Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Identifying nanodescriptors to predict the toxicity of nanomaterials: a case study on titanium dioxide

Sivakumar Murugadoss,*[a] Nilakash Das[b], Lode Godderis[c,d], Jan Mast[e], Peter H.Hoet[#a] and Manosij Ghosh[#a]

a.Laboratory of Toxicology, Unit of Environment and Health, Department of Public Health and Primary Care, KU Leuven, 3000 Leuven, Belgium

b.Laboratory of Respiratory Diseases and Thoracic Surgery (BREATHE), KU Leuven, 3000 Leuven, Belgium

c.Laboratory for Occupational and Environmental Hygiene, Unit of Environment and Health, Department of Public Health and Primary Care, KU Leuven, 3000 Leuven, Belgium

d.IDEWE, External Service for Prevention and Protection at work, Interleuvenlaan 58, 3001 Heverlee, Belgium.

e.Trace Elements and Nanomaterials, Sciensano, 1180 Uccle, Belgium

# - equal contribution

Corresponding author

Prof.Peter H.Hoet

Email:peter.hoet@kuleuven.be

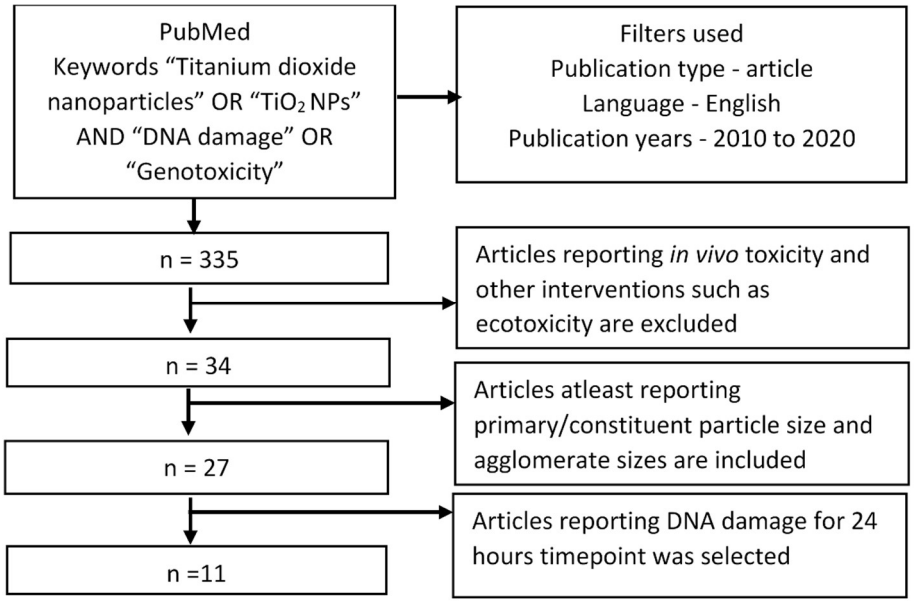


Fig.S1: Systematic selection of studies to extract data for case study 2

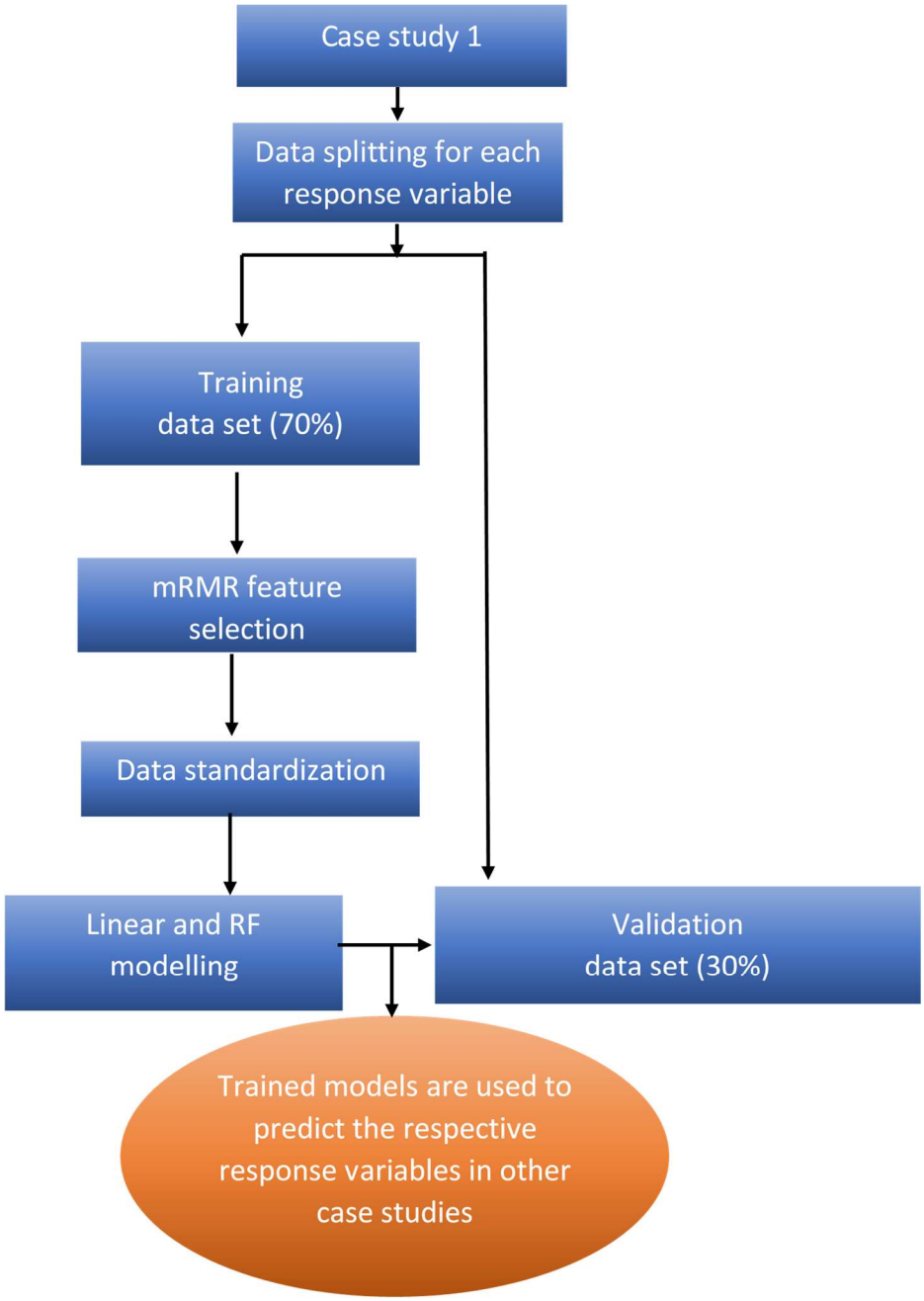
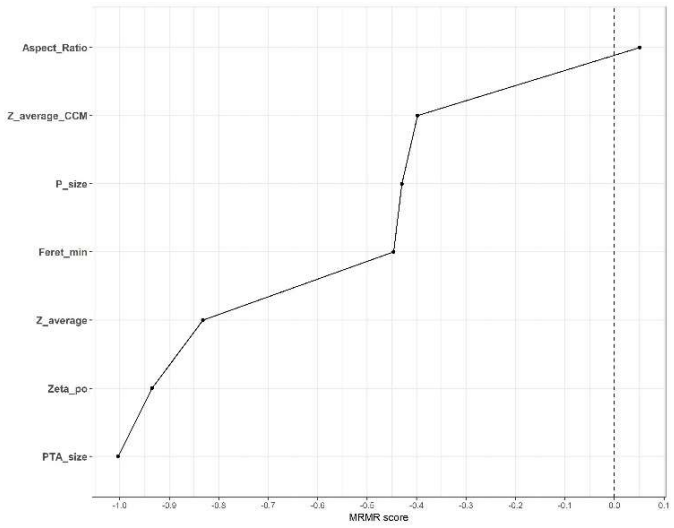
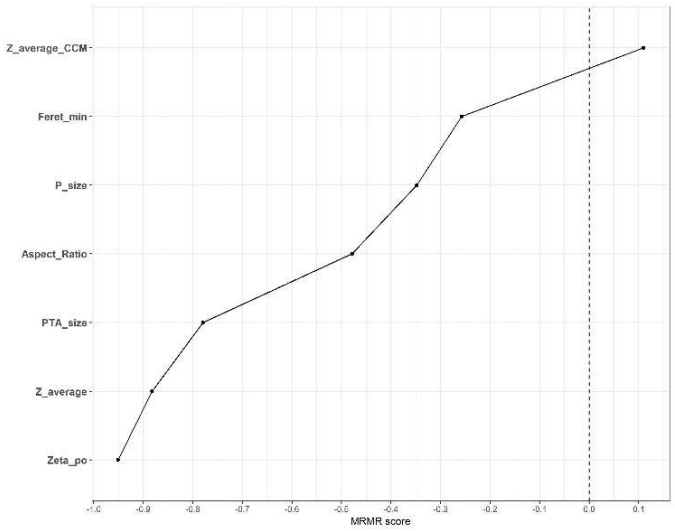


