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

Lutz Mädler

Department of Chemical and Biomolecular Engineering
6279 Boelter Hall
University of California, Los Angeles (UCLA)
Los Angeles, CA 90095 USA
Phone: 310-825-9926
Fax: 310-206-4107
Email: lutz@seas.ucla.edu
UC Lead Campus Program for Nanotoxicology Research and Training

Nanomaterial Science Chemistry

Nominated Standard Reference Materials

Exposure and Risk Assessment Environmental Tox

Public Policy Societal implications Economic forecasts

Nanobiology Nanotoxicology High Throughput screening

CNSI (UCLA & UCSB)
UCLA HSSEAS (Engineering)
Department of Chemistry & Biochemistry

Sch Public Health Southern Cal Particle Center
Bren School of the Envir

David Geffen Sch of Medicine Biological Sciences
Bio-imaging Jonsson Cancer Center
Howard Hughes Institute

Scientific Director
Andre Nel

Anderson School of Business Sociology
Bren School Enviroment (UCSB)
Cellular uptake & delivery using organic & inorganic NP to deliver cytotoxics, drugs, dyes, sensors

Hierarchical oxidative stress paradigm as screening tool for NP toxicity in mammalian cells and transgenic reporter gene animals

NP-induced DNA oxidative damage in genetically susceptible animals

High throughput screening for NP toxicity, including pathway identification & small molecule intervention

NP interactions with soil & aquatic substrates, including use of NP biocidal properties for H₂O purification

NP uptake, processing, fate & toxicity in bacteria

Environmental detox through metal binding ligands attached to vault NP

Nanotech-industry forecast for toxic risk analysis, exposure and life cycle assessment

Project-oriented NP nomination, synthesis, acquisition & NP physical-chemical characterization

UC Lead Campus Program for Nanotoxicology Research and Training at CNSI / UCLA

Lutz Mädler
**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
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

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


UV activation of electron hole pairs leading to bond splitting and radical formation

Material composition, e.g., discontinuous crystal planes and defects, generating active electronic configurations

Electron-donor/acceptor active groups

Redox cycling chemistry via coating metals (e.g., Fe) and organics (e.g., quinones)

Media interactions by particle dissolution, coating, passivation and hydrophobicity/hydrophilicity

Fenton chemistry

Example:

Reduction of $O_2$ to $O_2^-$

Coating may protect the surface, change the cellular uptake or release toxic chemicals

Passivation

Dissolution

Hydrophobicity $\rightarrow$ interactions with cell membranes and uptake

Hydrophilicity $\rightarrow$ water suspendability

$O_2$ $\rightarrow$ $O_2^-$

$Q$ $\rightarrow$ $Q^-$

$Fe^{++}$ $\rightarrow$ $Fe^{+}$

$H_2O$ $\rightarrow$ $H_2O_2$

$OH^-$ $\rightarrow$ $H_2O$

$O_2$ $\rightarrow$ $O_2^-$

$Q = $ quinone

$Q^- = $ semiquinone
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

<table>
<thead>
<tr>
<th>Product</th>
<th>Volume t/y</th>
<th>Ind. Process (dominant)</th>
<th>Use (exemplary)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon black</td>
<td>8 M</td>
<td>Vapor Flame</td>
<td>Inks, Rubber</td>
</tr>
<tr>
<td>Titania</td>
<td>2 M</td>
<td>Vapor Flame</td>
<td>Paints</td>
</tr>
<tr>
<td>Fumed Silica</td>
<td>0.2 M</td>
<td>Vapor Flame</td>
<td>Toothpaste, Tires</td>
</tr>
</tbody>
</table>

Chemical Economics Handbook, 2001; direct industrial quotes
Vapor Flame (Gas-to-particle)

