From transcriptomics to CNT-induced inflammation and beyond: The power of nano-QSARs

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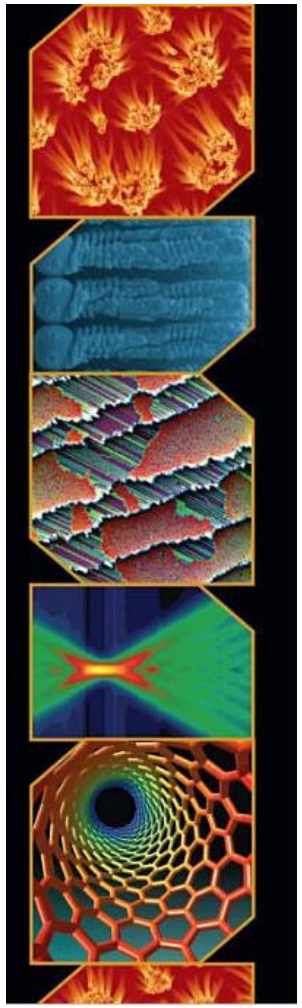
Outline

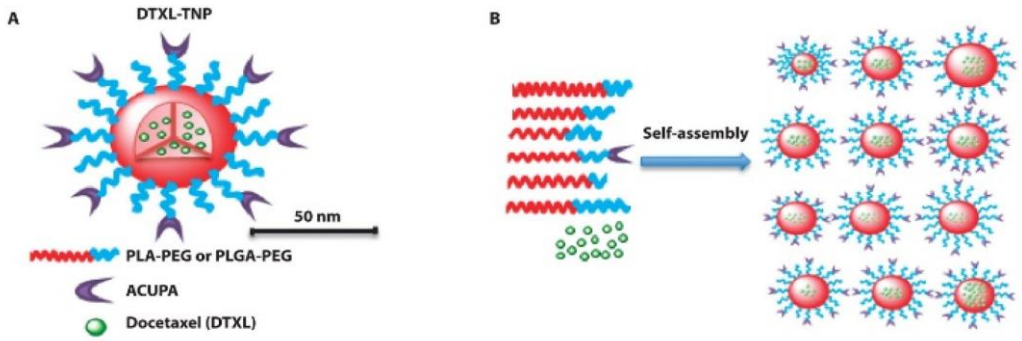


- Idea of integrating **Quantitative Structure-Activity Relationships for nanomaterials (Nano-QSAR)** and **Adverse Outcome Pathways for nanomaterials (Nano-AOP)** based on -omics data

- Proof-of-the-concept: MWCNTs induced inflammation and lung fibrosis (AOP 173)

QSAR for nanoparticles (Nano-QSAR)

Variance in the structure

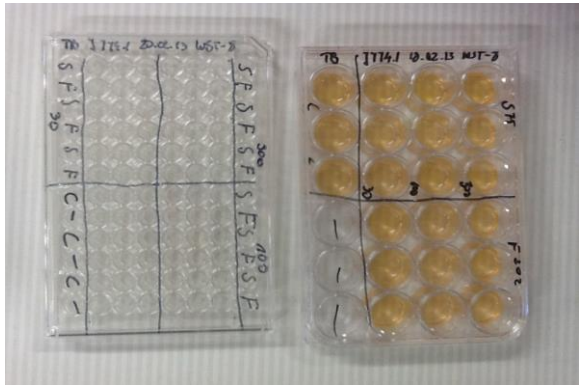


Pearce et al. (2019) *Bioconjugate Chem* 30, 2300-2311.

Structural features (descriptors):

- size
- shape
- coating
- structure functionalization
- system-dependent behavior
- …

Variance in the activity

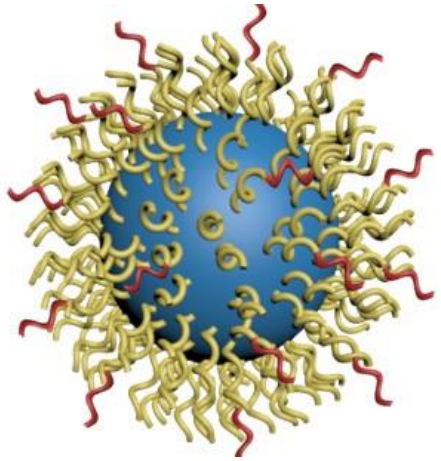


Phenotypic toxicity endpoints:

- in vitro: cell viability
- in vitro: cell death
- in vitro: mutagenicity
- in vivo: acute toxicity
- in vivo: organ toxicity
- …

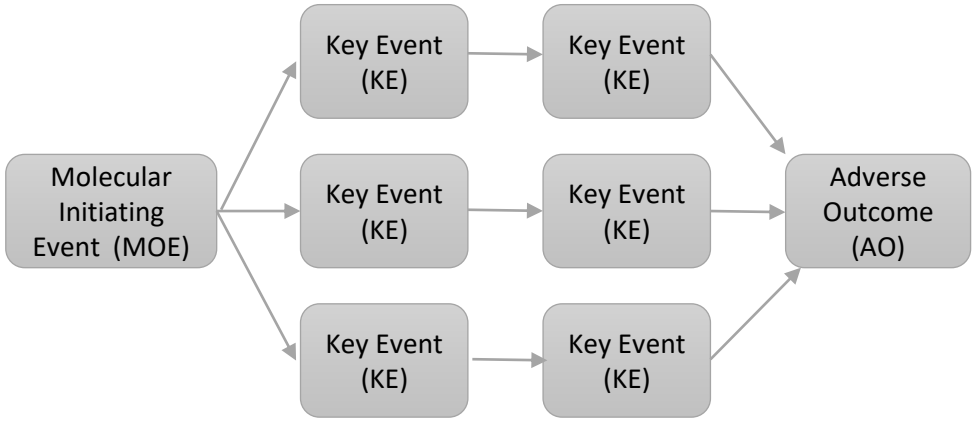
AOP for nanoparticles (Nano-AOP)

Presence of a NP



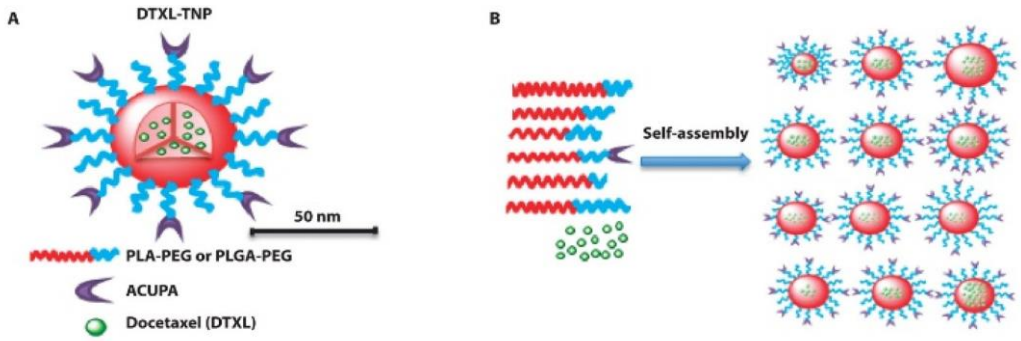
https://physicsworld.com/a/cloaking-nanoparticles-from-liver-cells-lengthens-blood-circulation/

