Mechanistic computational modeling of respiratory effects of ultrafine particle inhalation

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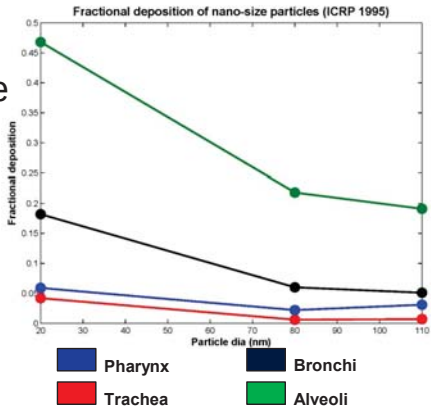
Motivation for modeling inhalable particle toxicology

- Ultrafine particles (< 100 nm) are ubiquitous in the environment and are generated from natural as well as man-made sources, with engineered nanoparticles becoming a part of an ever-growing number of consumer products
- Unlike PM10 and PM2.5, they are not regulated in the environment and data regarding content of engineered nanoparticles in consumer products is scarce
- Unlike larger particles, ultrafine particles do not get completely arrested in the respiratory airways and a large fraction of them travel to the alveoli and are also translocated to the blood circulation and ultimately to other organs (Kreyling *et al.*, 2009; MacCalman *et al.*, 2009)
- Due to the small size and large surface area of these particles, their interaction with cells and respiratory surfaces creates alterations in respiratory function, even at sub-toxic levels
- A mechanistic understanding of these processes occurring at multiple scales, requires mathematical modeling supported by experimental measurements of observable endpoints



Respiratory physiology at multiple scales

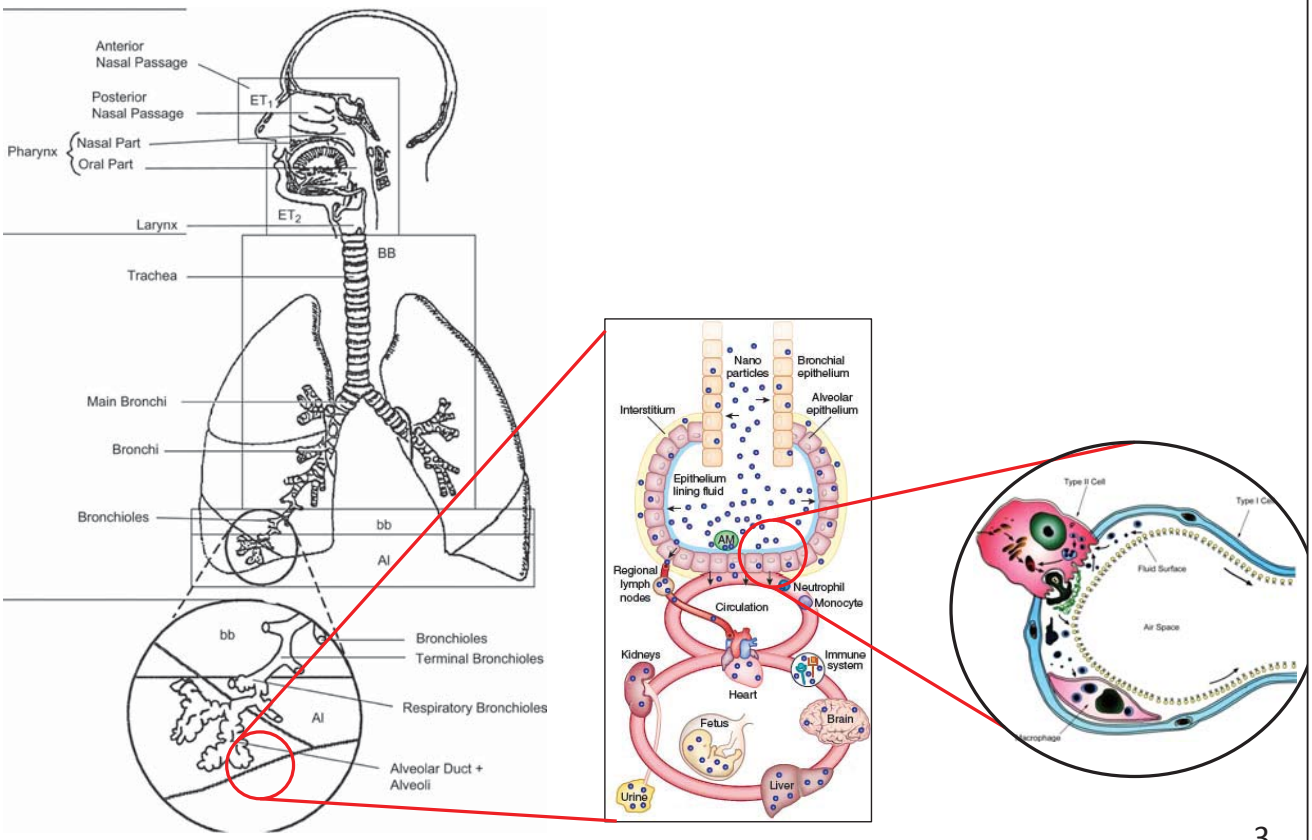
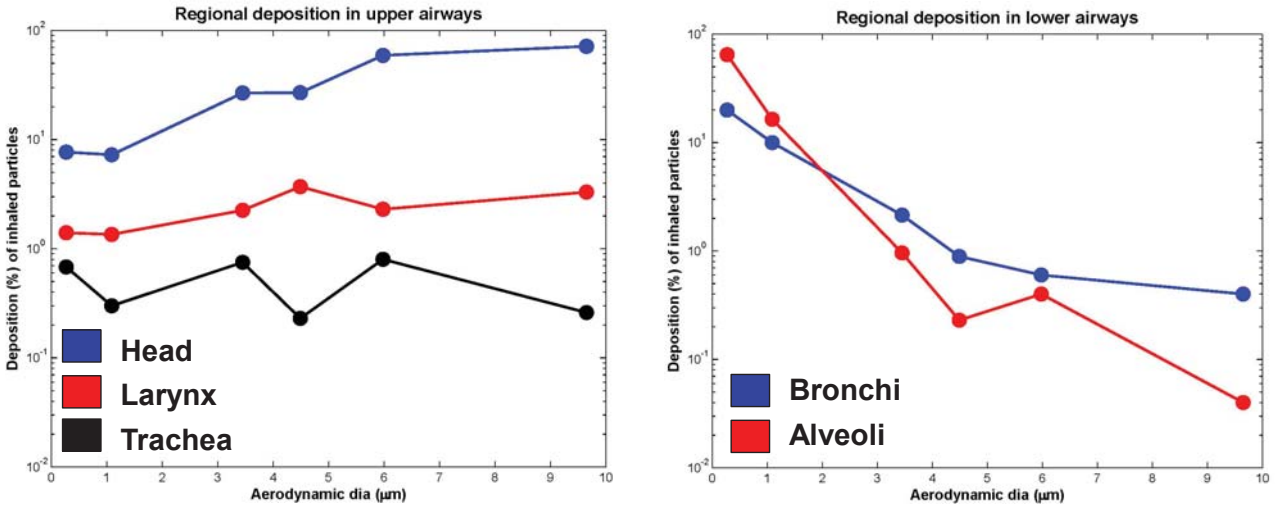


