EU-U.S.: Bridging NanoEHS Research Efforts Joint Workshop
Venice, Italy
12-13 march 2015

Characterization CoR
U.S. co-chair: Anil Patri, U.S. Food and Drug Administration
EU co-chair: Kenneth Dawson, University College Dublin

Agenda
• Perspectives on Nanomaterial Characterization:
  • 10 minutes each co-chair
• Round table discussion on 5 questions: 1 hour
• Wrap up: 10 minutes
Considerations for Characterization CoR

• Avoid duplication of other efforts
  – OECD
• Focus on assays for reproducible science through characterization
• Collaboration between US & E.U. Research communities
• Round robin studies
• Standards
NANOMATERIALS
And
ANALYSIS
Focus on Engineered Nanomaterial

- Exciting possibilities with Nanomaterial
- Various compositions, combinations and properties
- Challenging for regulatory assessment

Dendrimers

Fullerenes

Metal nanoparticles

Metal Oxides

Liposomes

CNTs

Core-shell particles

Nanoemulsions

Polymers

Nanorods

Quantum Dots
Physico-chemical and Functional Parameter Characterization to Consider for Nanomaterial Assessment

Nanoformulation Properties

- Size, Shape
- Surface ligand/coating
- Surface ligand density
- Surface charge
- Solubility
- Shape/Architecture
- Stability
- Purity

Functional Properties

- Therapeutic
  - Drug loading, stability, release
- Targeting
  - Conjugation, quantitation, activity
- Imaging
  - Stability
Analytical

- Size, Size distribution
- Topology, Shape
- Molecular weight
- Surface characteristics
  - Net charge
  - Zeta potential
- Functionality (Identification, Quantitation, Distribution)
  - Targeting agents
  - Imaging agents
  - Therapeutics
- Composition
  - Elemental
  - Core-shell
  - Coating

- Purity
  - Homogeneity/Inhomogeneity
  - Residual solvents
  - Free components
  - Free vs bound drug

- Stability
  - Thermal
  - pH
  - Photo
  - Freeze-thaw
  - Lyophilization
  - Centrifugation
  - Short-term storage
  - Long-term storage
  - Drug release kinetics
  - Stability of the coating
Instrumentation

**Spectroscopy**
- Mass Spectroscopy
- Nuclear Magnetic Resonance
- UV-Vis
- Infrared
- Raman
- Fluorescence
- Refractive Index
- XPS

**Chromatography/Separations**
- HPLC
- GC
- FPLC
- Size Exclusion
- Asymmetric Field-flow Fractionation
- Centrifugal FFF
- Disc Centrifuge
- Analytical Ultracentrifugation
- Capillary Electrophoresis
- Gel Electrophoresis

**Microscopy**
- Transmission Electron Microscopy
- Scanning Electron Microscopy
- Atomic Force Microscopy

**Scattering/Diffraction**
- Dynamic Light Scattering/ZP
- Static Light Scattering
- Electron Diffraction
- X-ray Diffraction
- Neutron Diffraction

**Other**
- Surface Plasmon Resonance
- Polarimetry
- Laser Diffraction
- Microchannel Resonator
- Nanoparticle Tracking (Nanosight)
- Coulter Counter (qNano)
- Liquid Surface Area (Acorn; NMR)
- Gas Adsorption System (BET)
- Charge Titration (ZP)
- TGA/DSC
Case study of variability

Protein analysis by 2D PAGE
TEM analysis of particles uptake by macrophages


Difference in surface characteristics can cause dramatically different in vivo outcomes.

How to quantify coating polymer for batch-to-batch consistency and stability assessment?
Nanomaterial can have different sizes, shapes, compositions, coatings, stabilities

- Evaluate which physico-chemical and functional property is critical for intended use
- Evaluate which parameter is important for desired efficacy and causing unintended toxicity
- Develop appropriate and robust reproducible assays to evaluate consistency
Welcome to caNanoLab

Welcome to the cancer Nanotechnology Laboratory (caNanoLab) portal. caNanoLab is a data sharing portal designed to facilitate information sharing across the international biomedical nanotechnology research community to expedite and validate the use of nanotechnology in biomedicine. caNanoLab provides support for the annotation of nanomaterials with characterizations resulting from physico-chemical, in vitro and in vivo assays and the sharing of these characterizations and associated nanotechnology protocols in a secure fashion.

Browse caNanoLab

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Standards

**Reference Material Development**
- NIST RMs 10, 30, 60 nm gold colloids
- Many other RMs in development

**Standard Protocols and Procedures**
- ASTM E56 Committee on Nanotechnology
- ISO TC229 Nanotechnologies

**Inter Laboratory Studies and round Robin Tests**
- DLS, AFM, TEM, SEM, Cytotoxicity, Hemolysis
- International Alliance for NanoEHS Harmonization (IANH)
- IRMM
- NRC Canada

E2524 (hemolysis)
E2525 (CFU-GM inhibition)
E2526 (kidney and liver cytotox)
Hydrodynamic size (DLS)
NANOMATERIALS
and
SYSTEM
COMBINED
Mapping protein binding sites on the biomolecular corona of nanoparticles

