New Tools for Nanosafety Assessment

Vladimir Lobaskin
University College Dublin

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Where we are

- Limited capacity to predict hazard for new materials as the properties of concern are not known

Standard NM characterisation is not sufficiently informative

Toxicity mechanisms are not known

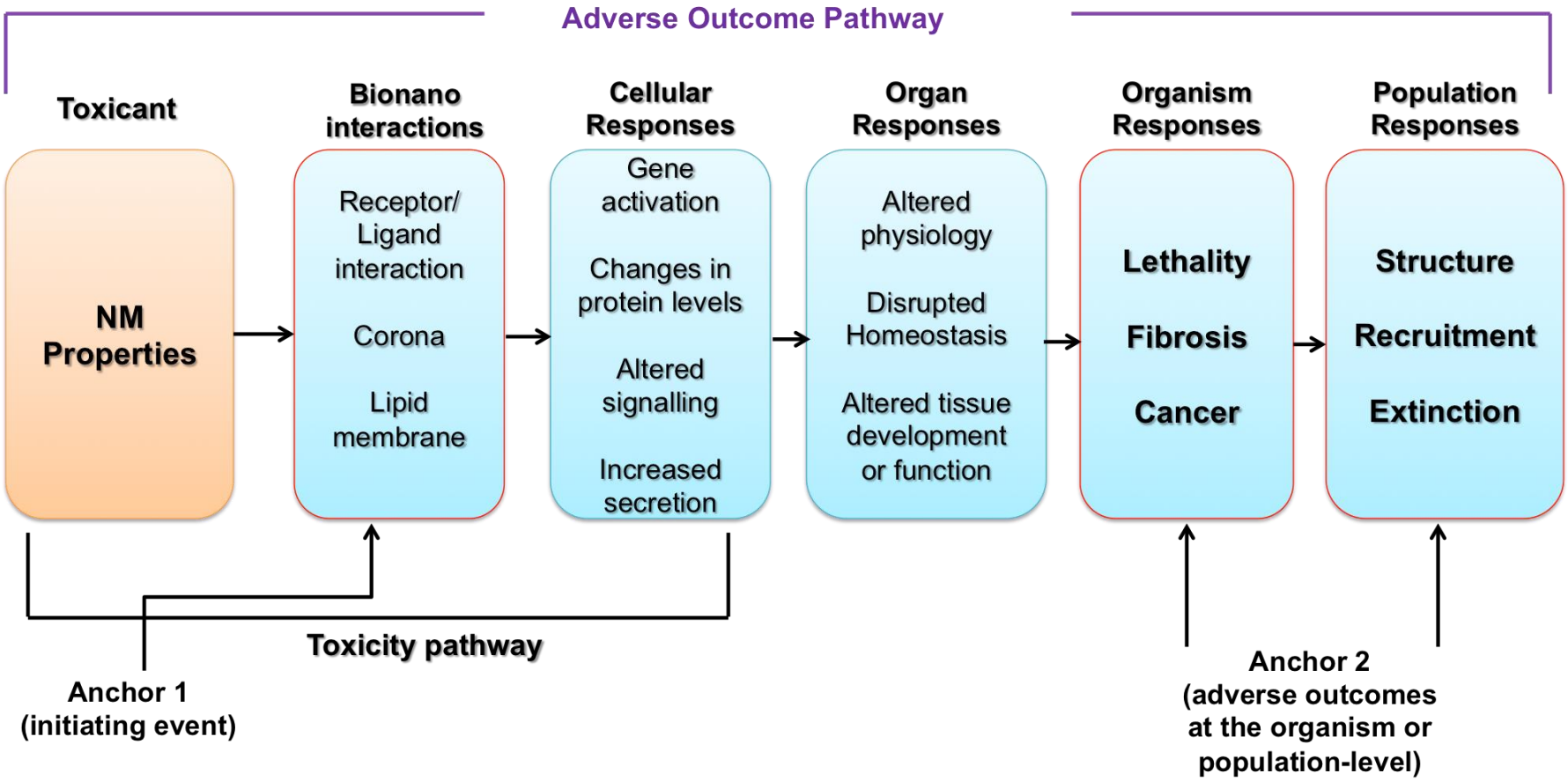
- Real dosage/NP state after uptake not known
- Many in vitro toxicity endpoints (e.g. EC50) irrelevant

