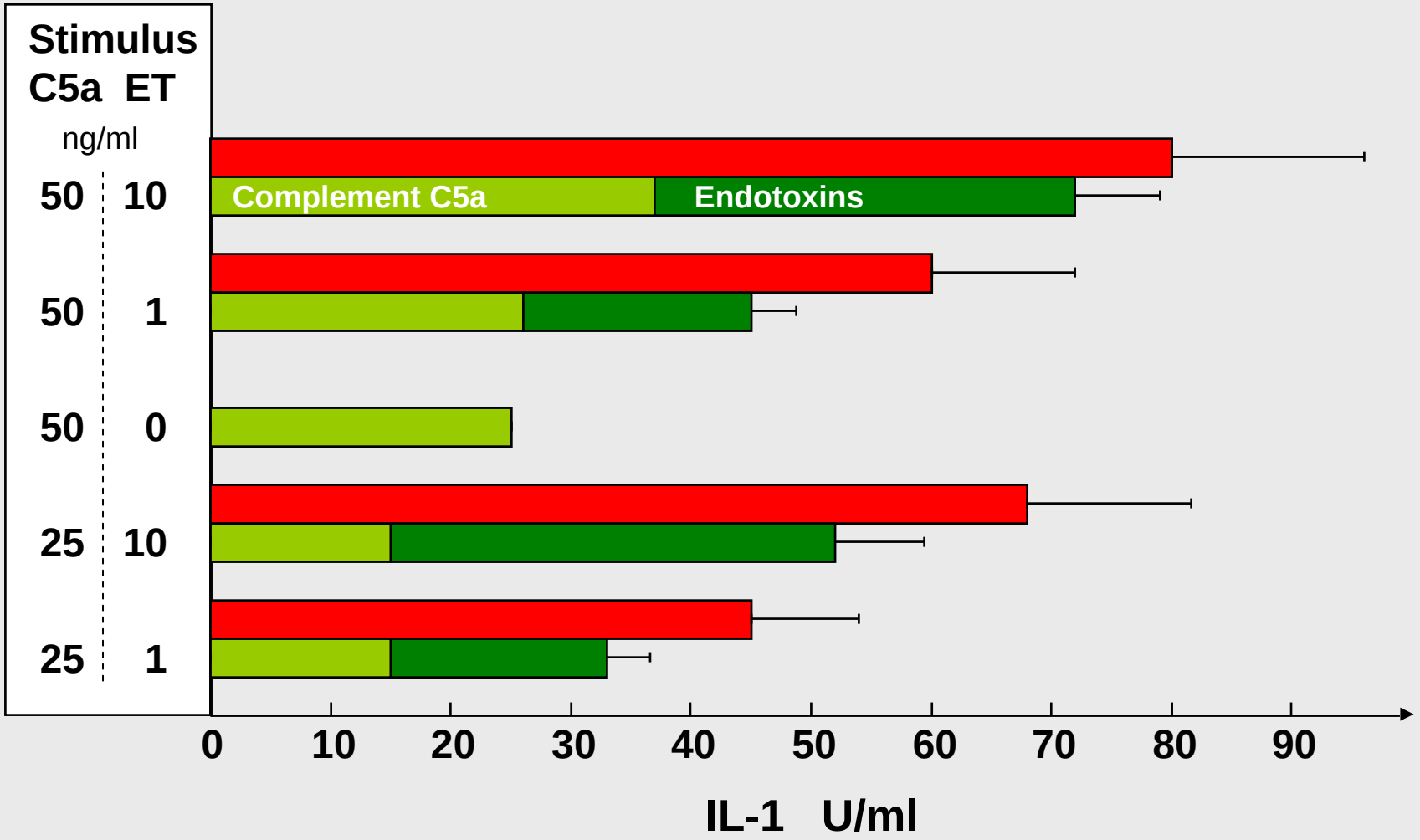


Source for C3: human serum (A113)

K4: no sugar, control for spontaneous complement activation

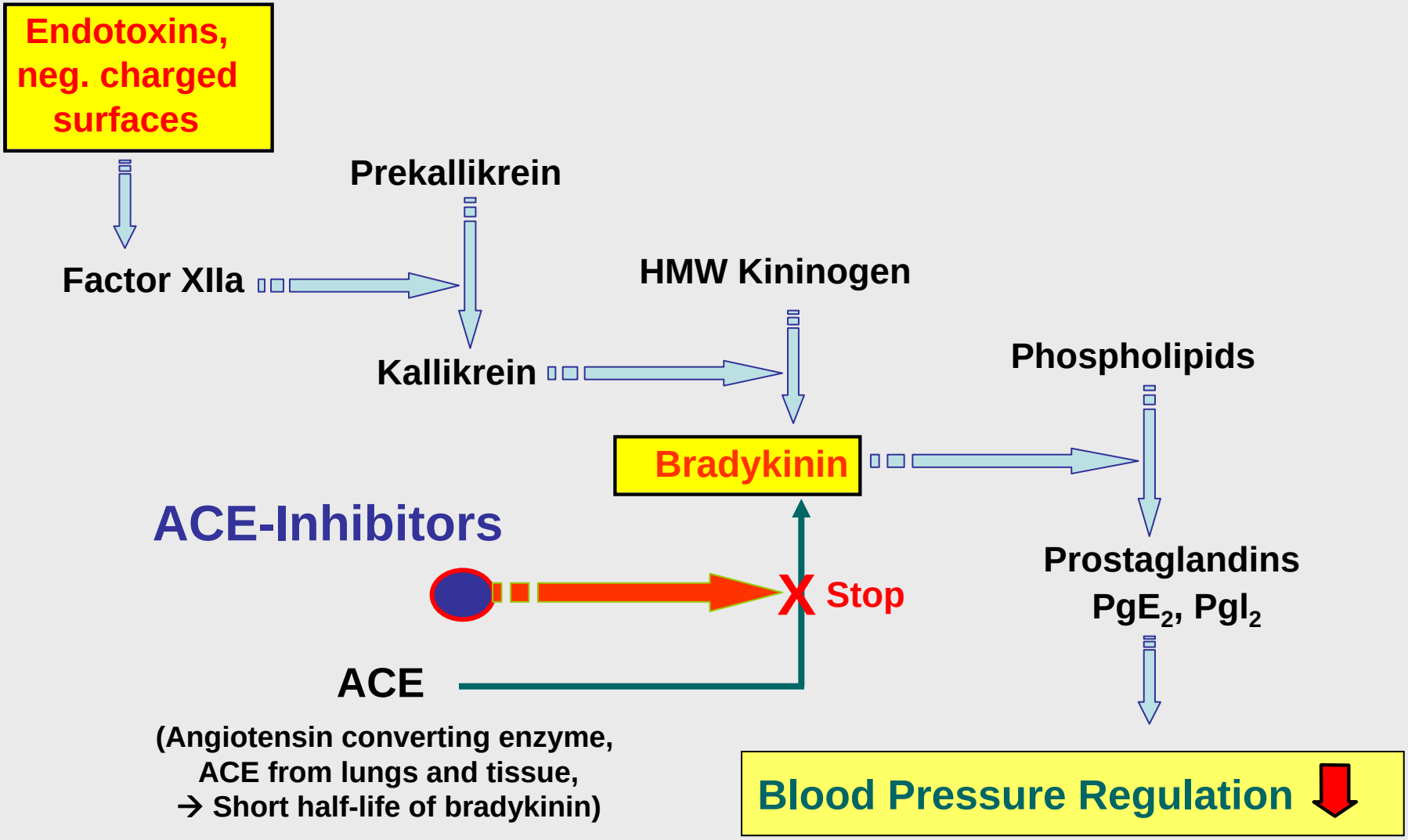
Synergistic Effects of C5a and Endotoxins (ET)

Need to avoid the simultaneous presence of both stimuli

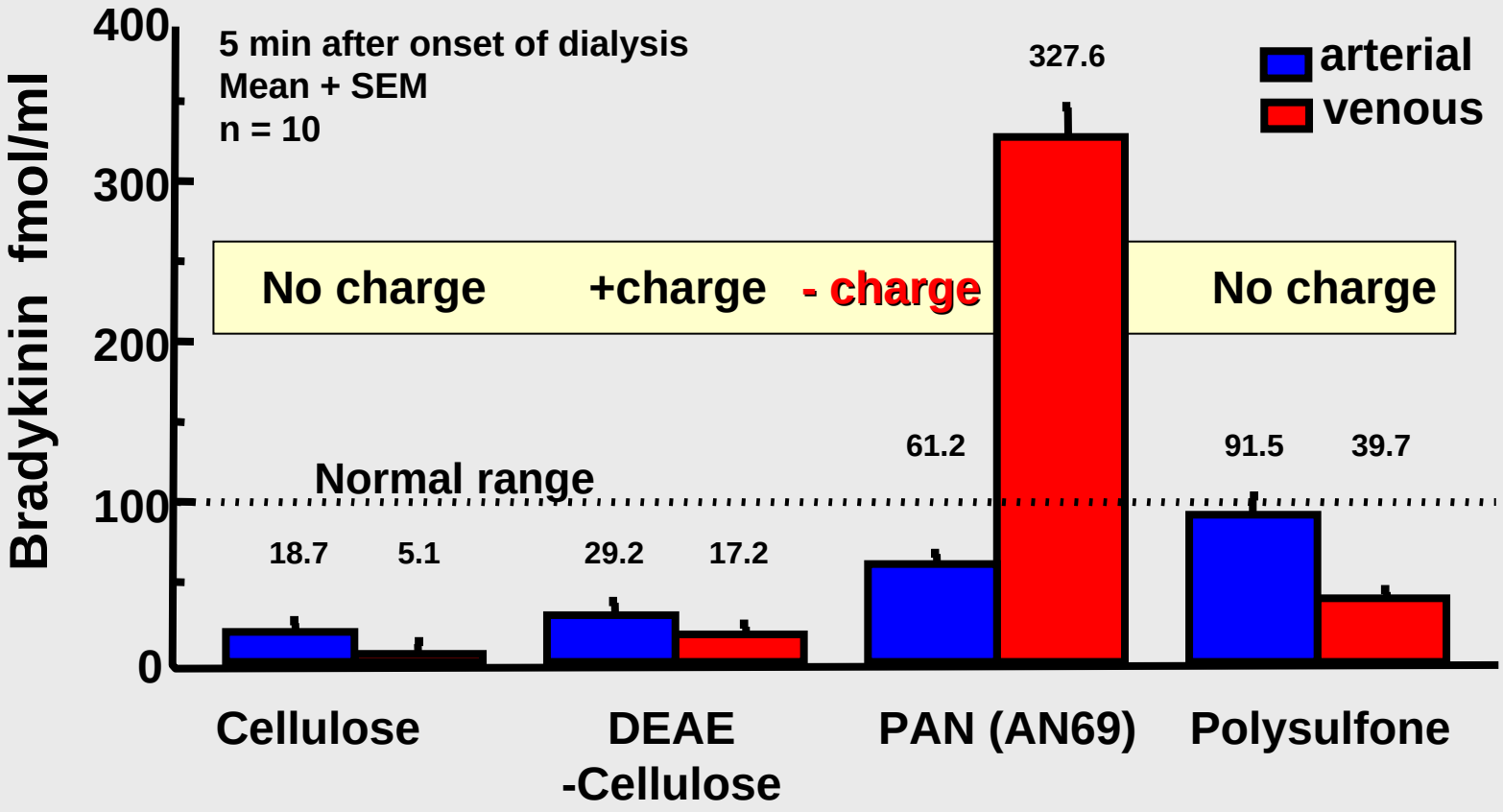


S. Okusawa et al,
J Immunol, 139:2635-2640 (1987)

Blood Pressure Regulation
through negatively charged surfaces and subsequent contact-phase activation