Flame provides:
- High $T_{\text{max}}$
- Large $\frac{\partial T}{\partial t}$

Chemical Reaction & Coagulation

Powder

Coagulation
Aggregation
Agglomeration

Coalescence
Product
Particles

Nucleation & Surf. Reaction

Product
Molecules
& Clusters

Chemical Reaction

Reactant
Vapor/Gas
Molecules

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

\[
-n(v,t)\int_0^\infty \beta(v,v')n(v',t)dv'
\]

\[
\frac{d a}{d t} = -\frac{1}{\tau} (a - a_s)
\]

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

\[-\frac{\partial}{\partial v}[g(v)n(v,t)]\]
Vapor flame materials

Periodic Table of the Elements

- H  He
- Li  Be
- Na  Mg
- 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
- Ac  Th  Pa  U  Np  Pu  Am  Cm  Bk  Cf  Es  Fm  Md  No  Lr

- 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

Lutz Mädler
Spray Flame (Droplet-to-particle)

Liquid supplies:
- Energy
- Material (precursor)

Liquid ensures:
- Mixing
- Release

Flame provides
- High $T_{\text{max}}$
- Large $\frac{\partial T}{\partial t}$

Flame Spray Pyrolysis (FSP) set-up


**Preliminary nanomaterial nominations**

<table>
<thead>
<tr>
<th>Name</th>
<th>Formula</th>
<th>Main use</th>
<th>Production (tons/year)</th>
<th>Primary synthesis method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon black</td>
<td>C amorphous</td>
<td>Tire manufacture, pigments</td>
<td>$8 \times 10^6$</td>
<td>Aerosol flame</td>
</tr>
<tr>
<td>Titania</td>
<td>TiO$_2$, anatase, rutile</td>
<td>Pigments, UV-absorber, catalyst</td>
<td>$2 \times 10^6$</td>
<td>Aerosol flame, sol gel</td>
</tr>
<tr>
<td>Zinc oxide</td>
<td>ZnO$_2$</td>
<td>Polymer filler, UV-absorber</td>
<td>$0.6 \times 10^6$</td>
<td>Polymer filler, UV-absorber, Aerosol evaporation/oxidation, sol gel</td>
</tr>
<tr>
<td>Ceria</td>
<td>CeO$_2$ / Ce$_2$O$_3$</td>
<td>Catalyst (cars), CMP polishing</td>
<td>$0.01 \times 10^6$</td>
<td>sol gel, serosol flame</td>
</tr>
<tr>
<td>Silica</td>
<td>SiO$_2$ amorphous</td>
<td>Dispersant, and flowing agent</td>
<td>$&gt;0.2 \times 10^6$</td>
<td>Aerosol flame, sol gel</td>
</tr>
<tr>
<td>Zirconia</td>
<td>ZrO$_2$</td>
<td>Ceramic, catalyst support</td>
<td></td>
<td>Aerosol flame, Sol gel</td>
</tr>
<tr>
<td>Quantum dots</td>
<td>CdSe/ZnS/InAs/InP/InGaP</td>
<td>Medical imaging</td>
<td>1-4 uM</td>
<td>Wet chemistry</td>
</tr>
<tr>
<td>Carbon nanotubes</td>
<td>C ordered structure</td>
<td>Composite filler, electronic applications</td>
<td>??</td>
<td>Gas phase synthesis on catalyst</td>
</tr>
<tr>
<td>Fullerenes</td>
<td>C ordered structure</td>
<td>medical applications</td>
<td>??</td>
<td>Wet chemistry</td>
</tr>
</tbody>
</table>
Increasing FUNCTION

Increasing COMPLEXITY

Increasing VALUE

Single-phase particles & aggregates

Mixed-phase & composition

Embedded, surface clusters & shells

Surface functionalization

Formulations

Immobilization, films & 2D coatings

Devices

NANOTOXICology
Research & Training Program

Lutz Mädler
### 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

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

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

- 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

Nanoparticle

Solid / liquid interface

Suspending media

L. Mädler workshop presentation on Bio-physicochemical Interactions of Engineered Nanomaterials September 9 - 11, 2007 at CNSI/UCLA - 18 -
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