Figure from ICRP, 1995

Figure adapted from Kreyling *et al.*, 2010

Inhaled particle dosimetry in the respiratory system

Regional ultrafine particle deposition in mice (data from Raabe et al., 1988)

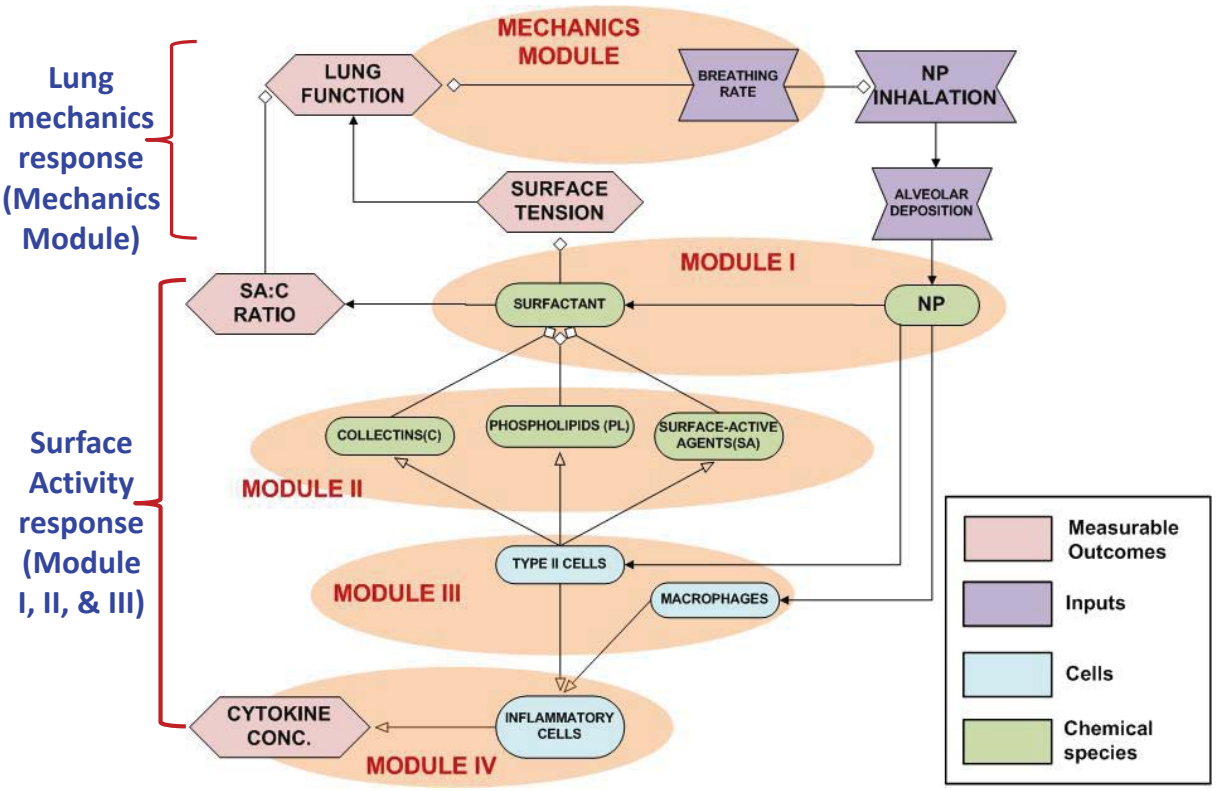


Fractional deposition for nano-sized particles in mice

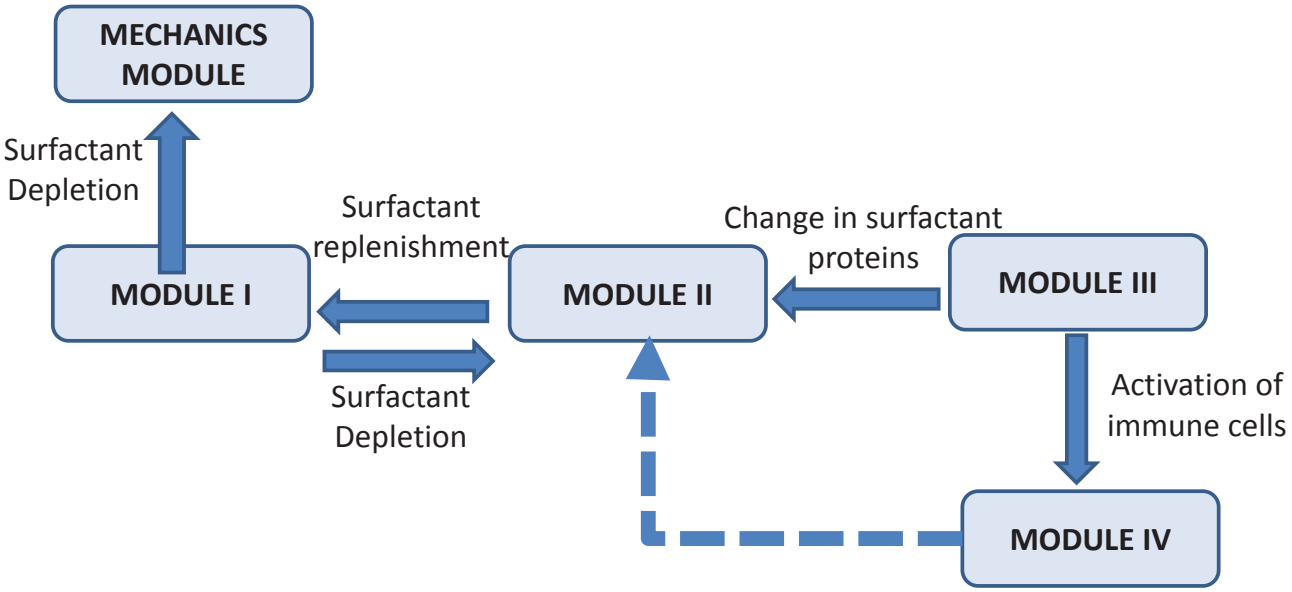
	Head	Larynx	Trachea	Bronchi	Alveoli
15nm Ag	2.206	1.507	1.237	35.653	40.264