Induction of AOP



Integration of Nano-QSAR and Nano-AOP

Variance in the structure



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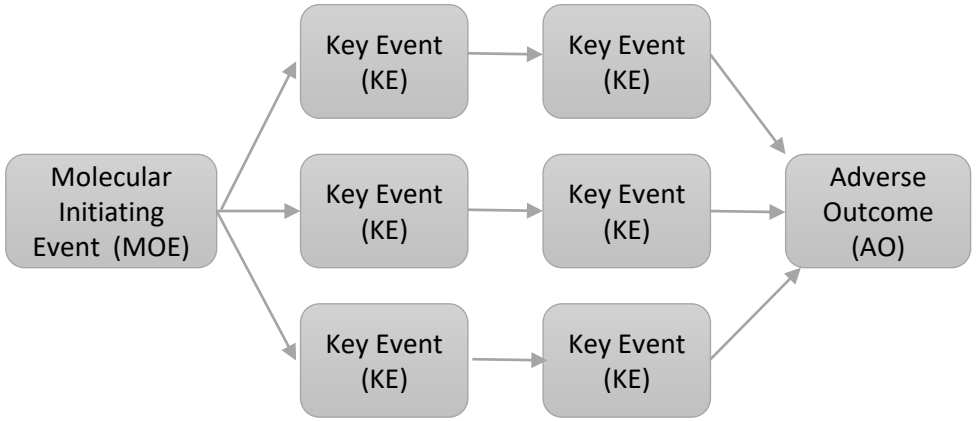
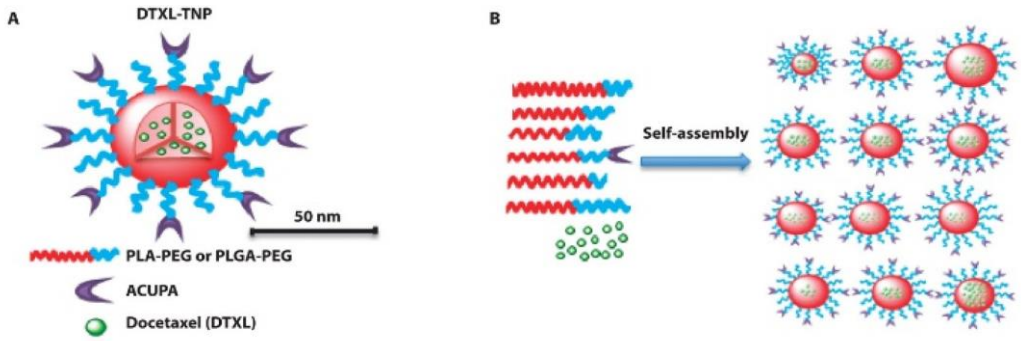
?

Variance in the AOP induction



Integration of Nano-QSAR and Nano-AOP

Variance in the structure



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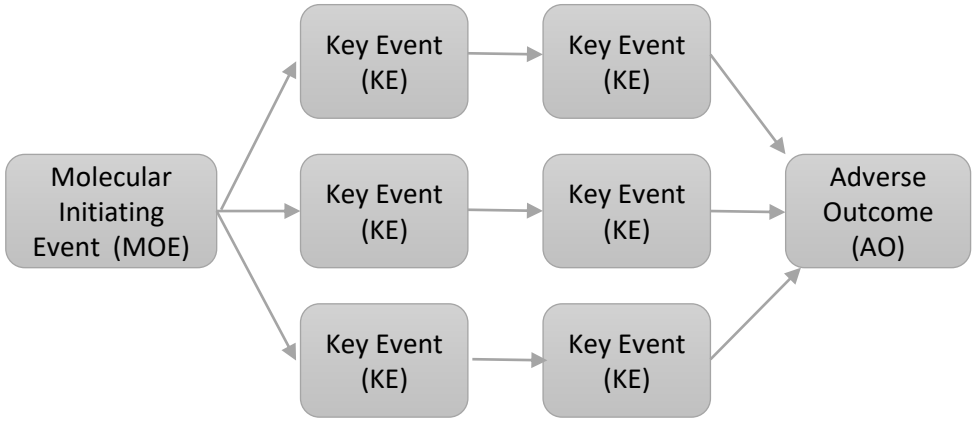
OMICS level

Variance in the AOP induction



Nanomaterials and the AOP

Transcriptomic response (BMDL* values for various pathways) in lungs of adult female C57BL/6 mice exposed for 24h on the following 10 MWCNT:

CNTs	Length [nm]	Diameter [nm]	Aspect ratio κ	BET [m^2/g]	Carbon purity [%]	OH [mmol/g]	COOH [mmol/g]
NRCWE-006	5700	65	87.69	26.00	99.00	0.08	0.04
NM-401	4050	20	202.50	140.00	99.70	0.03	0.02
NRCWE-048	1604	15.08	106.37	185.00	98.80	0.58	0.29
NRCWE-045	1553	28.07	55.33	119.00	96.30	0.63	0.31
NRCWE-044	1330	32.55	40.86	74.00	98.60	0.23	0.11
NRCWE-026	850	11	77.27	245.80	84.40	0.79	0.40
NRCWE-043	771.3	26.73	28.86	82.00	98.50	0.18	0.09
NRCWE-049	731.1	13.85	52.79	199.00	98.80	0.33	0.16
NRCWE-046	717.2	17.22	41.65	223.00	98.70	0.63	0.32
NRCWE-047	532.5	12.96	41.09	216.00	98.70	0.26	0.13

* BMDL – the statistical lower bound of the estimated benchmark dose (BMD)