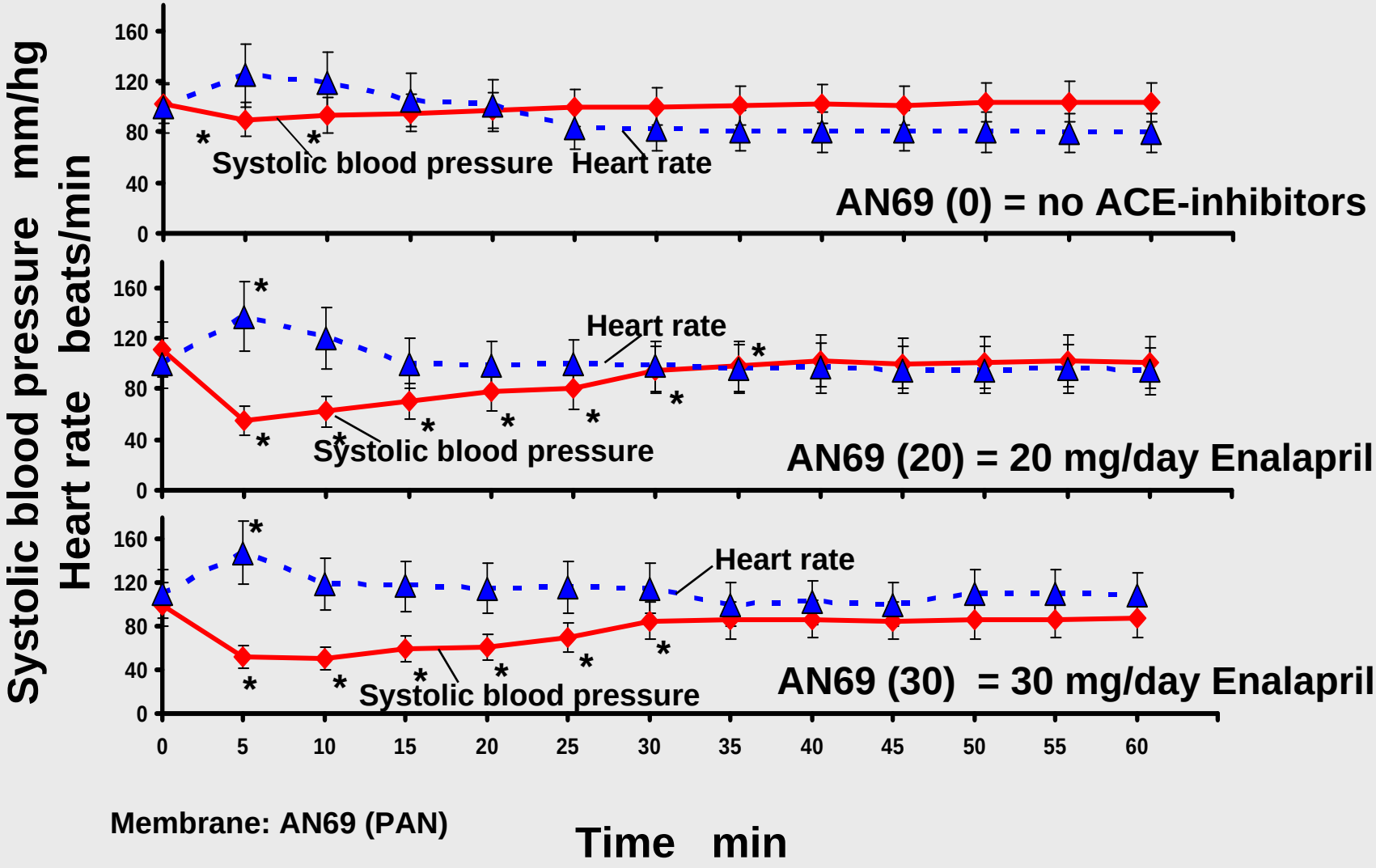
Bradykinin Generation in Plasma
by negatively charged surfaces



Verresen et al,
Kidney Int; 45: 1497-1503 (1994)

ACE-Inhibitors and Anaphylactoid Reactions

Sheep Model



Membrane: AN69 (PAN)

Krieter et al,
Kidney Int, 53: 1026-35 (1998)

ACE-Inhibitor Arelix

(negatively charged surfaces)

no PAN-methallylsulfonate
no dextransulfate

Aventis

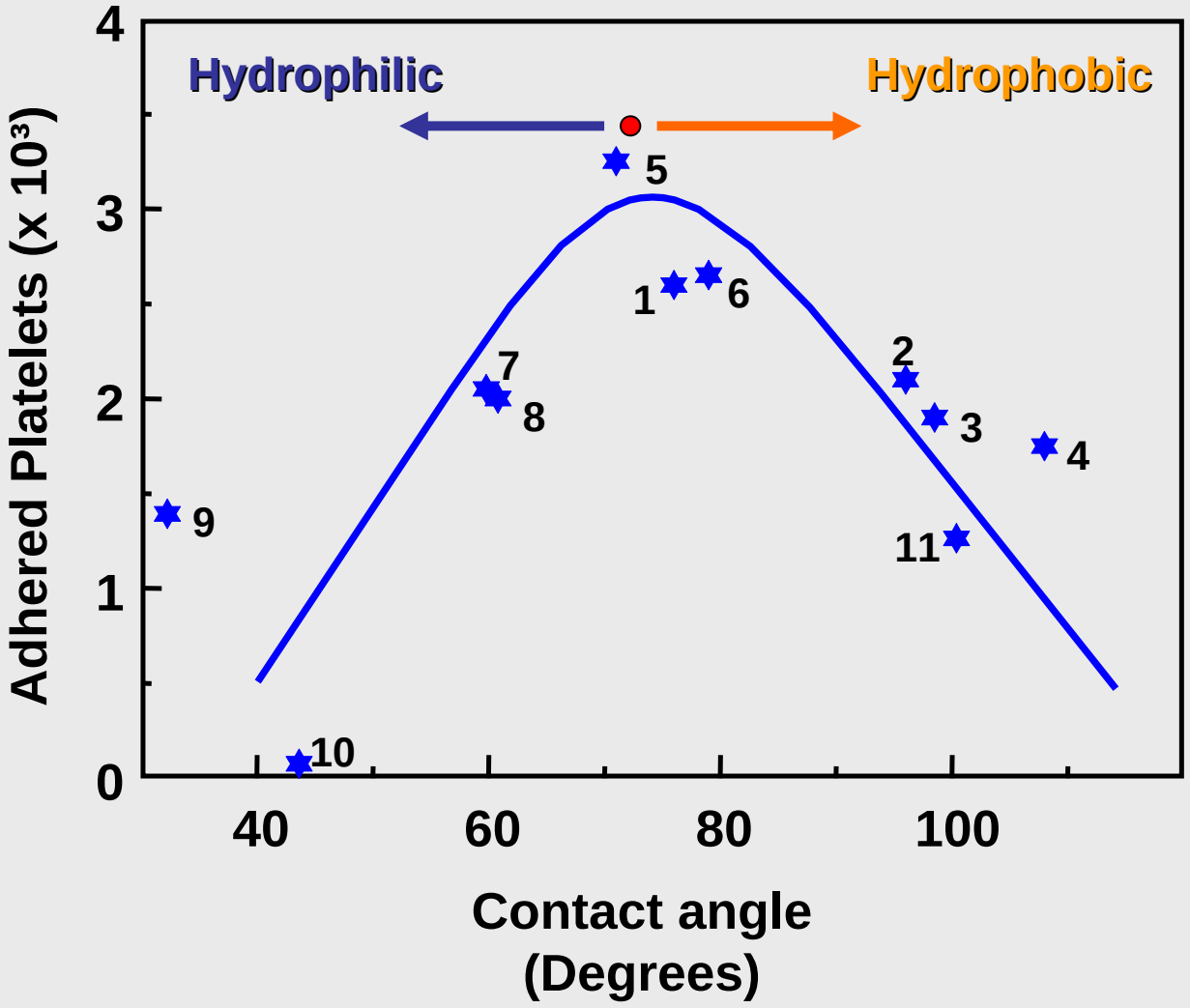
Sehr geehrte Patientin, sehr geehrter Patient, bitte lesen Sie die folgende Gebrauchsinformation – auch vor Anbruch jeder neuen Packung – aufmerksam durch. Sie enthält wichtige Informationen darüber, was Sie bei der Anwendung dieses Arzneimittels beachten sollen. Diese Packungsbeilage wird regelmäßig neuen Erkenntnissen angepasst (siehe unter »Stand der Information«). Wenden Sie sich bei Fragen bitte an Ihren Arzt oder Apotheker.

Warnhinweise: **Warning**

Bei der gleichzeitigen Anwendung von Arelix ACE und bestimmten Behandlungsmethoden (extrakorporale Therapieverfahren), die zum Kontakt von Blut mit negativ geladenen Oberflächen führen (z. B. Dialyse oder Hämofiltration mit bestimmten Dialysemembranen oder LDL-Apherese mit Dextransulfat), besteht die Gefahr, dass schwere Überempfindlichkeitsreaktionen (anaphylaktoide Reaktionen) bis hin zum lebensbedrohlichen Schock auftreten können. Dies gilt auch für eine Therapie zur Schwächung bzw. Aufhebung der allergischen Reaktionsbereitschaft (Desensibilisierungstherapie) gegen Insektengifte. Arelix ACE nicht zusammen mit Poly(acrylonitril, natrium-2-methylallylsulfonat)-high-flux-Membranen (z. B. »AN 69«), während einer LDL-Apherese mit Dextransulfat oder während einer Desensibilisierungsbehandlung mit Insektengiften anwenden (siehe auch unter »Gegenanzeigen«). Wenn plötzlich Gewebeschwellungen (angioneurotische Ödeme) während der Behandlung auftreten, muss Arelix ACE sofort abgesetzt werden. Ein durch ACE-Hemmer ausgelöstes angioneurotisches Ödem kann mit Beteiligung von Kehlkopf, Rachen und/oder Zunge verlaufen (siehe Gegenmaßnahmen unter »Nebenwirkungen«!)

Platelet - Adhesion

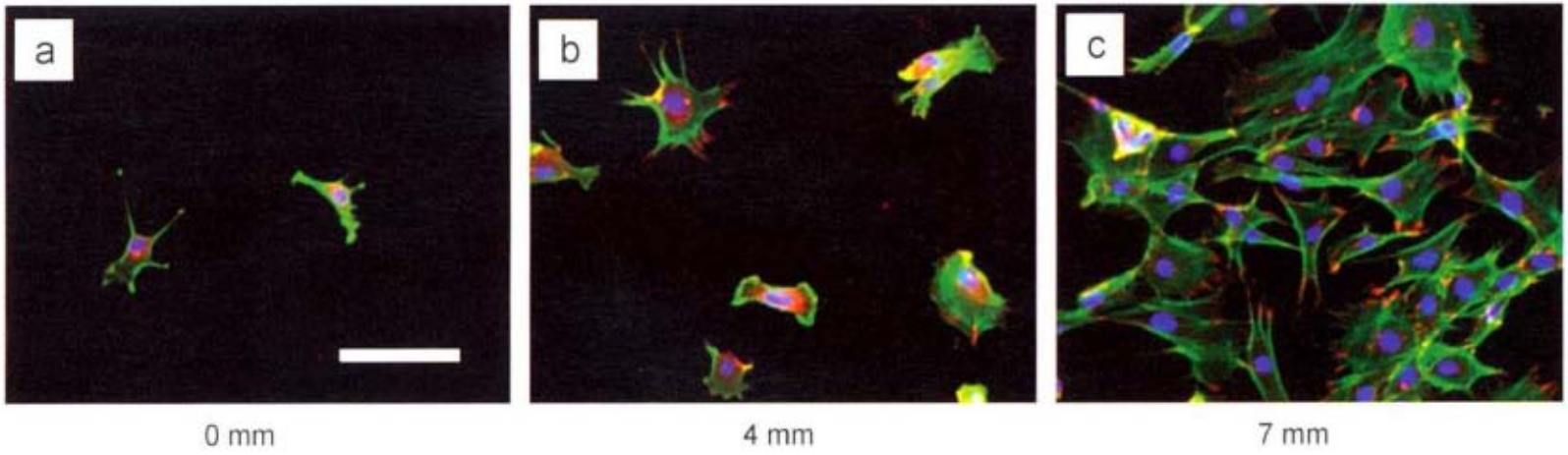
Various polymers

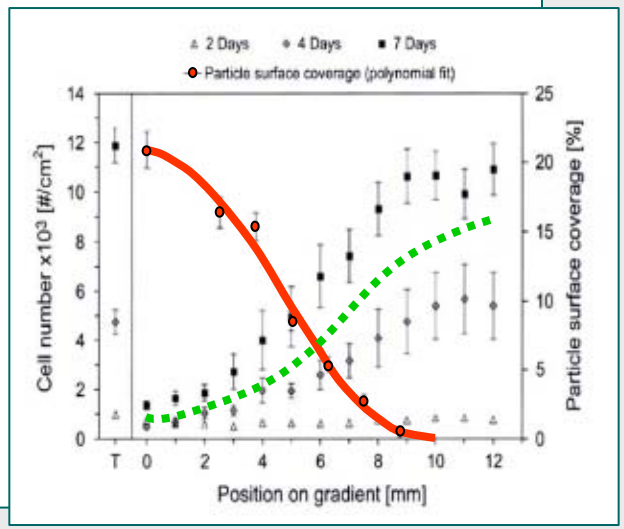


1. Polyethylene
2. Polypropylene
3. Polytetrafluoro-Ethylene
4. Hexafluoro-Propylene
5. Polyethylene Terephthalate
6. Nylon 6,6
7. Polymethyl-methacrylate
8. Vinyl-A, alcohol-ethylene copolymer
9. Cellulose
10. Polyvinylalcohol
11. Polydimethyl-Siloxane

Y Ikada,
Proc. 5. PIMS-Meeting, pp. 6/1-6/8 (1986)