5 nm TiO$_2$ NP

Surface energy of NP and media interaction

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

Surface energy of NP and media interaction

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

Same nanoparticle with surface-bound water

Importance of Particle Size

Stability

all nanoparticles are metastable

transformation always possible

strained surface alters total energy

\[ G_{\text{total}} = G_{\text{bulk}} + G_{\text{surface}} \]

quantify dissolution

B. Gilbert, L. Mädler, X. Tian, A. Nel (2007)
Importance of Particle Size

Stability

All nanoparticles are metastable

Transformation always possible

\[ G_{\text{total}} = G_{\text{bulk}} + G_{\text{surface}} \]

Strained surface alters total energy

Quantify dissolution

B. Gilbert, L. Mädler, X. Tian, A. Nel (2007)
Importance of Particle Size

Stability

all nanoparticles are metastable

transformation always possible

strained surface alters total energy

\[ G_{\text{total}} = G_{\text{bulk}} + G_{\text{surface}} \]

predict speciation

quantify dissolution

B. Gilbert, L. Mädler, X. Tian, A. Nel (2007)
ZnO NP of different sizes

(a) 1 mL/min
(b) 2 mL/min
(c) 3 mL/min
(d) 4 mL/min

ZnO dissolution and toxicity

Soluble nanoparticles

Dissolved-ions dissolution front

mammalian cell

cell membrane

endosomal compartment

a

b

c

non-acidifying compartment

acidifying compartment

B. Gilbert, L. Mädler, X. Tian, A. Nel (2007)
Oxidative Stress as a Stratified Response

## Tiered approach

<table>
<thead>
<tr>
<th></th>
<th>Abiotic ROS</th>
<th>Cellular ROS</th>
<th>Tier 1</th>
<th>Mito perturba</th>
<th>Cyto-toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient UFP</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>TiO₂</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbon Black</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fullerol</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>COOH-PS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NH₂-PS</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>++++</td>
</tr>
</tbody>
</table>

Control zinc oxide solubility by encapsulation

Modified FSP synthesis process

SiO₂ encapsulation (different loadings)

Controlled decrease in:
• ZnO exposed surface (partial or total encapsulation)
• Zn²⁺ release into media (cell)

Bio-consequences: determined by released Zn²⁺

L. Mädler, X. Tian, A. Nel (2007)
Encapsulation

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

Biological impact varies as a result of:
- Size
- Shape
- Adhesive area
- composition

Experimentation & exposure conditions should be as close to these units as possible

Particle dispersion methods are important

A. Nel and L. Mädler workshop presentation on Bio-physicochemical Interactions of Engineered Nanomaterials September 9 - 11, 2007 at CNSI/UCLA
NP agglomerate structure

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.
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

Nanoparticle photo activity

- Radiation properties:
  - wave length
  - power
  - duration

- Particle properties:
  - material (band gap)
  - size
  - crystallinity

- Media properties:
  - hole electron acceptor
  - conjugated surface
  - adsorption process

Time for:
- Excitation
- Recombination
- Adsorption
- Reaction

L. Mädler workshop presentation on Bio-physicochemical Interactions of Engineered Nanomaterials September 9 - 11, 2007 at CNSI/UCLA
Nanoparticle photo activity

Absorption of a photon

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


Lutz Mädler
Photo excitation in the visible range

**TiO₂**  **Fe-TiO₂**

Activation with **Visible light**

Electron hole reduction (e.g. biological molecules)

Activation with **UV light only**

Electron hole reduction (e.g. biological molecules)
Impurity energy levels by doping

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

Noble metal doping

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

Nanoparticles as catalysts for Protein Fibrillation

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.


Large NPs (blue) and an amyloid protein (green) in its monomeric and folded state.
Change of protein function by Adsorption

Chicken egg lysozyme
Activity ++++

Monolayer adsorption
a-helical structure
Activity +++

Multilayer adsorption
Loss of a-helicity
Activity +

Physical-chemical variables:
- size (curvature)
- shape
- surface energy

Surface Modification of Metal Oxides

Metal oxides coated with NH₂-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.

Funding supported by:

and KTI, DFG, Parsons, UC Discovery