AOP 173

MIE: Substance interaction with the resident lung cell membrane components

▼

KE1: increased, Pro-inflammatory and pro-fibrotic mediators

▼

KE2: Increased, recruitment of pro-inflammatory cells

▼

KE3: Loss of alveolar capillary membrane integrity

▼

KE4: T-helper type 2 cells activation

▼

KE5: Fibroblast and myofibroblasts proliferation

▼

KE6: ECM deposition

▼

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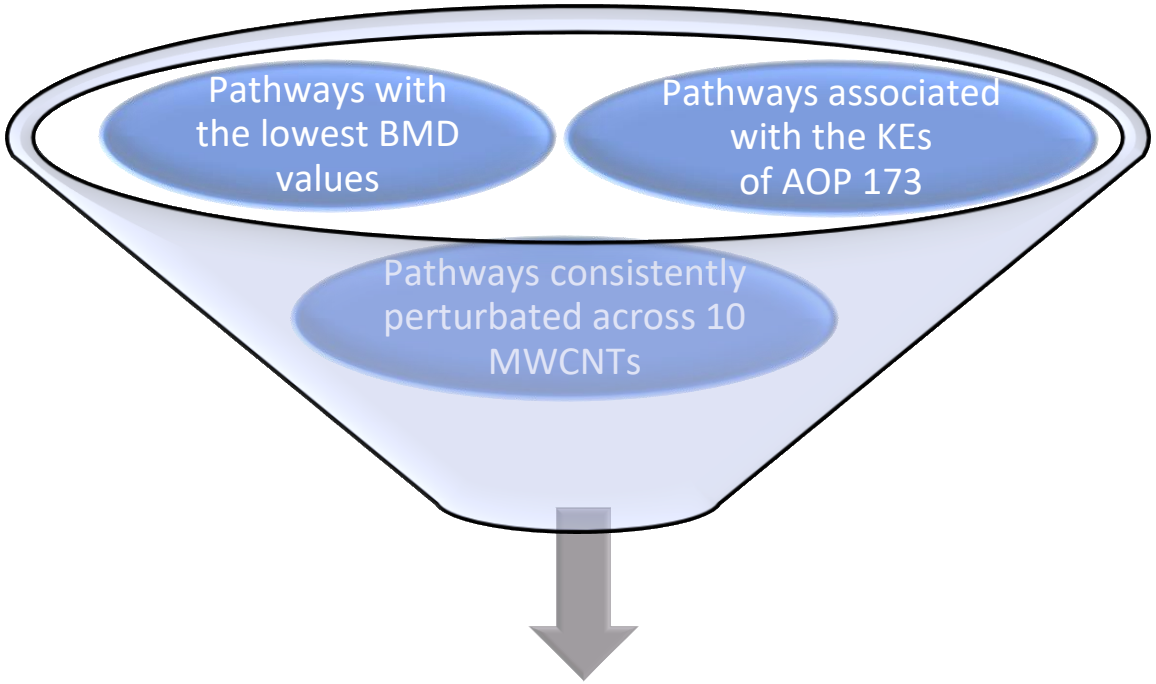
Pathway selection



17 different pathways

Three individual pathways selected

- Agranulocyte adhesion and diapedesis
- Granulocyte adhesion and diapedesis
- Acute phase signalling

Determination coefficient analysis

One pathway selected

- Agranulocyte adhesion and diapedesis

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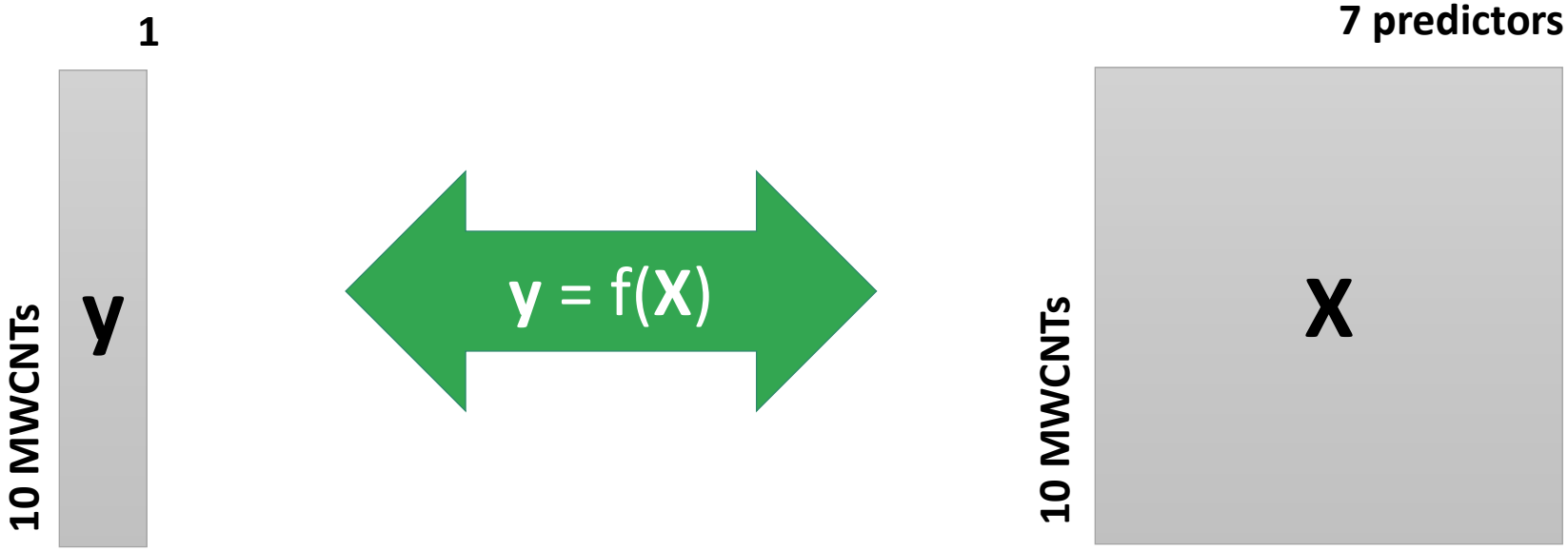
Nano-QSAR modeling for the selected pathway



BMDL values associated with agranulocytes adhesion and diapedesis pathway

Length, l [nm]
Diameter, f [nm]
Aspect ratio, K
BET [m^2/g]
OH [mmol/g]
COOH [mmol/g]
Chemical composition (carbon purity) [%]