Fig.S2: Flow chart for statistical analysis performed for each case studies

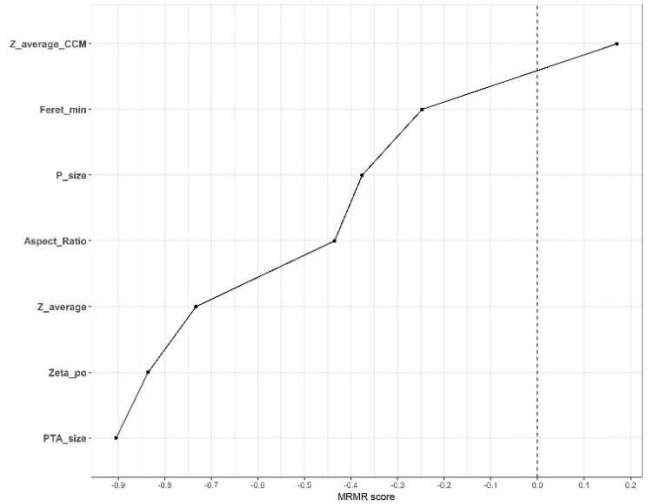
A


Aspect_Ratio
Z_average_CCM
P_size
Feret_min
Z_average
Zeta_po
PTA_size
MRMR score

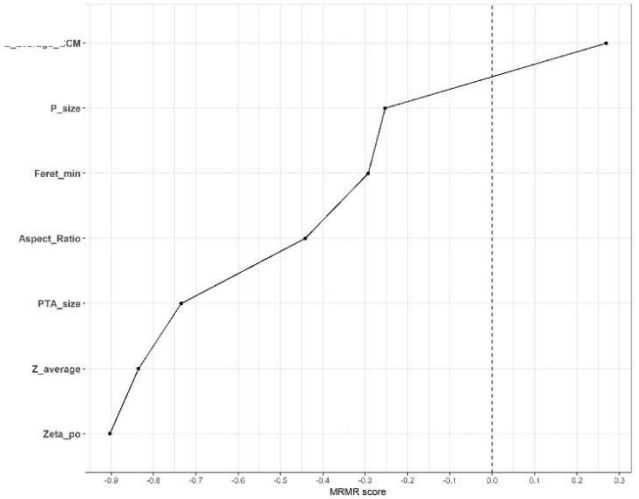
B


Z_average_CCM
Feret_min
P_size
Aspect_Ratio
PTA_size
Z_average
Zeta_po
MRMR score

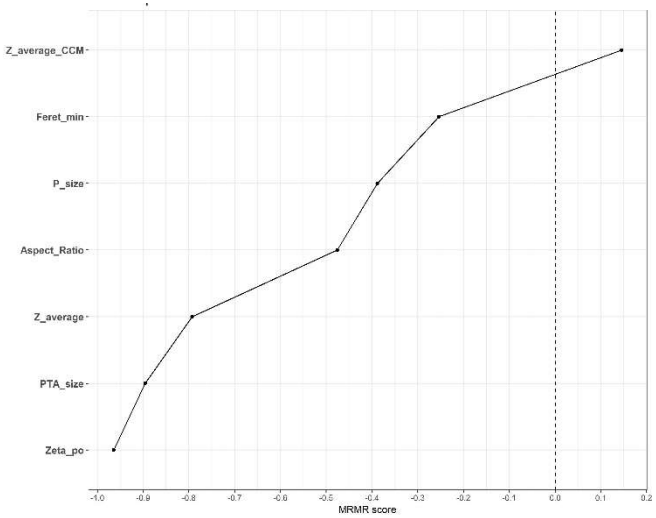
C


Z_average_CCM
Feret_min
P_size
Aspect_Ratio
Z_average
Zeta_po
PTA_size
MRMR score