- Optimization of Surfaces with Nanoparticl
- Attachment of Osteoblasts





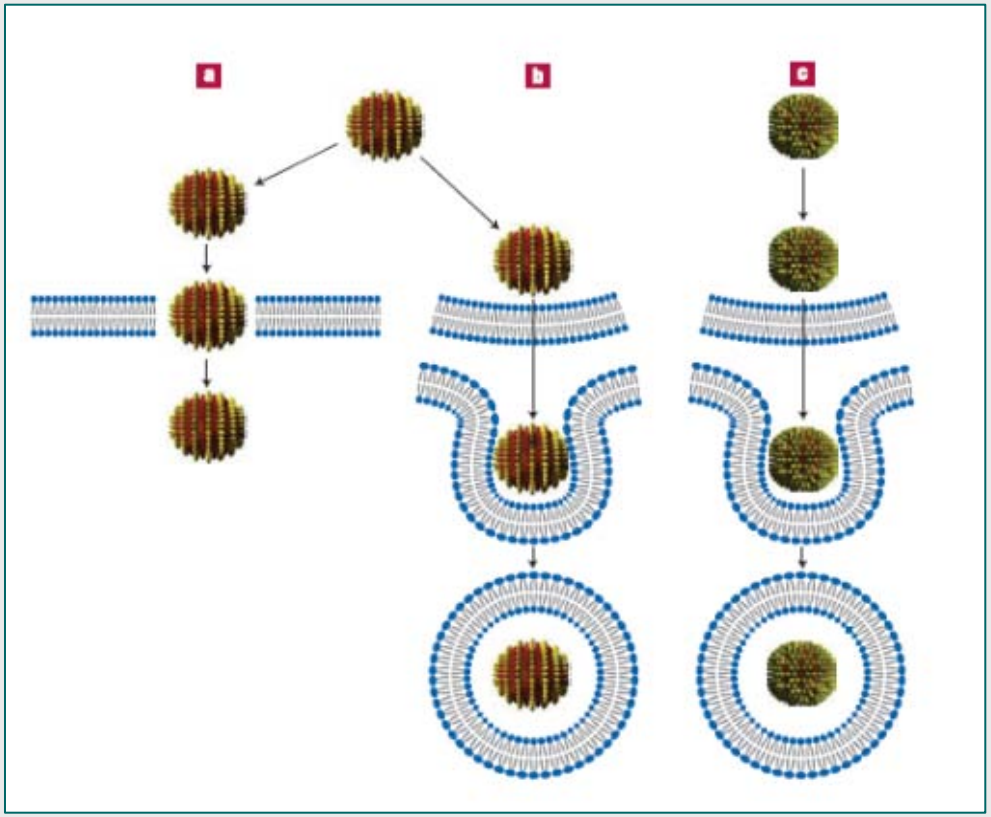
Cell seading with 3.500 RCO-cells/cm², cultivation: 7 days
Red: Vinculin, green: Actin, blue: cell nuclei

T Kunzler et al., Biomaterials, 28:5000-5006 (2007)

NANOBIOLOGY

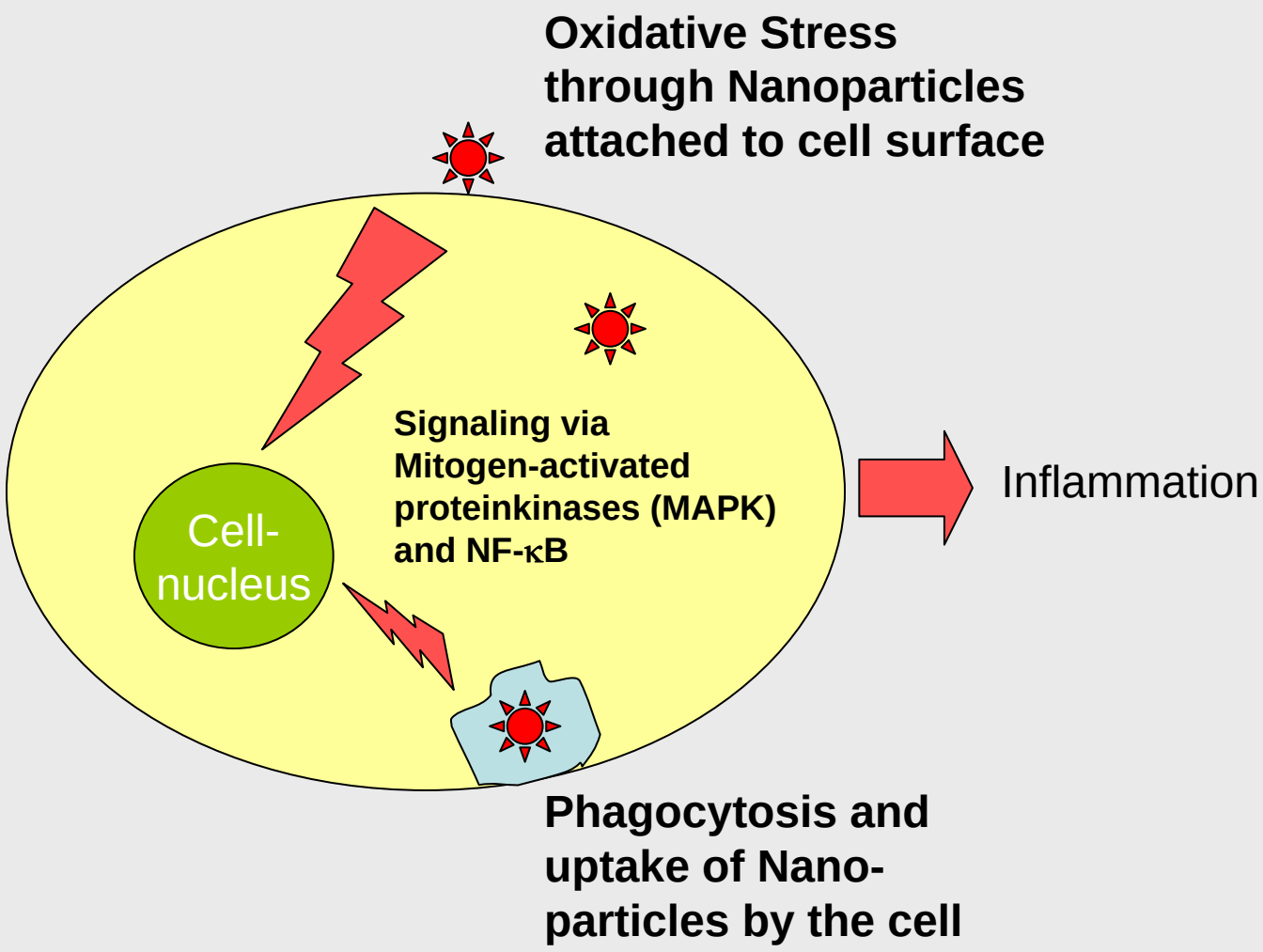
Particles slip cell security

Nanoparticles with alternating striations of hydrophobic and hydrophilic ligands cross the cell membrane by a direct mechanism — a route that delivers them to the main compartment of the cell while leaving the membrane undisrupted.

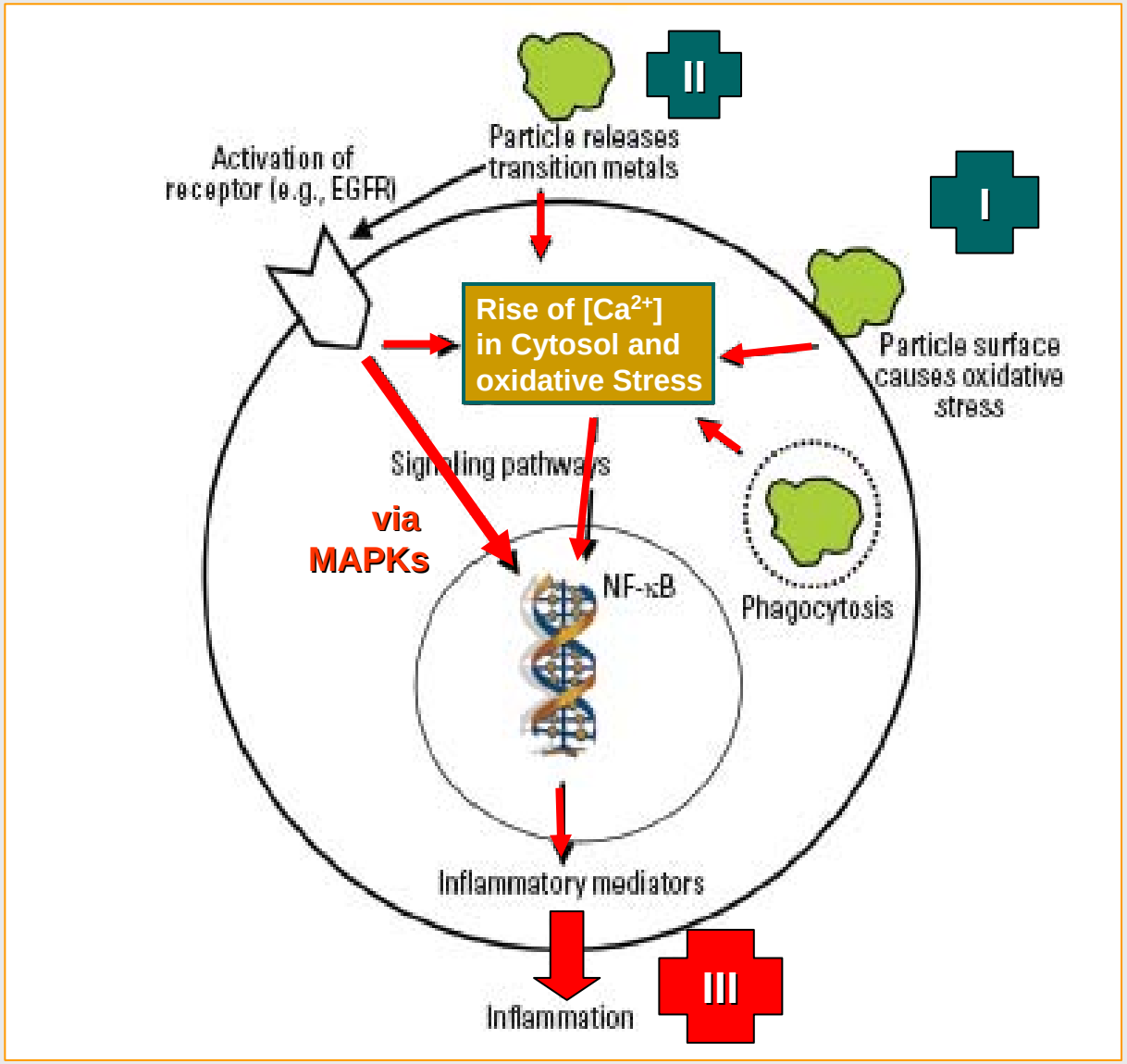


Re: T Xia et al.,
Nature Materials, 7:519-520 (2008)

Mechanisms of Cell-Activation by Nanoparticles



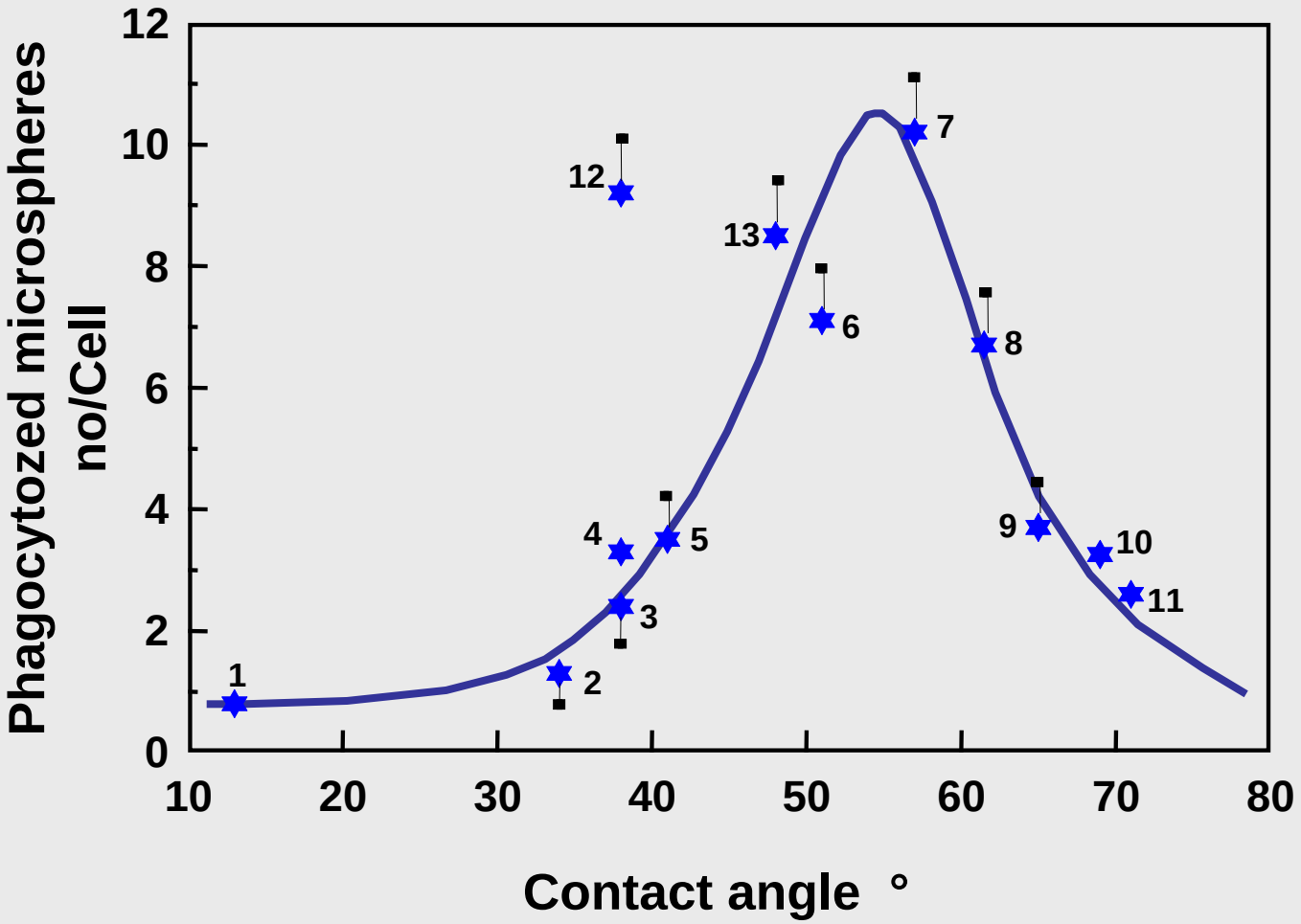
Postulated Mechanism for Inflammatory Reactions after cell exposition to Nanoparticles