Mechanistic understanding of nanotoxicity

New level of complexity:

- Knowing the nanomaterial chemistry and structure is not enough: coating, adsorbed materials, surface energy, dielectric properties may be important
- Nanoparticles use specific ways of systemic distribution, which are unavailable for individual molecules or micron-sized particles. Exposure route can be equally important
- Toxicity and adverse outcomes may be related to molecular perturbation of cell structures/pathways and not direct damage

Mechanistic Understanding of Toxicity



T. E. H. Allen et al., Defining Molecular Initiating Events in the Adverse Outcome Pathway Framework for Risk Assessment. *Chem. Res. Toxicol.* 2014, **27**, 2100−2112

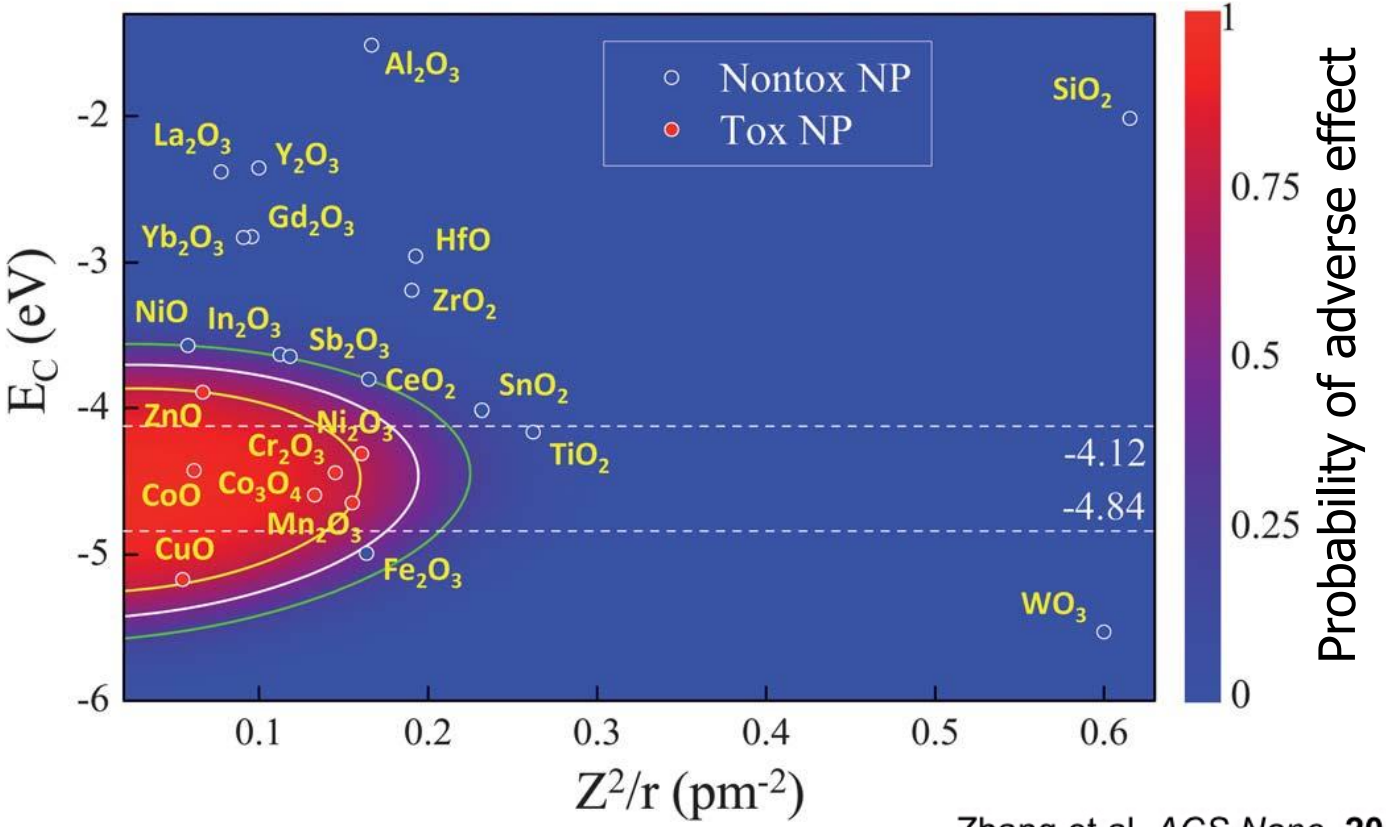
New toxicity assessment paradigm

Pathway-based assessment:

