Physico-chemical characterization of nanoparticles and its relation to their bio-interactions

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UC Lead Campus Program for Nanotoxicology Research and Training

Anderson School of
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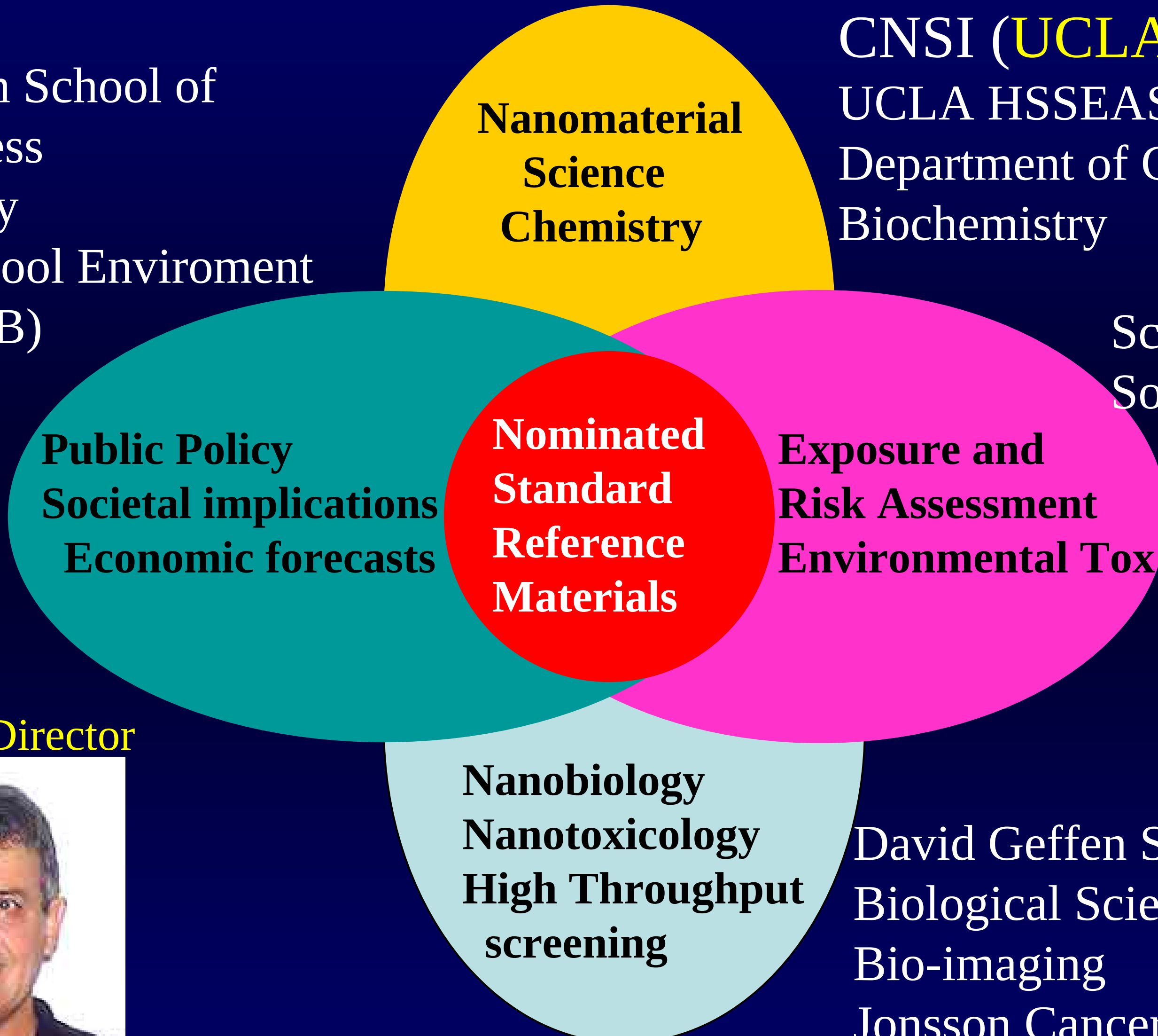
UCLA HSSEAS (Engineering)
Department of Chemistry &
Biochemistry



Sch Public Health
Southern Cal Particle
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Scientific Director

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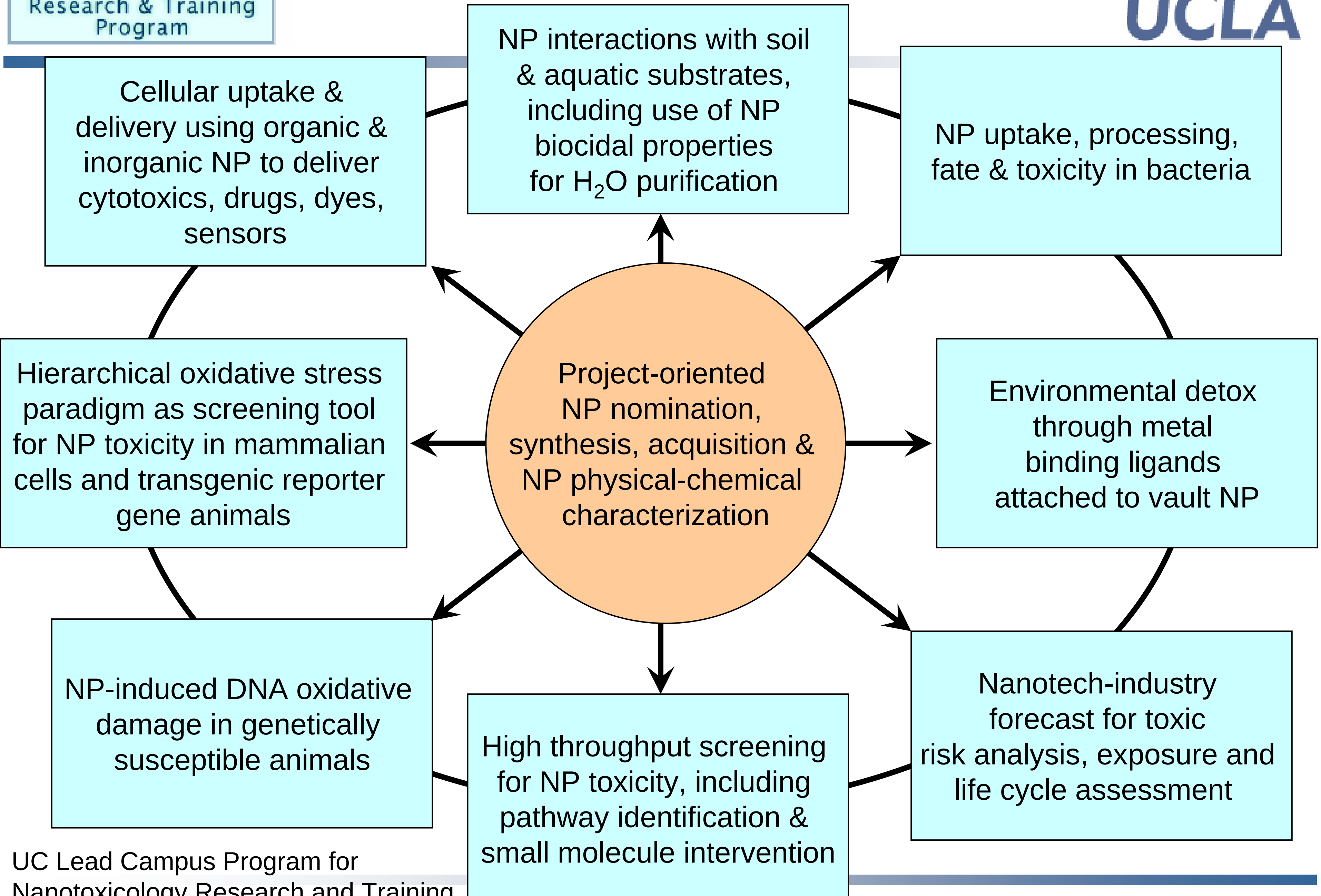
Cellular uptake & delivery using organic & inorganic NP to deliver cytotoxics, drugs, dyes, sensors
NP interactions with soil & aquatic substrates, including use of NP biocidal properties for H_2O purification
NP uptake, processing, fate & toxicity in bacteria
Hierarchical oxidative stress paradigm as screening tool for NP toxicity in mammalian cells and transgenic reporter gene animals
Project-oriented NP nomination, synthesis, acquisition & NP physical-chemical characterization
Environmental detox through metal binding ligands attached to vault NP
NP-induced DNA oxidative damage in genetically susceptible animals
High throughput screening for NP toxicity, including pathway identification & small molecule intervention
Nanotech-industry forecast for toxic risk analysis, exposure and life cycle assessment

Nanotoxicology

Definition: The study of the potential biological threat to humans and the ecosystem through widespread use of nanomaterials

Cornerstones: Nanomaterial testing, toxicological assays, exposure assessment, risk assessment, dosimetry, social policy, regulatory decision making

A. Nel workshop presentation on Bio-physicochemical Interactions of Engineered Nanomaterials September 9 - 11, 2007 at CNSI/UCLA

Classical Toxicological Approach

1. Nomination of the Chemical to be studied
2. Data gathering/profiling (in vitro, in vivo, clinical)
   …attempt to classify as potentially hazardous/safe
3. If considered potentially hazardous:
   a. Animals studies (time-length & dose exposures)
      Outcomes: General health, disease, pathology

   b. Tissue culture: mechanisms, biological pathways
   c. ADME (absorption, dose, metabolism, excretion)
4. Risk assessment = hazard + exposure

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How to test for Nanomaterial Toxicology: Some Sobering Thoughts in dealing with Industrial Chemical Compounds

80,000 chemicals registered for commercial use in the US

Only 530 have undergone long-term and 70 short-term testing by the National Toxicology Program

The resource-intensive nature of traditional tox studies puts the cost of each study at $2 to $4 million and takes > 3 years to complete (major cost factor animal studies)

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Can a scientific model be developed that predicts which materials are potentially hazardous ?

Mechanism/paradigms of toxicity

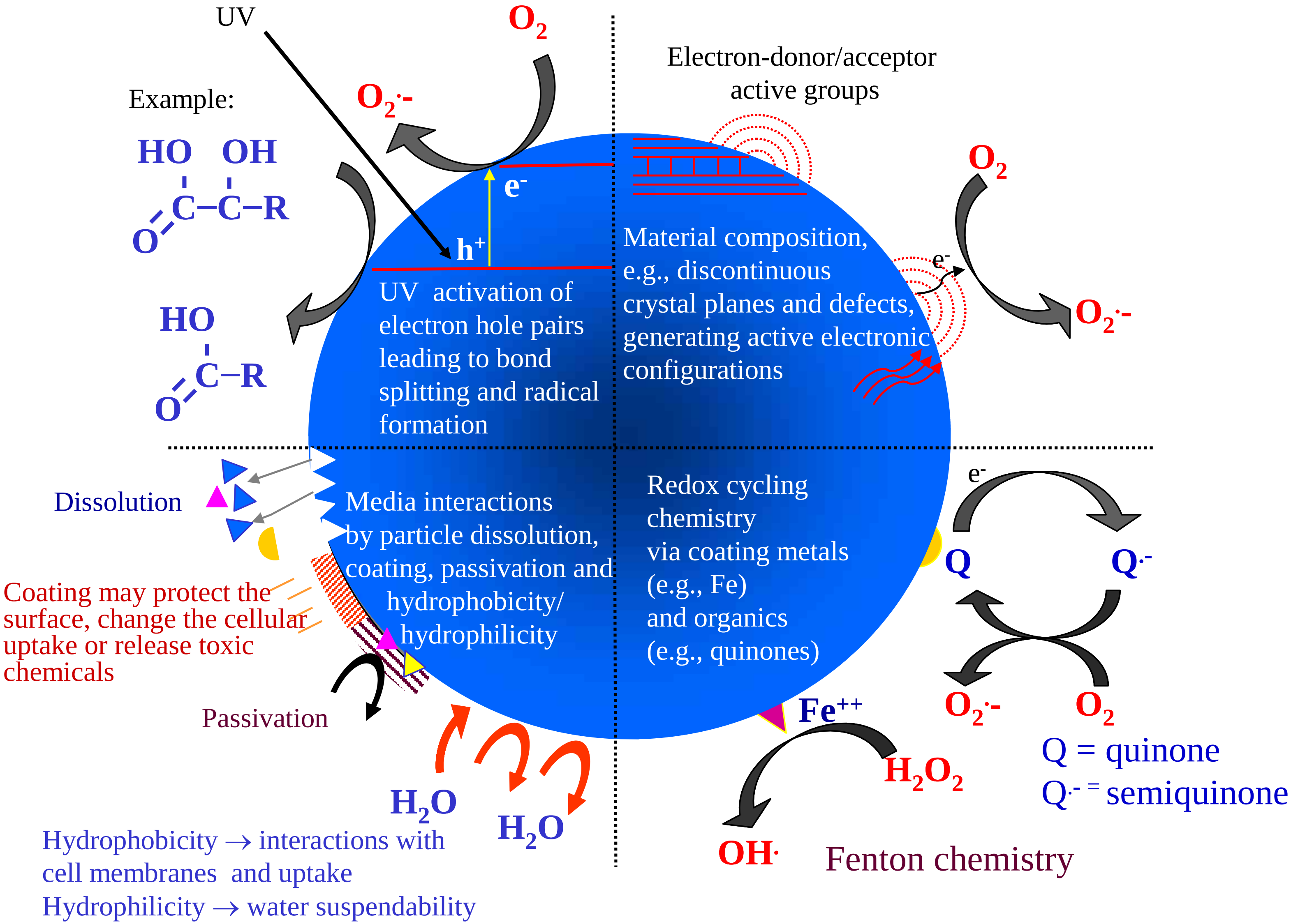
High throughput screening studies: abiotic, biotic

Biocompatible vs bioadverse response pathways

Potentially hazardous

Animal

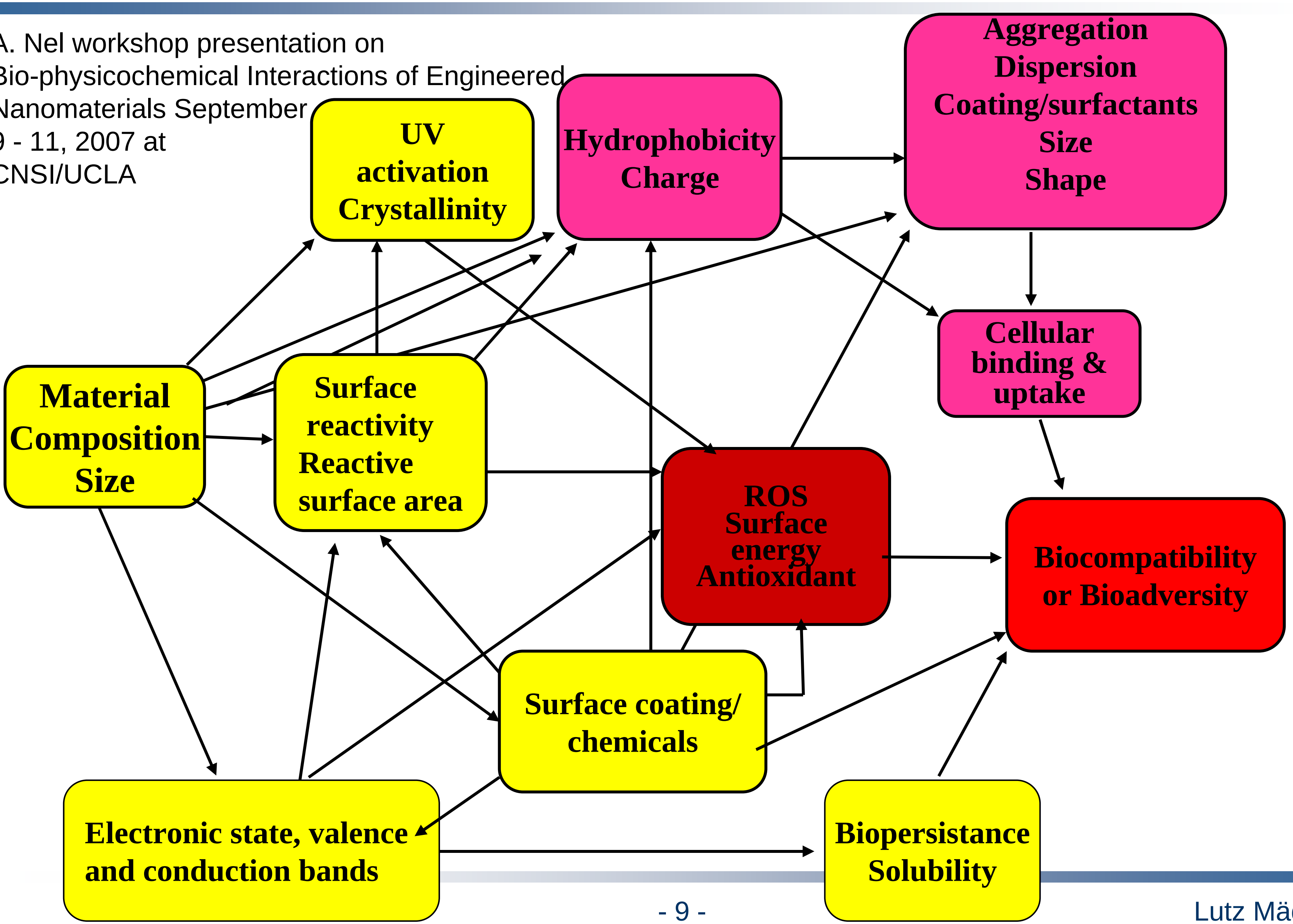
Toxic potential of materials at the nanolevel



Nel, A., Xia, T., Mädler, L., Li, N., *Science* **311** 622-627 (2006).