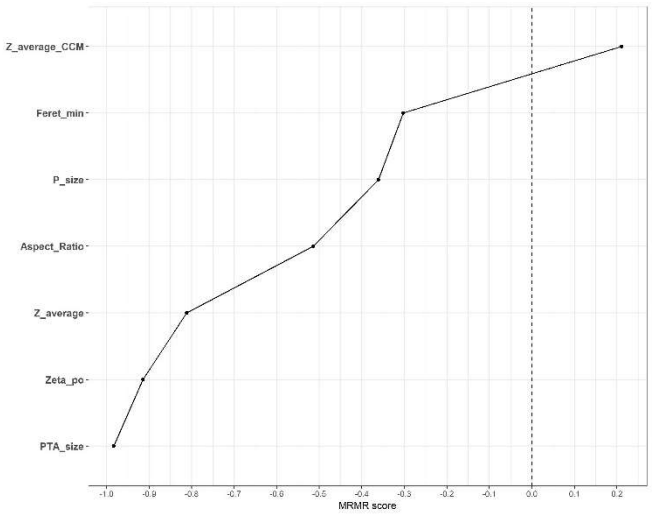
D


P_size
Feret_min
Aspect_Ratio
PTA_size
Z_average
Zeta_po
MRMR score

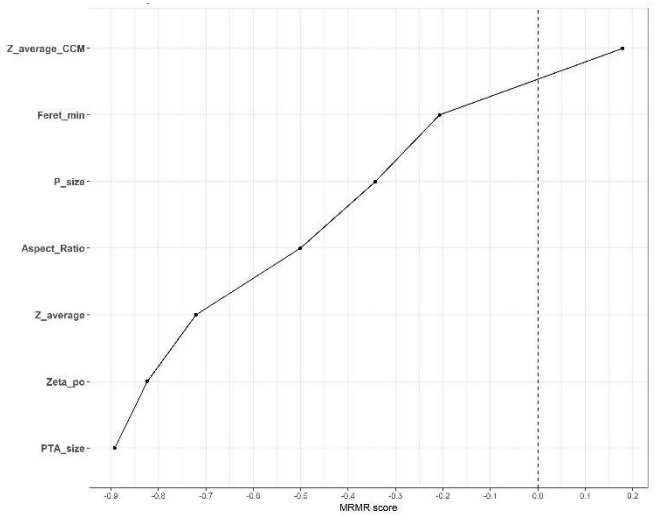
E


Z_average_CCM
Feret_min
P_size
Aspect_Ratio
Z_average
PTA_size
Zeta_po
-1.0 -0.9 -0.8 -0.7 -0.6 -0.5 -0.4 -0.3 -0.2 -0.1 0.0 0.1 0.2
MRMR score

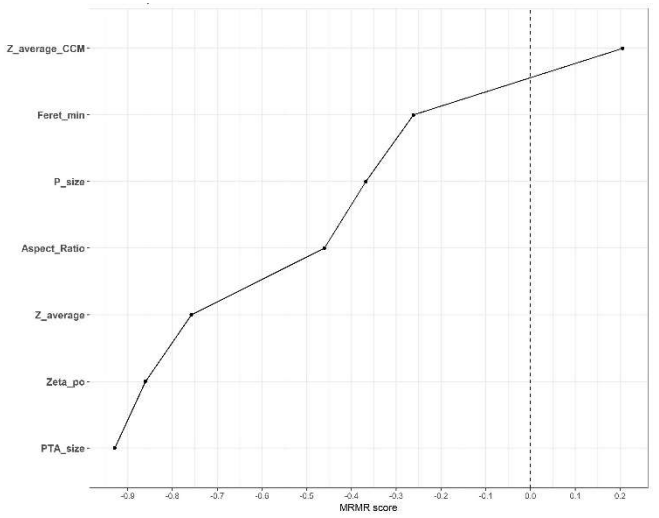
F


Z_average_CCM
Feret_min
P_size
Aspect_Ratio
Z_average
Zeta_po
PTA_size
-1.0 -0.9 -0.8 -0.7 -0.6 -0.5 -0.4 -0.3 -0.2 -0.1 0.0 0.1 0.2
MRMR score

G


Z_average_CCM
Feret_min
P_size
Aspect_Ratio
Z_average
Zeta_po
PTA_size
-0.9 -0.8 -0.7 -0.6 -0.5 -0.4 -0.3 -0.2 -0.1 0.0 0.1 0.2
MRMR score