Understanding of bionano interactions is needed to address Molecular Initiating Events, systemic transport

Known adverse effects/ properties of concern

Conduction bandgap/ redox potential – oxidative stress

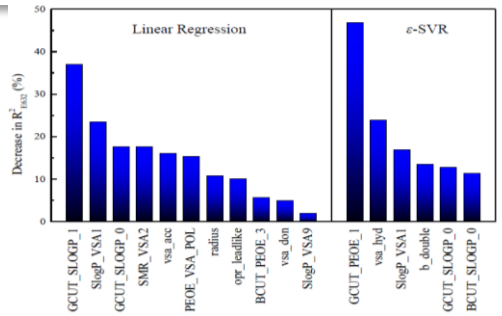


Zhang et al. *ACS Nano*, **2012**, 6(5):4349-4368

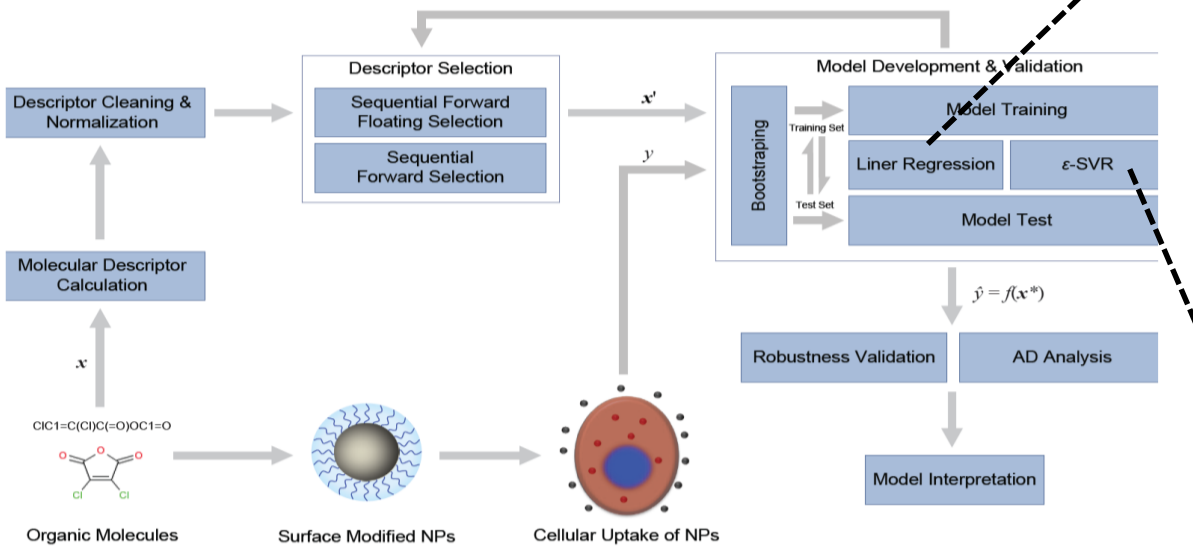
Bionano interactions

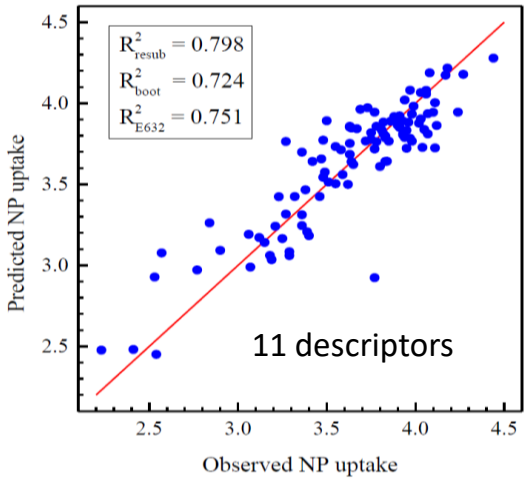
Quantitative Structure-Activity-Relationships for Cellular Uptake of Surface-Modified Nanoparticles