Module relating particle physical-chemical characteristics to biocompatibility and bioadversity

A. Nel workshop presentation on Bio-physicochemical Interactions of Engineered Nanomaterials September 9 - 11, 2007 at CNSI/UCLA



Gas phase synthesis of particles

Product Particles	Volume t/y	Ind.Process (dominant)	Use (exemplary)
Carbon black	**8 M**	**Vapor Flame**	**Inks, Rubber**
Titania	**2 M**	**Vapor Flame**	**Paints**
Fumed Silica	**0.2 M**	**Vapor Flame**	**Toothpaste, Tires**

Chemical Economics Handbook, 2001; direct industrial quotes

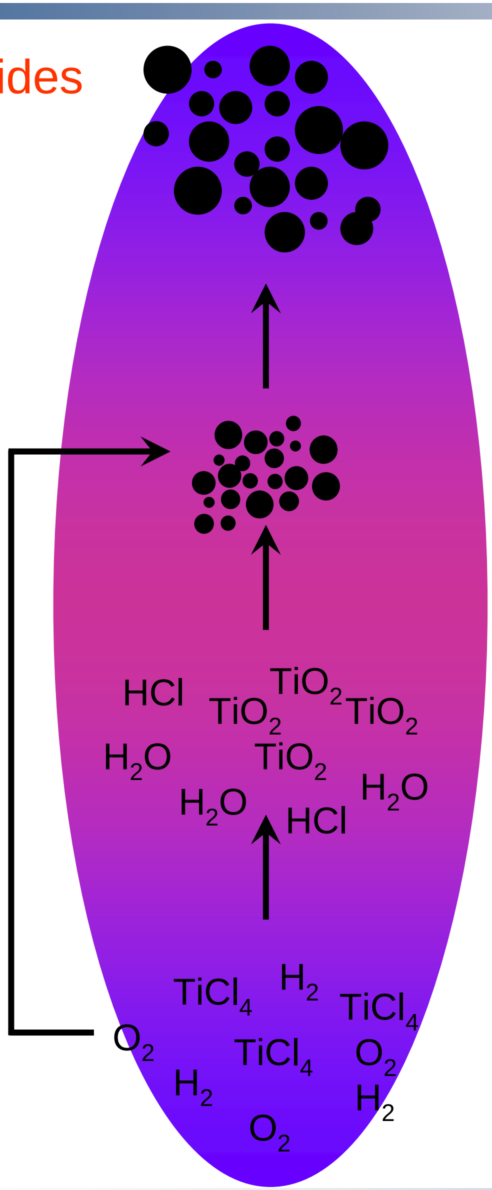
Vapor Flame (Gas-to-particle)

Flame provides

High T_{max}

Large $\frac{\partial T}{\partial t}$

Chemical Reaction & Coagulation



Powder

Coagulation
Aggregation
Agglomeration

$$\frac{\partial n(v,t)}{\partial t} = \frac{1}{2}\int_0^v \beta(v', v-v')n(v',t)n(v-v',t)dv' - n(v,t)\int_0^\infty \beta(v,v')n(v',t)dv'$$

Coalescence

$$\frac{da}{dt} = -\frac{1}{\tau}(a - a_s)$$

Product Particles

Nucleation & Surf. Reaction

$$\frac{\partial n(v,t)}{\partial t} = S\delta(v - v_0) - \frac{\partial}{\partial v}[g(v)n(v,t)]$$

Product Molecules & Clusters

Chemical Reaction

Reactant Vapor/Gas Molecules

Vapor flame materials

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac															

La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

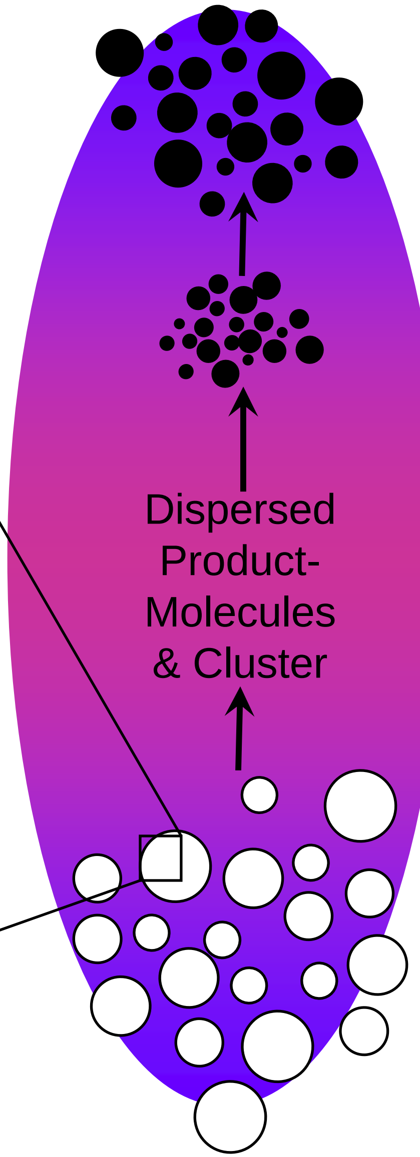
Spray Flame (Droplet-to-particle)

Liquid supplies:

- Energy
- Material (precursor)

Liquid ensures:

- Mixing
- Release



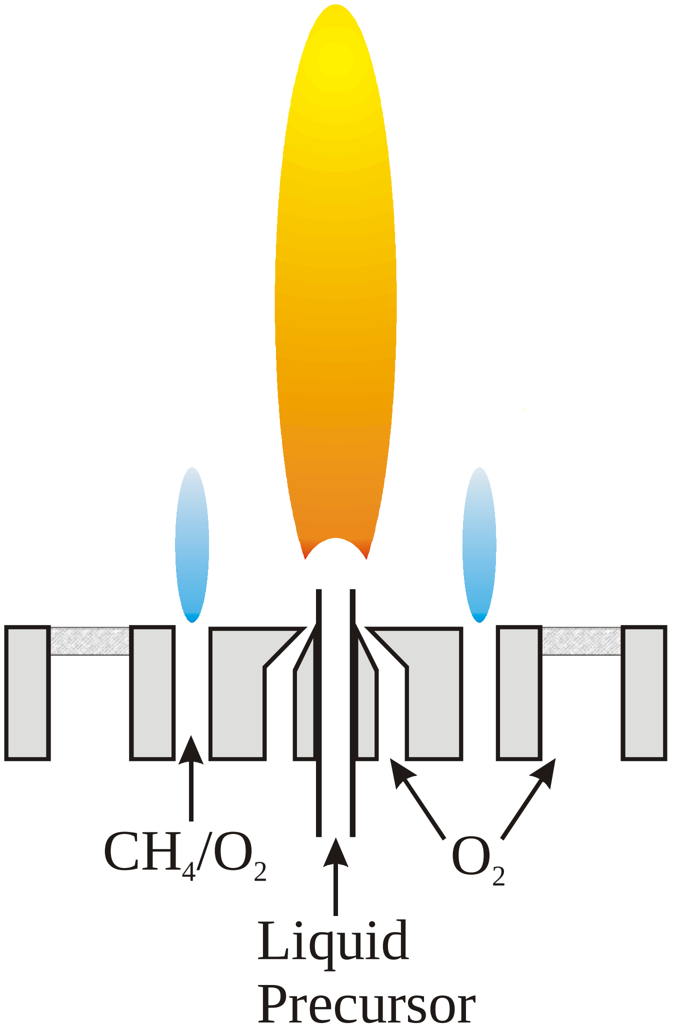
Flame provides

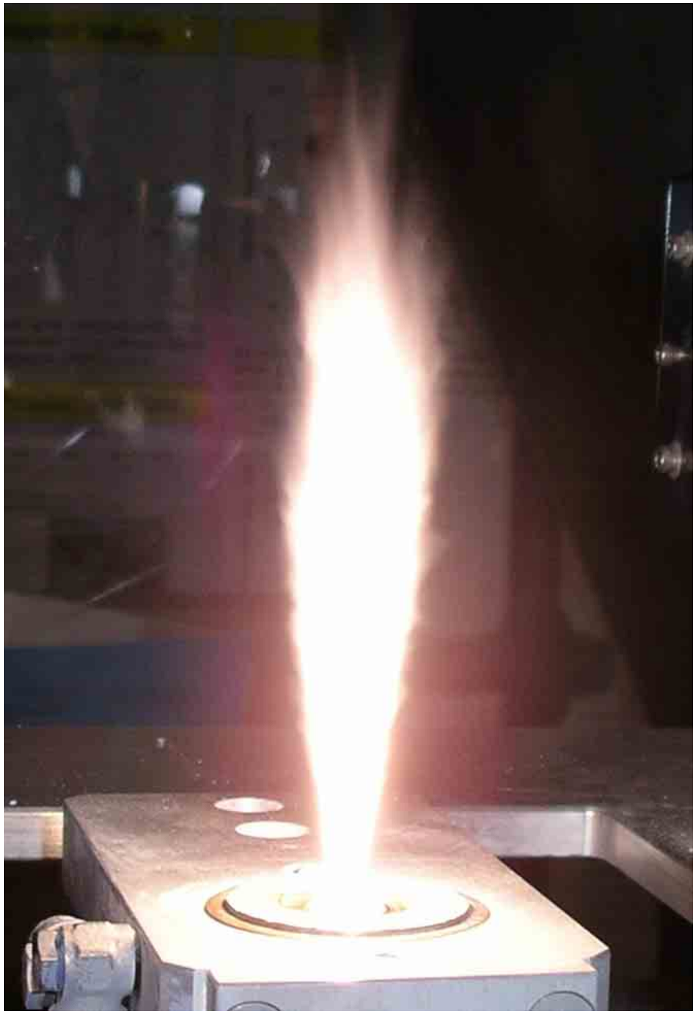
High T_{max}

Large $\frac{\partial T}{\partial t}$

Mädler, L., KONA, **22** 107-120 (2004).

Flame Spray Pyrolysis (FSP) set-up

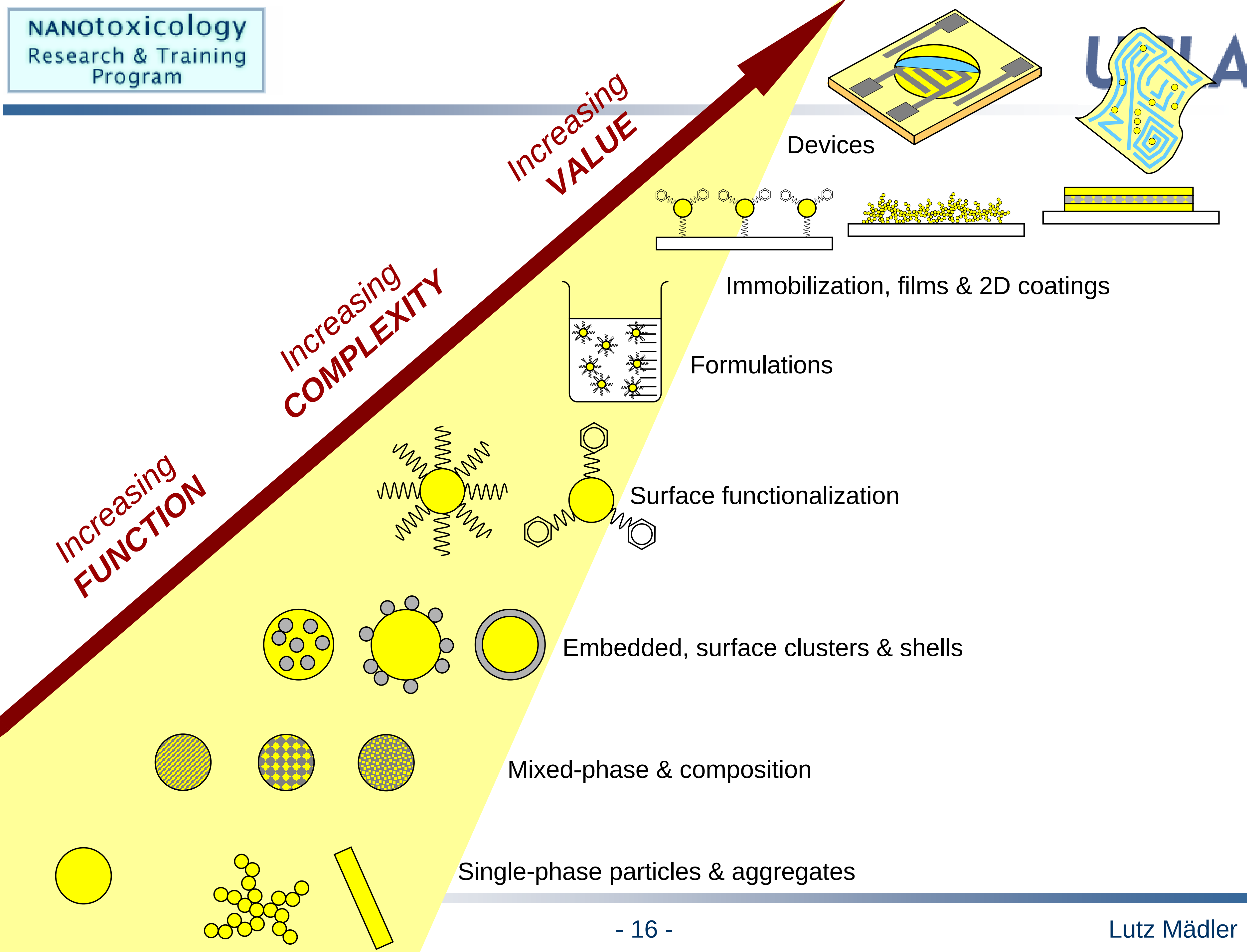




Mädler, L., Kammler, H.K., Mueller, R., and Pratsinis, S.E., *J. Aerosol Sci.* **33** (2) 369-389 (2002).

Preliminary nanomaterial nominations

Name	Formula	Main use	Production (tons/ year)	Primary synthesis method
Carbon black	C amorphous	Tire manufacture, pigments	8×10^6	Aerosol flame
Titania	TiO_2, anatase, rutile	Pigments, UV-absorber, catalyst	2×10^6	Aerosol flame, sol gel
Zinc oxide	ZnO_2	Polymer filler, UV-absorber	0.6×10^6	Polymer filler, UV-absorber Aerosol evaporation/ oxidation, sol gel
Ceria	CeO_2 / Ce_2O_3	Catalyst (cars), CMP polishing	$0.01\ 10^6$	sol gel, serosol flame
Silica	SiO_2 amorphous	Dispersant, and flowing agent	$> 0.2\ 10^6$	Aerosol flame, sol gel
Zirconia	ZrO_2	Ceramic, catalyst support		Aerosol flame, Sol gel
Quantum dots	CdSe/ZnS/InAs/InP/InGaP	Medical imaging	1-4 uM	Wet chemistry
Carbon nanotubes	C ordered structure	Composite filler, electronic applications	??	Gas phase synthesis on catalyst
Fullerenes	C ordered structure	medical applications	??	Wet chemistry


Increasing
FUNCTION
Increasing
COMPLEXITY
Increasing
VALUE
Devices
Immobilization, films & 2D coatings
Formulations
Surface functionalization
Embedded, surface clusters & shells
Mixed-phase & composition
Single-phase particles & aggregates

New view on Characterization

Traditional Approach

- Composition (molecular structure
- Melting Point
- Boiling Point
- Vapor Pressure
- pH
- Solubility
- Octanol/Water Part.
- Soil/Water Part.

Nanoparticle characterization

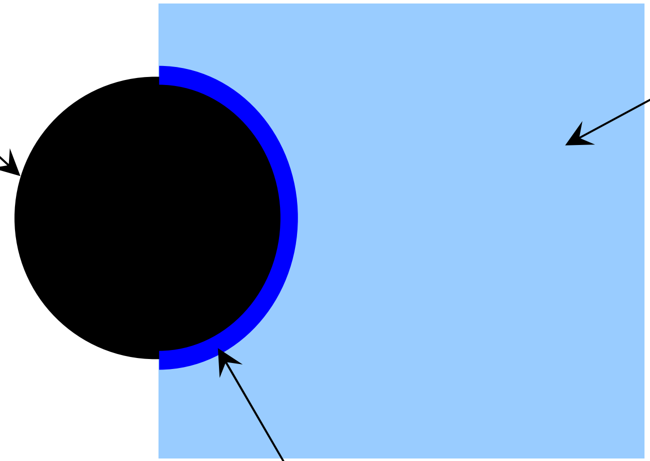
- Surface Area and Porosity
- Particle size distribution
- Solubility
- Aggregation
- Hydrated surface analysis
- Zeta Potential
- Wettability
- Adsorption Potential
- Shape, size of interactive surface

partly from F. Klaessig (degussa) and from the workshop summary on
Bio-physicochemical Interactions of Engineered Nanomaterials
September 9 - 11, 2007 at CNSI/UCLA

Surface properties of nanoparticles and solution chemistry

Nanoparticle

- Surface charge
- Surface porosity, roughness
- Functional groups
- Conjugated organic molecules



Suspending media

- Hydration
- Ion adsorption
- Double layer formation
- Charge and number of counter ions
- Steric molecules

Solid / liquid interface

- lyophobic / lyophilic systems
- Surfactant adsorption
- Net charge of particle (iso-electric point)

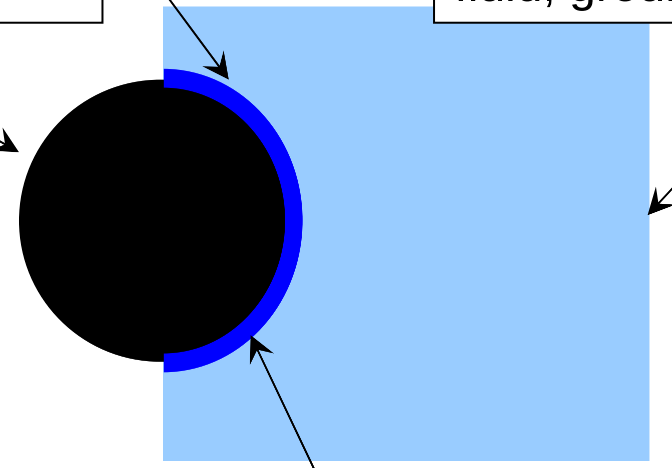
↔

- Surface energy / surface curvature
- Bio-molecule binding
- Modified surface charge
- Leading to particle-particle attraction or repulsion

Surface properties of nanoparticles and solution chemistry

- Can we predict the interface by knowing the particle and media properties

- What media can mimic important interactions (cell compartments, lung fluid, groundwater)?



