This arena is already EU-US

Australia!
Nanoparticles travel into cell via Existing energy dependent pathways

Recognition Motifs and ‘Identity’ Matters

Salvati, A. Time and space resolved uptake study of silica nanoparticles by human cells. Mol Biosyst, 7, 371-378
Interactions with Living World Occur Via Interface

Size, shape, other Factors matter
Surface (interface) Main element of ‘Biological’ identity

This cell has 2 square microns Of interface; in lysosomes-for ever

50 nm silica entering A549 cell

Cedervall, T. Understanding the nanoparticle-protein corona
Hypothesis of Biological Identity
Defined by exchange at the In situ Interface

Cozzarelli Prize NAS 2008

PNAS, 2007, 104, 2050-2055
NATURE NANO, 2009, 4, 546
JACS, 2010, 2011
Typical Engineered Nanoparticle Physiochemical Nature of the Interface with Living World

‘Hard Corona’
Nanoparticles are surface covered by proteins

Particles in plasma

Particles after washing

Lighter shell is a rudimentary visualization of the corona
Protein coatings persist in time, and do change
Washing the particles does not remove the corona

Interactions of Nanoparticle with Cells, others surfaces, and extracellular matrix Determined by Hard Corona

Protein Array (>10,000 proteins)
Used to assess interactions of nano Particle-corona with ‘all’ human proteins

Pristine Surface of Nanoparticle Interacts with ‘everything’
Particle in Presence of Biological (and Environmental) more specific

Non-Specific Interactions of Nanoparticles ubiquitous
Fibronectin on Hard Corona Increases increasing plasma At the expense of other proteins; Corona Identity Changes

% Protein in hard Corona

10%  25%  55%

40nm COOH Mass Spectrometry

Histidine-rich glycoprotein  Fibronectin
Human Plasma at increasing concentration
Receptor for Fibronectin Targeted In Vivo conditions
FNDCA3 Signal Intensity

Without Protein Huge
Non-Specific nanoparticle
Surface-surface Interactions

‘In between-here 10%-Interactions ‘cancel’

In presence of High Protein, Specific interactions

Nanoparticle Corona Fibronectin Receptor interactions

PBS
10% Plasma
55% Plasma
75% Plasma
The Nanoparticle-Corona Biomolecular Complex Interactome

Increasing Plasma Concentration

Key Target Proteins in Interaction Network
(Not all need to be biological Targets)
Hard corona Changes
With different Biological (environmental) Fluid
*Identity is Context Dependent*

NPs incubated with different plasma concentrations to mimic *in vitro* and *in vivo* conditions

Here Biological Identity Changes going
*From experimental in cell to in vivo conditions*

<table>
<thead>
<tr>
<th>Gel band Mw</th>
<th>Acc number</th>
<th>Protein Identity</th>
<th>Spectral Counts</th>
<th>NSpC [10% plasma]</th>
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Nucleation and growth of protein fibrils.

Particle protein disruption-unfolding at the surface

Two More Examples
Trojan Horse effect of corona
Corona determines biodistributions?

‘positive’ Particles apoptosis
Corona screens positive charge
Which is re-expressed at destination

Protein corona determines
Destination of nanoparticles
In vivo

Highly Swollen Lysosomes

A. Salvati and B. Wong

W. Kreyling picture
Messages

• **ACUTE HAZARDS IN WHOLE FIELD LESS THAN EXPECTED**

• ‘REAL’ IN SITU IDENTITY OF NANOPARTICLE-fundamental for
  - Hazard classification
  - Biokinetics, Biodistributions
  - Parameters for ADME and QSARS
  - Might not be as complex as we think?!

• **SURFACE ADSORBED SPECIES (ENVIRONMENT AND LIFE CYCLE ISSUE)**

• **FRAME RESEARCH TO EVALUATE HYPOTHESES (CHARACTERISE RIGHT THINGS ETC)**

• **IF ‘INTERFACE’ IS (OFTEN) WHAT MATTERS, NEED NEW ‘TOOLS’**

*By understanding these issues we have the potential to make innovation safe for a generation*