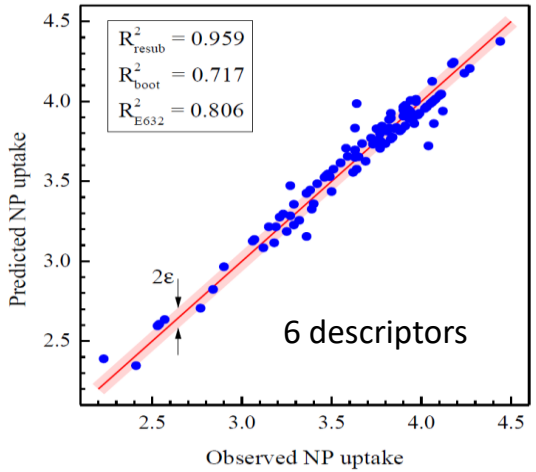
Rong Liu[1,2], Robert Rallo[3], Muhammad Bilal[2] and Yoram Cohen[*,1,2,4]



Selected descriptors mostly related to lipophilicity and Van der Waals Surface Area



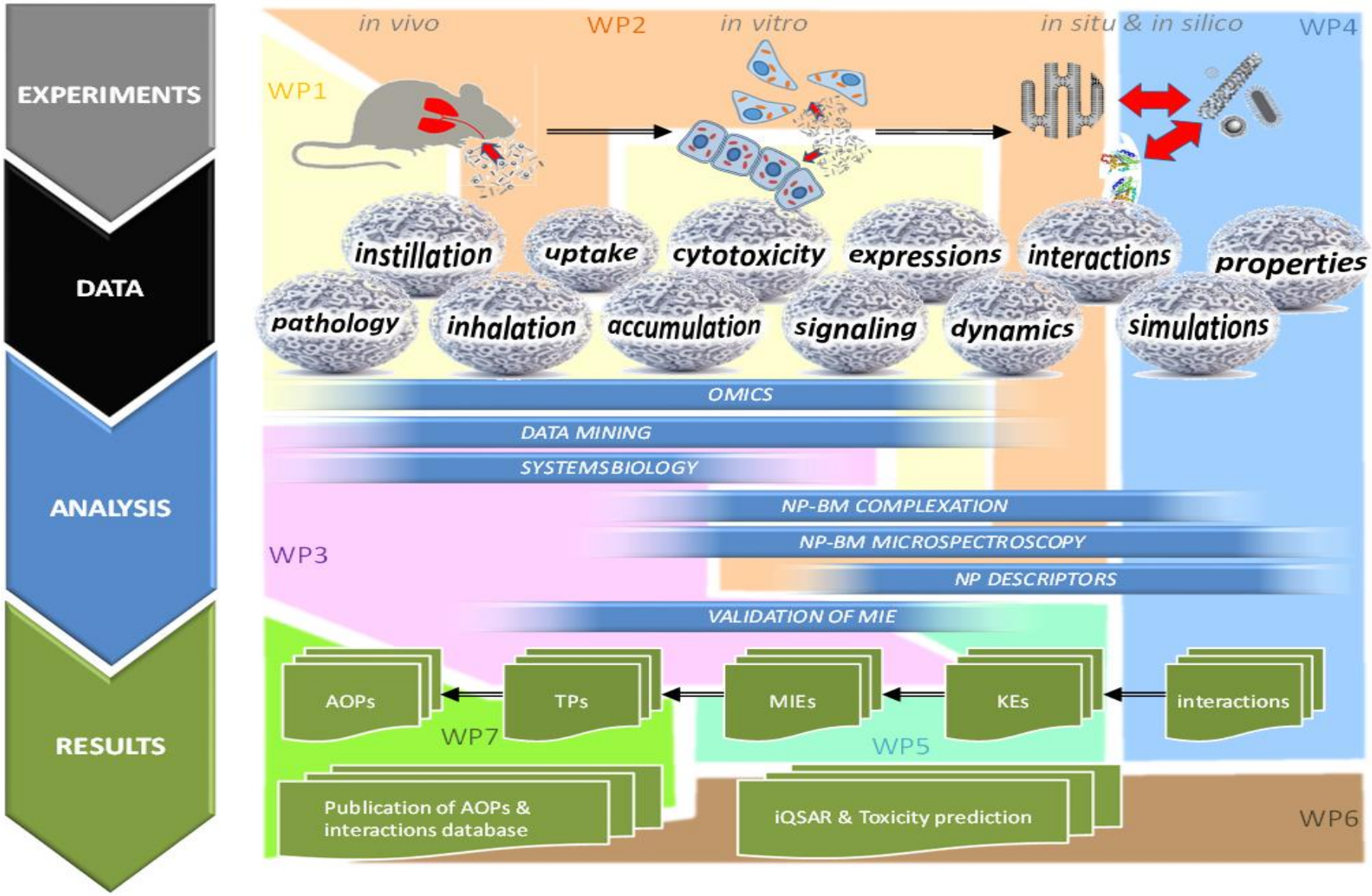




Dataset:109 NPs for biomedical applications
Core of 3 nm Iron oxide + dextran layer (38 nm) with different organic modifications