Oberdörster et al., Environ Health Perspect, 113:823-839 (2005)

Phagocytosis and Contact Angles

Cell type: Macrophages



Modification of Microspheres:

1. none
2. Methylamine
3. Ethylamine
4. n-Propylamine
5. n-Butylamine
6. n-Hexylamine
7. n-Octylamine
8. n-Decylamine
9. n-Dodecylamine
10. n-Hexadecylamine
11. n-Octadecylamine
12. Anilin
13. Cyclohexylamin

Y Ikada,
Biomaterials, 15: 725-36 (1994)

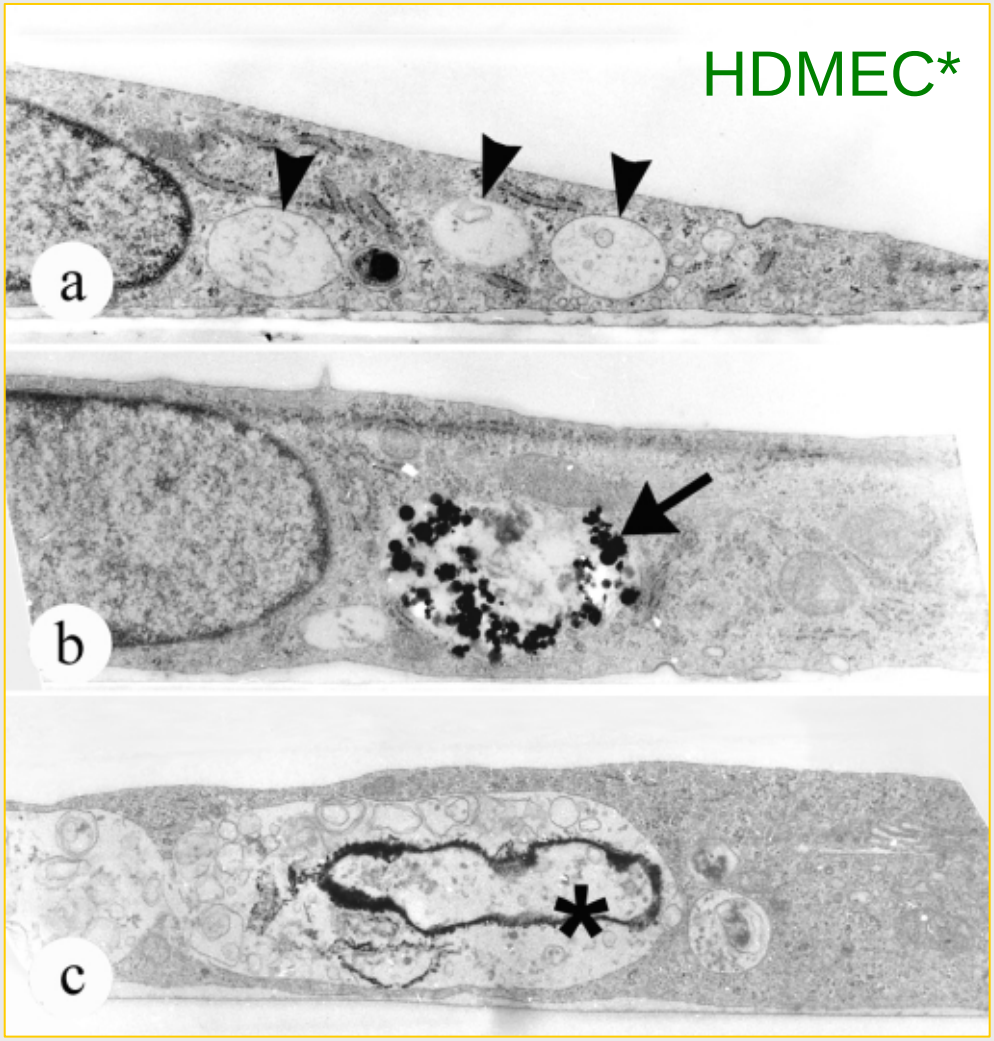
Nanoparticles and Endothelial Cells

TEM (48 h Nanoparticle Exposition)

Untreated control

TiO_2 Particles

Co - Particles

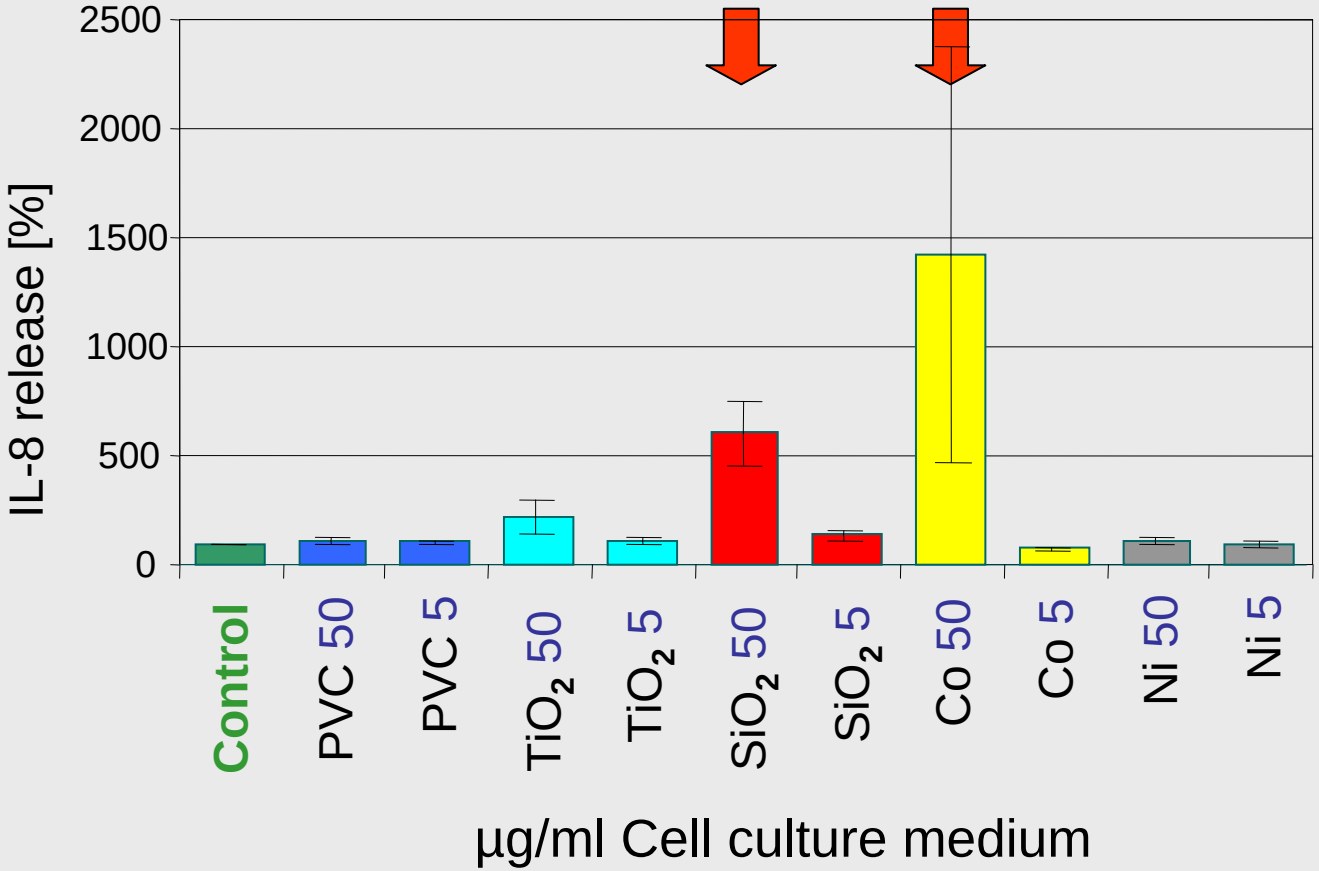


*Human mckrovascular skin-endothelial cells
„Human dermal microvascular endothelial cells“

Re: Peters et al., JMS MIM 15:319-323 (2004)

HDMEC and Nanoparticles

Pro-inflammatory Cytokines (IL-8 release)



Nanoparticle Polymeres

PVC (130 nm)

TiO_2 (20-160nm)

SiO_2 (4 – 40 nm)

Co (50 -200nm)

Ni (40- 420nm)

Re: Peters et al., JMS MIM (2004) 15: 319-323

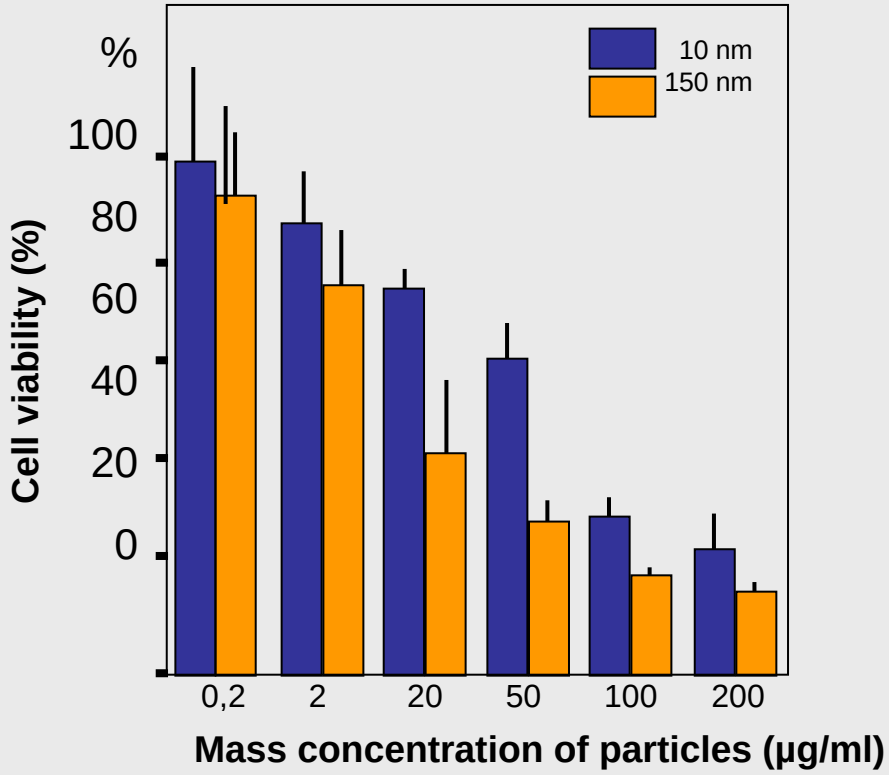
ARTICLES
PUBLISHED ONLINE: 6 NOVEMBER 2011 | DOI: 10.1038/NNANO.2011.188
nature nanotechnology