- What do we need to measure?
- How accessible is the surface for measurements?
- How accurate are current theoretical models?

Possible contribution of surface energy to the NanoBio Interface

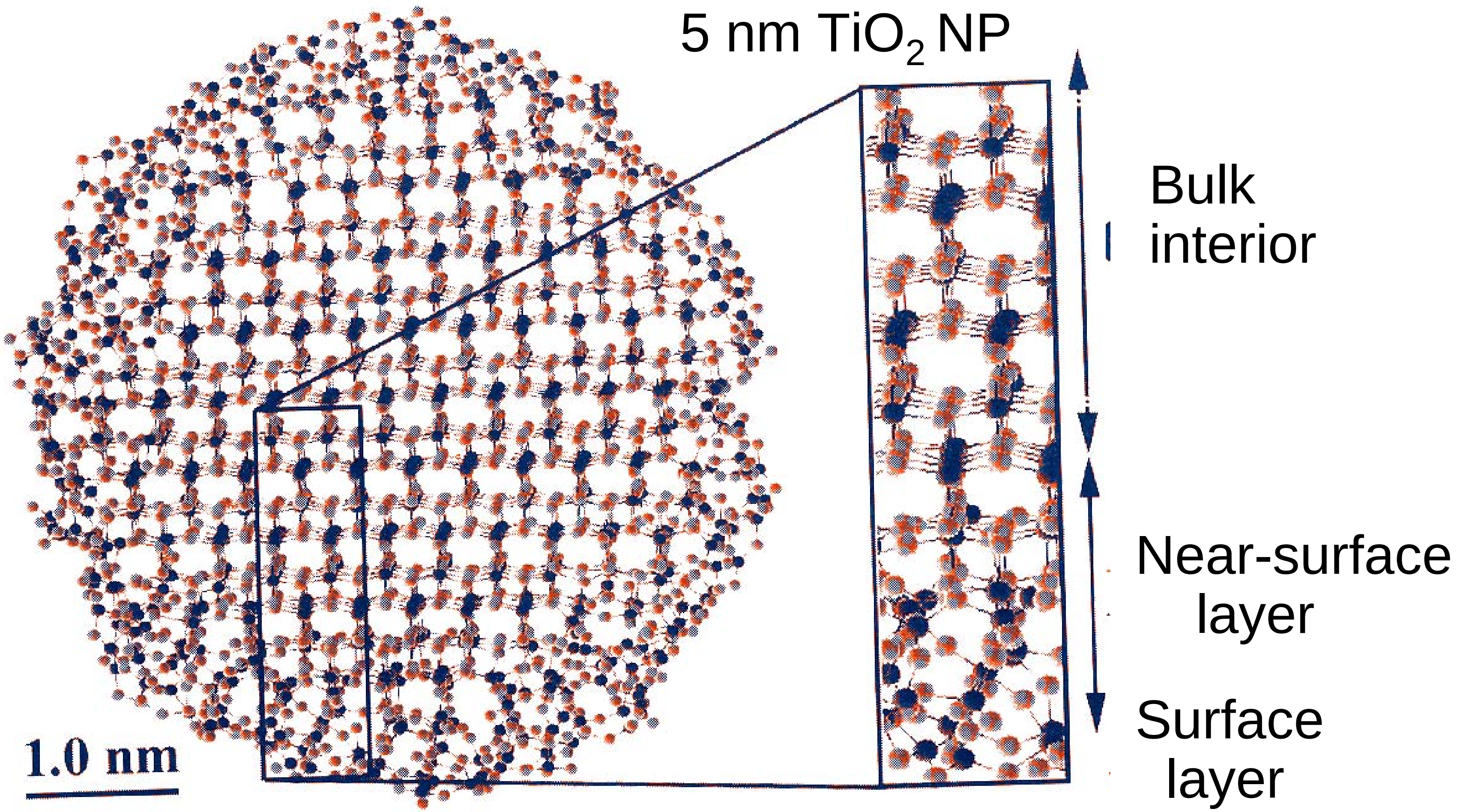
Destabilization and dissolution of the nanoparticle surface

Adsorption onto the surface releases energy that may be imparted to the interacting molecule/organelle/membrane.

Consequences:

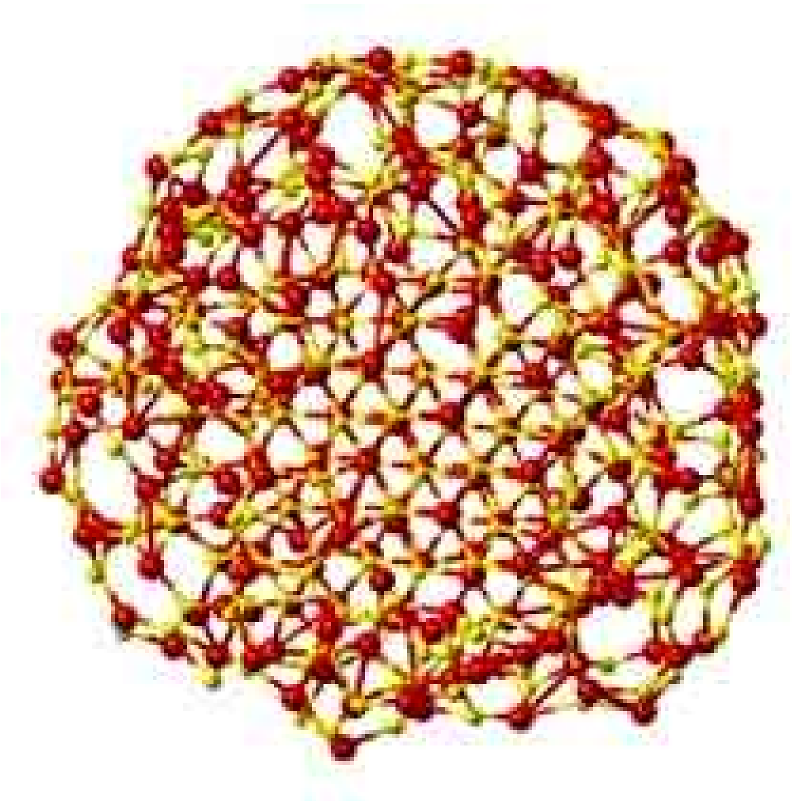
- Proteins adsorption onto the NP surface, denaturation and loss of function
- Nanoparticle binds to and damage a vital biological component, e.g., membrane structure, DNA or mitochondrion

Surface energy of NP



J. F. Banfield & H.Z. Zhang, Reviews in mineralogy and geochemistry 2001, vol. 44, pp. 1-58

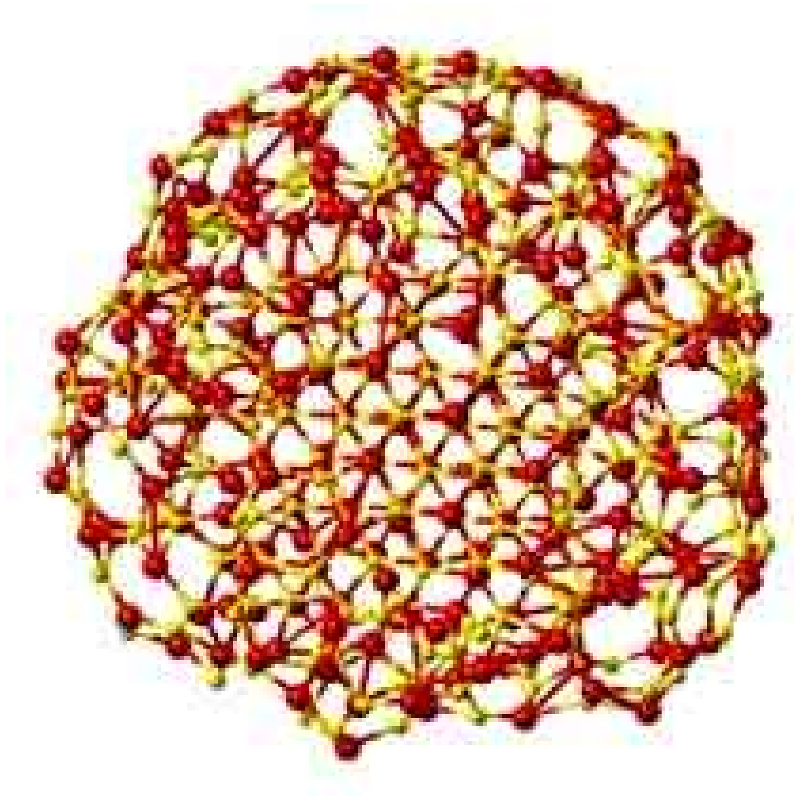
Surface energy of NP and media interaction



3 nm zinc sulphide (ZnS) NP without water bound to its surface

H. Zhang, B. Gilbert, F. Huang & J. F. Banfield.
Nature, 424, 1025-1029, 2003

Surface energy of NP and media interaction



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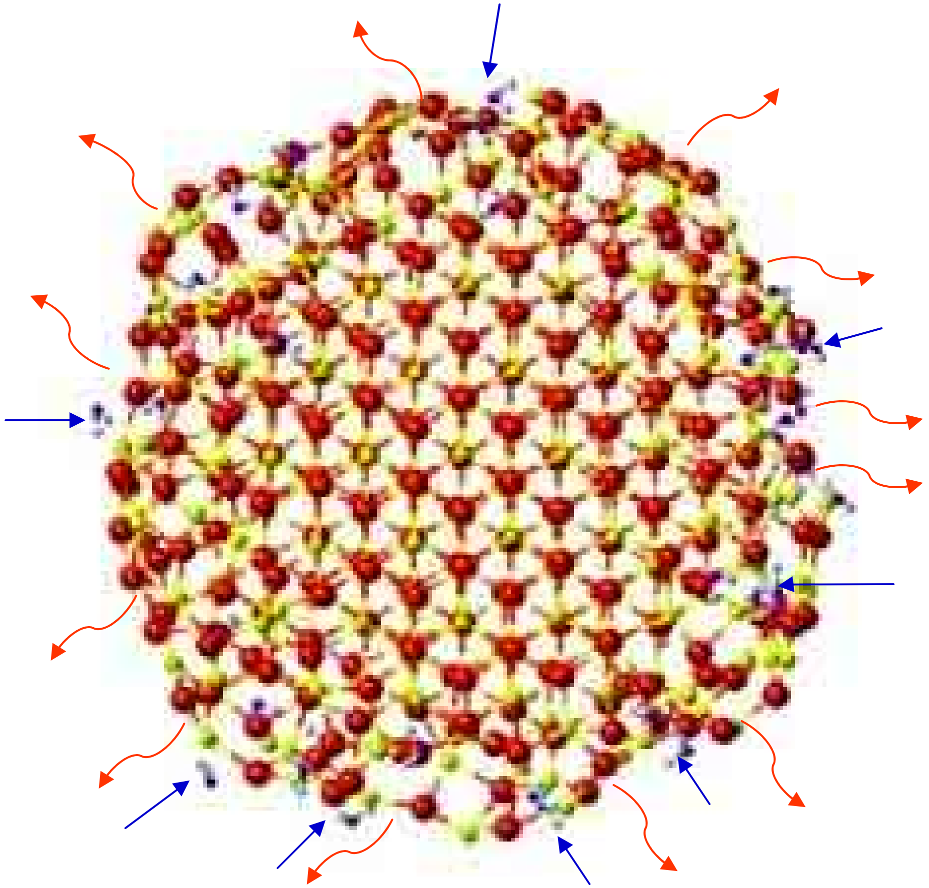


Same nanoparticle with surface-bound water

H. Zhang, B. Gilbert, F. Huang & J. F. Banfield. Nature, 424, 1025-1029, 2003

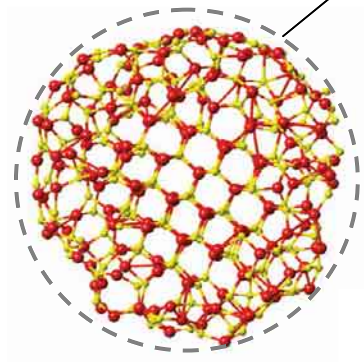
Importance of Particle Size

Stability

all nanoparticles are **metastable**

transformation always possible

strained surface alters total energy

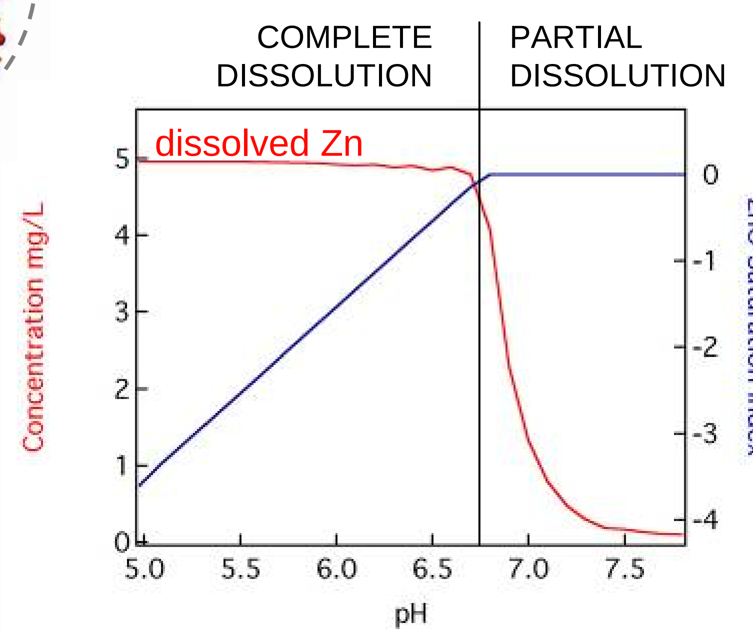


$$G_{total} = G_{bulk} + G_{surface}$$

DISSOLUTION

Zn^{2+}

quantify dissolution



B. Gilbert, L. Mädler, X. Tian, A. Nel (2007)

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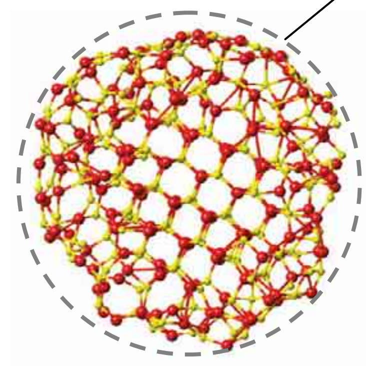

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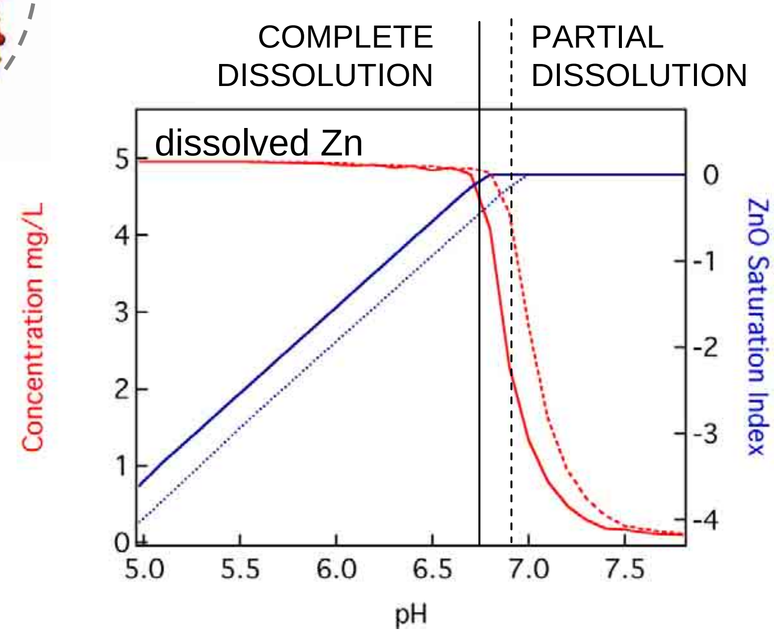