Combinatorial Chem & HTS, 2015 18(4):365-375

H2020 SmartNanoTox

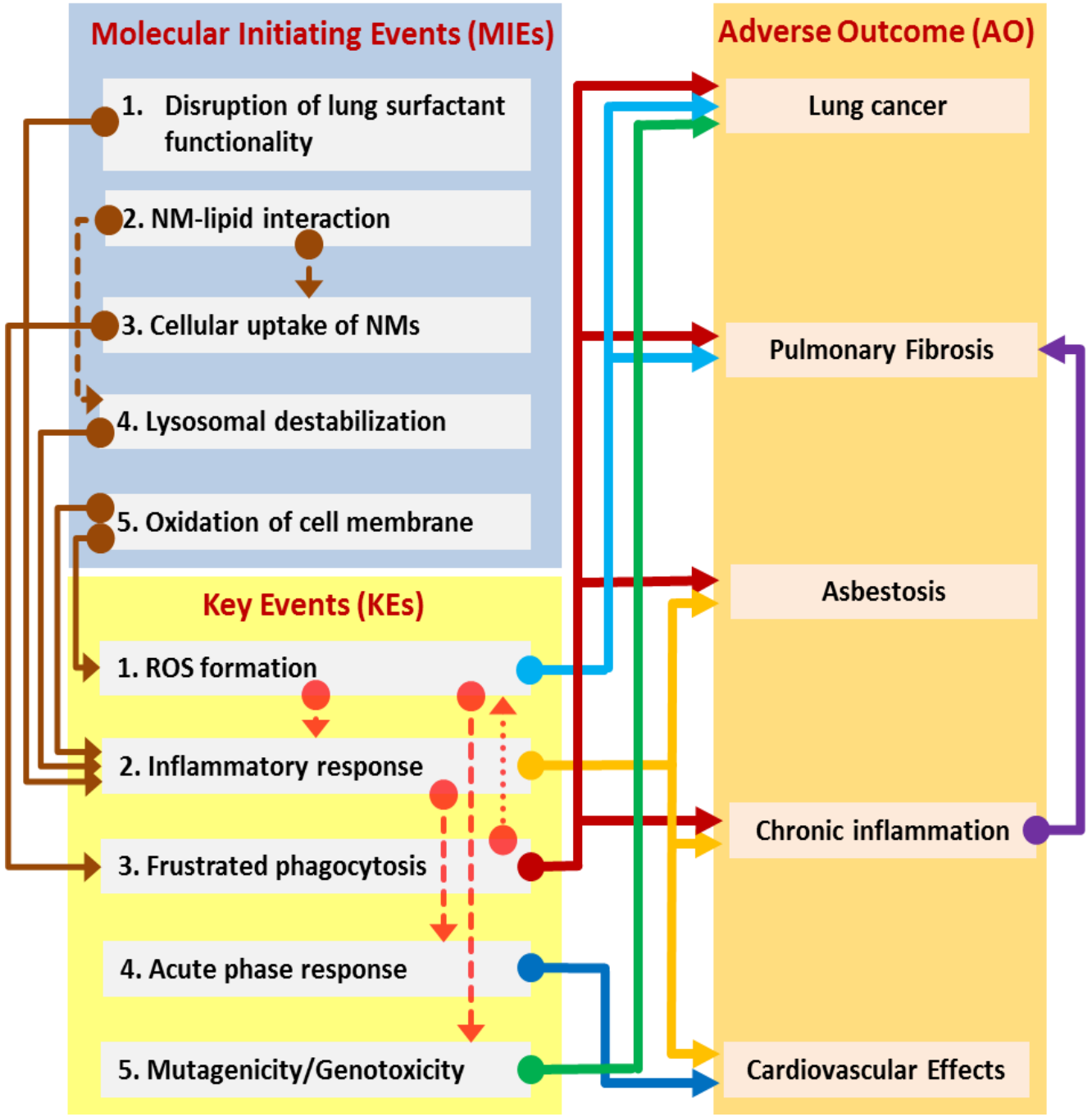


SmartNanoTox
Smart Tools for Gauging Nano Hazards

projected impacts

- Described and validated respiratory AOPs
- Database of bionano interactions for 60+ NMs with proteins and lipids
- Identified NM properties of concern
- Mechanism-aware toxicity assessment tools
- Methods for NM tracking inside the tissues and post-uptake characterization
- Replacement of animal experiments by in vitro/in silico tests