H


Z_average_CCM
Feret_min
P_size
Aspect_Ratio
Z_average
Zeta_po
PTA_size
-0.9 -0.8 -0.7 -0.6 -0.5 -0.4 -0.3 -0.2 -0.1 0.0 0.1 0.2
MRMR score

I

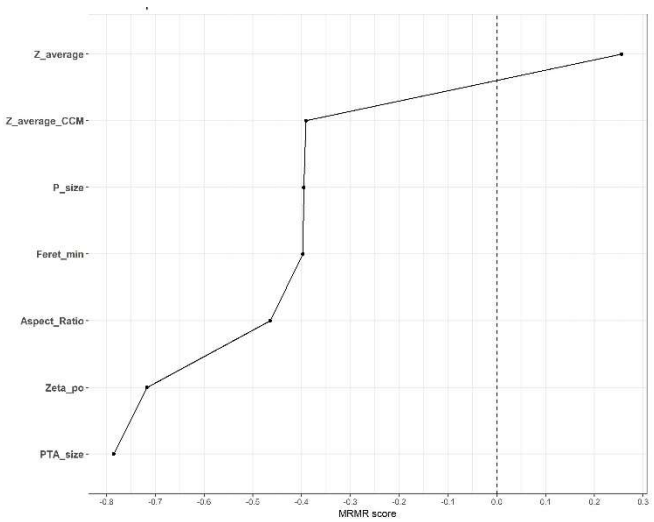
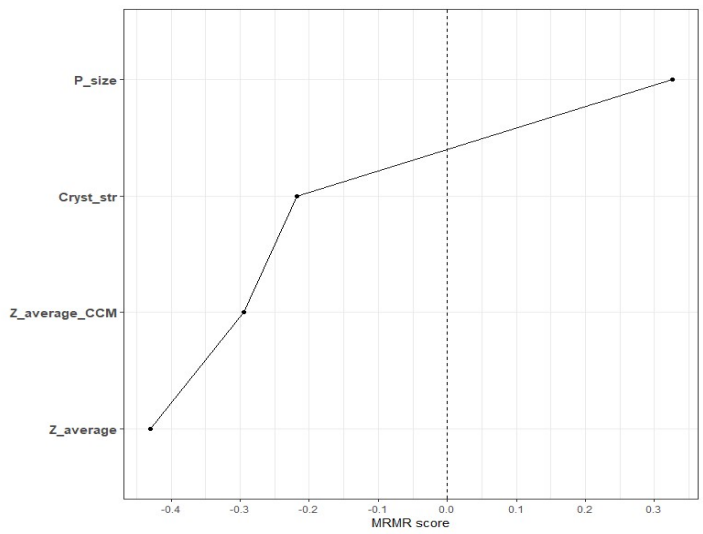
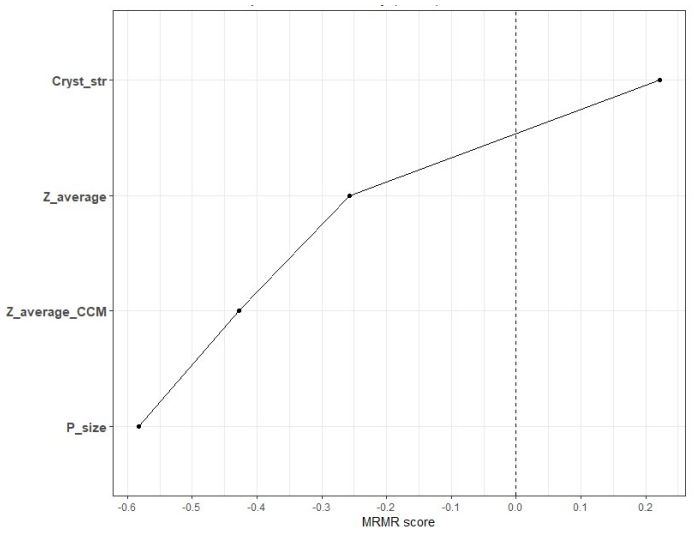


Fig.S3: Ranking of nanodescriptors from case study 1 by mRMR method according to their association with cell metabolic activity (A), cell viability (B), glutathione depletion (C), transepithelial electrical resistance (D), IL-8 (E), IL-6(F), TNF-α(G), IL-1β(H) and DNA damage (I). Z-average and Z-average_CCM - Hydrodynamic size measured by DLS in stock and in cell culture medium; P_size- constituent (primary) particle size; Feret_min-Feret minimum in stock dispersions; Aspect_Ratio - Aspect ratio in stock dispersions; Zeta_po - Zeta potential in stock dispersions; PTA _size -Hydrodynamic size measured by PTA in stock dispersions.