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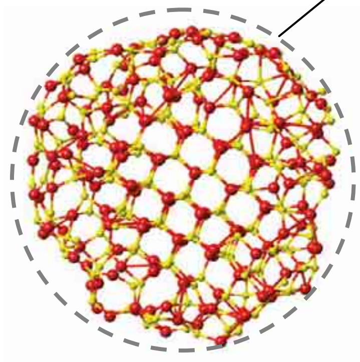
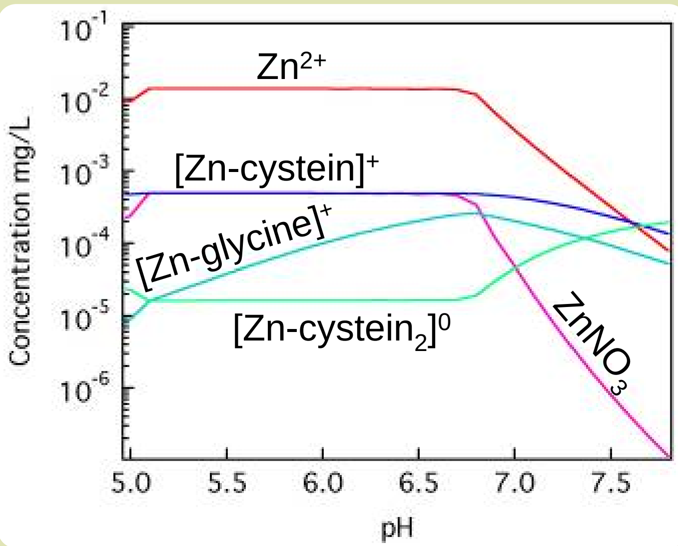


$$G_{total} = G_{bulk} + G_{surface}$$

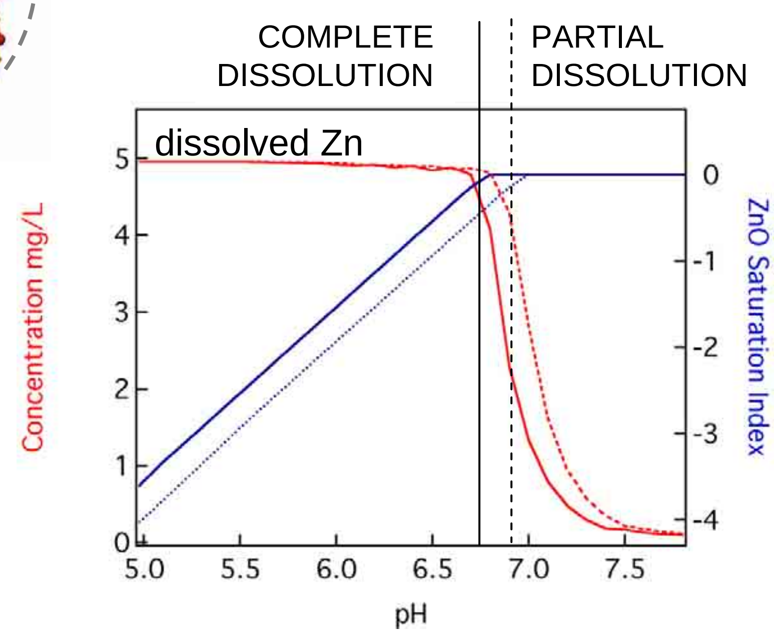
predict speciation



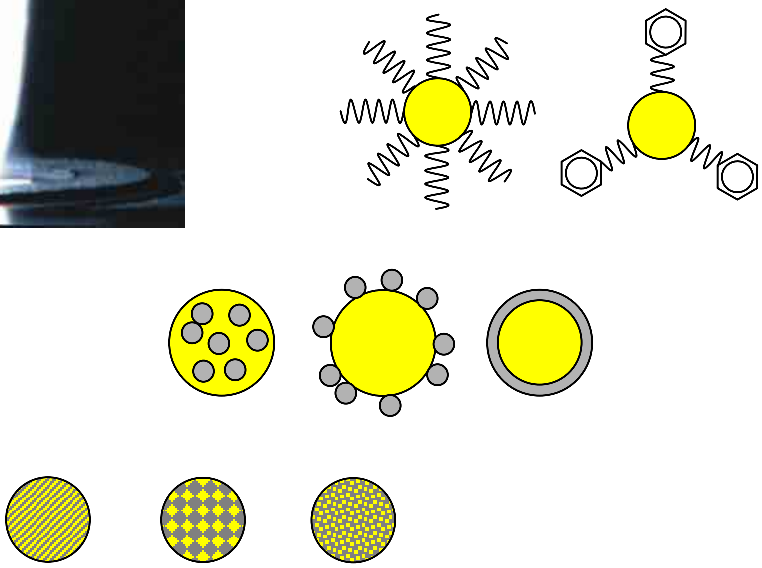
DISSOLUTION

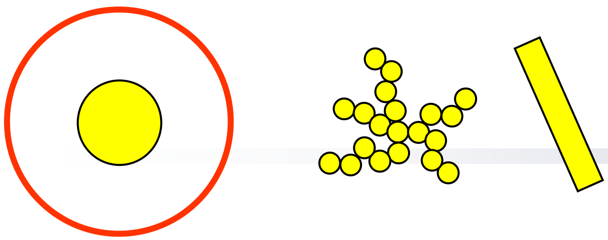
Zn^{2+}

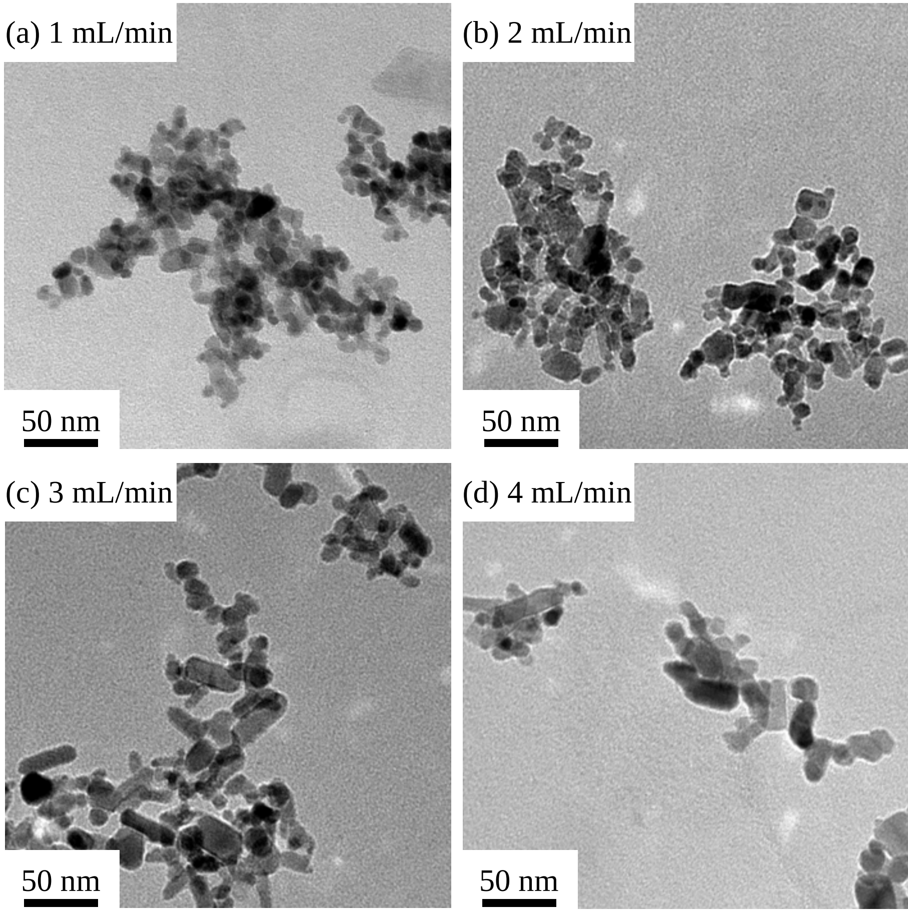
quantify dissolution



ZnO NP of different sizes

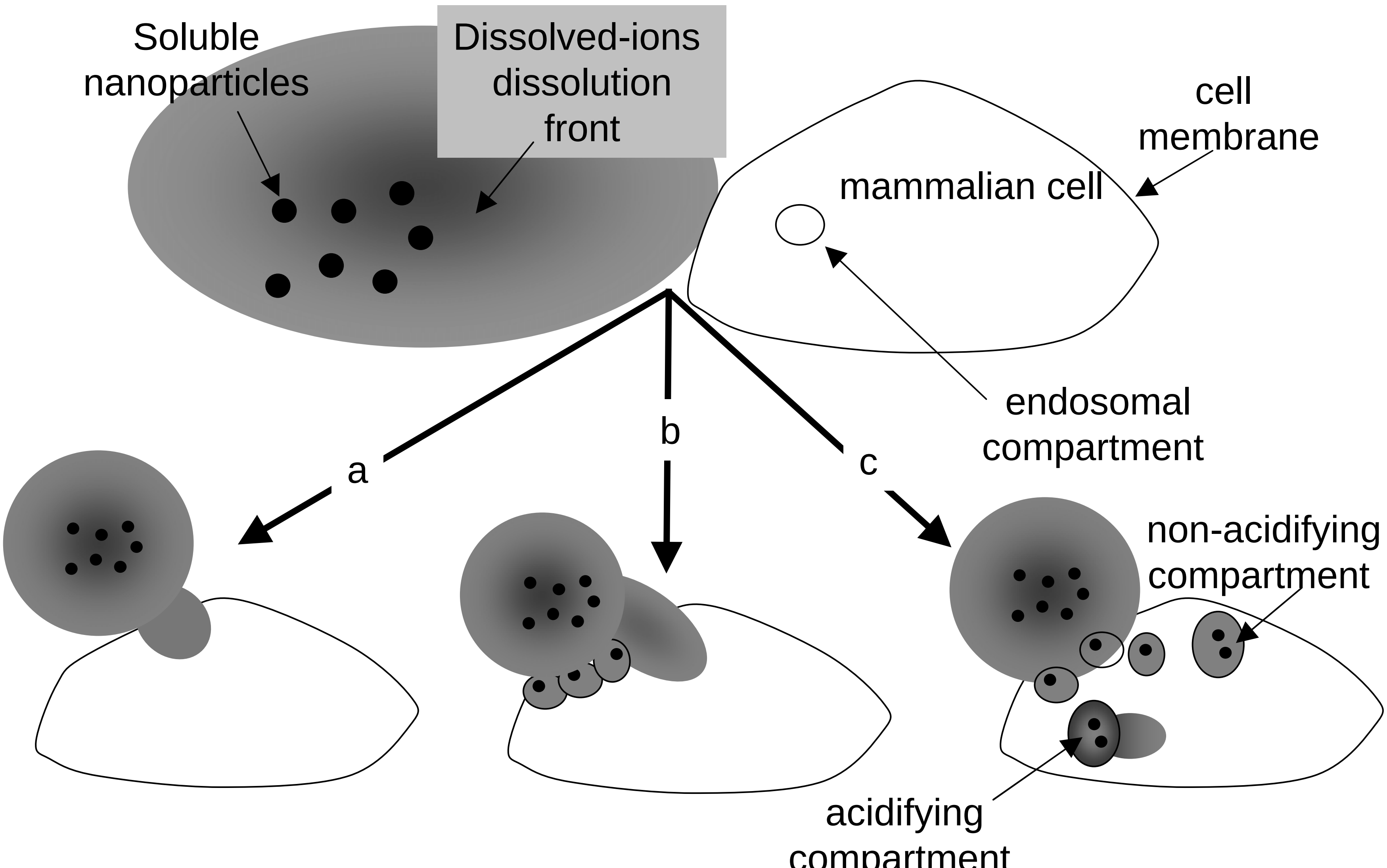






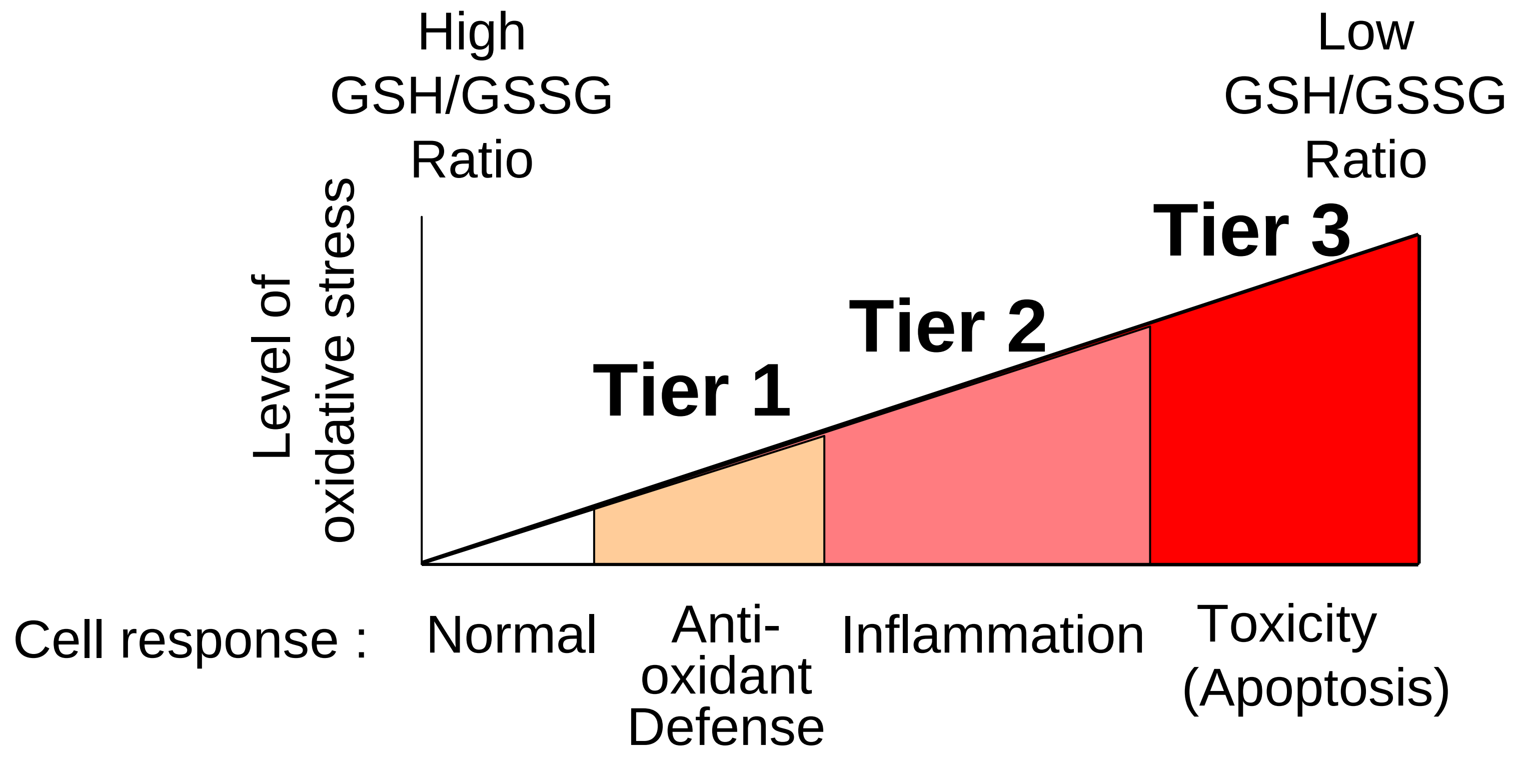
Tani, T., Mädler, L., and Pratsinis, S. E. (2002). *Journal of Nanoparticle Research*, 4(4), 337-343.

ZnO dissolution and toxicity



B. Gilbert, L. Mädler, X. Tian, A. Nel (2007)

Oxidative Stress as a Stratified Response



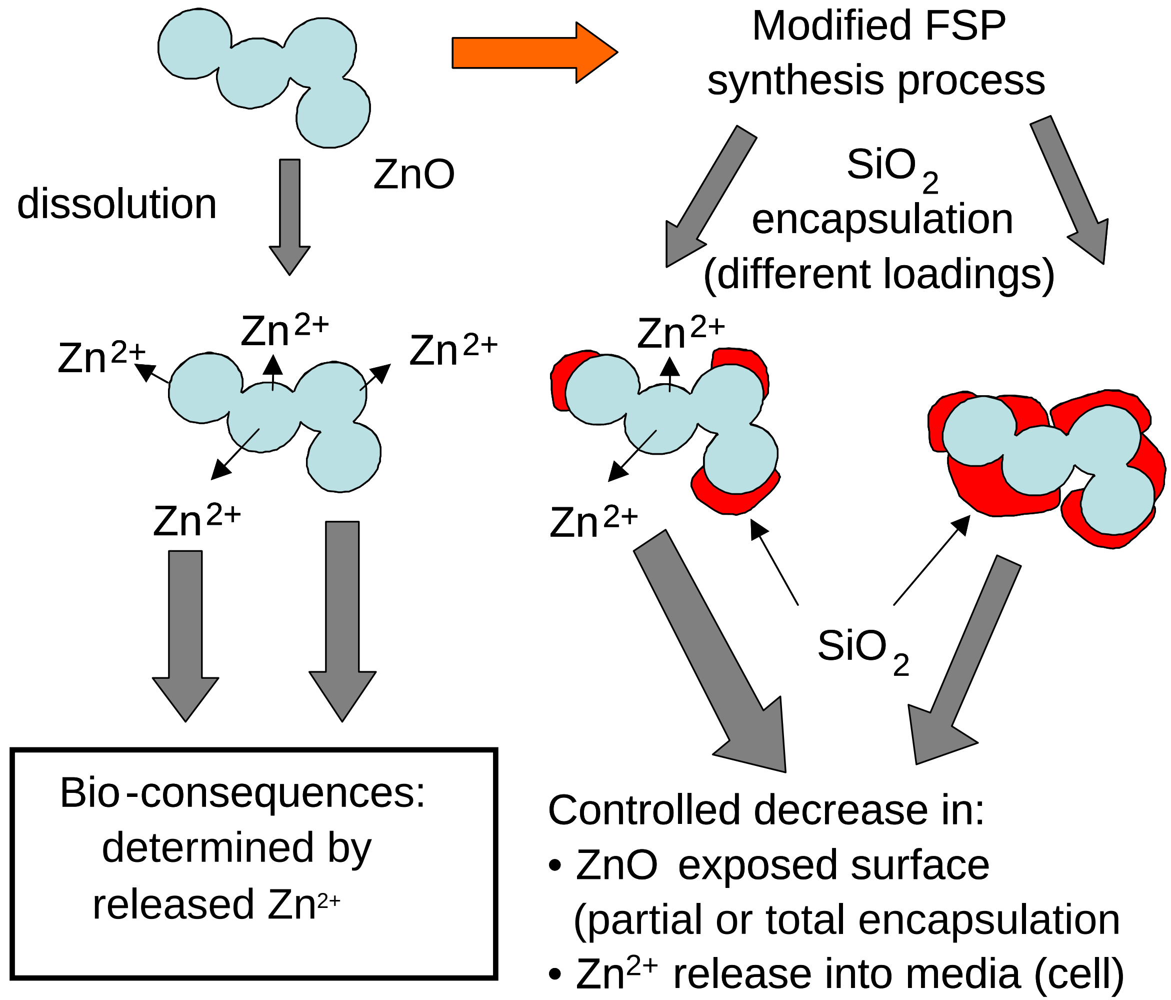
Xiao, G. G., Wang, M. Y., Li, N., Loo, J. A., and Nel, A. E. (2003).
Journal of Biological Chemistry, 278(50), 50781-50790.