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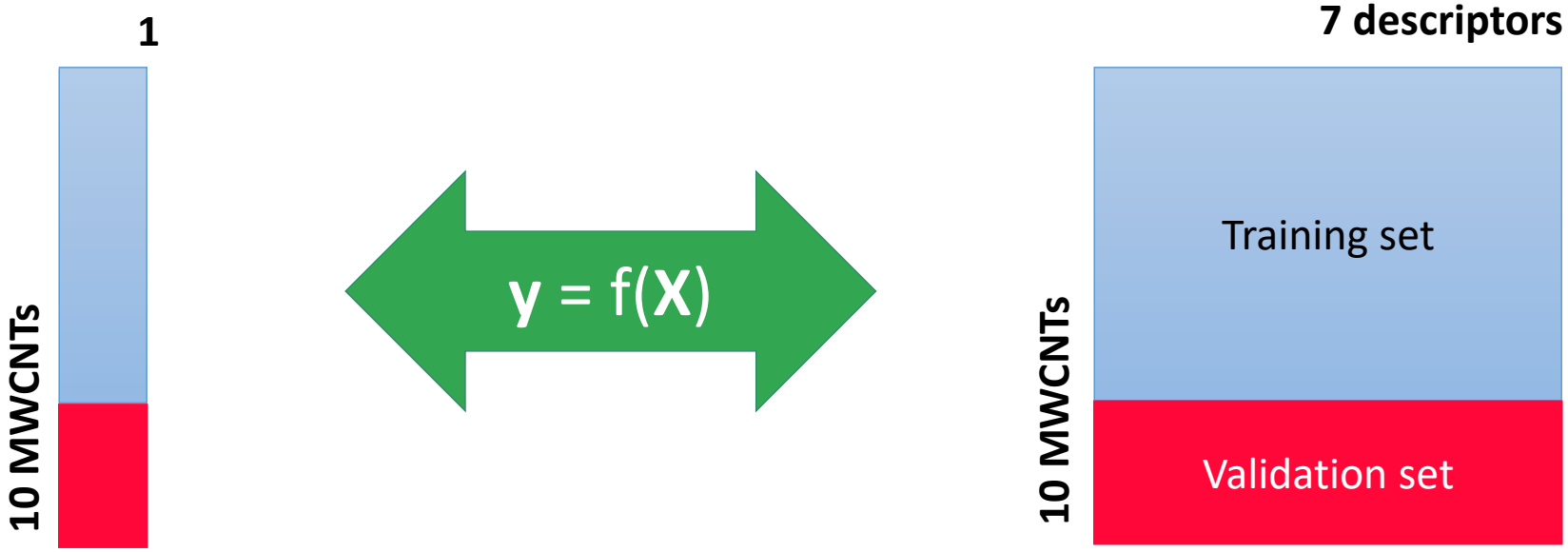

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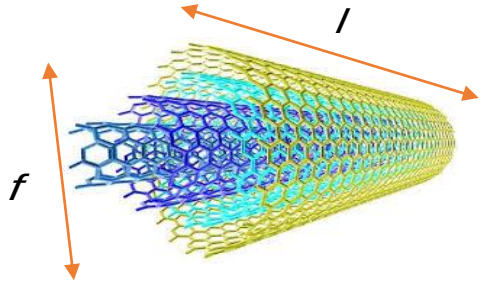

Aspect ratio (K)

$$k = \frac{l}{f}$$

l - length

f - diameter

Model	Equation	R^2	$RMSE_C$	Q^2_{CV}	$RMSE_{CV}$	$Q2_{EXT}$	$RMSE_{Ext}$
Model #0	BMDL = 15.01 – 0.071 K	0.85	1.63	0.78	1.99	0.69	2.39
Model #1	BMDL = 15.31 – 0.072 K	0.84	1.76	0.74	2.22	0.74	2.23
Model #2	BMDL = 14.92 – 0.075 K	0.81	2.07	0.47	3.47	0.92	1.34
Model #3	BMDL = 15.06 – 0.071 K	0.85	1.63	0.77	2.03	0.68	2.4
Consensus model	BMDL = 15.07 – 0.070 K	0.86	1.63	-	-	0.62	2.34

$$R^2 = 1 - \frac{\sum_{i=1}^{n}\left(y_i^{obs} - y_i^{pred}\right)^2}{\sum_{i=1}^{n}\left(y_i^{obs} - \tilde{y}_i^{obs}\right)^2} \qquad RMSE_C = \sqrt{\frac{\sum_{i=1}^{n}\left(y_i^{obs} - y_i^{pred}\right)^2}{n}} \qquad Q^2_{EXT(F2)} = 1 - \frac{\sum_{j=1}^{k}\left(y_j^{obs} - y_j^{pred}\right)^2}{\sum_{j=1}^{k}\left(y_j^{obs} - \tilde{y}_j^{obs}\right)^2} \qquad RMSE_{EXT} = \sqrt{\frac{\sum_{j=1}^{k}\left(y_j^{obs} - y_j^{pred}\right)^2}{k}}$$

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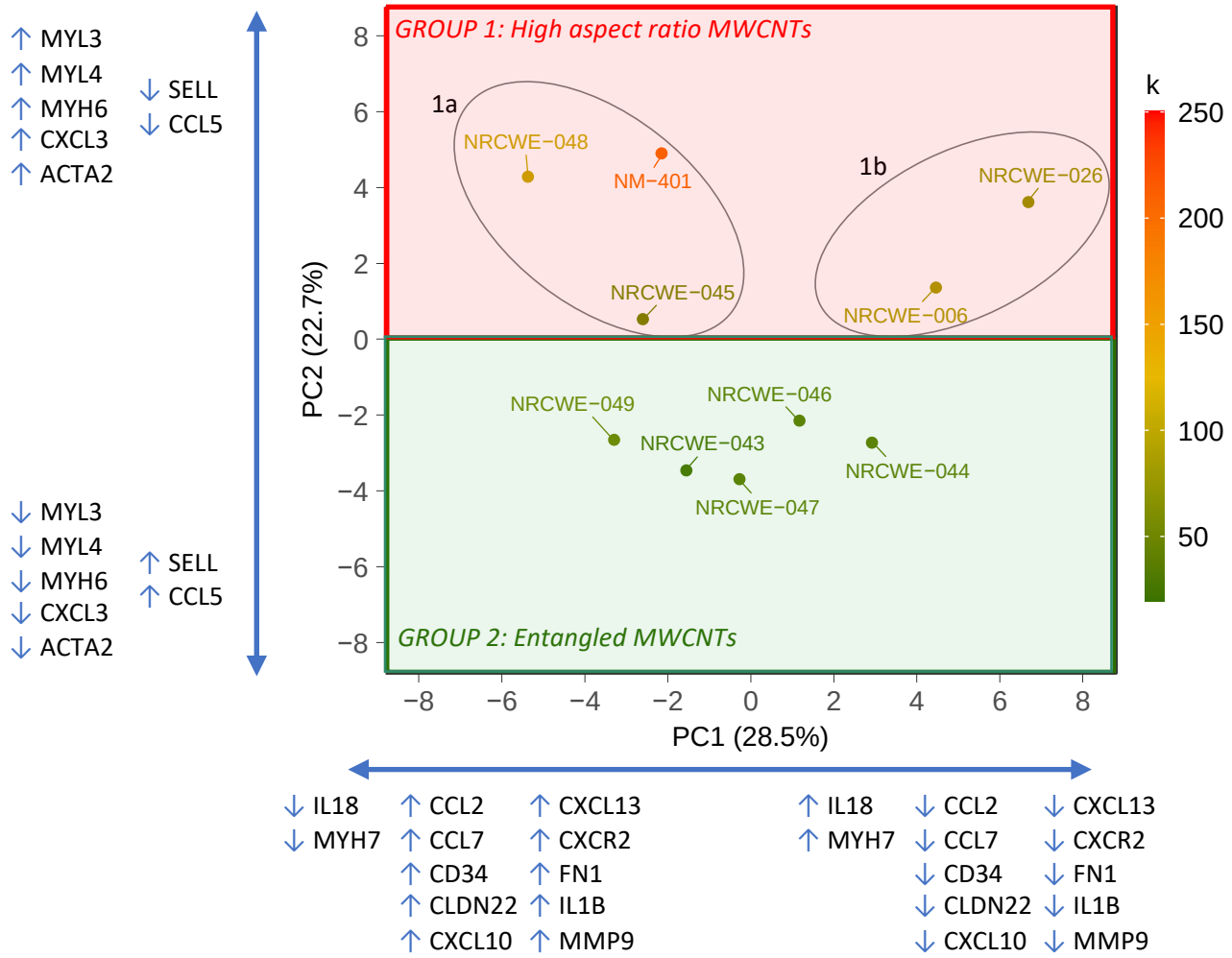
Grouping based on the structure-activity relationships