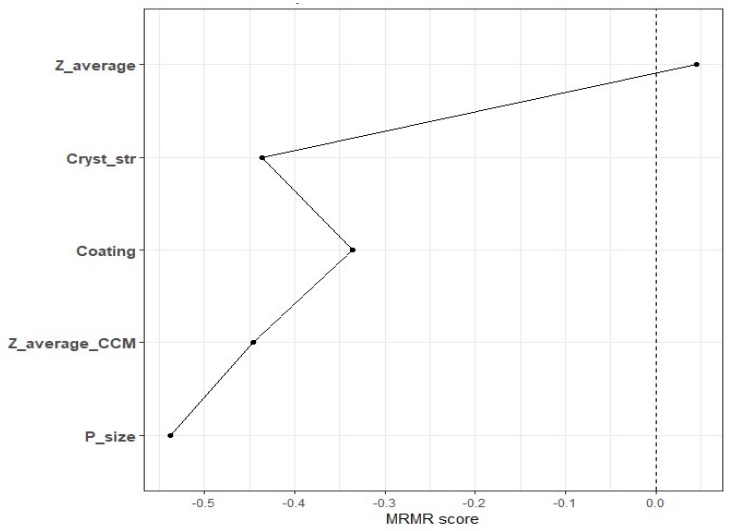
A


P_size
Cryst_str
Z_average_CCM
Z_average
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
MRMR score

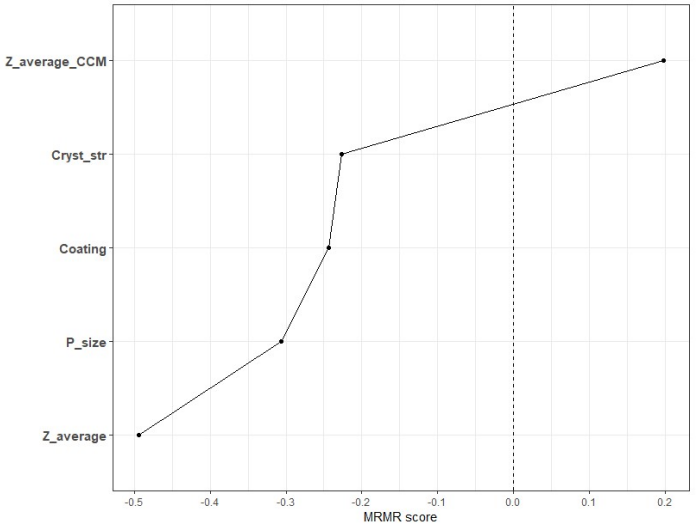
B


Cryst_str
Z_average
Z_average_CCM
P_size
-0.6
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
MRMR score

C


Z_average
Cryst_str
Coating
Z_average_CCM
P_size
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
MRMR score

D


Z_average_CCM
Cryst_str
Coating
P_size
Z_average
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
MRMR score