Tiered approach

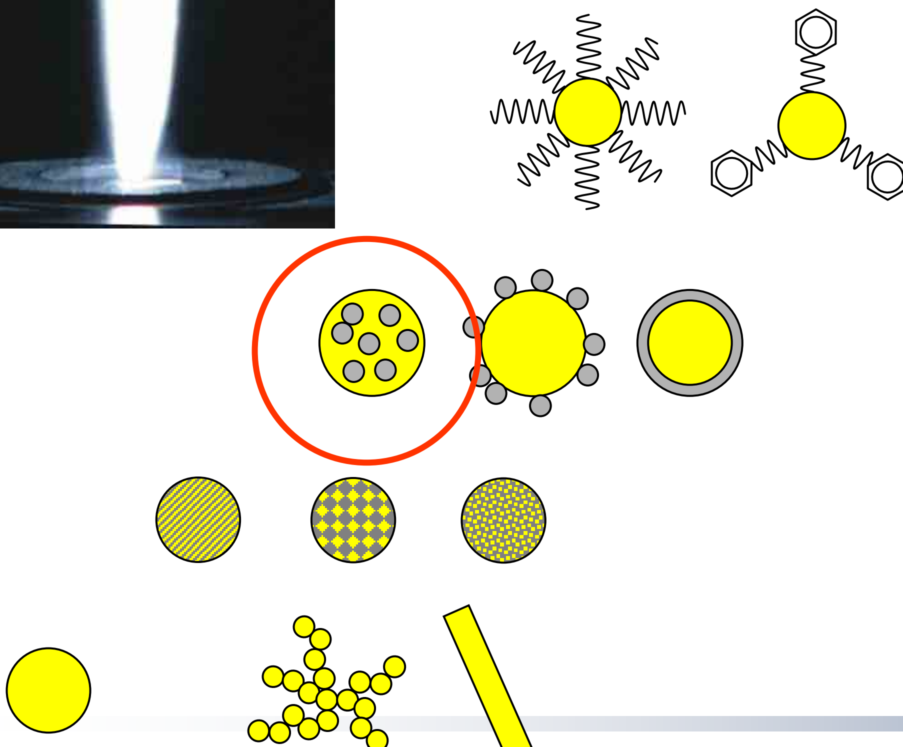
	Abiotic ROS	Cellular ROS	Tier 1	Mito perturba	Cyto-toxicity
Ambient UFP	+++	+++	++	+++	+++
TiO_2	++++	+	+	-	-
Carbon Black	-	-	-	-	-
Fullerol	+++	+	+	±	-
COOH-PS	-	-	-	-	-
NH_2-PS	-	+++	-	++++	++++

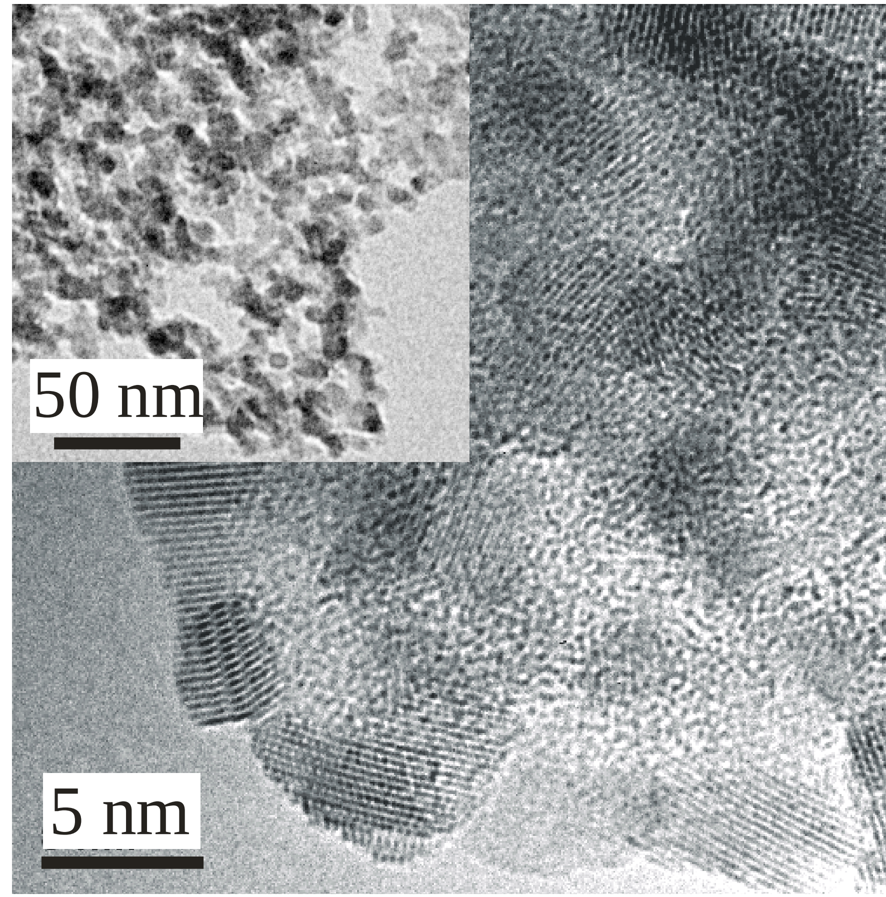
Xia, Kovochich et al. Nanoletters. 2006.

Control zinc oxide solubility by encapsulation



Encapsulation





Mädler, L., Stark, W.J., Pratsinis. S.E., *J. Appl. Phys.*, **92** (12) 6537-6540 (2002).

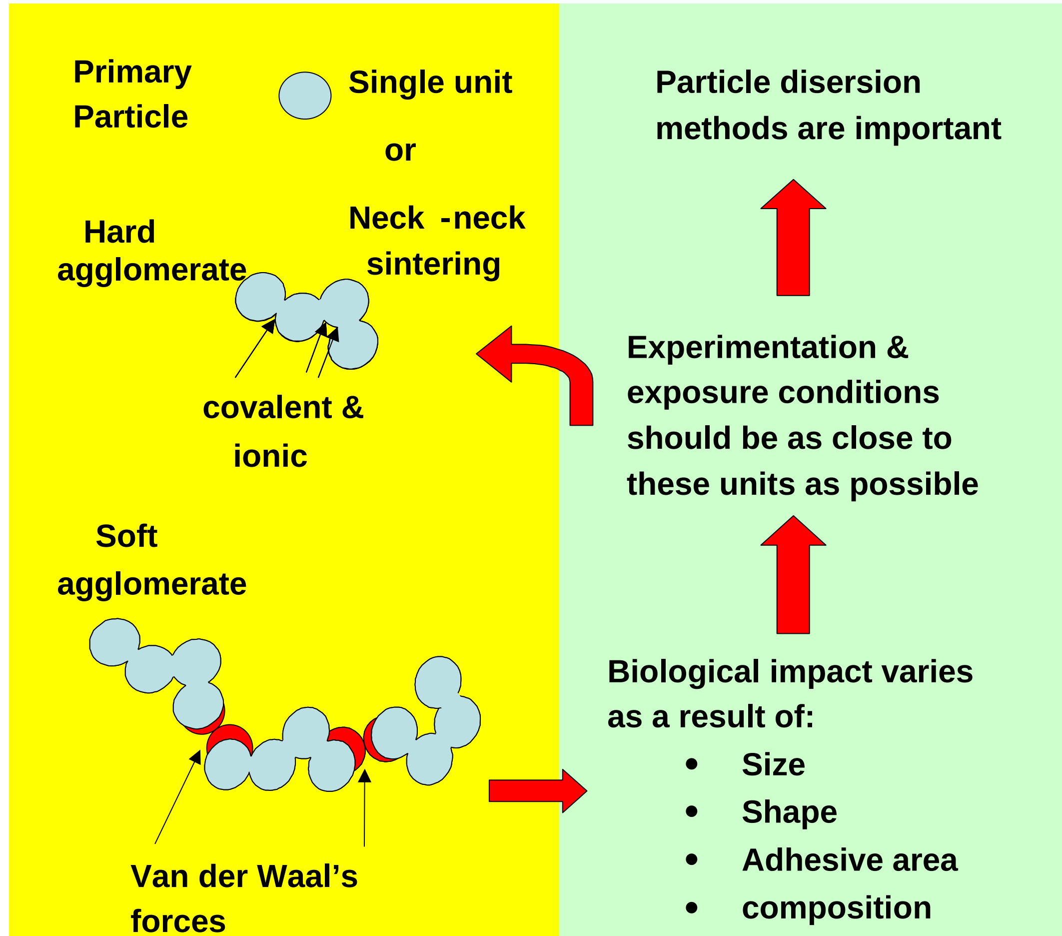
Hard and soft agglomerates

Aggregation is determined by:

- Electrostatic forces
- van der Waals bonds
- Hydration forces (hydrophobicity)
- Steric forces surfactant/polymer layer

Dispersion by:

- phospholipids
- detergents
- proteins
- engineered coatings



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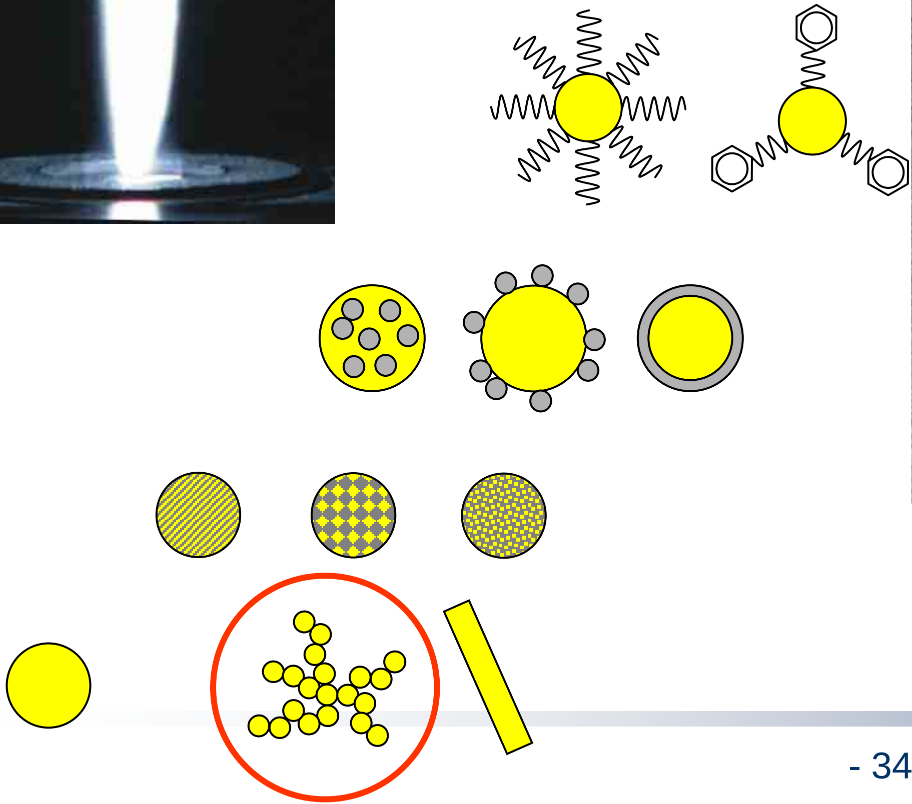
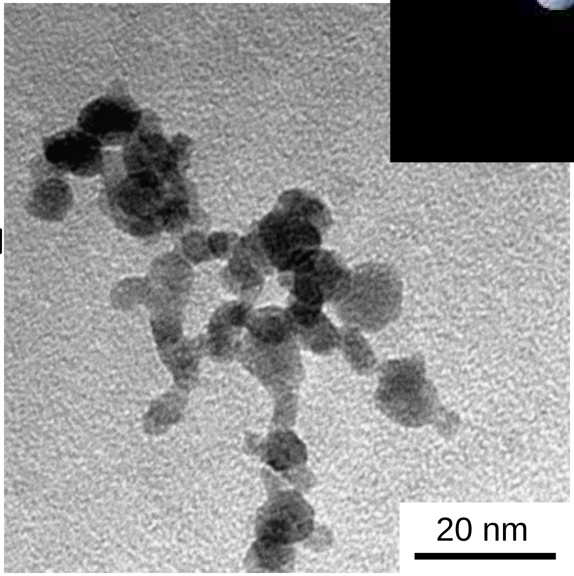
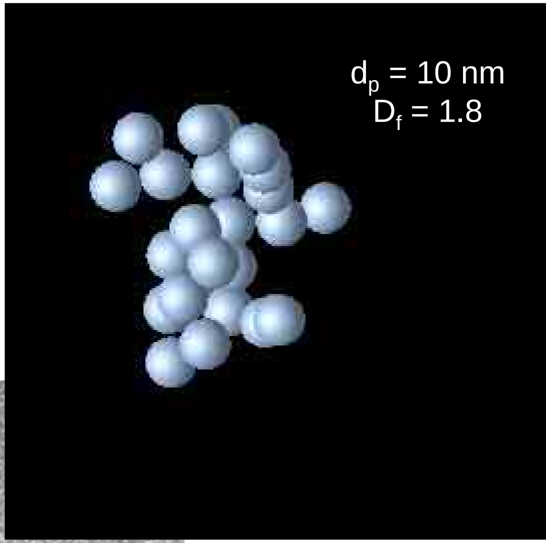
NP agglomerate structure







Mädler, L., Lall, A. A., and Friedlander, S. K. *Nanotechnology* **17** (19) 4783-4795 (2006).

Bio-interactions of nanoparticle aggregates

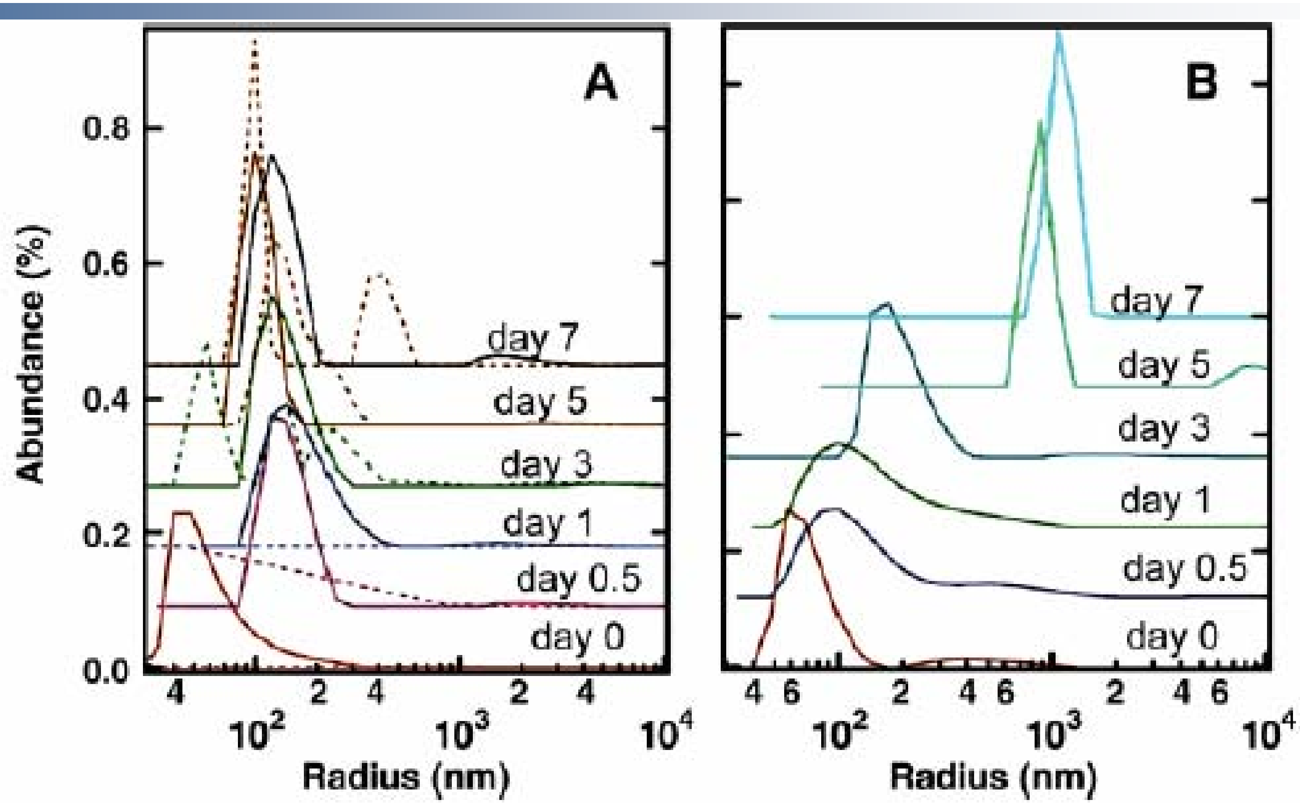
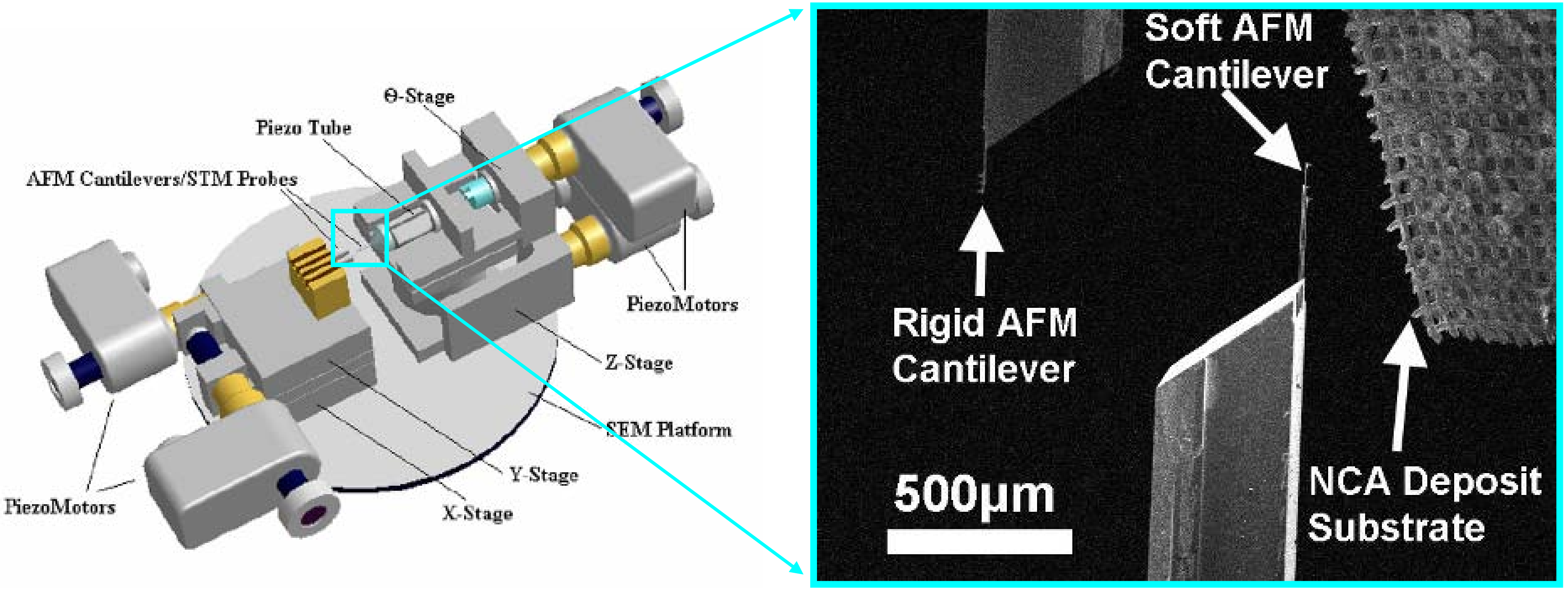