Obtained by extrapolation of data from Raabe *et al.*, 1988

Schematic for multiscale toxicodynamic model for particle inhalation



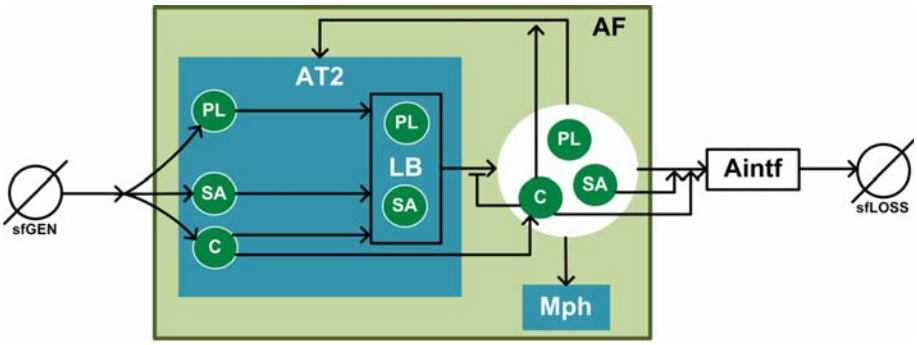
Interaction of various modules



Modular modeling of alveolar dynamics in the presence of NPs

Schematic diagram for Module II

Surfactant dynamics (Secretion, surface-adsorption, recycling of surfactant components)



Model compartments:

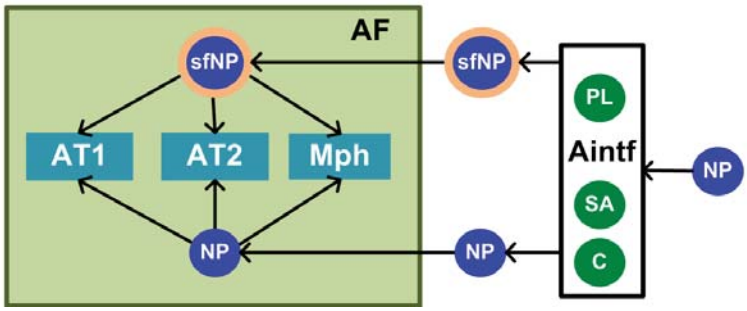
- Alveolar type I cell (AT1)
- Alveolar type II cell (AT2)
- Alveolar fluid (AF)
- Alveolar macrophages (Mph)
- Alveolar interface (Aintf)
- Airway Loss (sfLoss)
- Lamellar Bodies (LB)
- Surfactant Generation (sfGen)

Model chemicals:

- Surfactant phospholipids (PL)
- Surface-active proteins (SA)
- Collectins (C)
- Nanoparticles (NP)
- Surfactant-bound NPs (sfNP)

Schematic diagram for Modules I & III

NP binding with surfactant and uptake by cells



Assumptions:

- Intratracheal NP dose is assumed to fully reach alveolar surface
- NP aggregation not considered
- Mph number considered constant at basal levels
- SA & C binding considered to occur at same rate as for PL due to their close association as part of tubular myelin

Modules I & III – interactions of NPs with alveolar fluid and cells

PL adsorption on nanoparticles

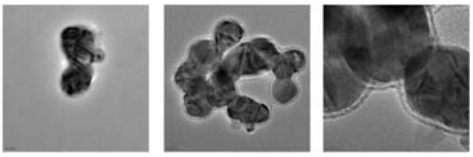
$$\frac{dm_{PL}}{dt} = -\frac{V_A A}{K_A + A}$$

$$\frac{dA}{dt} = \frac{dm_{PL}}{dt}\left(\frac{1}{1000h\rho}\right)$$

m_{PL} is the moles of free PL at the alveolar interface, A is the uncoated surface of NPs present, h is the thickness of PL layer on NPs (estimated as ~5 nm from Project 1 experiments), ρ is the density of PL (1.04 at 20°C from Shelley *et al.*, 1975), and V_A and K_A are the Michaelis-Menten parameters

Parameters estimated from Kendall *et al.*, 2004 for 25 nm carbon NPs for two different surface properties

	V_A(mg/ml)	K_A(m²/ml)
Oxidized surface	5.1×10^{-3}	1.03×10^{-2}
Non-oxidized surface	3.581×10^{-3}	1.131×10^{-2}



PL layer thickness around Ag NPs estimated to be ~5 nm (Data from Alexandra Porter, Mary Ryan, Milo Shaffer, RESAC Project 1)

Nanoparticle uptake by cells

Process considered as composed of two steps: Particle deposition on cellular surface & Particle endocytosis

Particle deposition

Probability of nanoparticle deposition on cellular surface, given by: $k_f = k_c \frac{3(1-\varepsilon)}{2\varepsilon d_c}\eta_s u$ From Su *et al.*, 2010

ε is the tissue porosity, d_c is the cellular diameter, u is the tissue fluid velocity and η is the collection efficiency

$\eta_s = \eta_0 \times \eta_e, \quad \eta_0 = f(d_p), \quad \eta_e = f(\zeta)$ d_p is the NP diameter, ζ is the zeta potential of the particle

The functional forms are different for epithelial cells and macrophages and are empirically estimated using data from Su *et al.*, 2010

Particle endocytosis

NP uptake rates (both w/o surfactant) by cells estimated from:
Type I cells: Kemp *et al.*, 2008
Type II cells: Chithrani *et al.*, 2006 (without surfactant), Verma & Stellacci, 2009 (with surfactant)
Macrophages: Beduneau *et al.*, 2009 (without surfactant), Zahr *et al.*, 2006 (with surfactant)

Module II – surfactant dynamics

Model compartments:
- Alveolar type II cell (Type2)
- Alveolar fluid (hypophase) (AF)
- Alveolar macrophages (Mph)
- Alveolar interface (Aintf)
- Airway Loss (sfLoss)
- Lamellar Bodies (LB)
- Surfactant Generation (sfGEN)