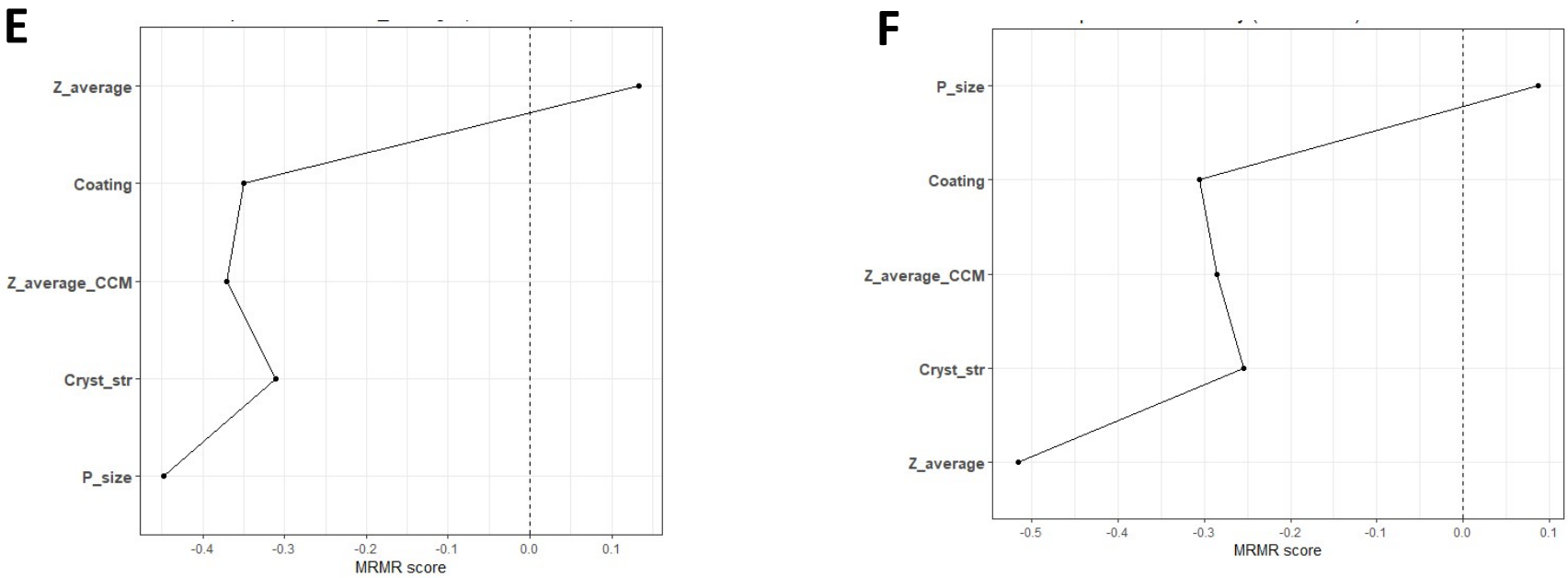


Fig. S4: Ranking of nanodescriptors for case study 2 (A and B), case study 3 (C and D) and all case studies combined (E and F) by mRMR method according to their association with effect on DNA damage (A,C,E) and cell viability (B,D,F). Z_average and Z-average_CCM - Hydrodynamic size measured by DLS in stock and in cell culture medium; P_size – Constituent (primary) particle size; Cryst_str - Crystal phase; Coating - Surface coating.

Table S1: Summary of biological endpoints reported in articles selected for case study 2.

Reference	Cytotoxicity /viability	DNA damage	Oxidative stress	Inflammatory mediators
El yamani et al 2017	✓	✓	X	X
Gea et al 2019	✓	✓	X	X
Wang et al 2015	✓	✓	X	X
Bessa et al 2017	✓	✓	X	X
Zijno et al 2016	✓	✓	X	X
Ursini et al 2014	✓	✓	X	✓
Prasad et al 2013	✓	✓	X	X
Patel et al 2017	✓	✓	X	X
Shukla et al 2011	✓	✓	✓	X
Shi et al 2015	✓	✓	✓	X
Clier et al 2017	✓	✓	X	X

Table S2. Dispersion protocols reported in studies used for case study 2

El yamani et al 2017	Nanogenotox Protocol
Gea et al 2019	Dimethylsulfoxide (DMSO 1% in water) was added to the TiO_2 NMs dispersions(final concentration 2.5 mg/ml); the dispersions were homogenized using an ultra-sonication procedure
Wang et al 2015	TiO_2 NMs were suspended in culture medium at a final concentration of 200 µg/mL and ultrasonicated (Branson Sonifier,USA) at 300 W for 10 min.
Bessa et al 2017	Prior to each toxicity treatment and interference analysis, TiO_2 NMs suspensions were sonicated in water bath for 5 min
Zijno et al 2016	TiO_2 NMs were suspended in cell culture medium without foetal calf serum (FCS) and then sonicated using ultrasonic bath (35 kHz, 320 W; Baldelin, RK100, Germany)
Ursini et al 2014	A stock solution (2 mg/mL) of TiO_2 NMs was prepared in ultrapure sterile water, vortexed for 1 min and sonicated for 5 min to disperse NMs
Prasad et al 2013	For the KB or KF dispersion, preweighed TiO_2 NMs were suspended in KGM medium with 0.1% BSA (KB) or KGM with 10% FBS (KF) at 1 mg/mL and probe sonicated at 7W for 2 min on ice
Patel et al 2017	TiO_2 NMs were suspended in deionized water (Milli-Q) to get a 5 mM stock solution and dispersed by sonication for 10 min
Shukla et al 2011	TiO_2 NMs (160 µg/mL) were suspended in IMEM (incomplete minimum essential medium; without FBS) and probe sonicated (Sonics Vibra cell, Sonics & Material Inc., New Town, CT, USA) for 10 min (1.5-min pulse on and 1-min pulse off for four times)
Shi et al 2015	TiO_2 NMs was sterilized by heating to 120 ◦C for 2 h, and freshly suspended into PBS, vigorous stirred and then sonicated for 20 min at 60W (KQ3200E ultrasonic disintegrator, Kunshan Ultrasonic Instrument Co., Ltd.) before use
Clier et al 2017	TiO_2 NMs were dispersed in ultrapure water as previously described by ultrasonication for 30 min at 4°C on a Vibra Cell 75043 sonicator (Bioblock Scientific) operated in pulse mode (1 s on/1 s off) at 28% amplitude , i.e. 16.7 W