Fig. 4. Size distribution curves from DLS data acquired in ZnS nanoparticle aggregation experiments. (**A**) Control experiments. 10 μM ZnS nanoparticles alone (solid lines) aggregate within 1 day to form ~100-nm-radius clusters that exhibit little further growth over a 5-day period. 100 μM cysteine alone (dashed lines) gives a very weak DLS signal, with no consistent trend in size distribution. (**B**) In the presence of both 10 μM ZnS and 100 μM cysteine, sustained aggregation occurs over the 7-day period, resulting in aggregates that are more than one order of magnitude larger than the initial clusters. DLS correlation functions from which size distributions were derived are shown in fig. S7.

Moreau, J. W., Weber, P. K., Martin, M. C., Gilbert, B., Hutcheon, I. D., and Banfield, J. F. (2007). *Science*, 316(5831), 1600-1603.

AFM nano-manipulator inside SEM



(Yu et al., *Nanotechnology,* 1999)

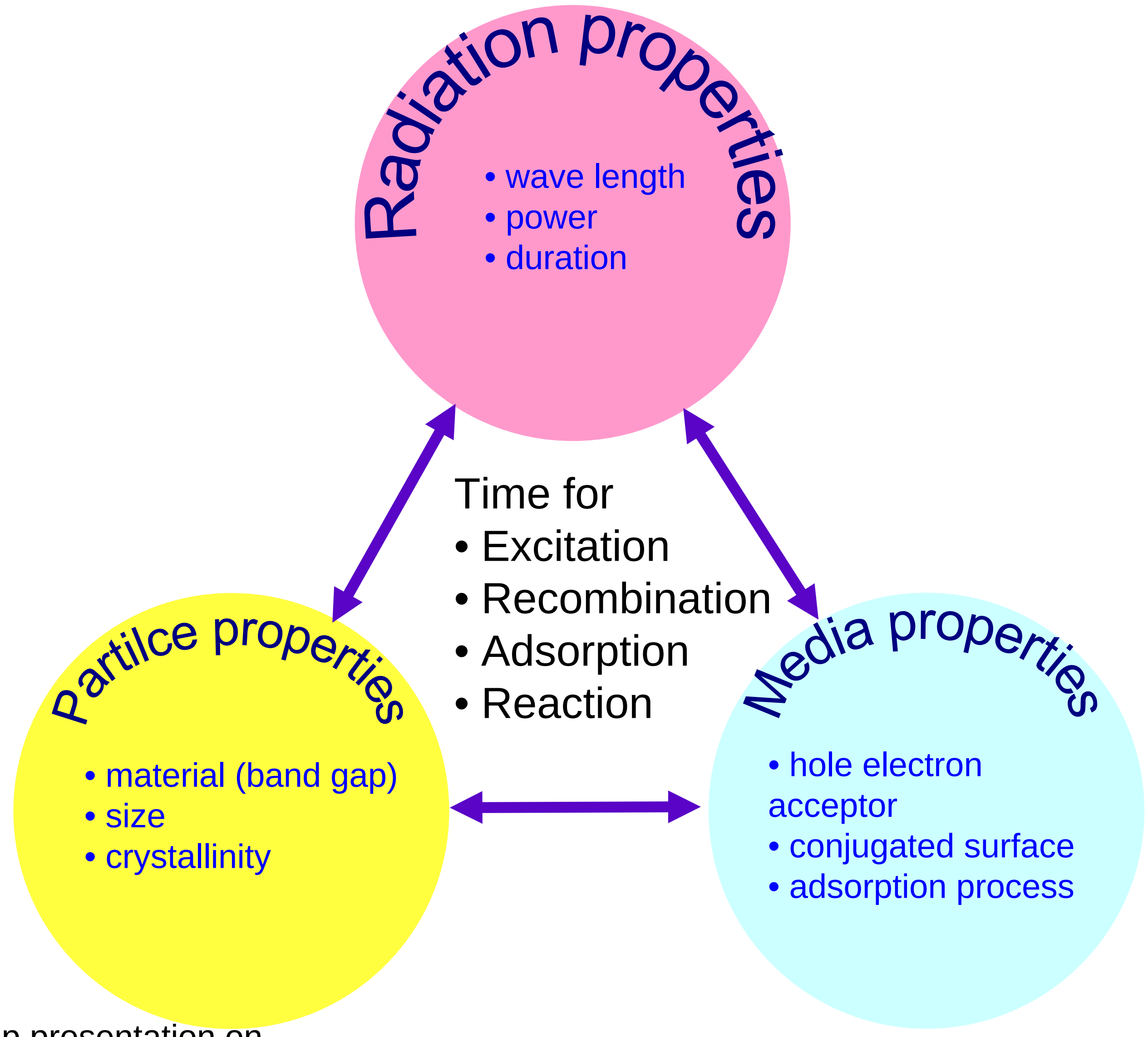
COLLABORATION WITH PROF. RUOFF'S GROUP, NORTHWESTERN UNIVERSITY.

Please click on the link below to access a short video clip:

http://ftp.dtsc.ca.gov/sppt/Nanotechnology_Symposium_20071003_maedler_slide37.asx

Rong, W. Z., Ding, W. Q., Madler, L., Ruoff, R. S.,
and Friedlander, S. K. (2006). *Nano Letters*, 6(12), 2646-2655.

Nanoparticle photo activity



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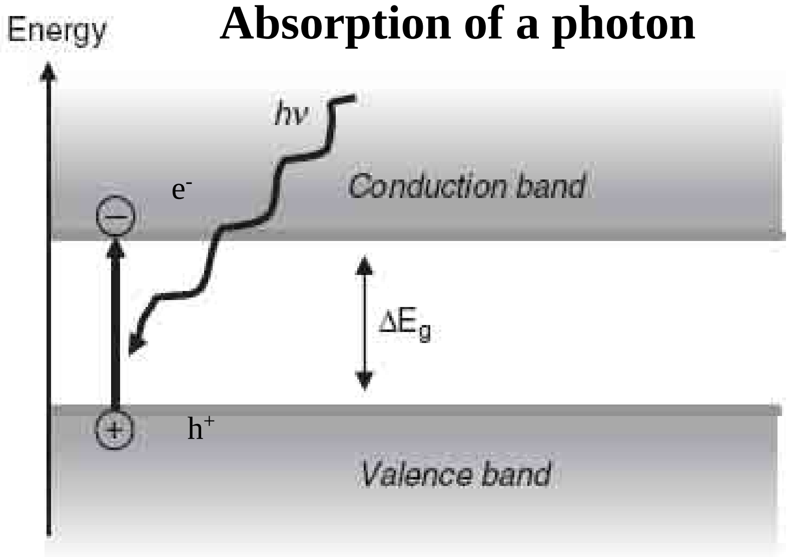
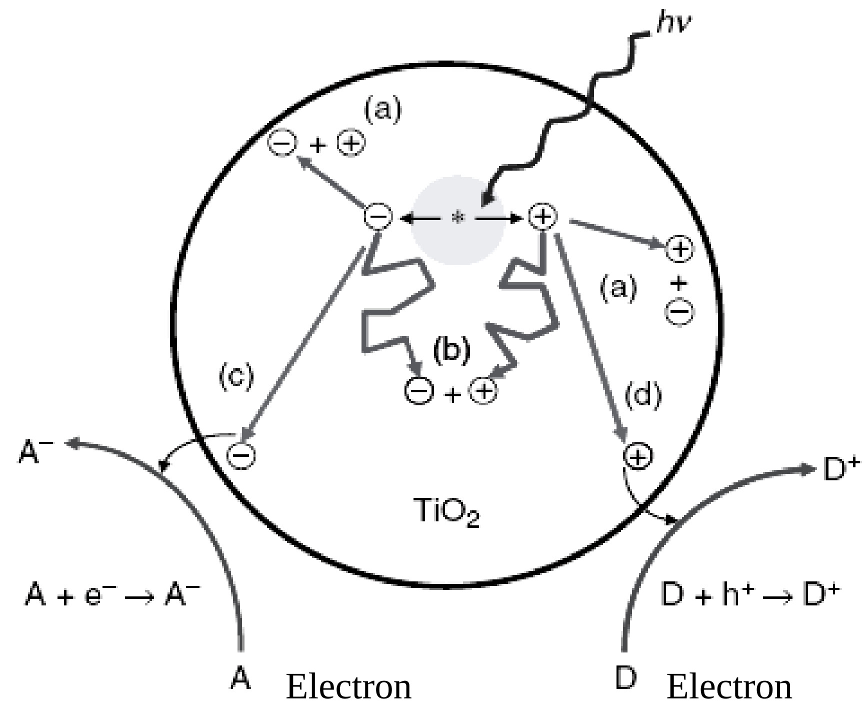
Nanoparticle photo activity

Absorption of a photon





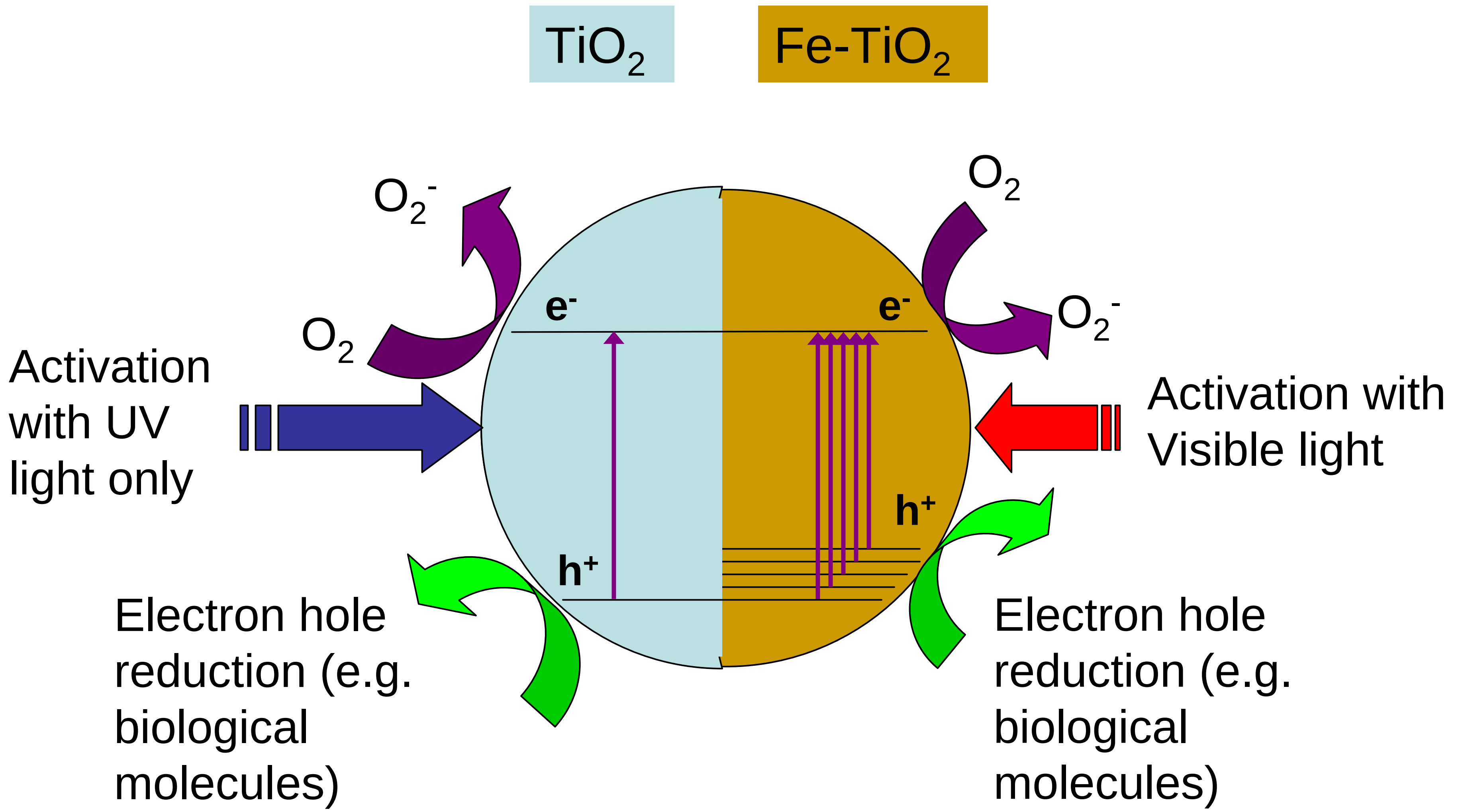
Electron acceptor

Electron donor

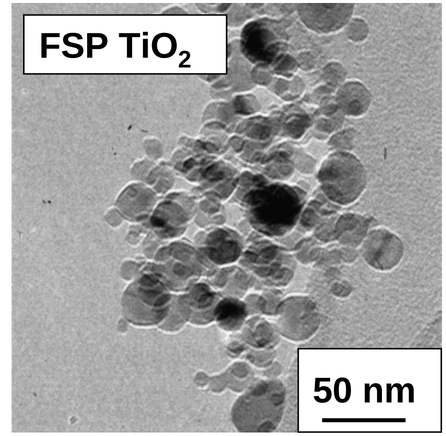
a = direct band-gap recombination
b = indirect recombination from trapped states
c = e^- and h^+ migrate to surface where they undergo e^- transfer reactions

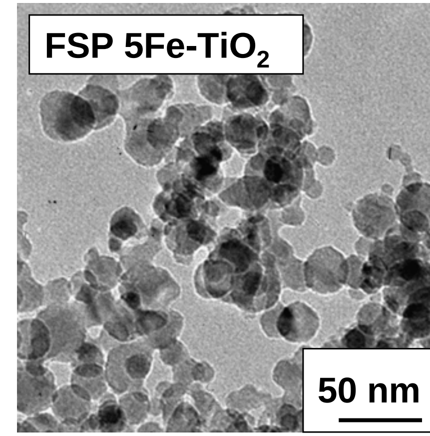
Wiesner, M., and Bottero, J.-Y. (2007). *Environmental Nanotechnology*, McGraw-Hill Professional