Model chemicals:
- Surfactant phospholipids (PL)
- Surface-active proteins (SA)
- Collectins (C)
- Nanoparticles (NP)
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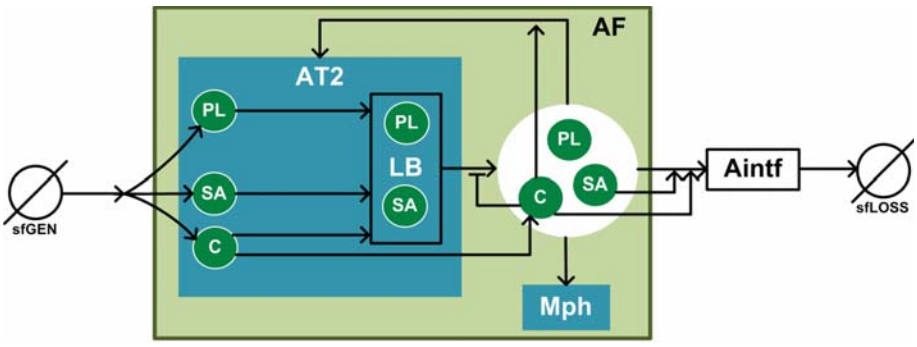


- Rate of PL secretion is estimated from Martini *et al.*, 1999 (study in pigs), SA secretion from Gobran & Rooney, 2001, and C secretion from Rooney *et al.*, 1993
- Rate of PL adsorption to alveolar interface estimated from Walters *et al.*, 2000
- SA and C considered to adsorb at the same rate as PL
- Loss of surfactant estimated to be 3% based on study by Pettenazzo *et al.*, 1988
- Alveolar surface tension estimated as function of surfactant concentration based on Hill-type equation

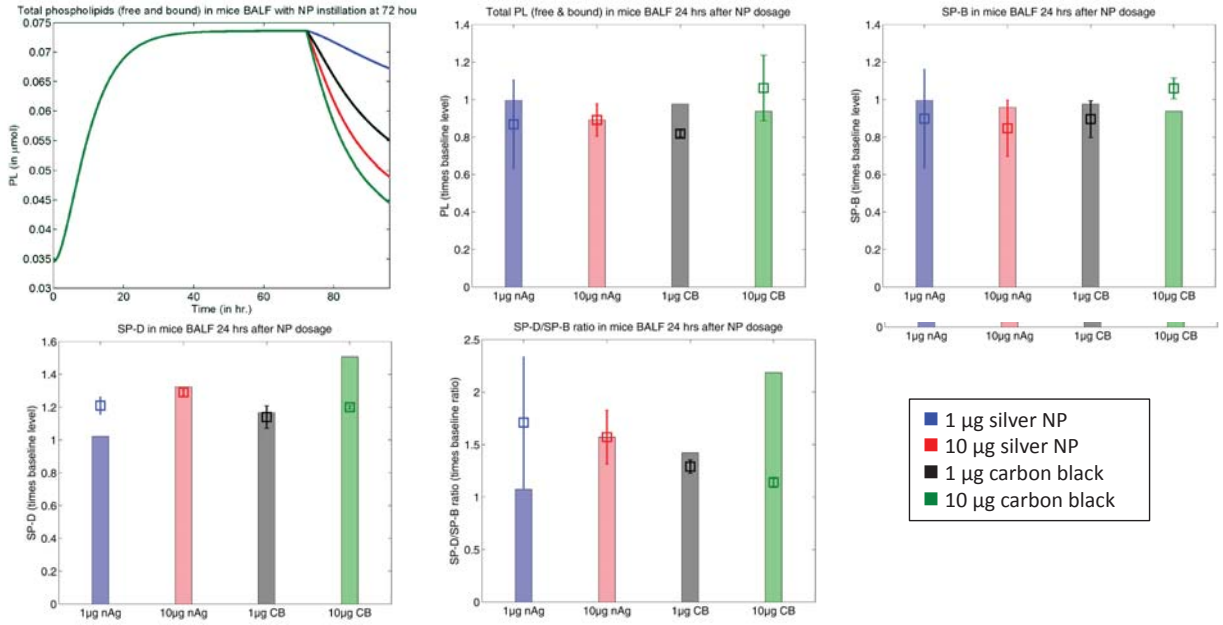
$$\gamma = \gamma_{\max}\left(1 - \frac{C_s^n}{K + C_s^n}\right)$$

$\gamma_{\max} = 28.1$ mN/m; K=18.647; n=2.81

$K_{ads} = 1.649$ ml/μmol.min; $C_{seq} = 634.78\,\mu mol/m^2$

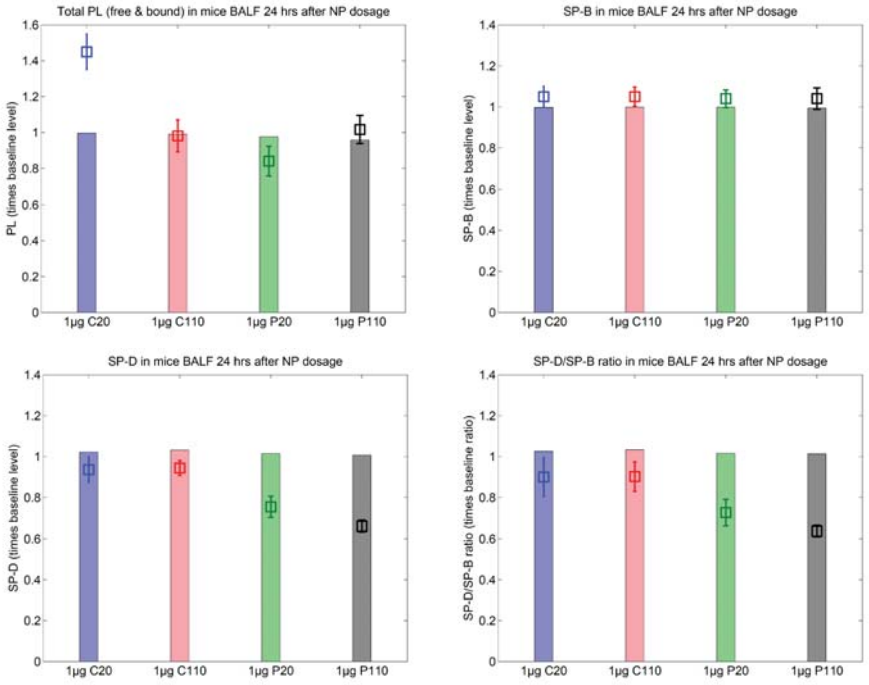
$$\frac{dC_s}{dt} = C_b K_{ads}(C_{seq} - C_s)$$

Results of simulations employing Modules I, II, & III – compared with *in vivo* measurements in mice using 15 nm nanoparticles



Simulation results of the toxicodynamic model involving modules I, II, & III. The model was run for the first 72 hours without NPs to allow surfactant levels to reach steady-state and then the effects of intratracheal instilled dose were simulated for 24 hours. Figure (a) shows the time profile of total phospholipids while in (b-e) the bars represent the simulation results at the end of 24 hours after dosage and the squares and error bars show mean and SD of results of lung lavage analysis of mice 24 hours after NP instillation.