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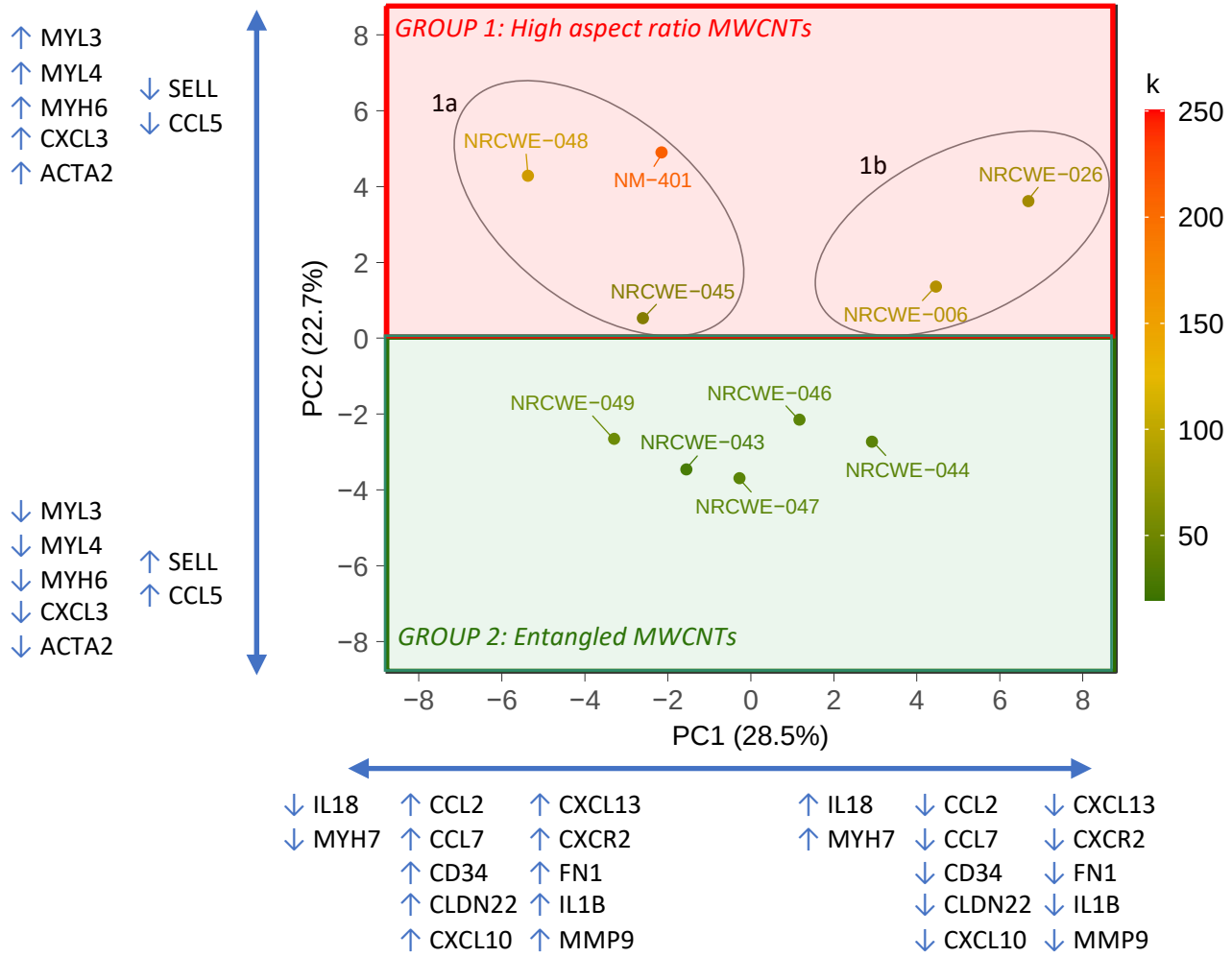
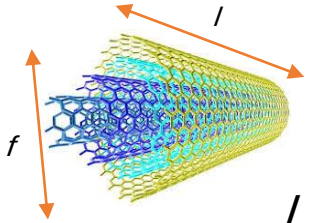


PC2 > 0

PC2 < 0

Consensus model:

PC2 = -7.54 – 0.11 k



$$k = \frac{l}{f}$$

$R^2 = 0.85$ $RMSE_C = 1.26$

$Q^2_{EXT} = 0.62$ $RMSE_{EXT} = 1.51$

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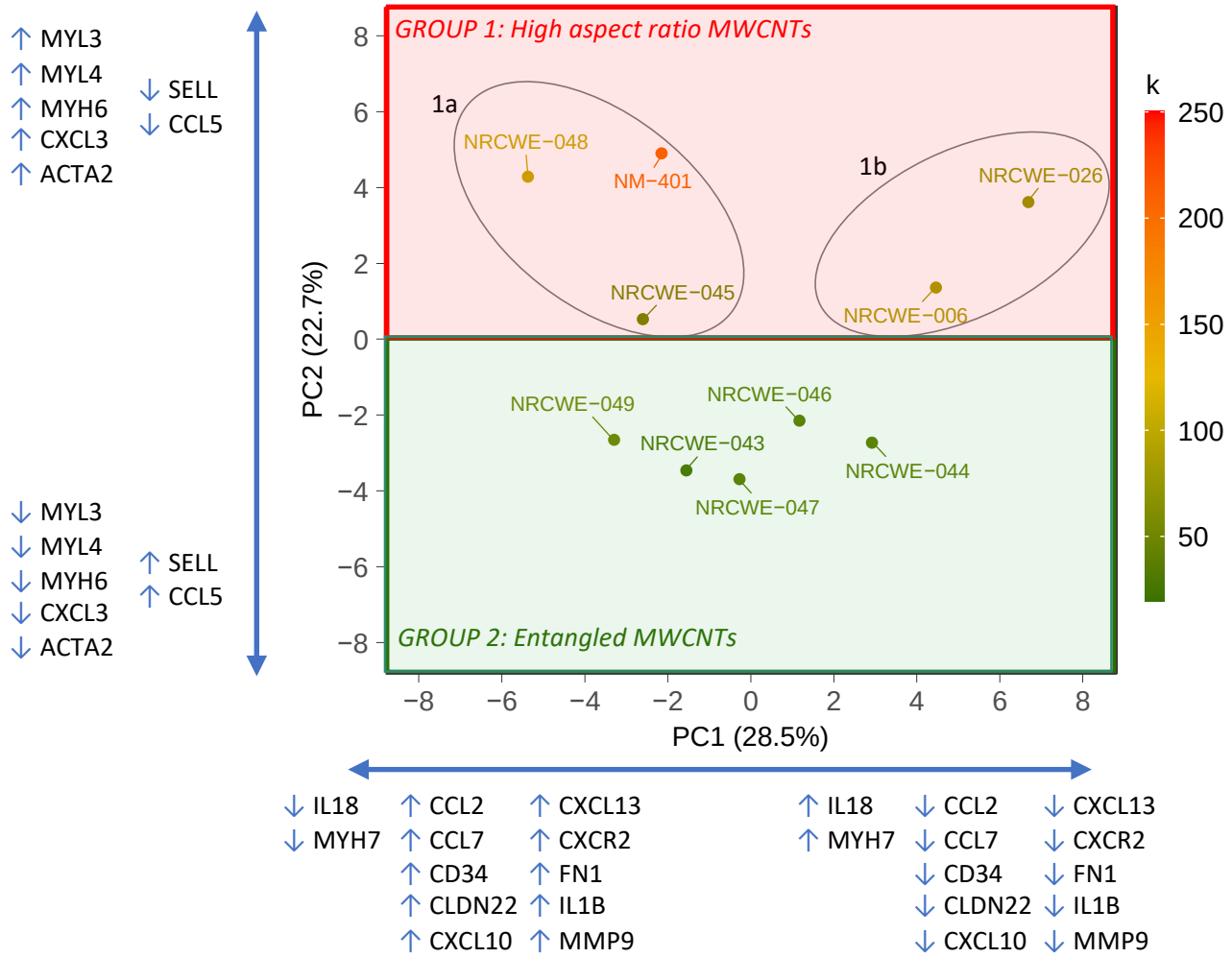
Grouping based on the structure-activity relationships



AOP 173

MIE: Substance interaction with the resident lung cell membrane components

KE1: increased, Pro-inflammatory and pro-fibrotic mediators

KE2: Increased, recruitment of pro-inflammatory cells

KE3: Loss of alveolar capillary membrane integrity

KE4: T-helper type 2 cells activation

KE5: Fibroblast and myofibroblasts proliferation

KE6: ECM deposition

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Jagiello et al (2020), Submitted

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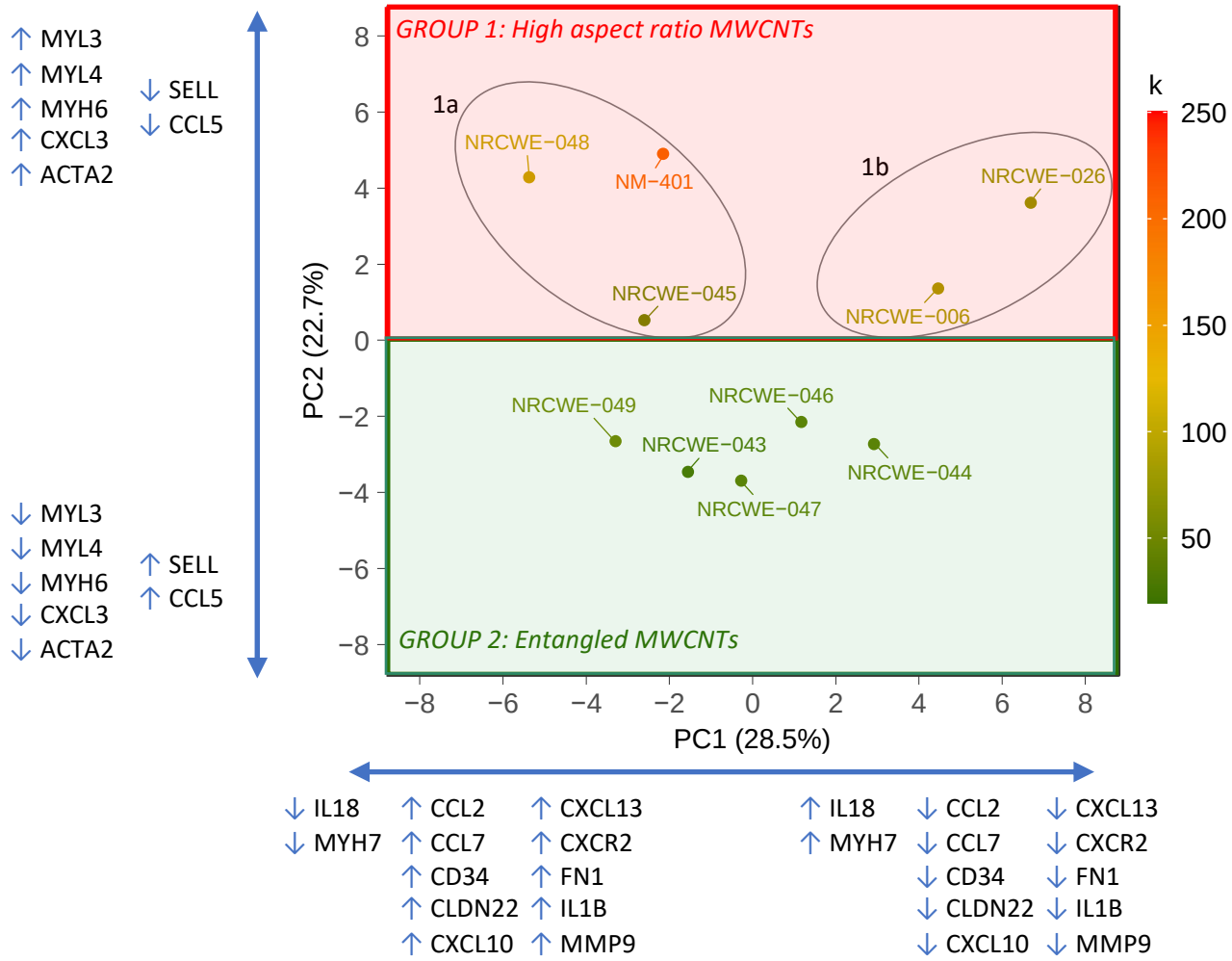

L-Selectin (*SELL*) is an adhesion molecule expressed on most of leukocytes and regulates accumulation and activation of neutrophils and monocytes at the site of injury and inflammation.