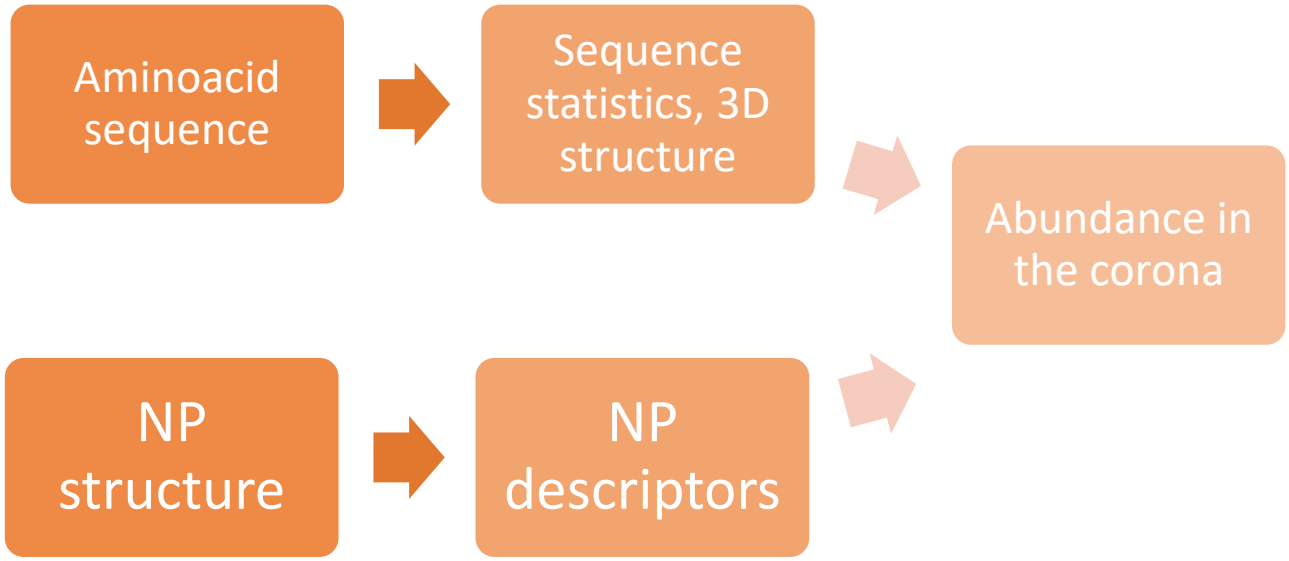
AOP
MIE
KE


Molecular Initiating Events (MIEs)
1. Disruption of lung surfactant functionality
2. NM-lipid interaction
3. Cellular uptake of NMs
4. Lysosomal destabilization
5. Oxidation of cell membrane
Key Events (KEs)
1. ROS formation
2. Inflammatory response
3. Frustrated phagocytosis
4. Acute phase response
5. Mutagenicity/Genotoxicity
Adverse Outcome (AO)
Lung cancer
Pulmonary Fibrosis
Asbestosis
Chronic inflammation
Cardiovascular Effects