Results of simulations employing Modules I, II, & III – compared with *in vivo* measurements in mice using 20nm and 110 nm nanoparticles



C20 - Citrate-stabilized 20 nm NPs
C110 - Citrate-stabilized 110 nm NPs
P20 - PVP-stabilized 20 nm NPs
P110 - PVP-stabilized 110 nm NPs

NP dosage is 1 ug for all particles
PVP: Poly Vinyl Pyrrolidone

Relating intratracheal dose to inhalation exposure

Regional deposition of nanoparticles in mice

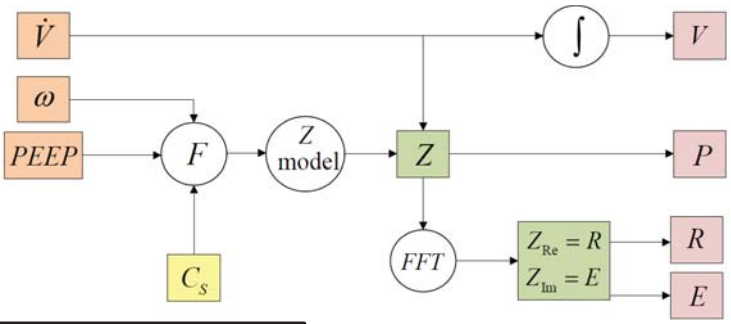
	Head	Larynx	Trachea	Bronchi	Alveoli
15nm Ag	2.206	1.507	1.237	35.653	40.264

Obtained by extrapolation of data from Raabe *et al.*, 1988

Total percentage of inhaled particles reaching alveoli = 40.26 %

Nanoparticle	Intratracheal dose	Predicted inhaled dose
15 nm	1 µg	2.484 µg
	10 µg	24.84 µg

Reduced Lung Mechanics Module (operational version)



Independent or input variables

Output variables

Variables obtained from other modules

Squares denote variables and parameters involved in the model. Circles denote functions which act on the variables and parameters. R and E are the overall lung resistance and elastance, respectively. (FFT stands for Fast Fourier Transform)

Constant Phase Model (CPM)
(Hantos *et al.*, 1992)

$$Z_{\mathrm{Re}} = R_{aw} + G/\omega^{\alpha}$$

$$Z_{\mathrm{Im}} = I\omega - H/\omega^{\alpha}$$

$$\omega = 2\pi f;\ \alpha = \tan^{-1}(H/G)$$

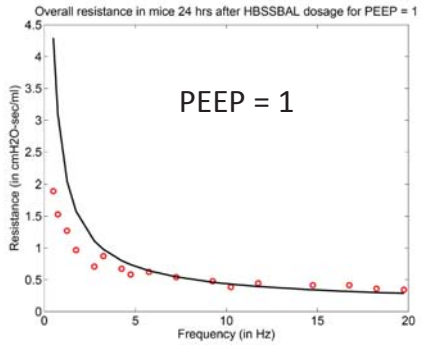
$$G^* = G(1+S_G),\ \ S_G = k_G C_S / C_{S0},\ \ k_G = -1.527(PEEP)^2 + 16.34(PEEP) + 8.121$$

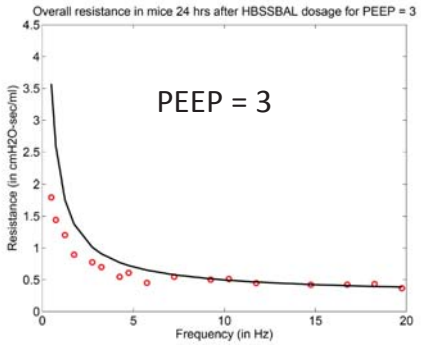
$$H^* = H(1+S_H),\ \ S_H = k_H C_S / C_{S0},\ \ k_H = -0.7(PEEP)^2 + 9.204(PEEP) + 36.02$$

R_{aw} = Airway resistance; G = Tissue damping; H = Tissue Elastance; I = Inertance; S_G = Surfactant effect on G; S_H = Surfactant effect on H; f = breathing frequency; Z = Impedance; $PEEP$ = Positive End Expiratory Pressure; C_S = alveolar surface concentration of phospholipids

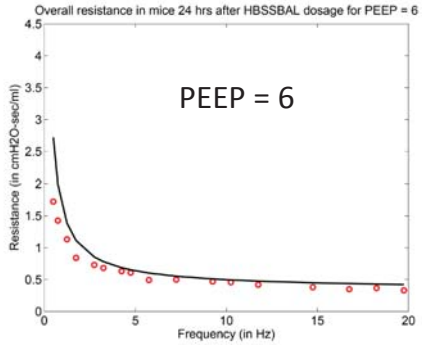
- S depends on available surfactant concentration, C_S and PEEP which affects alveolar recruitment
- The PEEP dependency is lumped in parameters k_G and k_H controlling surface-active modulation
- Parameter estimation performed based on nAg data only; carbon black causes other physiological effects in lungs and Module IV (cell recruitment and inflammation) cannot be isolated
- Surfactant-depletion does not occur in a dose-dependent manner - need to consider more detailed size distribution of nanoparticles and particle agglomeration

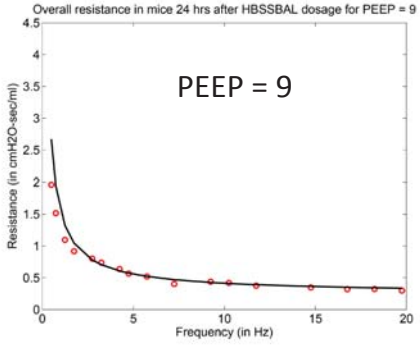
Model results compared to impedance measurements (control mice)





Mice intratracheally dosed with nanoparticle suspensions, were subjected to forced oscillation breathing maneuvers after 24 hours, and their lung function measured using impedance measurements