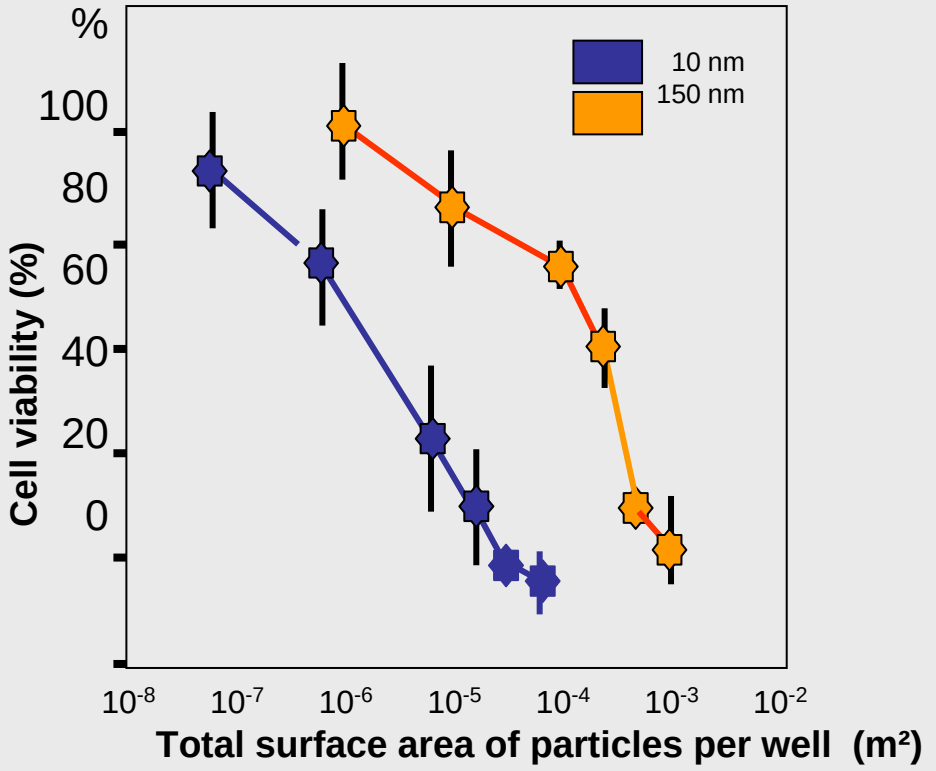
Signalling of DNA damage and cytokines across cell barriers exposed to nanoparticles depends on barrier thickness

A. Sood[1†], S. Salih[1†], D. Roh[2], L. Lacharme-Lora[1], M. Parry[1], B. Hardiman[1], R. Keehan[1], R. Grummer[3], E. Winterhager[3], P. J. Gokhale[4], P. W. Andrews[4], C. Abbott[5], K. Forbes[6], M. Westwood[6], J. D. Aplin[6], E. Ingham[7], I. Papageorgiou[7], M. Berry[8], J. Liu[8], A. D. Dick[8], R. J. Garland[9], N. Williams[9], R. Singh[10], A. K. Simon[11], M. Lewis[12], J. Ham[12], L. Roger[13], D. M. Baird[13], L. A. Crompton[14], M. A. Caldwell[14], H. Swalwell[15], M. Birch-Machin[15], G. Lopez-Castejon[16], A. Randall[17], H. Lin[18], M-S. Suleiman[18], W. H. Evans[19], R. Newson[20] and C. P. Case[1]*

The use of nanoparticles in medicine is ever increasing, and it is important to understand their targeted and non-targeted effects. We have previously shown that nanoparticles can cause DNA damage to cells cultured below a cellular barrier without crossing this barrier. Here, we show that this indirect DNA damage depends on the thickness of the cellular barrier, and it is mediated by signalling through gap junction proteins following the generation of mitochondrial free radicals. Indirect damage was seen across both trophoblast and corneal barriers. Signalling, including cytokine release, occurred only across bilayer and multilayer barriers, but not across monolayer barriers. Indirect toxicity was also observed in mice and using *ex vivo* explants of the human placenta. If the importance of barrier thickness in signalling is a general feature for all types of barriers, our results may offer a principle with which to limit the adverse effects of nanoparticle exposure and offer new therapeutic approaches.

Nano-Particle Size
- with impact on cell viability -

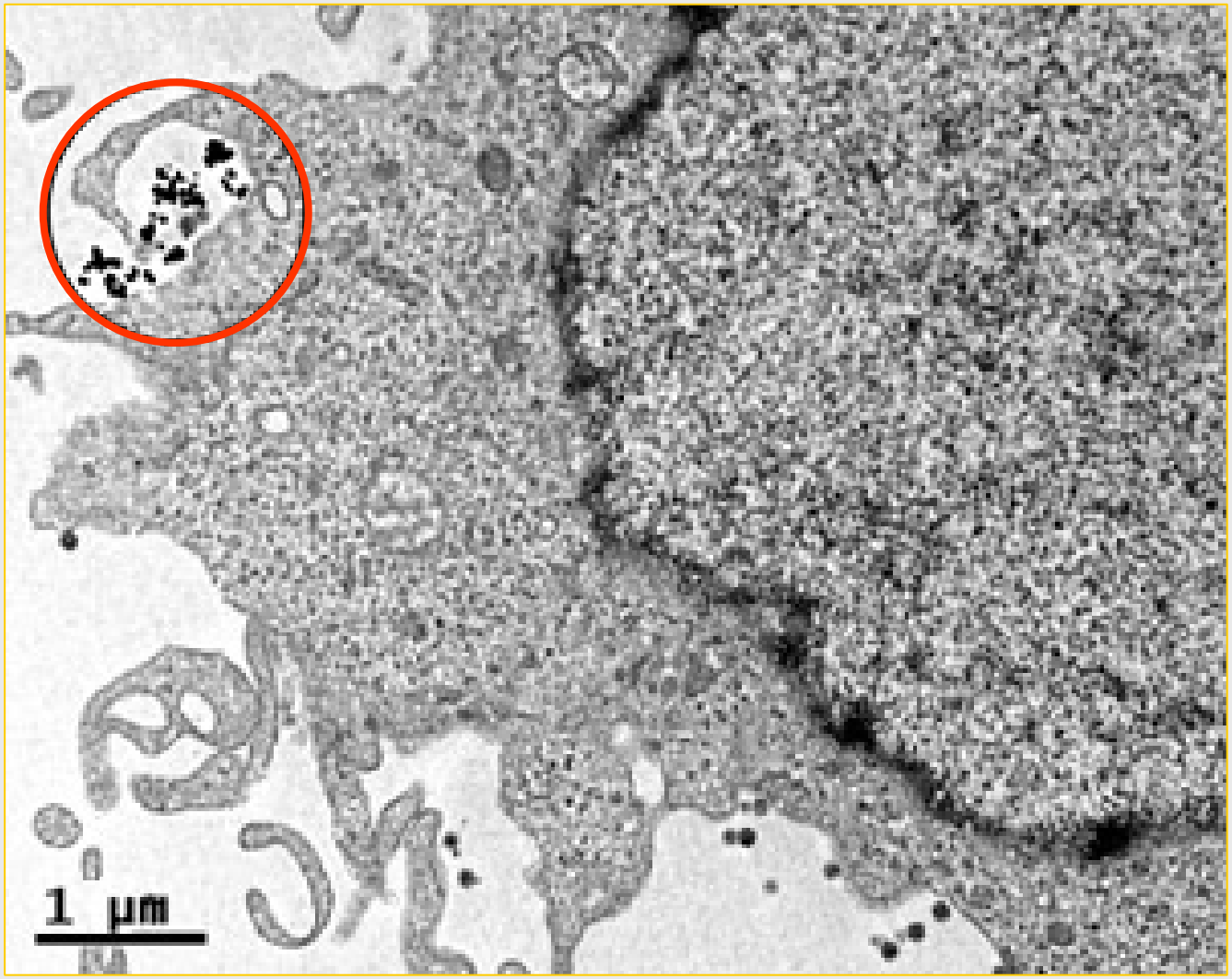




H Yin et al.,
Biomaterials, 26:5818-5826 (2005)

Phagocytosis of TiO_2 - Nanoparticles

TiO_2-Particles
∅: 30 nm

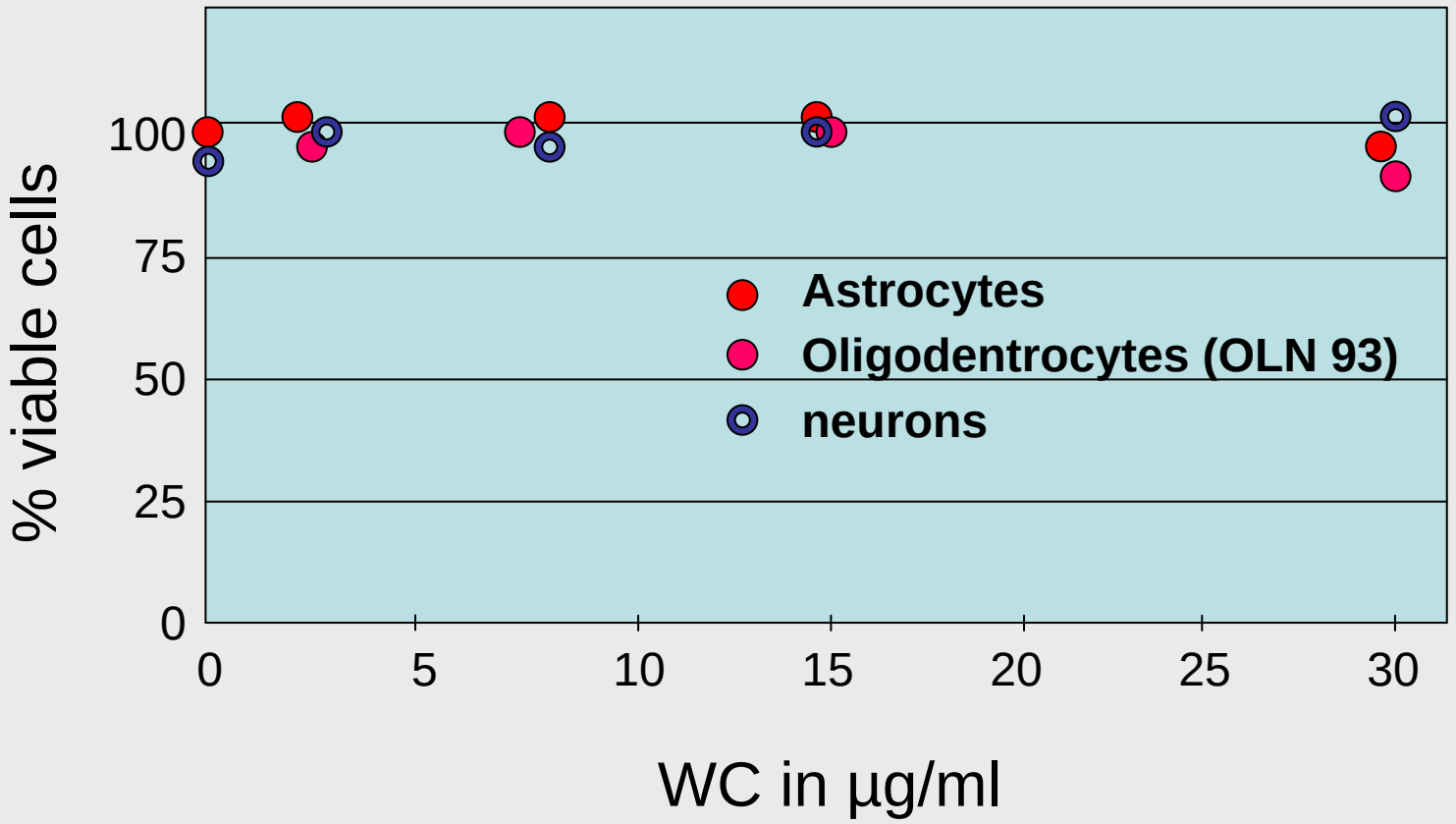


Gliacell

Re: FAZ-Sonntagszeitung
25.06.2006

Viability of WC particles exposed to rat brain cells

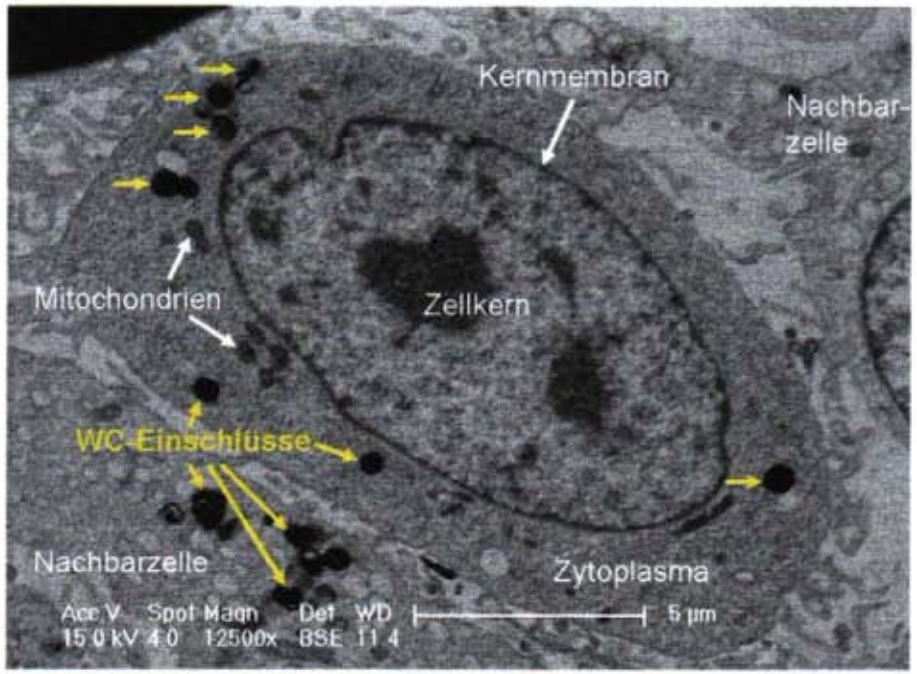
- short term viability, 2 days exposure -