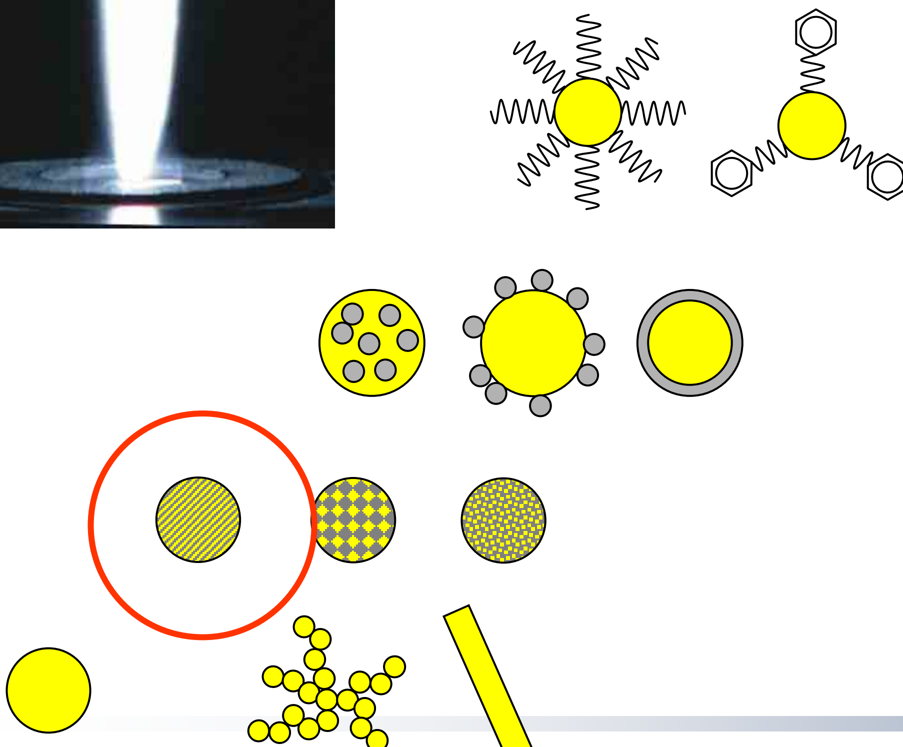
Photo excitation in the visible range

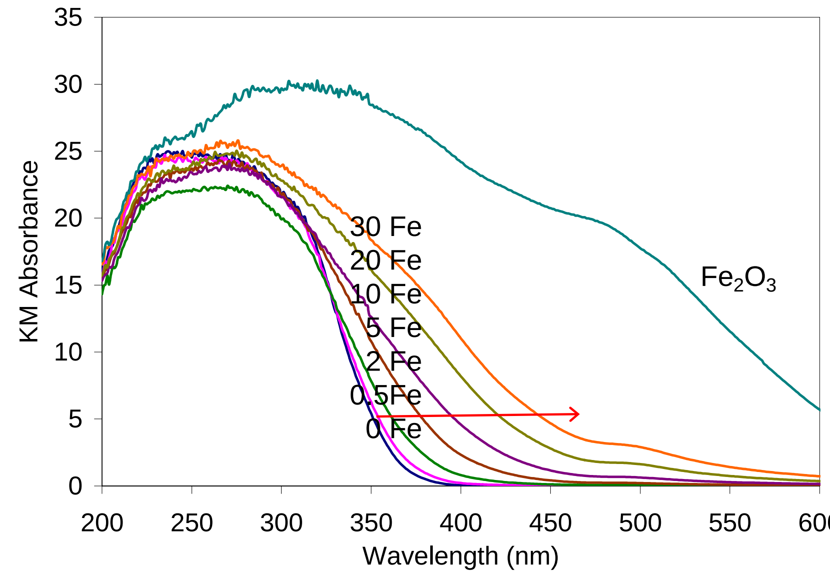


Impurity energy levels by doping



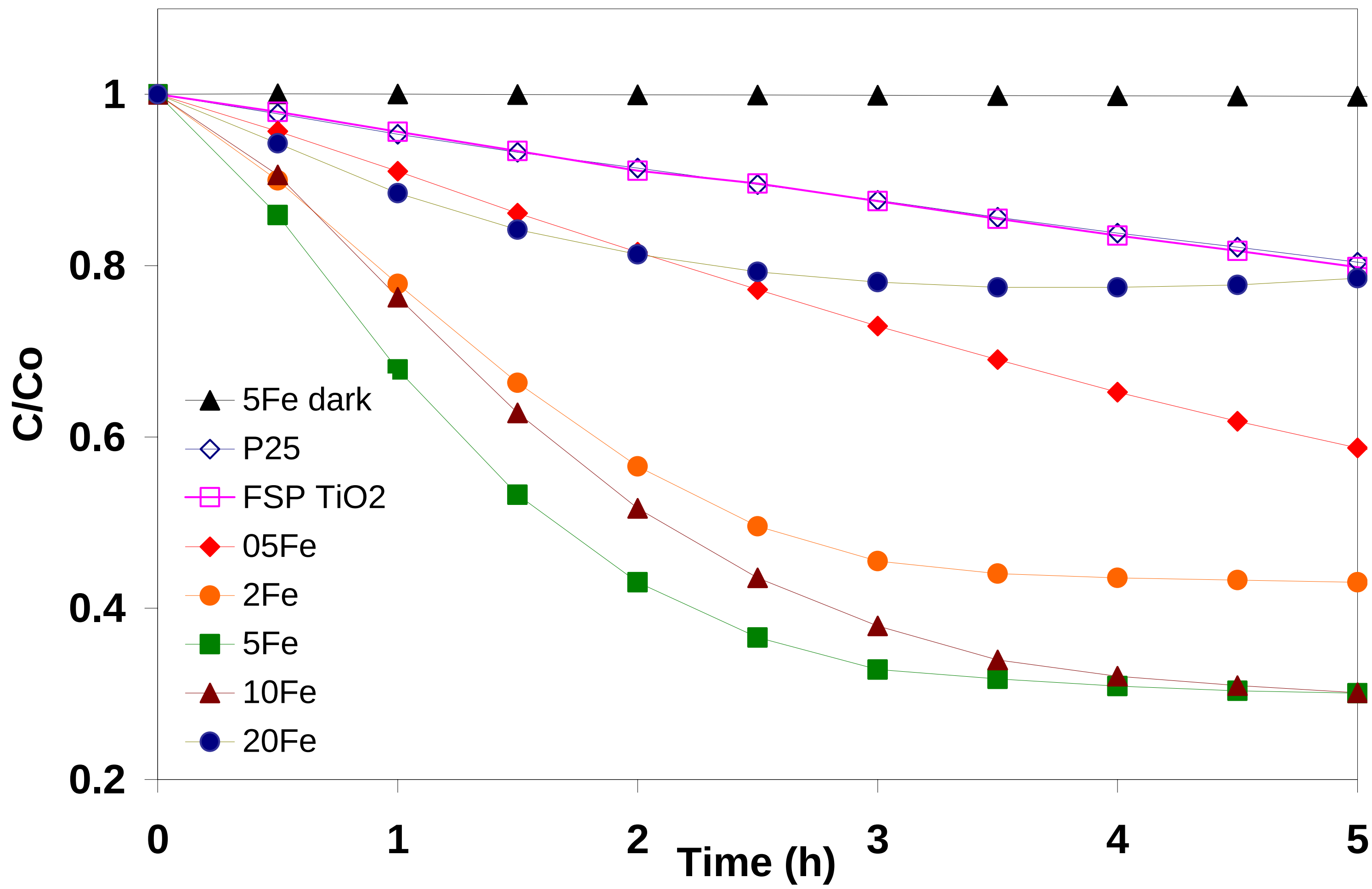






Teoh, W.Y., Amal, R., Mädler, L., Pratsinis, S.E., *Catalysis Today* **120** (2) 203-213 (2007).

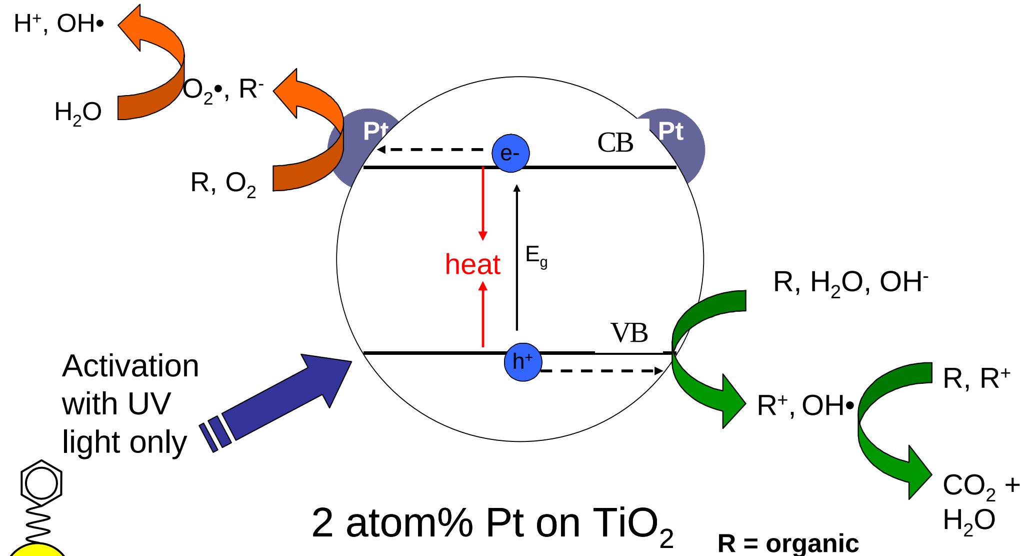
Effect of Fe loading on photo-activity in the visible range

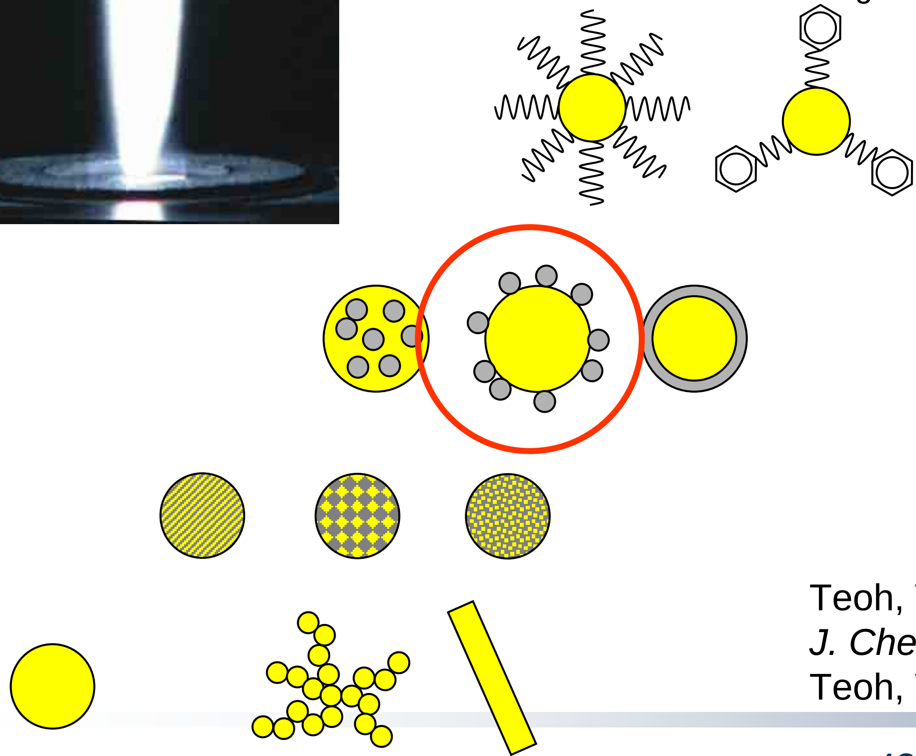


10 ppm C oxalic acid, pH 3.5, visible light

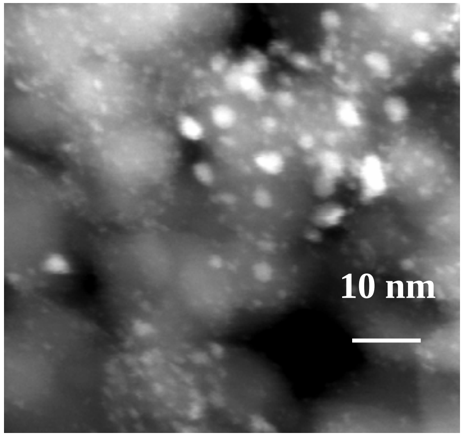
Teoh, W.Y., Amal, R., Mädler, L., Pratsinis, S.E., *Catalysis Today* **120** (2) 203-213 (2007).

Noble metal doping



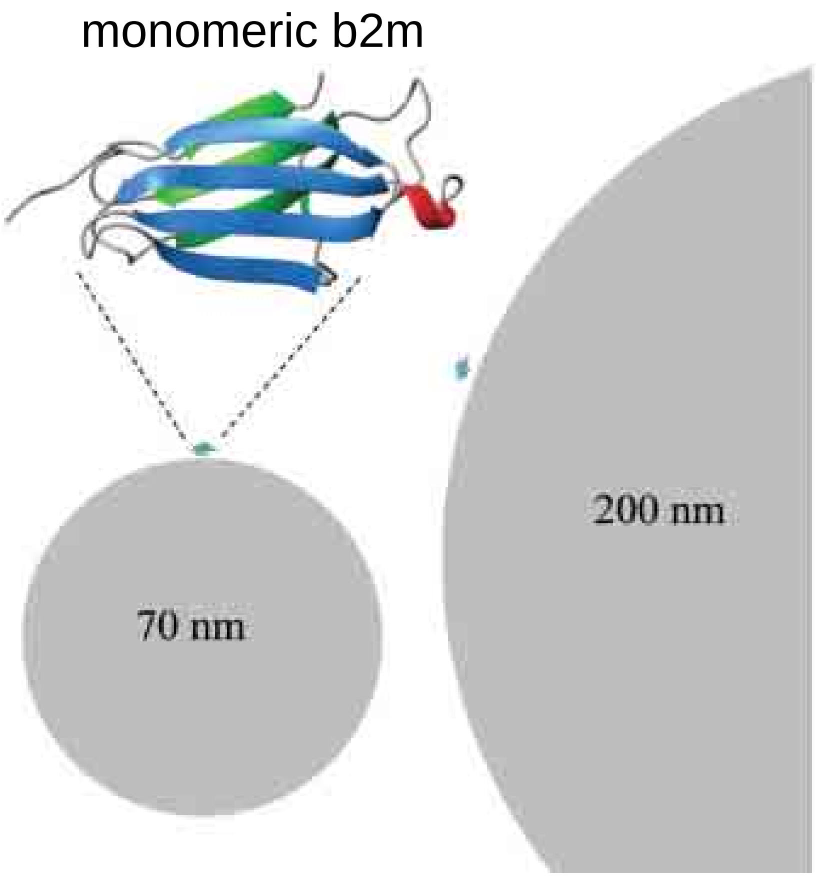


2 atom% Pt on TiO_2



Teoh, W.Y., Mädler, L., Beydoun, D., Pratsinis, S.E., Amal, R. *J. Chem. Eng.* **60** 5852-5861 (2005).
Teoh, W.Y., Mädler, L., Amal, R., *J. Catal.*, in press (2007).

Protein interaction with the Nanoparticle Surface



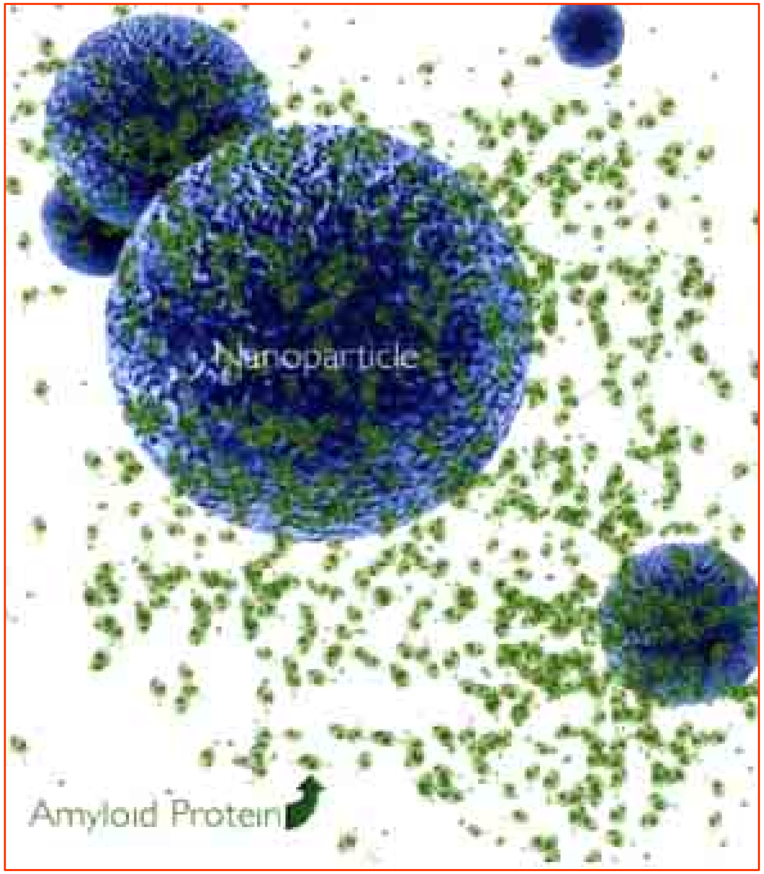
Passive adsorption -no effect

Protein unfolding
- loss of enzyme activity
- exposure cryptic epitopes
- cell stress response

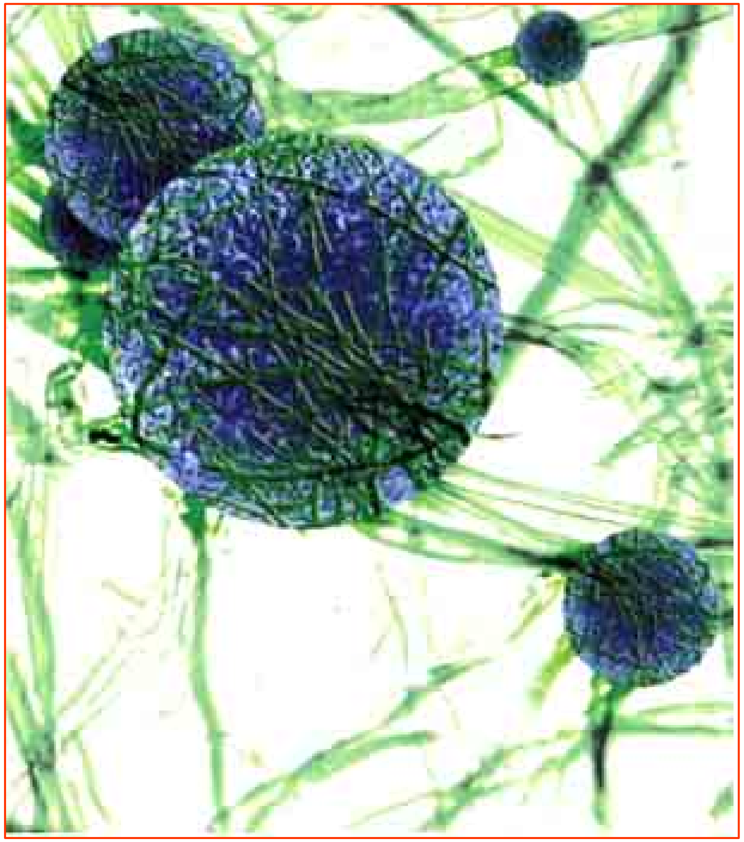
Protein fibrillation-plaque/ amyloid

Vertegel, A. A., Siegel, R. W., and Dordick, J. S. (2004). *Langmuir*, 20(16), 6800-6807.