MIEs / KEs	Detection Assays	In Vitro Lung Models	
		Cell free (Surfactant)	Alveol. cells
Disruption of lung surfactant (LS) functionality	Constrained drop surfactometer, MD simulation	✓	
NM-lipid interaction	Microscopy (FRET, FRET-FMS, pFMS, DLS, EPR), MD simulation	✓	✓
Cellular uptake of NMs	Microscopic localization (pFMS)	✓	✓
Lysosomal destabilization	Membrane leakage, cell viability, MD simulation	✓	✓
Oxidation of cell membrane	Antioxidant depletion, 'Band Gap' calculation	✓	
ROS formation	Redox sensitive dyes & marker genes, Electron paramagnetic resonance (EPR)	✓	✓
Inflammatory response	Inflammatory gene & protein expression		✓
Frustrated phagocytosis	Lysosomal damage, inflammasome activation		✓
Acute phase response	Release of acute phase reactants		✓
Mutagenicity/Genotoxicity	Marker gene & protein expression		✓

NP-protein interactions
Bio/Nanoinformatics approach

Prediction of corona content using NP and protein descriptors:

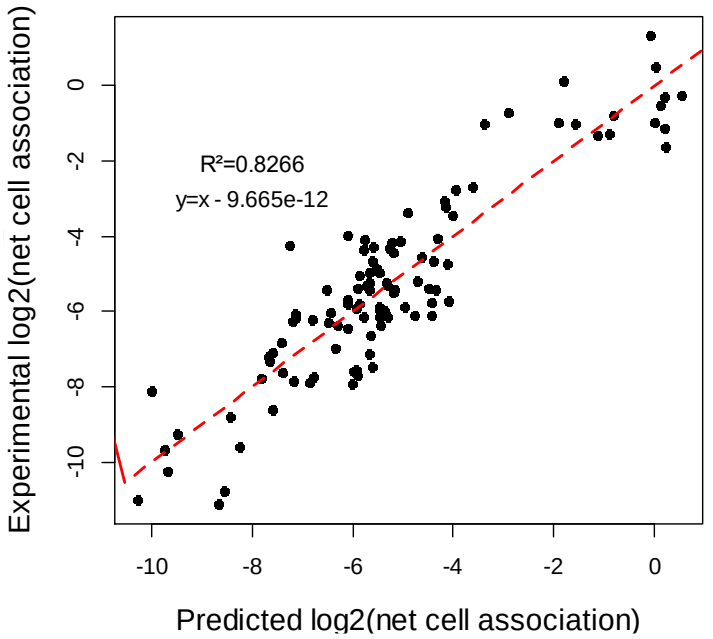


Sequence descriptors (PepStat), 3D structure (I-TASSER)

NP-protein interactions
Bio/Nanoinformatics approach

Prediction of Key Events of the AOP using protein descriptors:

Protein descriptors → Relative abundance

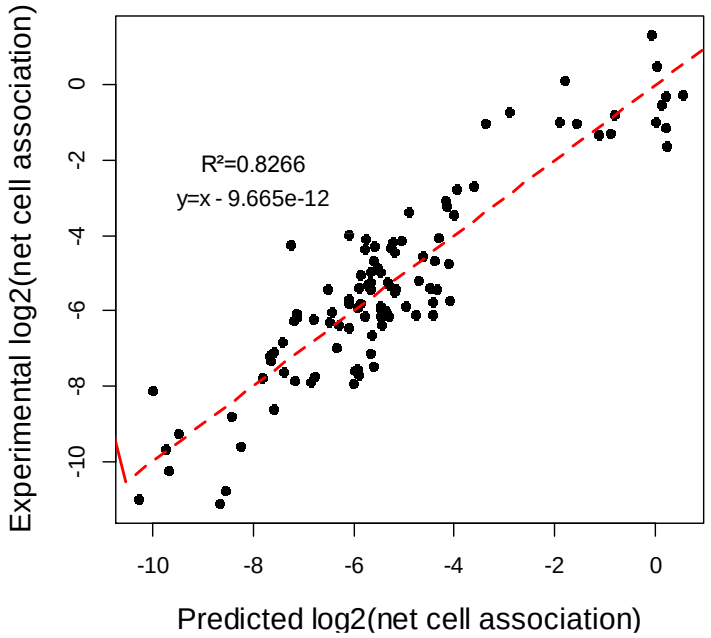


Experimental data from Walkey et al. ACS Nano 2014.
Kamath et al. Current Topics in Medicinal Chemistry, 2015