High aspect ratio MWCNTs down-regulate SELL as they may competitively bind to the ligands required for activation of selectins and inhibit their interaction, which in turn, may impact activation of agranulocytes and their diapedesis.

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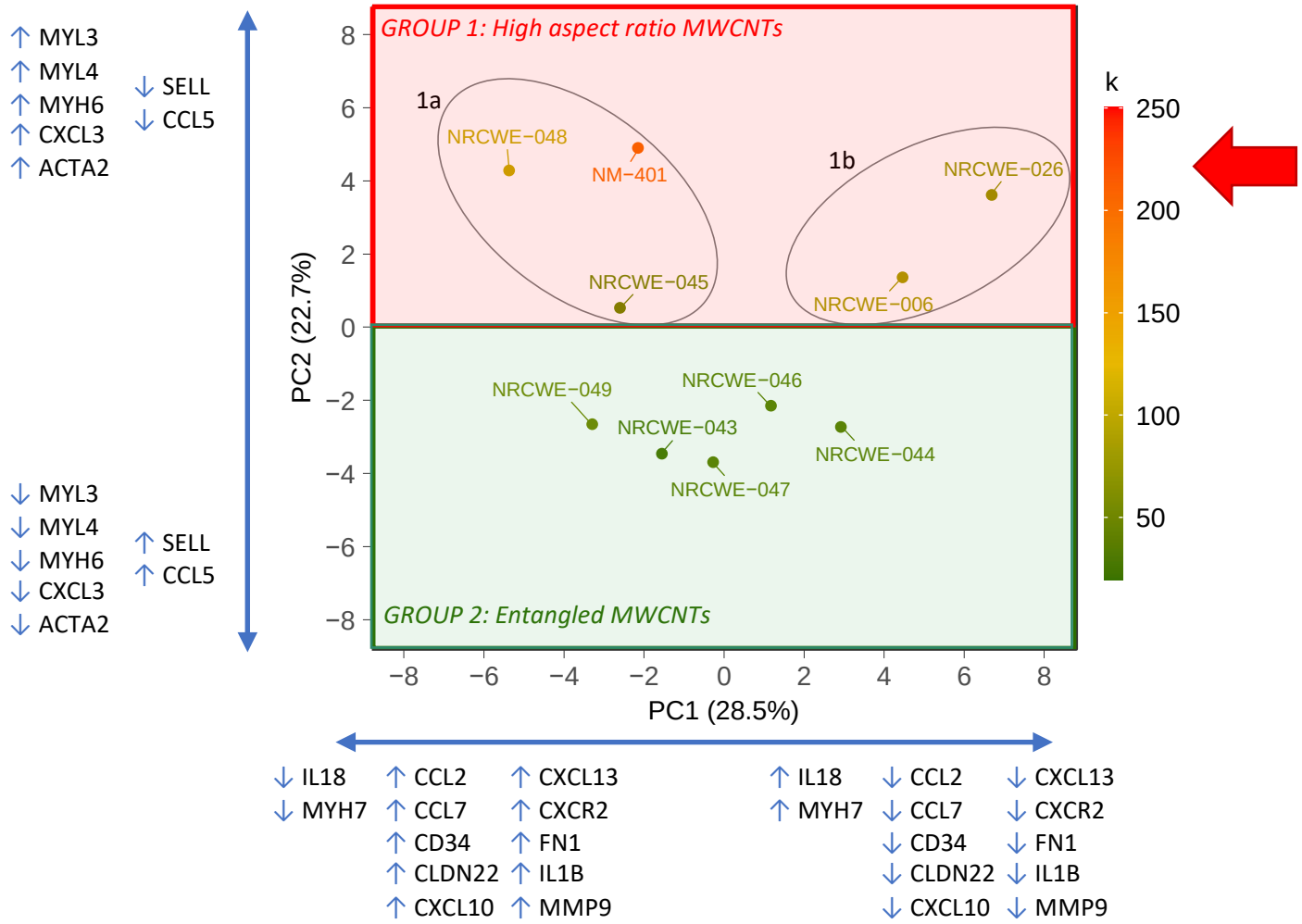
Grouping based on the structure-activity relationships



Myosins (*MYs*) are associated with the cytoskeletal remodelling. Following induction of adhesion molecule expression, an increase in endothelial migration and endothelial monolayer permeability are observed, which are controlled by the actin cytoskeleton. The cytoskeleton is rearranged when leukocytes bind to endothelial cells.

The expression of myosin genes was largely altered following exposure to long nanotubes, which may suggest that the long tubes affect the cytoskeleton arrangement, impeding the diapedesis process.