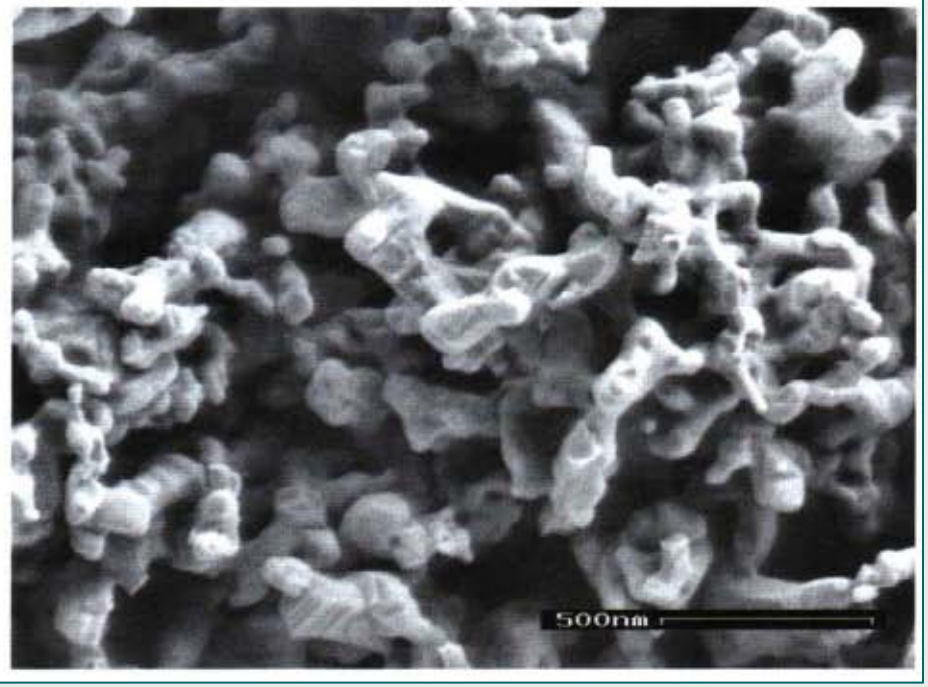


Re: A Potthoff et al.,
Solid State Phen, 15:183-189 (2009)

Gliacell and Uptake of Tungsten carbide Nanoparticles,

Synergistic effects between W and Co





Magnification: x 12.500

Tungsten carbide particles without acute toxicity when present alone.

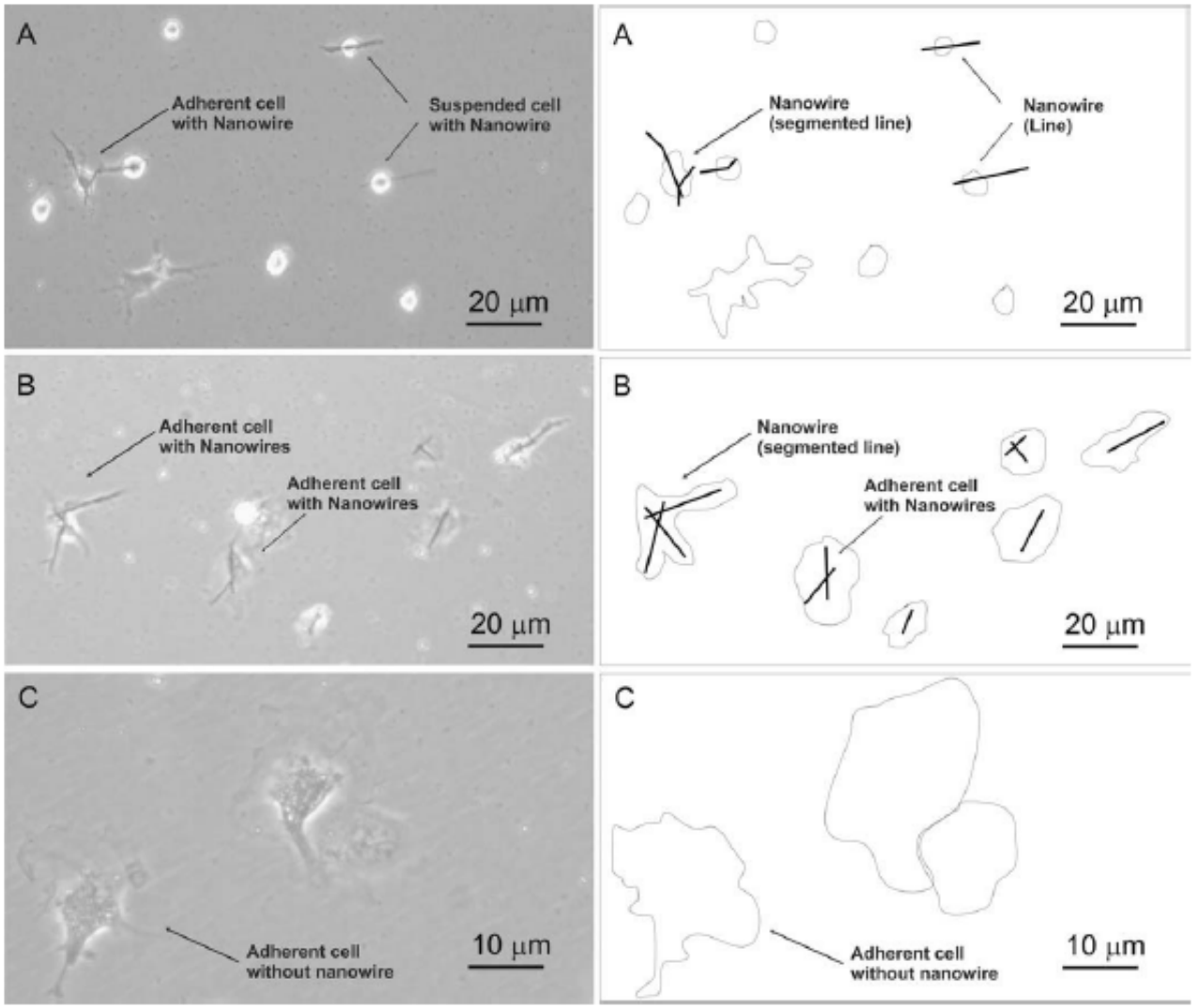
Toxicity observed, however, if Cobalt nanoparticles present.

Rat Lung Cell

Attempts to ingest a carbon nanotube

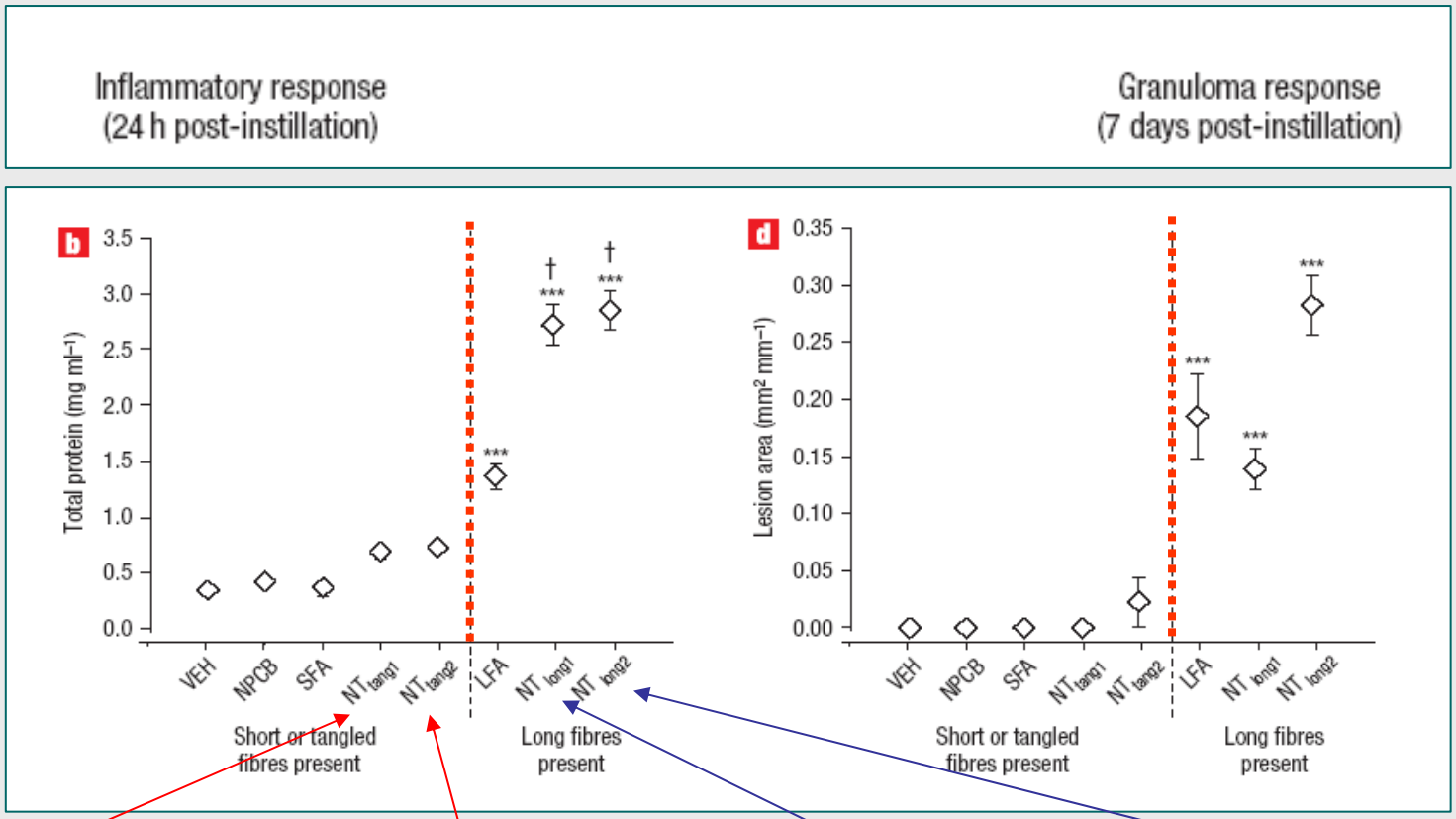
Re: R Service
Science,321:1036-37 (2008)

Internalisation of Nanofibres by living cells



Re: A Prina-Mello et al., J Nanobiotechnol, 4:9 (2006)

Fibre-length and -diameter of Nanotubes:
Determinants for inflammatory processes



	NTtang1	NTtang2	NTlong1	NTlong2
Diameter as supplied by the manufacturer (nm, mean ± s.e.m.)	15 ± 5	15 ± 5	40–50	20–100
Diameter as determined by authors (nm, mean ± s.e.m.)	14.84 ± 0.50	10.40 ± 0.32	84.89 ± 1.9	165.02 ± 4.68
Length as supplied by the manufacturer (μm)	1–5	5–20	Mean 13	Max 56

*** Carbon nanotubes inserted in the abdominal cavity of rats**
**** NT nanotubes**

Re: CA Poland et al
Nature Nanotech, 3: 423-428 (2008)