References:

1. Yamani N El, Collins AR, Rundén-Pran E, Fjellsbø LM, Shaposhnikov S, Zienolddiny S, et al. In vitro genotoxicity testing of four reference metal nanomaterials, titanium dioxide, zinc oxide, cerium oxide and silver: Towards reliable hazard assessment. Mutagenesis. 2017;32:117–26.
2. Gea M, Bonetta S, Iannarelli L, Giovannozzi AM, Maurino V, Bonetta S, et al. Shape-engineered titanium dioxide nanoparticles (TiO 2 -NPs): cytotoxicity and genotoxicity in bronchial epithelial cells. Food Chem Toxicol. Elsevier; 2019;127:89–100.
3. Wang Y, Cui H, Zhou J, Li F, Wang J, Chen M, et al. Cytotoxicity, DNA damage, and apoptosis induced by titanium dioxide nanoparticles in human non-small cell lung cancer A549 cells. Environ Sci Pollut Res. 2015;22:5519–30.
4. Bessa MJ, Costa C, Reinosa J, Pereira C, Fraga S, Fernández J, et al. Moving into advanced nanomaterials. Toxicity of rutile TiO2 nanoparticles immobilized in nanokaolin nanocomposites on HepG2 cell line. Toxicol Appl Pharmacol. Elsevier Inc.; 2017;316:114–22.
5. Zijno A, De Angelis I, De Berardis B, Andreoli C, Russo MT, Pietraforte D, et al. Different mechanisms are involved in oxidative DNA damage and genotoxicity induction by ZnO and TiO2 nanoparticles in human colon carcinoma cells. Toxicol Vitr. Elsevier Ltd; 2015;29:1503–12.
6. Ursini CL, Cavallo D, Fresegna AM, Ciervo A, Maiello R, Tassone P, et al. Evaluation of cytotoxic, genotoxic and inflammatory response in human alveolar and bronchial epithelial cells exposed to titanium dioxide nanoparticles. J Appl Toxicol. 2014;34:1209–19.
7. Prasad RY, Wallace K, Daniel KM, Tennant AH, Zucker RM, Strickland J, et al. Effect of treatment media on the agglomeration of titanium dioxide nanoparticles: Impact on genotoxicity, cellular interaction, and cell cycle. ACS Nano. 2013;7:1929–42.
8. Patel S, Patel P, Bakshi SR. Titanium dioxide nanoparticles: an in vitro study of DNA binding, chromosome aberration assay, and comet assay. Cytotechnology. Springer Netherlands; 2017;69:245–63.
9. Shukla RK, Kumar A, Gurbani D, Pandey AK, Singh S, Dhawan A. TiO2 nanoparticles induce oxidative DNA damage and apoptosis in human liver cells. Nanotoxicology. 2013;7:48–60.
10. Shi Z, Niu Y, Wang Q, Shi L, Guo H, Liu Y, et al. Reduction of DNA damage induced by titanium dioxide nanoparticles through Nrf2 in vitro and in vivo. J Hazard Mater. Elsevier B.V.; 2015;298:310–9.
11. Biola-Clier M, Beal D, Caillat S, Libert S, Armand L, Herlin-Boime N, et al. Comparison of the DNA damage response in BEAS-2B and A549 cells exposed to titanium dioxide nanoparticles. Mutagenesis. 2017;32:161–72.