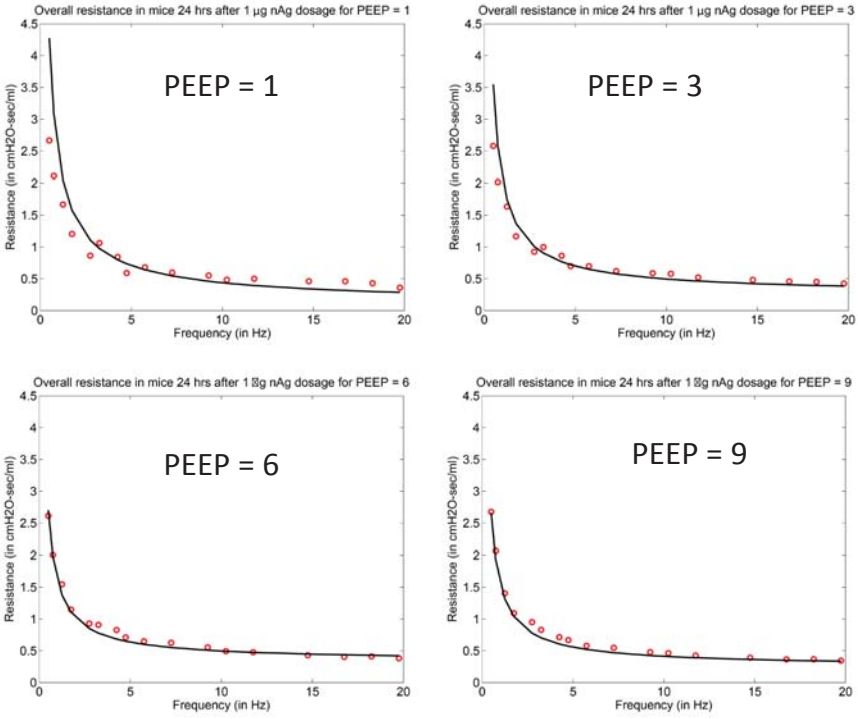




Measurements from Dr. Andrew Gow's lab. (HBSSBAL refers to surfactant treated mice without NPs) Each data point is the mean of measurements from 12 mice.

PEEP = Positive End Expiratory Pressure

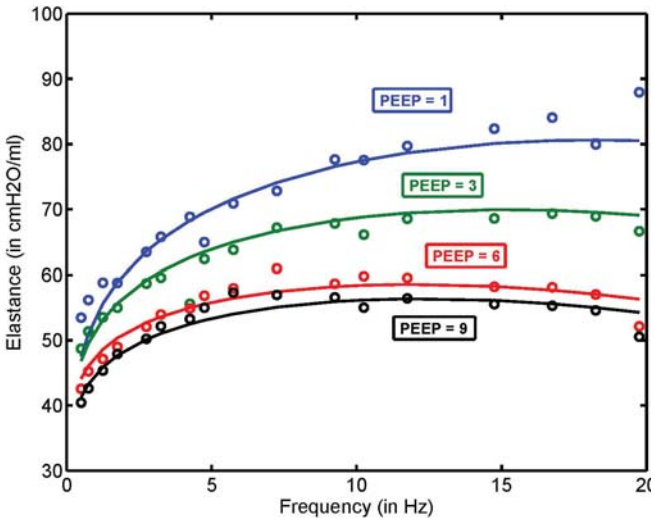
Model results compared to impedance measurements (10µg nAg)



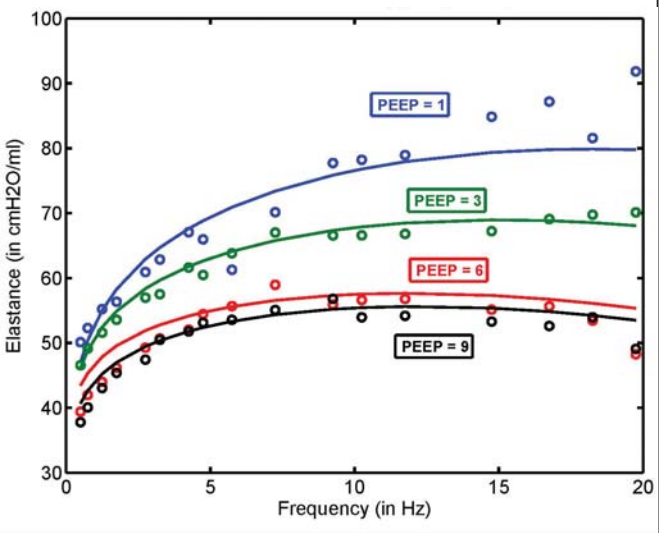
Measurements from Dr. Andrew Gow's lab. Each data point is the mean of measurements from 12 mice.