Philip M. Kelly, Christoffer Åberg, Ester Polo, Ann O’Connell, Jennifer Cookman, Jonathan Fallon, Željka Krpetić* and Kenneth A. Dawson*

Nanoparticles in a biological milieu are known to form a sufficiently long-lived and well-organized ‘corona’ of biomolecules to confer a biological identity to the particle. Because this nanoparticle–biomolecule complex interacts with cells and biological barriers, potentially engaging with different biological pathways, it is important to clarify the presentation of functional biomolecular motifs at its interface. Here, we demonstrate that by using antibody-labelled gold nanoparticles, differential centrifugal sedimentation and various imaging techniques it is possible to identify the spatial location of proteins, their functional motifs and their binding sites. We show that for transferrin-coated polystyrene nanoparticles only a minority of adsorbed proteins exhibit functional motifs and the spatial organization appears random, which is consistent, overall, with a stochastic and irreversible adsorption process. Our methods are applicable to a wide array of

The “Sweet” Side of the Protein Corona: Effects of Glycosylation on Nanoparticle–Cell Interactions

Sha Wan,† Philip M. Kelly,‡ Eugene Mahon,‡ Henning Stöckmann,‡ Pauline M. Rudd,‡ Frank Caruso,§ Kenneth A. Dawson,† Yan Yan,*,‡ and Marco P. Monopoli*,†

†Centre for BioNano Interactions, School of Chemistry and Chemical Biology, University College Dublin, Dublin 4, Ireland, ‡NIBRT, GlycoScience Group, NIBRT—The National Institute for Bioprocessing Research and Training, Fosters Avenue, Mount Merrion, Blackrock, Co. Dublin, Ireland, and §ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, and the Department of Chemical and Biomolecular Engineering, The University of Melbourne, Parkville, Victoria 3010, Australia
Engineered Nanoscale written in our biology
new medicine-new science; ADME Models will not work

Chemicals Partition but Nanoparticles processed-energy of cell used

Salvati et al.,
Nature Nanotech (2013)
Mahon et al.,
Nanoscale (2014)
Concept of Nanoparticle Libraries and Homologous Series
Reference Library: Gold Library of Shaped NPs (UCD)
2D TEM -> 3D model -> Wireframe model

materials of the future
Nanoparticle collisions with the cell membrane in presence of biological fluids

Many particle trajectories
Most unsuccessful in entering cell

There are few that enter
And they do so by regulated pathways (later)
A ‘TOXIC’ MODEL PARTICLE; OUTCOME DEPENDS ON PRESENCE OF ‘MILEU’

*In vitro* conditions: massive cell death

*In vivo* conditions: completely benign

EM confirms higher uptake and some NPs free in the cytosol in absence of serum

REASON FOR MUCH CONTROVERSY
Wrong conditions
Meaningless outcome?

RECOGNITION


Cozzarelli Prize NAS 2008

TIME TO FIND OUT HOW ALL THIS WORKS

EACH SPECIFIC FUNCTIONAL ELEMENT OF EACH PROTEIN ON THE CORONA CAN NOW BE MAPPED OUT PROVIDING A PROPOSAL FOR THE LIKELY INTERACTIONS OF NANOPARTICLES IN THAT EXPOSURE MEDIUM WITH THOSE CELLS

Immunogold (or other) probe for specific (adsorbed) biomolecule epitopes
Epitope Mapping

Polyclonal Tf receptor binding
- 318 ± 22
- 407 ± 37
- 259 ± 42
- 141 ± 20
- 76 ± 22
- 41 ± 7

Monoclonal Tf near receptor binding site

Progressive binding and epitope SATURATION monitored using DCS and electron microscopy - counting of epitopes

Transferrin Epitopes:
- TfR - Yellow binding
- Monoclonal Green (aa. 142-145)

‘Distributions’ replace concept of fixed structures - averaged numbers of Ig bound from EM

In this example most particles similar

Immunogold (or other) probe for specific (adsorbed) biomolecule epitopes
THE CONCEPT OF ‘GEOMETRY’ IN MOLECULAR SCIENCES WILL BE REPLACED BY DISTRIBUTIONS OF DISTANCES BETWEEN FUNCTIONAL EPITOPEs OF NANOPARTICLES - ULTIMATELY THIS COMPLETELY DEFINES RELEVANT PROPERTIES OF ENSEMBLE OF NANOPARTICLES
Population analysis yields the same result as mass spec.

Ratio of Tf to IGG
Mass Spec = 93 %
DCS = 89 %

Single particle analysis shows the individual biological Identity

Kelly, P.M. et al
Questions

1. Which properties/aspects of nanomaterials characterization are key in defining their biological impacts from a data reproducibility standpoint?
2. How can those properties be identified, measured qualitatively and quantitatively by current techniques, thereby providing the characterization of those materials in the biological/nanosafety context?
3. Which aspects of the total systems (nanoparticle properties, exposure conditions, environment etc.) must be fixed or controlled to render the system reproducible, and fully characterized from the point of view of biology/nanomedicine?
4. If new methods are required to accomplish these tasks, what are the technical challenges and how should they be developed into robust reproducible assays?
5. What standards are needed to facilitate regulatory review and commercialization?