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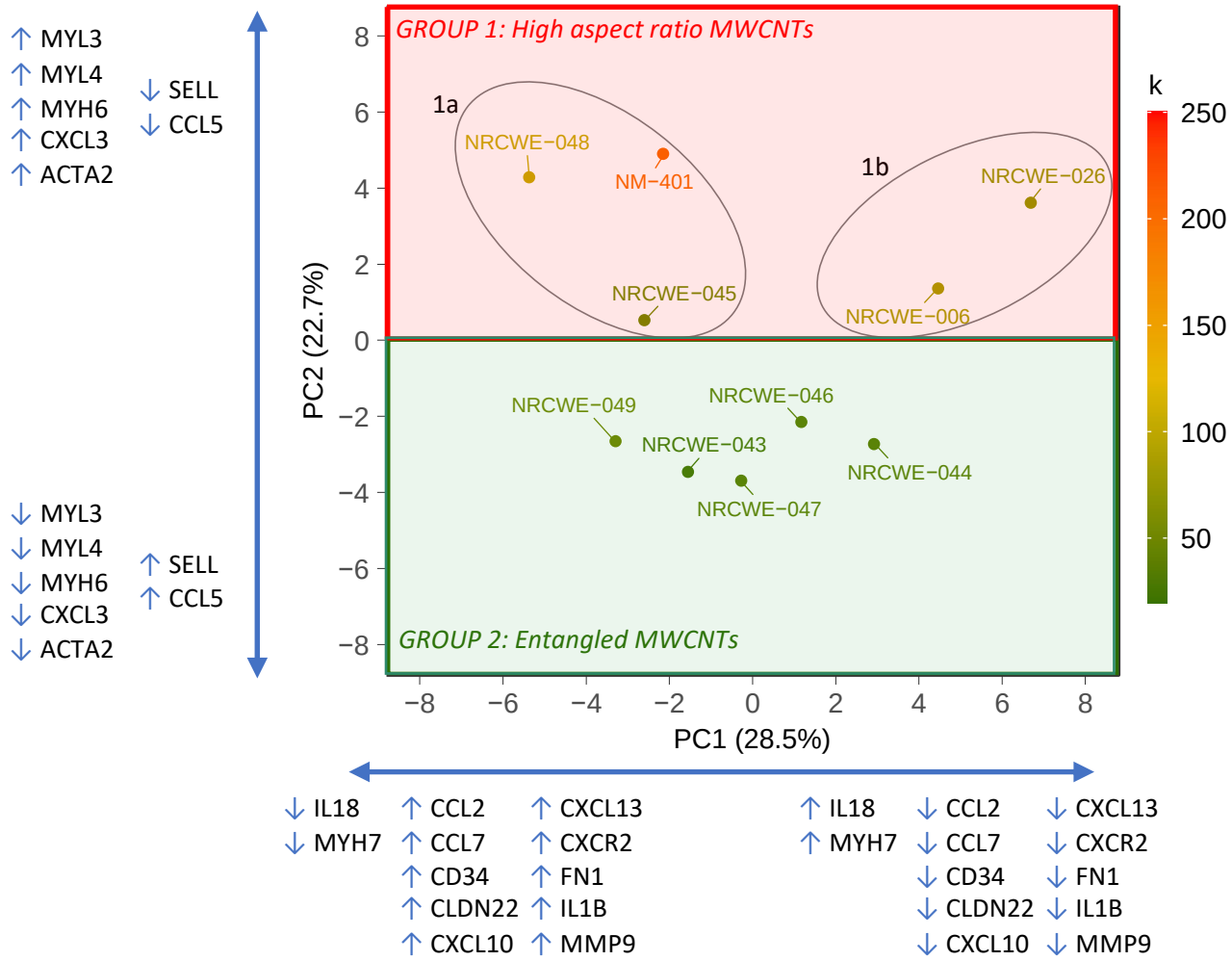
Grouping based on the structure-activity relationships



Similar differences were found in the expression of several **cytokines and chemokines** that signal and coordinate the diapedesis process.

Long MWCNTs do not allow timely rolling of leukocytes and thus impede inflammation process, which is also in alignment with the literature, suggesting that adverse effects of exposure to pathogenic MWCNTs could be partly initiated by impaired ability of the organism to mount the acute inflammatory response and timely clearance of MWCNTs.

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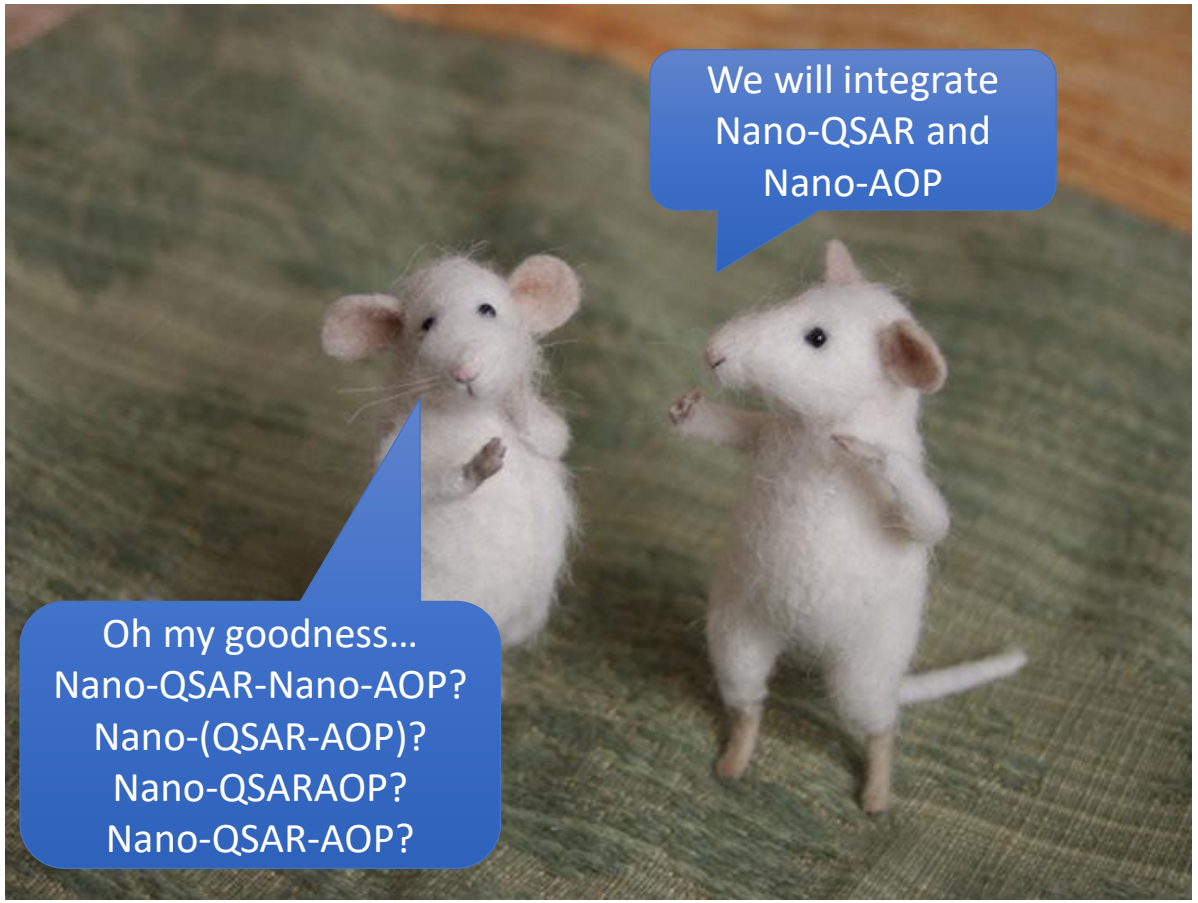
Summary



- When Nano-QSAR models are based on phyenotypic data, molecular mechanisms remain unclear.
- When developing Nano-AOPs, one can only conclude that “the presence of a given nanoparticle initiates the AOP”.
- Omics response can serve as an interface for integrating Nano-QSAR and Nano-AOP methodologies.
- The integration helps explaining the influence of the structural variation of nanoparticles (differences in chemistry) on the variation in the AOPs induction (differences in biology).

Acknowledgements

PATROLS
Advanced Tools for NanoSafety Testing

Funding has been received from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 760813, project title: Physiologically Anchored Tools for Realistic nanOmateriaL hazard aSsessment (PATROLS).

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