Model results compared to impedance measurements

Elastance 24 hrs after dose of 1µg nAg



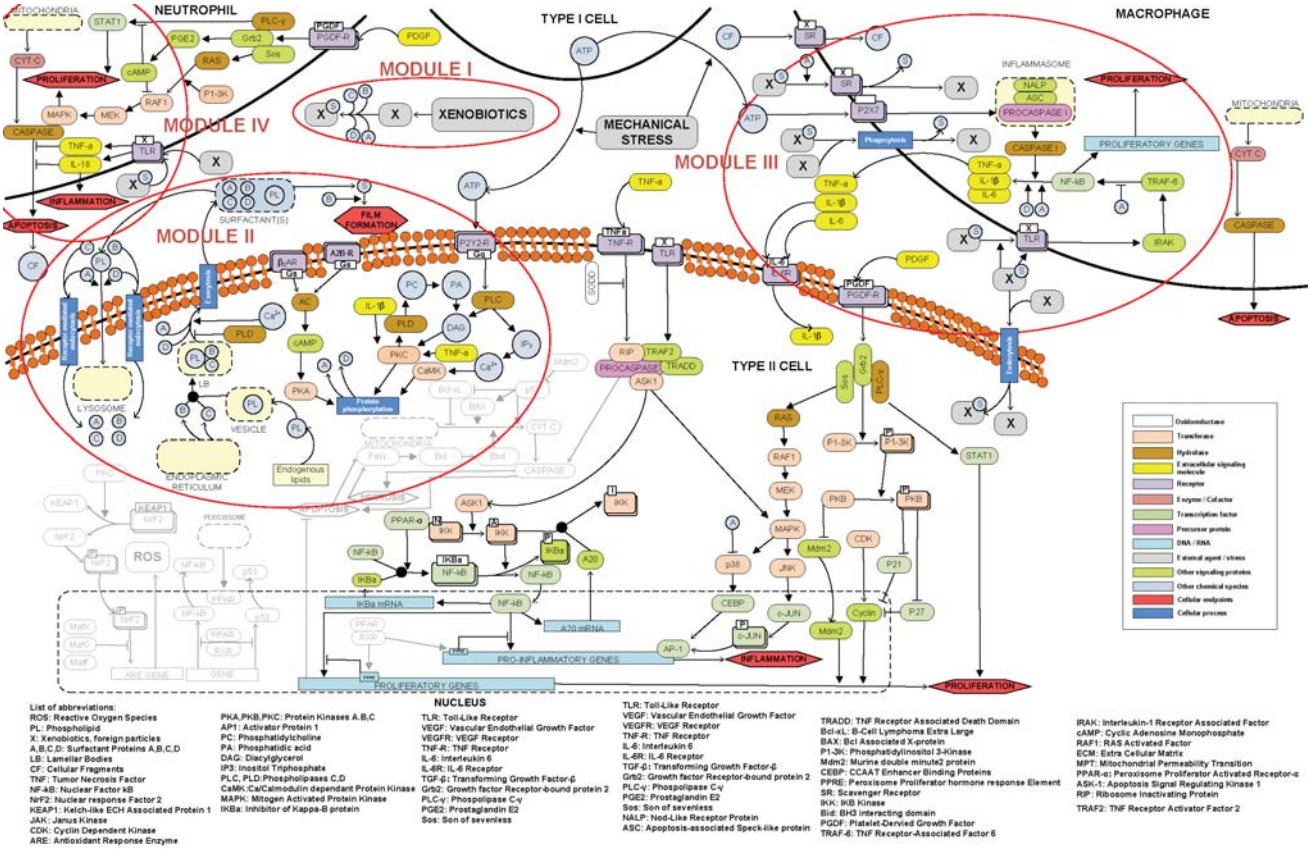
Elastance 24 hrs after dose of 10µg nAg



Measurements from Dr. Andrew Gow's lab. Each data point is the mean of measurements from 12 mice

PEEP = Positive End Expiratory Pressure

Cell-level toxicodynamic framework for nanoparticles reaching the alveoli after inhalation exposure



List of abbreviations:
ROS: Reactive Oxygen Species
PL: Phospholipid
X: Xenobiotics, foreign particles
A,B,C,D: Surfactant Proteins A,B,C,D
LB: Lamellar Bodies
CF: Cellular Fragments
TNF: Tumor Necrosis Factor
NF-kB: Nuclear Factor kB
NrF2: Nuclear response Factor 2
KEAP1: Kelch-like ECH Associated Protein 1
JAK: Janus Kinase
CDK: Cyclin Dependent Kinase
ARE: Antioxidant Response Enzyme

PKA,PKB,PKC: Protein Kinases A,B,C
AP1: Activator Protein 1
PC: Phosphatidylcholine
PA: Phosphatidic acid
DAG: Diacylglycerol
IP3: Inositol Triphosphate
PLC, PLD:Phospholipases C,D
CaMK:Ca/Calmodulin dependant Protein Kinase
MAPK: Mitogen Activated Protein Kinase
IkBa: Inhibitor of Kappa-B protein

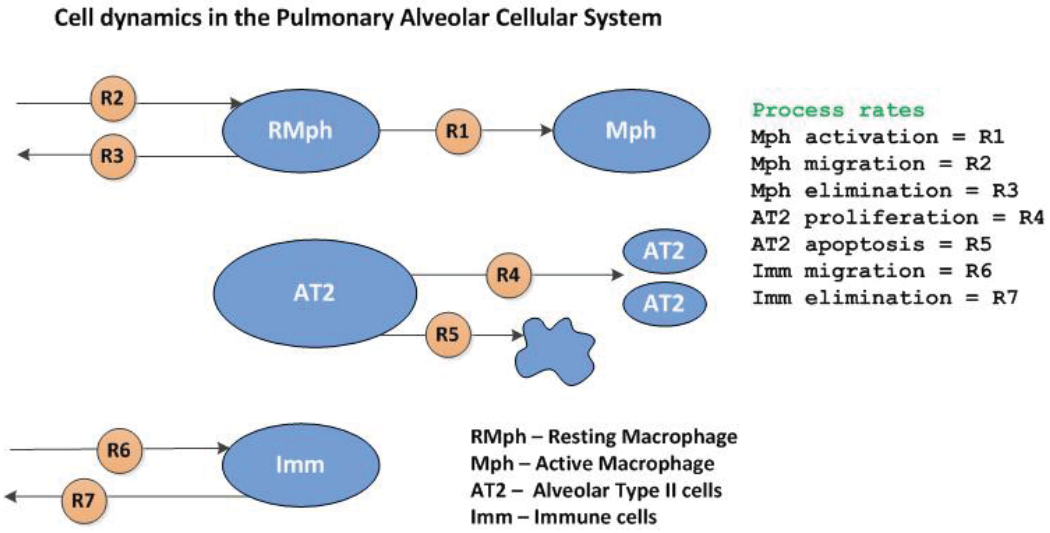
TLR: Toll-Like Receptor
VEGF: Vascular Endothelial Growth Factor
VEGFR: VEGF Receptor
TNF-R: TNF Receptor
IL-6: Interleukin 6
IL-6R: IL-6 Receptor
TGF-ß: Transforming Growth Factor-ß
Grb2: Growth factor Receptor-bound protein 2
PLC-γ: Phospolipase C-γ
PGE2: Prostaglandin E2
Sos: Son of sevenless

TLR: Toll-Like Receptor
VEGF: Vascular Endothelial Growth Factor
VEGFR: VEGF Receptor
TNF-R: TNF Receptor
IL-6: Interleukin 6
IL-6R: IL-6 Receptor
TGF-ß: Transforming Growth Factor-ß
Grb2: Growth factor Receptor-bound protein 2
PLC-γ: Phospolipase C-γ
PGE2: Prostaglandin E2
Sos: Son of sevenless
NALP: Nod-Like Receptor Protein
ASC: Apoptosis-associated Speck-like protein

TRADD: TNF Receptor Associated Death Domain
Bcl-xL: B-Cell Lymphoma Extra Large
BAX: Bcl Associated X-protein
P1-3K: Phosphatidylinositol 3-Kinase
Mdm2: Murine double minute2 protein
CEBP: CCAAT Enhancer Binding Proteins
PPRE: Peroxisome Proliferator hormone response Element
SR: Scavenger Receptor
IKK: IKB Kinase
Bid: BH3 interacting domain
PGDF: Platelet-Dervied Growth Factor
TRAF-6: TNF Receptor-Associated Factor 6