Nanoparticles as catalysts for Protein Fibrillation



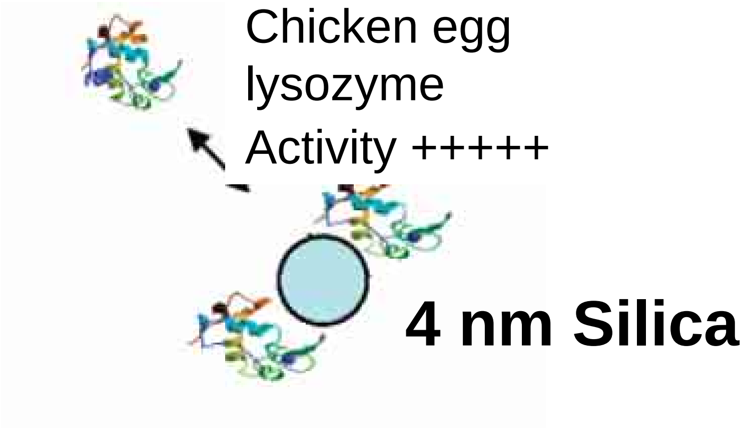
Large NPs (blue) and an amyloid protein (green) in its monomeric and folded state.



Association of the amyloid protein with the NP surfaces, with the generation of small oligomers, which are the precursors to fibrils. In solution, larger protein fibrils appear as their growth is enhanced by the surface association of proteins.

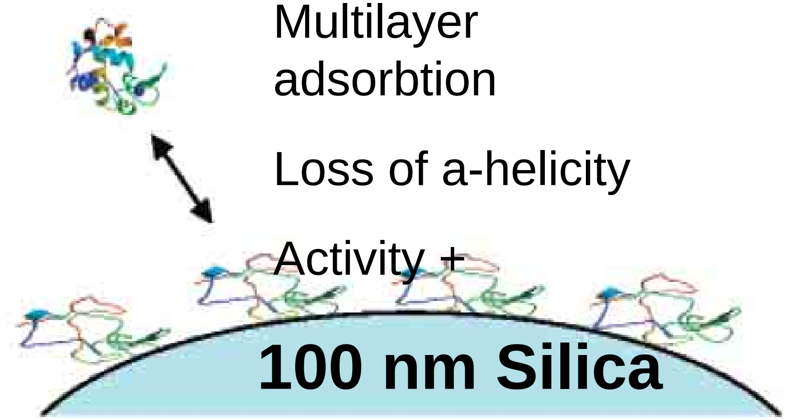
Colvin, V. L., and Kulinowski, K. M. (2007). PNAS, 104(21), 8679-8680.

Change of protein function by Adsorbtion



Chicken egg lysozyme

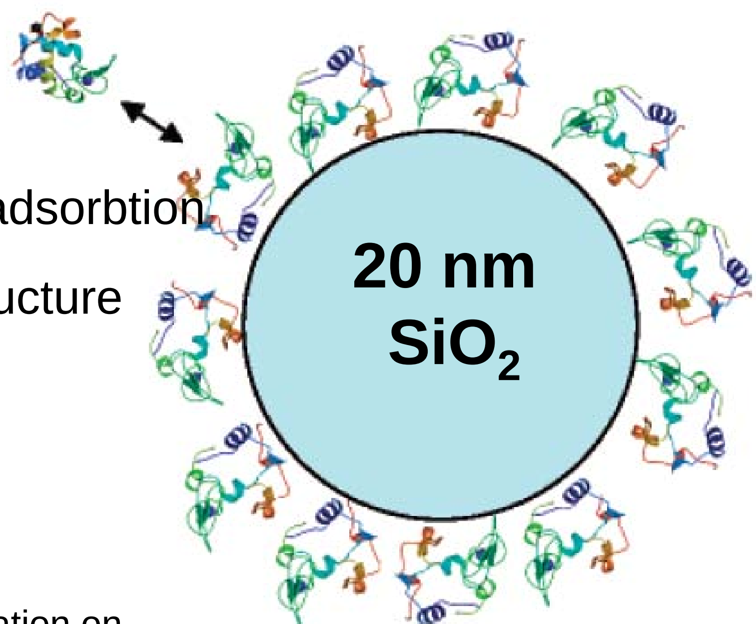
Activity +++++



Multilayer adsorbtion

Loss of a-helicity

Activity +



Monolayer adsorbtion

a-helical structure

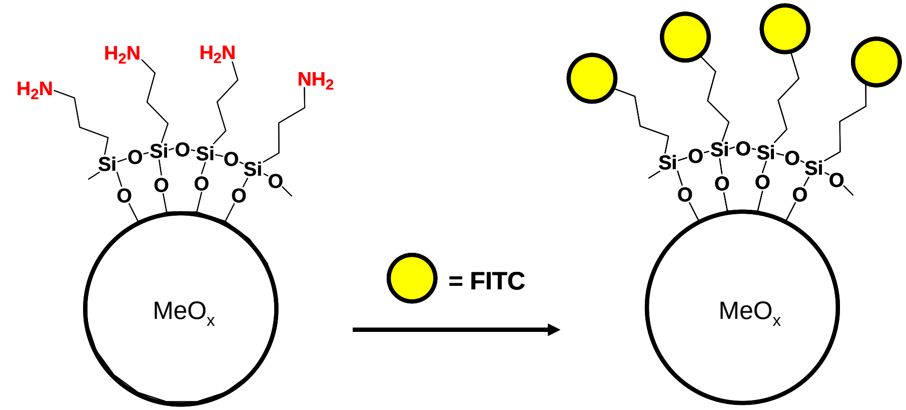
Activity +++

Physical-chemical variables:

- size (curvature)
- shape
- surface energy

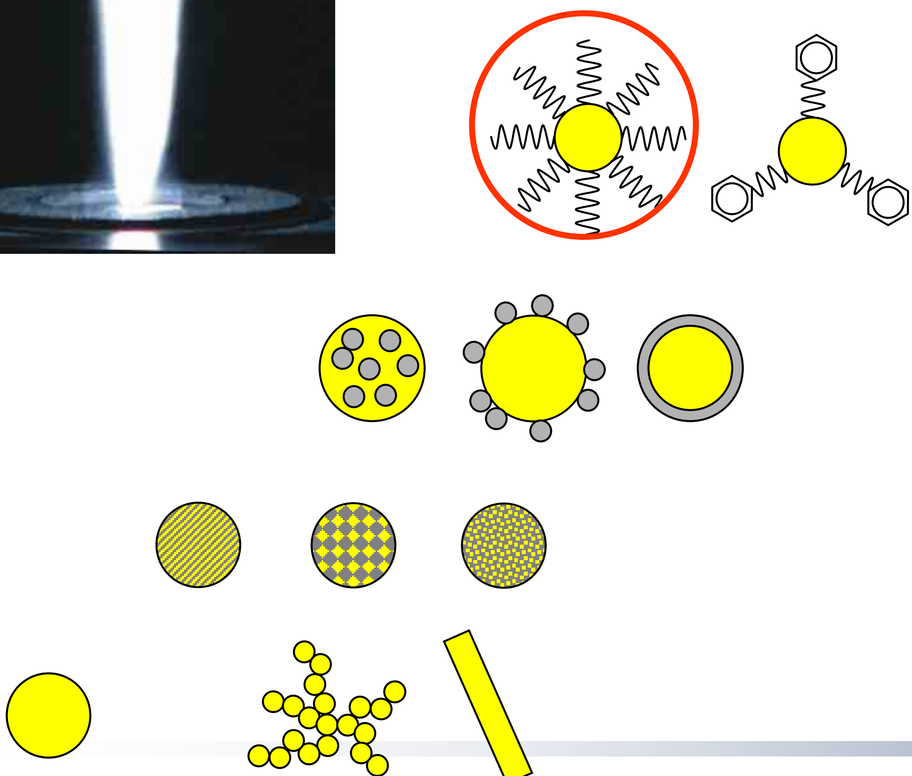
Vertegel, A. A., Siegel, R. W., and Dordick, J. S. (2004). *Langmuir*, 20(16), 6800-6807.

Surface Modification of Metal Oxides



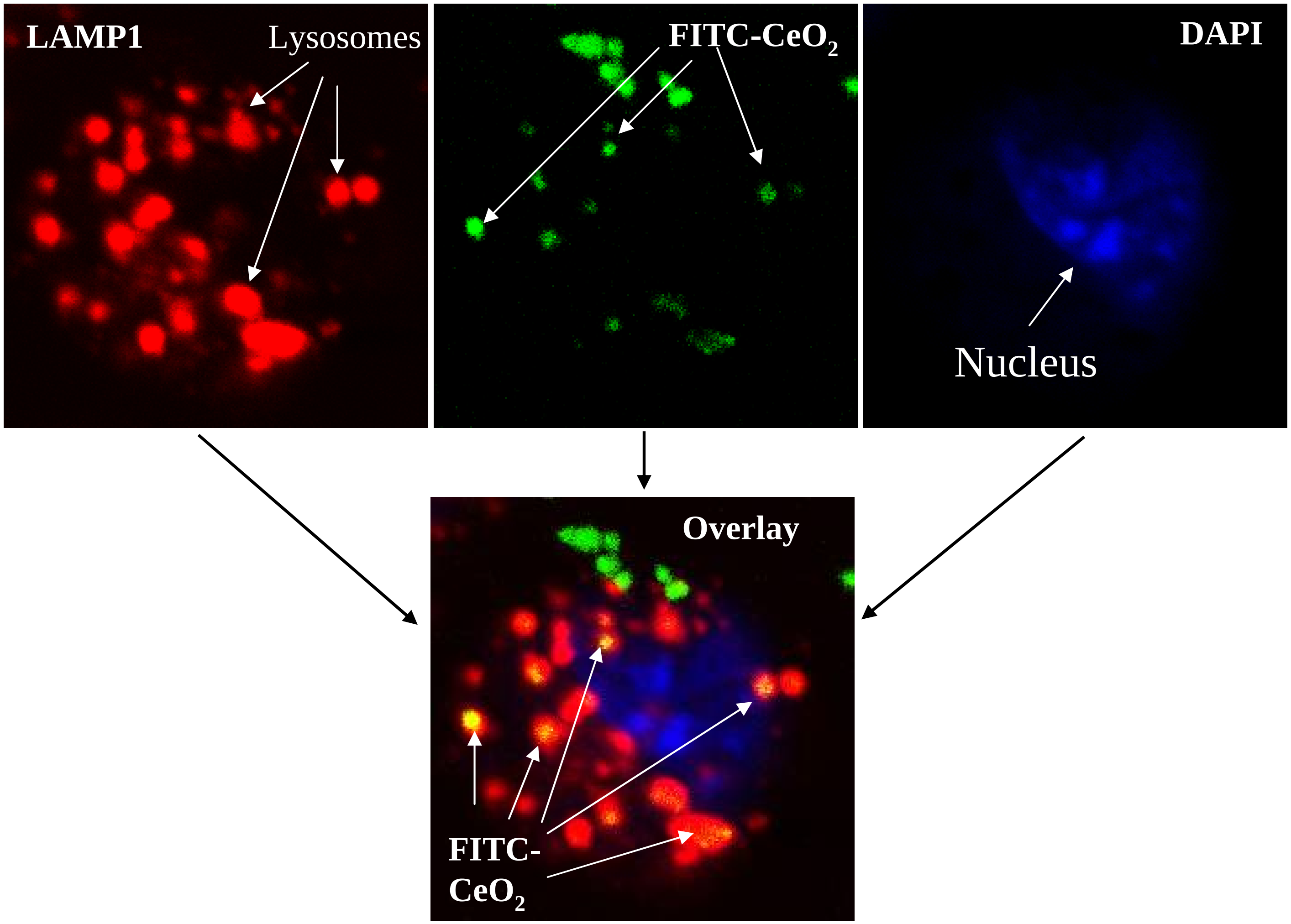
Metal oxides coated with NH_2-silane

FITC-labeled metal oxides



Data in collaboration with M. Liong, J. Zink, T. Xia, A. Nel (TSRTP, CNSI/UCLA)

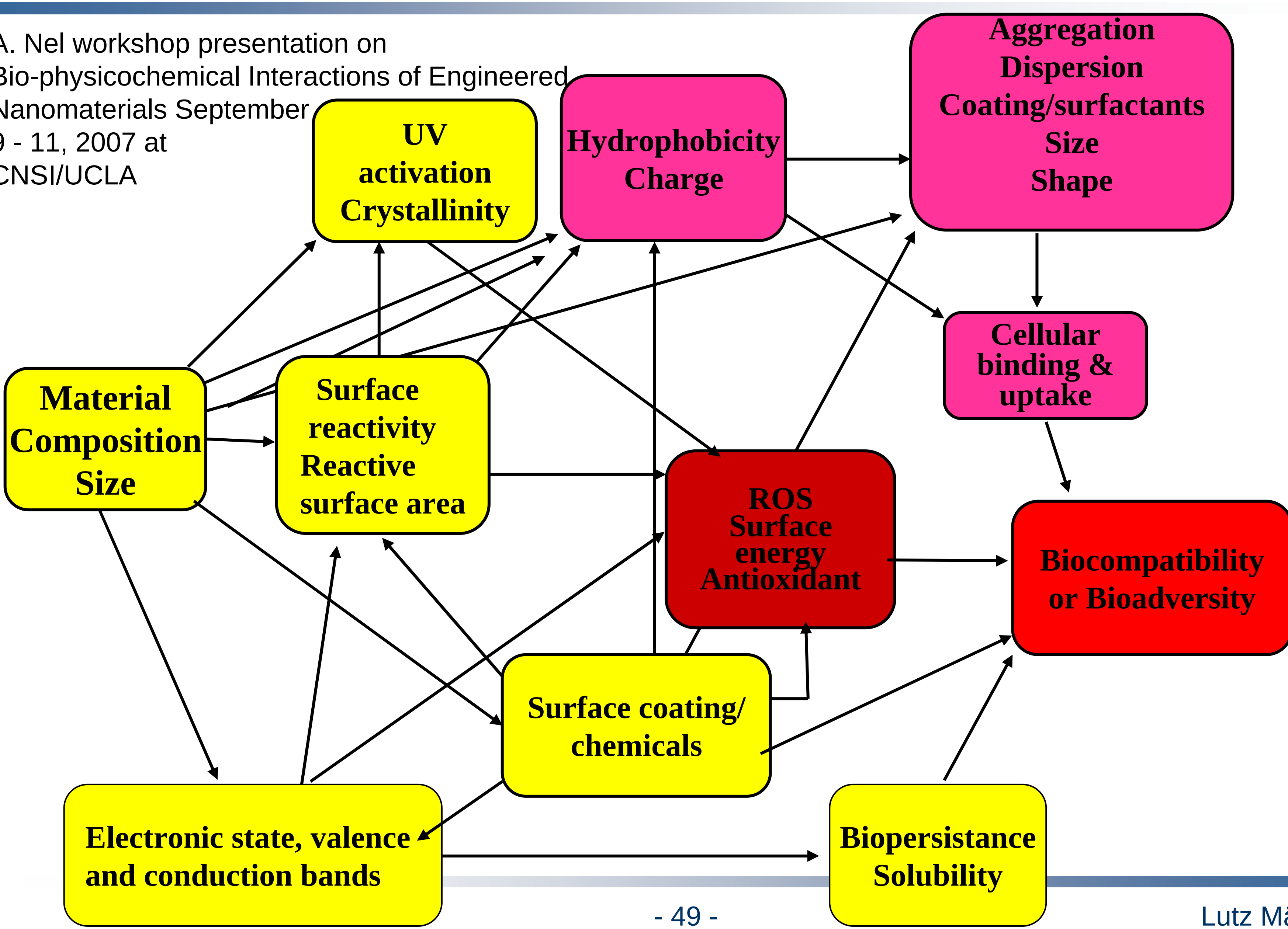
CeO_2 co-localize with lysosomes in RAW 264.7 cells



Data in collaboration with M. Liong, J. Zink, T. Xia, A. Nel (TSRTP, CNSI/UCLA)

Module relating particle physical-chemical characteristics to biocompatibility and bioadversity

A. Nel workshop presentation on Bio-physicochemical Interactions of Engineered Nanomaterials September 9 - 11, 2007 at CNSI/UCLA



Acknowledgments

A. Nel et al.
J. Zink et al.

S.K. Friedlander et al.

P. Holden et al.

S. E. Pratsinis et al.
F. Krumeich
A. Baiker et al.

R. Amal et al.

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