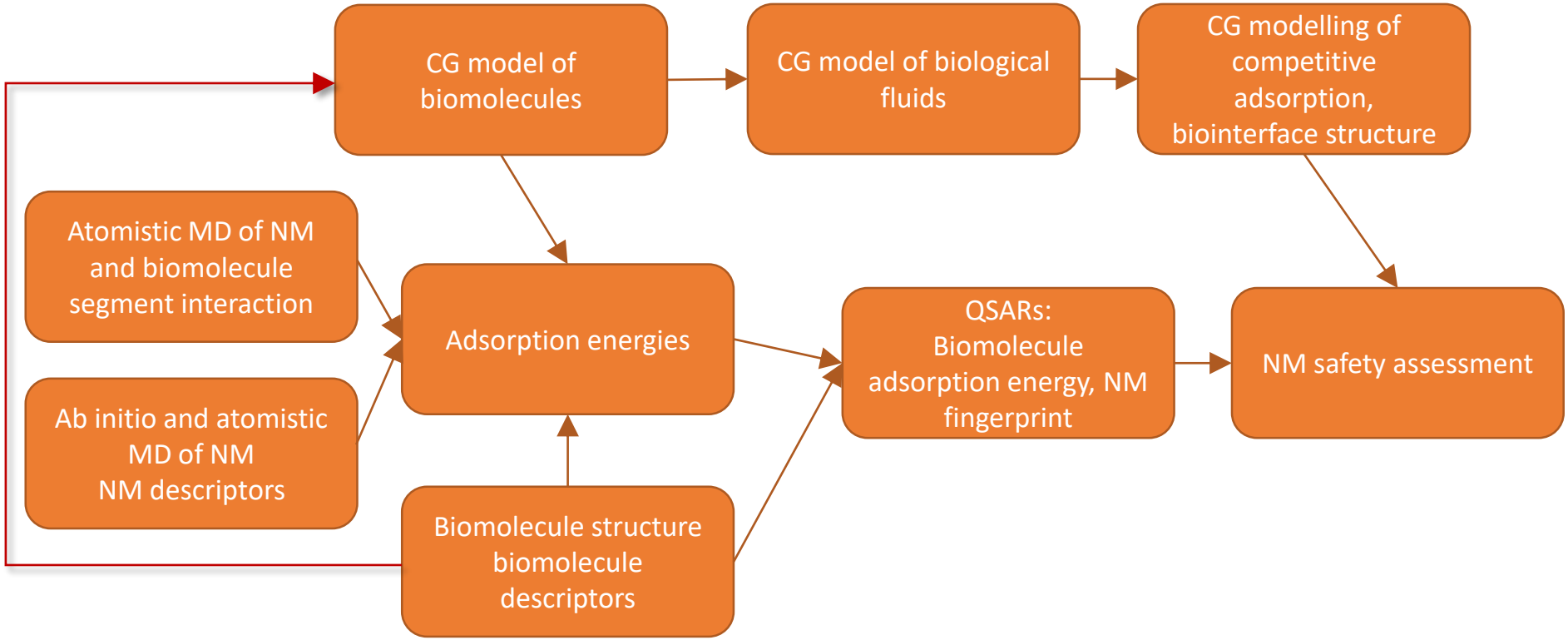
NP-protein interactions
Bio/Nanoinformatics approach

Prediction of Key Events using protein descriptors:

$$\text{Log2(net cell association)} = -4.924$$
$$+11.86 \times \text{Molecular weight} - 3.03 \times \text{Charge}$$
$$+2.542 \times \text{Isoelectric point}$$
$$-1.522 \times \text{Probability of expression in inclusion bodies}$$
$$+3.233 \times \text{Aromatic amino acids percentage}$$



NP-protein interactions
Bio/Nanoinformatics approach



Other possibilities

- Work with collected toxicity databases
- Add more advanced / more appropriate descriptors:
  - Band gap
  - Ionisation potential
  - Dissolution rate
  - Hydration energy
  - Surface energy
  - Protein binding affinity
  - …
- Reanalyse the existing data (NANoREG, MARINA, …)