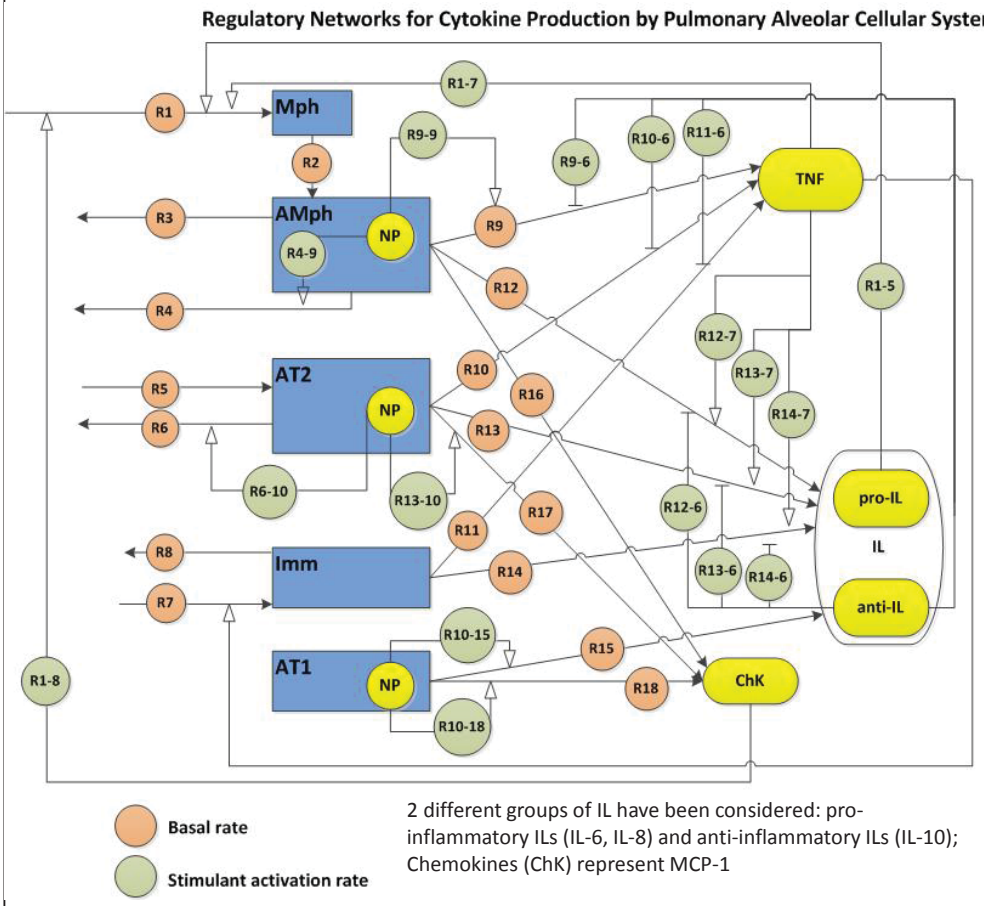
IRAK: Interleukin-1 Receptor Associated Factor
cAMP: Cyclic Adenosine Monophosphate
RAF1: RAS Activated Factor
ECM: Extra Cellular Matrix
MPT: Mitochondrial Permeability Transition
PPAR-α: Peroxisome Proliferator Activated Receptor-α
ASK-1: Apoptosis Signal Regulating Kinase 1
RIP: Ribosome Inactivating Protein
TRAF2: TNF Receptor Activator Factor 2

Summary of cellular processes considered in the model



- Mph proliferation in the alveolar hypophase is considered but do matured Mph proliferate in the alveoli ?
- Should Mph be considered both in their active and non-active states ?
- Migration rate of Mph, R3 is composed of 2 rates: migration from resident interstitial Mph in the lung and Mph obtained from migration and differentiation of extra-pulmonary monocytes
- Elimination rate of Mph, R4 is composed of 2 rates: elimination by transport by mucus up the airways, and elimination into the lymphatic and circulatory system
- Immune cell proliferation or apoptosis is not considered, only migration and elimination is considered

Modeling pathways for inflammatory response due to nanoparticles



2 different groups of IL have been considered: pro-inflammatory ILs (IL-6, IL-8) and anti-inflammatory ILs (IL-10); Chemokines (ChK) represent MCP-1

```
Process IDs
ID.Mph_Migr = 1
ID.Mph_Act = 2
ID.Mph_Apo = 3
ID.Mph_Elimi = 4
ID.AT2_Prof = 5
ID.AT2_Apop = 6
ID.Imm_Migr = 7
ID.Imm_Elimi = 8
ID.TNF_Mph = 9
ID.TNF_AT2 = 10
ID.TNF_Imm = 11
ID.IL_Mph = 12
ID.IL_AT2 = 13
ID.IL_Imm = 14
ID.IL_AT1 = 15
ID.ChK_Mph = 16
ID.ChK_AT2 = 17
ID.ChK_AT1 = 18

Compartment IDs
CID.Mph = 1
CID.AT1 = 2
CID.AT2 = 3
CID.Imm = 4
CID.pro-IL = 5
CID.anti-IL = 6
CID.TNF = 7
CID.ChK = 8
CID.NP_Mph = 9
CID.NP_AT1 = 10
CID.NP_AT2 = 11
```

Mph – Inactive Macrophage
AMph – Activated Macrophage
AT2 – Alveolar Type II cells
Imm – Immune cells
ChK – Chemokines

Summary of regulation effects considered in Module IV

		No. of AT2 cells	No. of Mph	No. of Imm	NP in AT2	NP in Mph	Extracellular TNF conc.	Extracellular pro-IL conc.	Extracellular anti-IL conc.
AT2	Proliferation								
	Apoptosis				+				
Mph	Migration			+		+	+	+	
	Elimination					+			
	Apoptosis					+			
Imm	Migration		+	+			+	+	
	Elimination								
TNF secretion	AT2				+		+	+	-
	Mph					+	+	+	-
	Imm						+	+	-
pro-IL secretion	AT2				+		+	+	-
	Mph					+	+	+	-
	Imm						+	+	-
anti-IL secretion	AT2						+	+	
	Mph						+	+	
	Imm						+	+	

On the rows are shown the various processes which take place in presence or absence of nanoparticles and on the columns are the various stimuli which regulate the processes. A plus (**+**) signifies an activation and a minus (-) signifies an inhibition.

- Anti-inflammatory effects of anti-IL is considered only in the secretion of TNF and pro-IL
- Pro-inflammatory effects of TNF and pro-IL is considered in their own secretion (auto-activation) and in the migration of Mph and Imm

Conclusions and ongoing work

- Toxicodynamic modeling produces predictions of phospholipids and surfactant protein levels after NP exposure that are comparable to in vivo measurements in mice
  - The changes in surfactant properties predicted by the model were used to estimate changes in macroscopic parameters of the lung (resistance and elastance); these estimates compared well with measurements in mice obtained using forced oscillation technique
  - Nanoparticle properties such as size, coating chemistry, and zeta potential were incorporated explicitly in the model, and can be used to assess their influence on toxicodynamic effects
- Ongoing work includes:
  - Steady-state analysis of cytokine balance model already accomplished
  - Parameter estimation of the inflammatory pathway model using experimental in-vitro and in-vivo measurements
  - Incorporating nanoparticle size distributions and effects of particle agglomeration and dissolution into the model

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