The New England
Journal of Medicine

VOLUME 346 FEBRUARY 14, 2002 NUMBER 7

PURE RED-CELL APLASIA AND ANTIERYTHROPOIETIN ANTIBODIES IN PATIENTS TREATED WITH RECOMBINANT ERYTHROPOIETIN

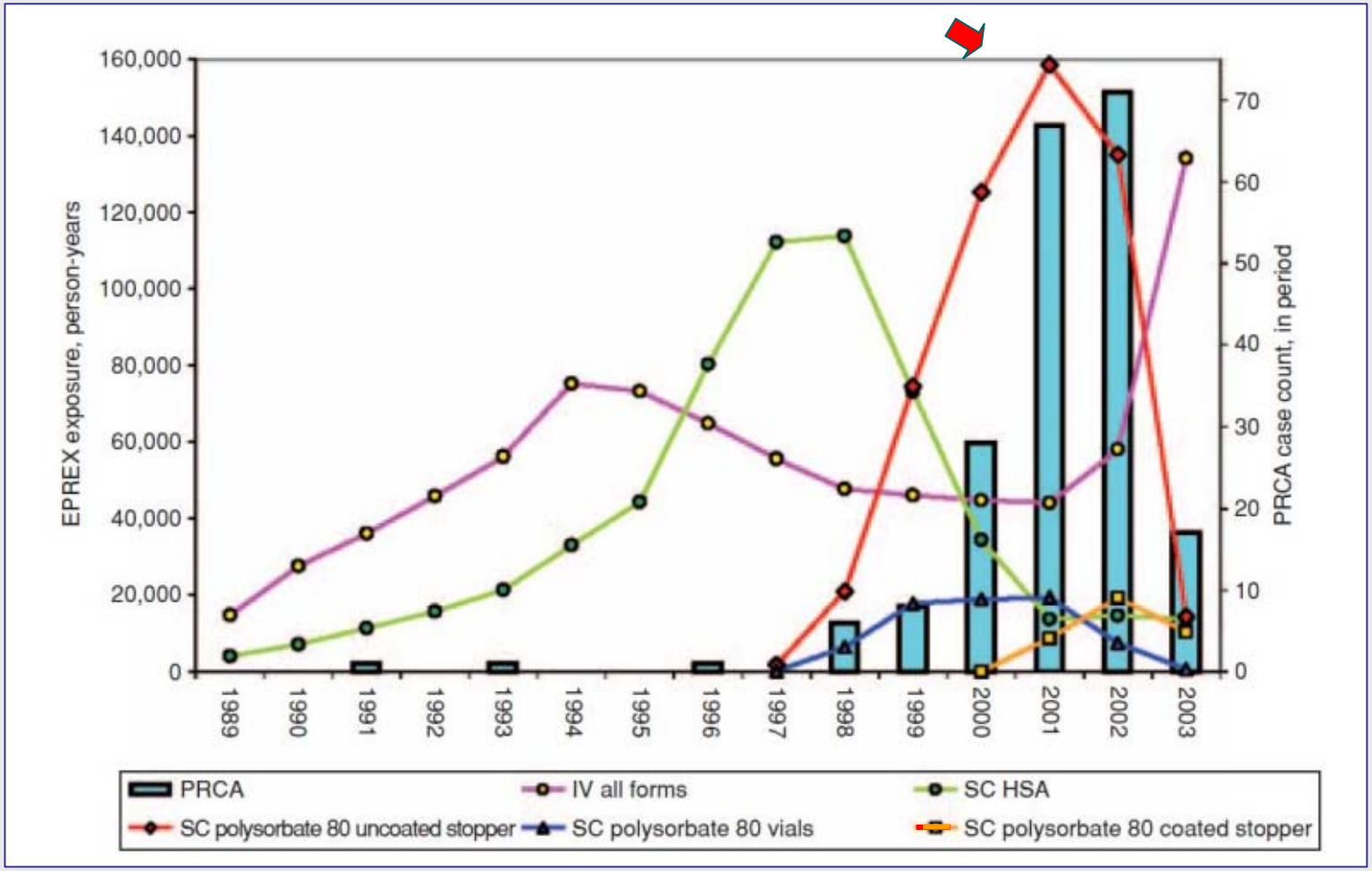
NICOLE CASADEVALL, M.D., JOELLE NATAF, M.D., BÉATRICE VIRON, M.D., AMIR KOLTA, M.D., JEAN-JACQUES KILADJIAN, M.D., PHILIPPE MARTIN-DUPONT, M.D., PATRICK MICHAUD, M.D., THOMAS PAPO, M.D., VALÉRIE UGO, M.D., IRÈNE TEYSSANDIER, B.S., BRUNO VARET, M.D., AND PATRICK MAYEUX, PH.D.

Conclusions Neutralizing antierythropoietin antibodies and pure red-cell aplasia can develop in patients with the anemia of chronic renal failure during treatment with epoetin. (N Engl J Med 2002;346: 469-75.)

The increased incidence of pure red cell aplasia with an Eprex formulation in uncoated rubber stopper syringes

KATIA BOVEN, SCOTT STRYKER, JOHN KNIGHT, ADRIAN THOMAS, MARC VAN REGENMORTEL, DAVID M. KEMENY, DAVID POWER, JEROME ROSSERT, and NICOLE CASADEVALL

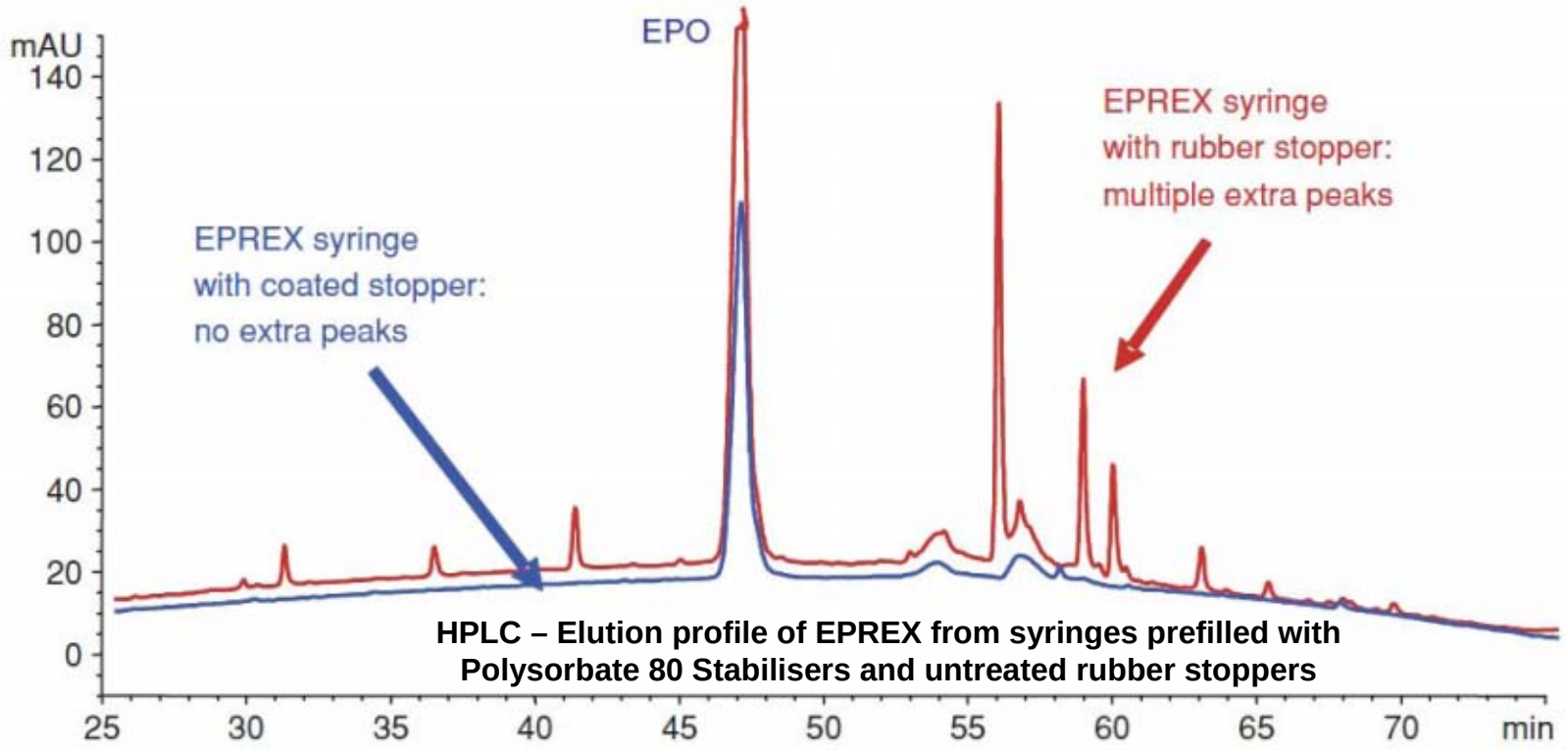
Johnson and Johnson, Pharmaceutical Research and Development, L.L.C, Raritan, New Jersey; Centre National de la Recherche Scientifique, Ecole Supérieure de Biotechnologie de Strasbourg, France; Department of Microbiology, National University of Singapore, Singapore; Kidney Laboratory, Austin Research Institute, Austin, Australia; Service de Néphrologie, Hôpital Tenon, Paris, France; and Service d'Hématologie Biologique, Hôpital Hôtel-Dieu, Paris, France



K Boven et al.,
Kidney Int, 67:2346 - 2353 (2005)

Aplasia (PRCA) in Dialysis Patients

- Analysis of Extractables by „Reverse Phase HPLC“ -



HPLC – Elution profile of EPREX from syringes prefilled with Polysorbate 80 Stabilisers and untreated rubber stoppers

PRCA:

- **acute severe isolated Anaemia**
- **no red precursor cells in bone marrow**
- **Reticulocyte number < $10x10^9$ / L**

K Boven et al., Kidney Int, 67:2346-2353 (2005)

Kidney International, Vol. 67 (2005), pp. 2346–2353

The increased incidence of pure red cell aplasia with an Eprex formulation in uncoated rubber stopper syringes

KATIA BOVEN, SCOTT STRYKER, JOHN KNIGHT, ADRIAN THOMAS, MARC VAN REGENMORTEL, DAVID M. KEMENY, DAVID POWER, JEROME ROSSERT, and NICOLE CASADEVALL

Johnson and Johnson, Pharmaceutical Research and Development, L.L.C, Raritan, New Jersey; Centre National de la Recherche Scientifique, Ecole Supérieure de Biotechnologie de Strasbourg, France; Department of Microbiology, National University of Singapore, Singapore; Kidney Laboratory, Austin Research Institute, Austin, Australia; Service de Néphrologie, Hôpital Tenon, Paris, France; and Service d'Hématologie Biologique, Hôpital Hôtel-Dieu, Paris, France

The increased incidence of pure red cell aplasia with an Eprex formulation in uncoated rubber stopper syringes.

Background. The incidence of pure red cell aplasia (PRCA) in chronic kidney disease patients treated with epoetins increased substantially in 1998, was shown to be antibody mediated, and was associated predominantly with subcutaneous administration of Eprex®. A technical investigation identified organic compounds leached from uncoated rubber stoppers in prefilled syringes containing polysorbate 80 as the most probable cause of the increased immunogenicity.

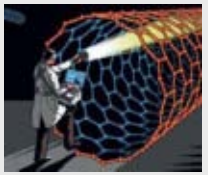
Methods. This study investigated whether the incidence of PRCA was higher for exposure to the product form contain-

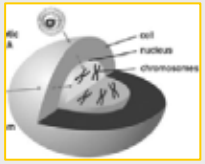
Pure red cell aplasia (PRCA) is a rare disorder that manifests itself as a severe, isolated anemia of sudden onset, characterized by an almost complete absence of red cell precursors in the bone marrow and a reticulocyte count below 10×10^9/L [1]. Many potential causes for PRCA have been reported, but most concern only isolated case reports, and about 50% of cases have no known cause [2]. Over the decade following its introduction in 1989, three cases of PRCA were associated with recombinant human erythropoietin (epoetin) treatment for

Conclusion. The epidemiologic data, together with the chemical and immunologic data, support the hypothesis that leachates from uncoated rubber syringe stoppers caused the increased incidence of PRCA associated with Eprex. Currently, all Eprex prefilled syringes contain fluoro-resin coated stoppers, which has contributed to decreased incidence of PRCA with continued surveillance.

Toxicity of nano-structured biomaterials at the cellular level

- Biomaterials in medical application
  - Facts and figures
- Biomaterial properties
  - Surface-related observations
  - Effects on the cellular level
- Conclusion





Possible Pathological Risks of Nanoparticles

Experimental NM effects	Possible pathophysiological outcomes
ROS generation*	Protein, DNA and membrane injury,* oxidative stress†
Oxidative stress*	Phase II enzyme induction, inflammation,† mitochondrial perturbation*
Mitochondrial perturbation*	Inner membrane damage,* permeability transition (PT) pore opening,* energy failure,* apoptosis,* apo-necrosis, cytotoxicity
Inflammation*	Tissue infiltration with inflammatory cells,† fibrosis,† granulomas,† atherogenesis,† acute phase protein expression (e.g., C-reactive protein)
Uptake by reticulo-endothelial system*	Asymptomatic sequestration and storage in liver,* spleen, lymph nodes,† possible organ enlargement and dysfunction
Protein denaturation, degradation*	Loss of enzyme activity,* auto-antigenicity
Nuclear uptake*	DNA damage, nucleoprotein clumping,* autoantigens
Uptake in neuronal tissue*	Brain and peripheral nervous system injury
Perturbation of phagocytic function,* "particle overload," mediator release*	Chronic inflammation,† fibrosis,† granulomas,† interference in clearance of infectious agents†
Endothelial dysfunction, effects on blood clotting*	Atherogenesis,* thrombosis,* stroke, myocardial infarction
Generation of neoantigens, breakdown in immune tolerance	Autoimmunity, adjuvant effects
Altered cell cycle regulation	Proliferation, cell cycle arrest, senescence
DNA damage	Mutagenesis, metaplasia, carcinogenesis

A Nel et al.
Science, 311:622-627 (2006)

Questions to be answered

- How to standardise test-procedures and assays for risk analysis?
- Are there dose-response principles?
  - Individual particles / Agglomerates?
  - Mass or particlenumber?
  - Limits for cell activation?
  - *In vitro* / *in vivo* differences
  - Biokinetiks? Bioburden?

- **Criteria for approval of medical devices: Should they refer to case-related situation or be defined by a general approach?**

Impact factors for market success of nanoscaled medical devices

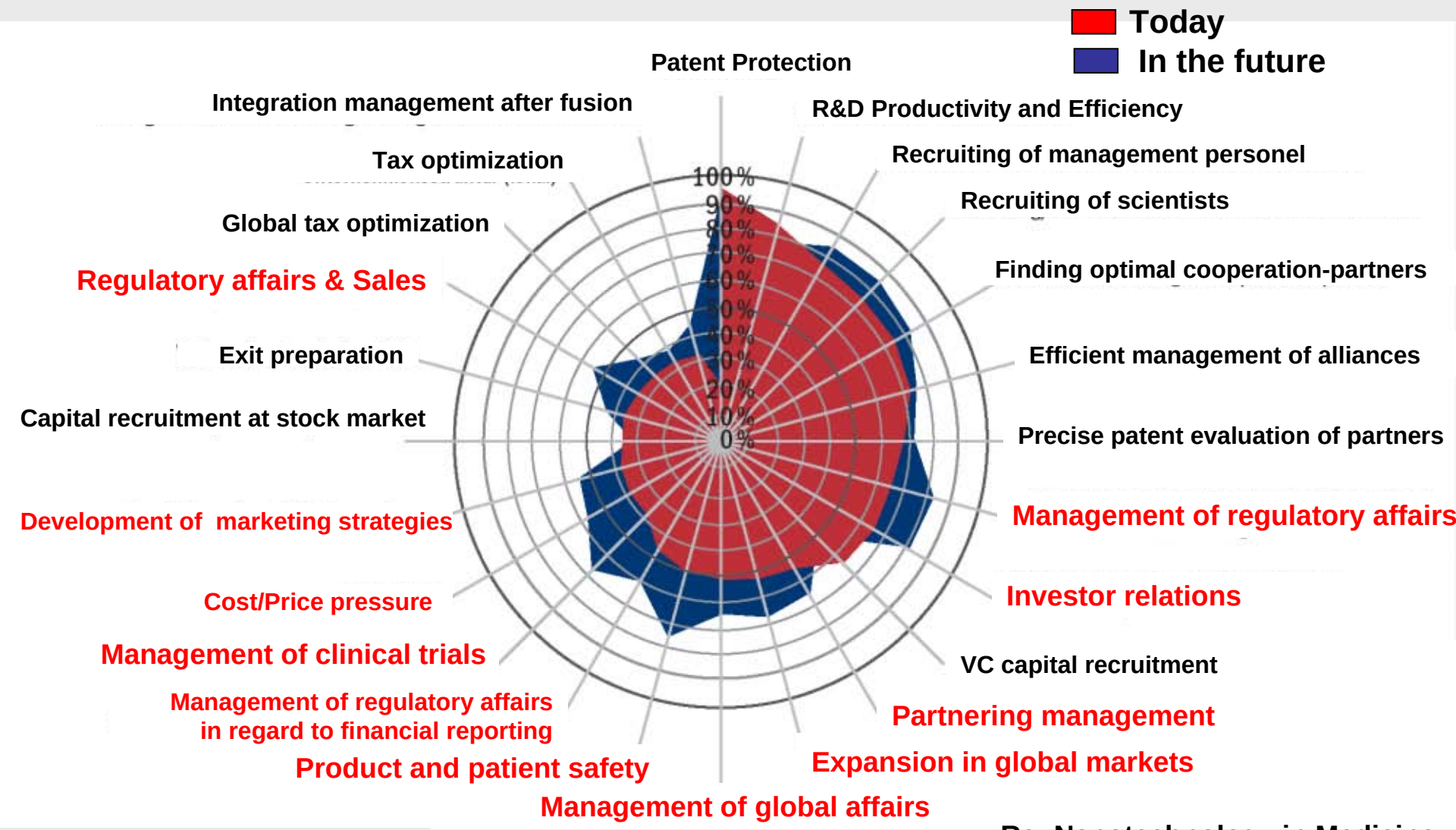
Approval:
Harmonisation of approval procedures and shortening of approval time

Safety: Acute *vs* chronic effects

Definitions:
A nanoparticle must have been engineered
vs
A nanoparticle can be a leachable

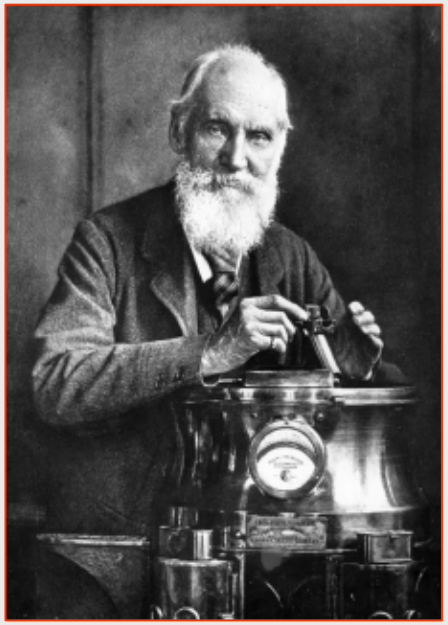
Funding Spin-off companies / SMEs
Exclusivity reasonable?
or: model to be envisaged that involves large multinational companies

Challenges for NanoMed-Tech Companies Strategy and Operations, an enquiry.



Re: Nanotechnology in Medicine
Report Ernst & Young 2007

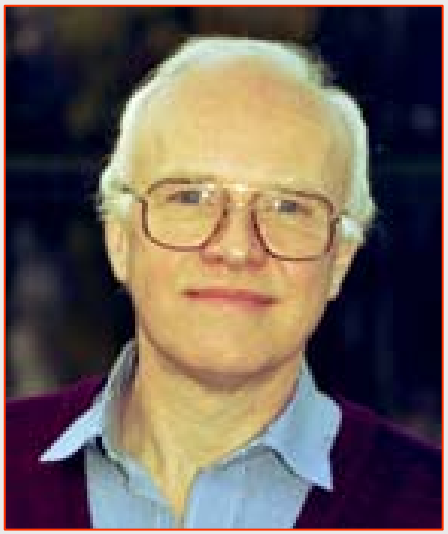
The Experts´ Opinion
“Who measures is right!”



“When you can measure what you are speaking about, and express it into numbers, you know something about it!

When you cannot express it in numbers your knowledge is of meager and unsatisfactory kind.“

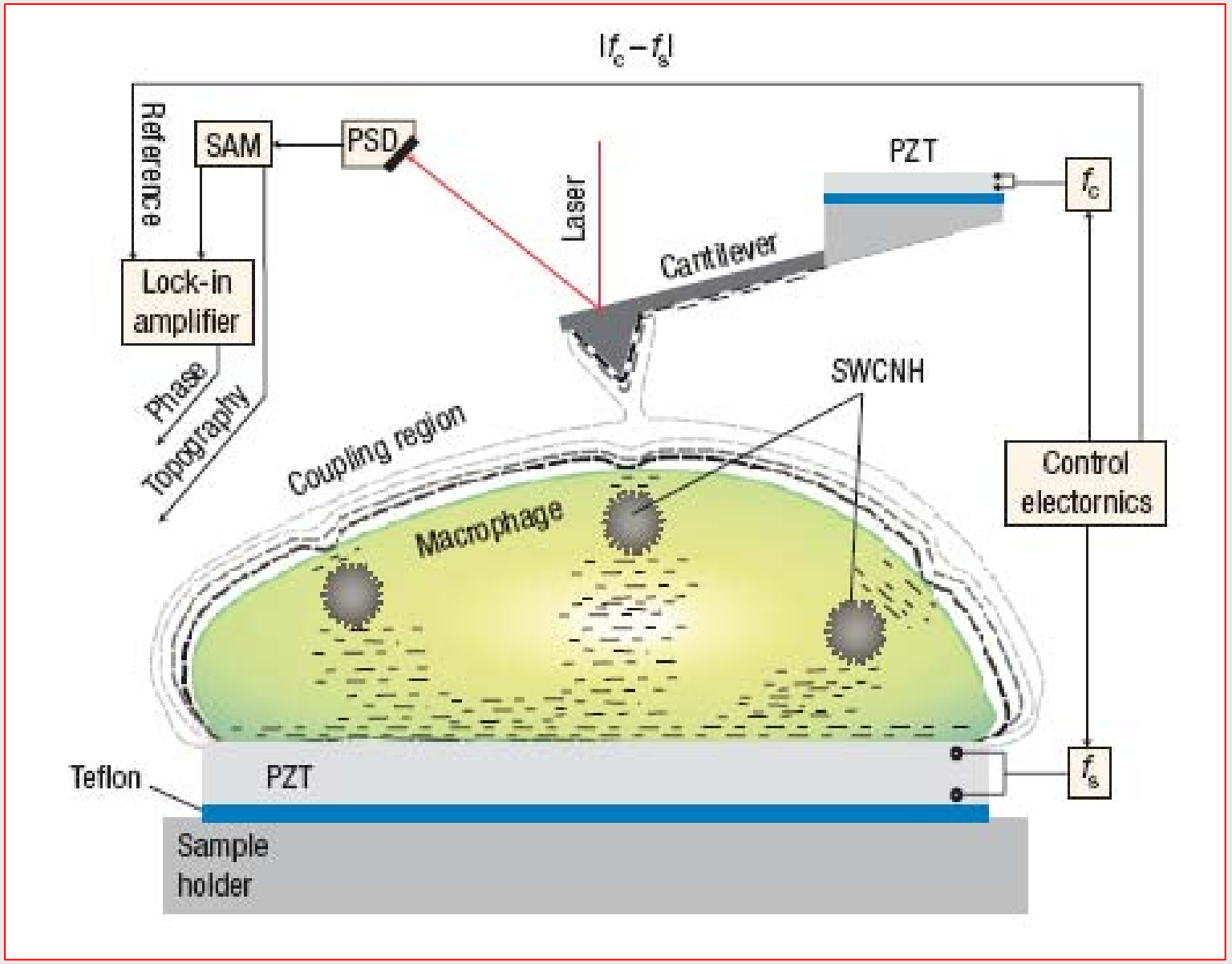
Lord Kelvin, physicist (1824 – 1907)



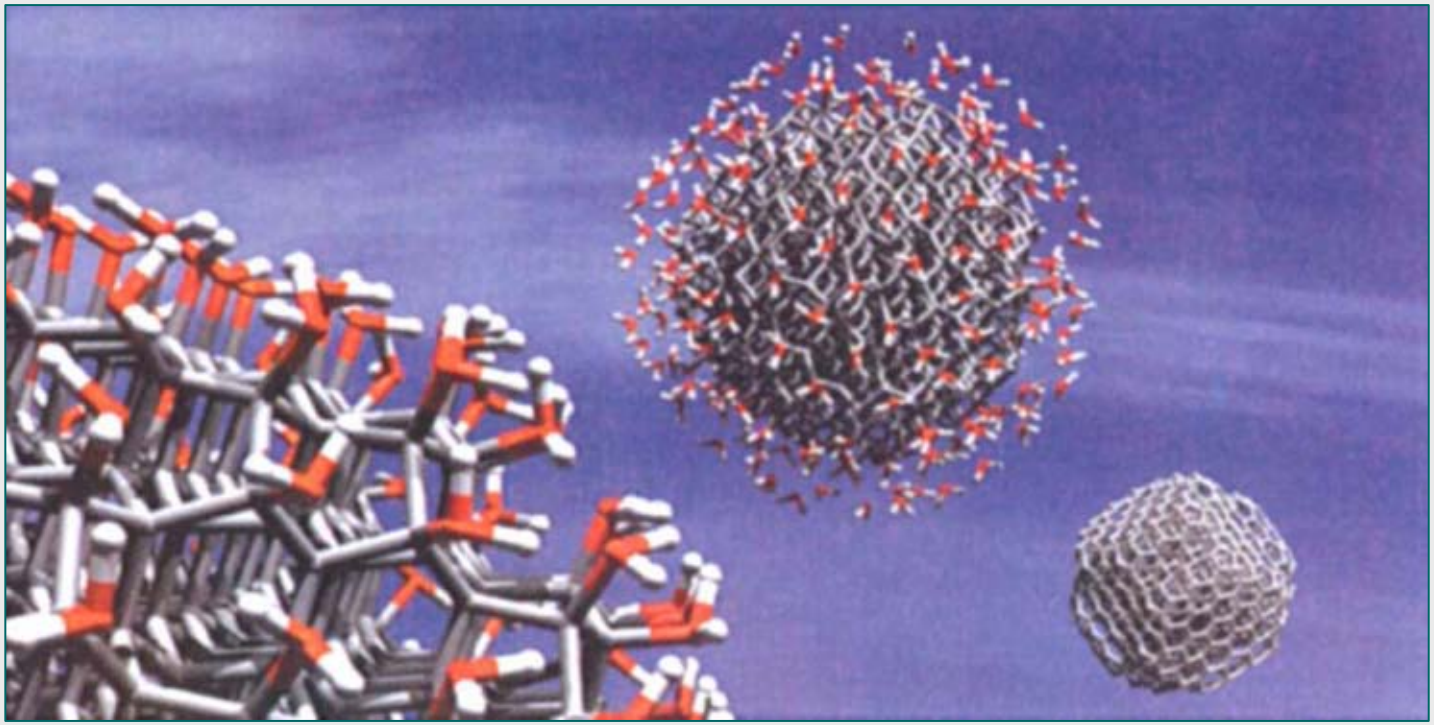
"You cannot **control** what you cannot **measure**."

Tom deMarco, modern software-guru, (*1940)

Detection of Nanoparticles in Biological Cells



Re: L Tetard et al., Nature Nanotechn, 3:505-505 (2008)



A Riskfactor is not a disease!

R Glassock & C Winearls
Nephron Clin Pract, 110:c39-c47 (2008)