Electronic Supplementary Material (ESI) for Toxicology Research.

Supplementary Information for:

Label-free Monitoring of the Nanoparticle Surface Modification Effects on Cellular Uptake, Trafficking and Toxicity

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S1. SEM images of 'as received' ZnO NP.

Confirmation of the expected, approximately size range of the ZnO NP as specified by the manufacturer (10-30nm size range) was confirmed using a Zeiss SEM, operating at 20kV voltage. Representative SEM micrograph is shown in **Fig. S2.**, below.

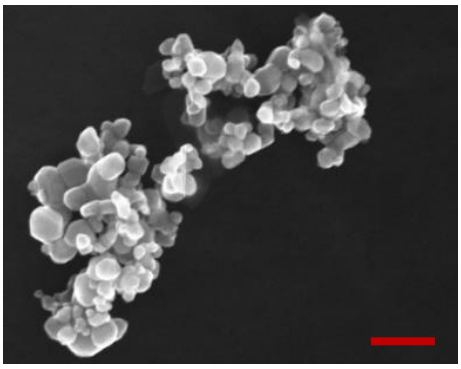

Figure S1. SEM image showing size range of ZnO NP as received from manufacturer. *Scale bar = 100nm*

S2. FTIR spectra of APTMS and APTMS-BSA ZnO NP.

FTIR spectra (**Fig. S3.**) from APTMS and APTMS-BSA capped ZnO, as well as BSA (fraction V) and APTMS ligands, all deposited on ATR crystal. Spectra were recorded with Perkin Elmer FTIR. APTMS ligand shows symmetric and asymmetric features of CH_2 chain stretch (2840 and 2930), amine bands (1650 and 1450), characteristic symmetric and asymmetric Si-O-Si bands (1070 and 1200) and NH bond deformation (750-810), whilst APTMS capped ZnO NPS show features of chemisorbed APTMS with the shift in symmetric and asymmetric Si-O-Si bands to around 1020 and 1100 wavenumber. BSA spectrum shows a typical broad band in the OH and amine NH stretch region (3200/3300 - 3500), symmetric and asymmetric CH_2 chain stretch (2840 and 2930) and typical features of amide I and II (500-1800)[1], whilst the fingerprint of APTMS-BSA capped NP was different than those observed for APTMS and BSA NP.

As opposed to plain and BSA capped NP, ζ-potential of APTMS and APTMS-BSA NP remains stable in cell culture media (main text), also the overall number of ligands does not change (main text), hence, these particles are not likely to suffer from extensive surface ligand exchange reactions.

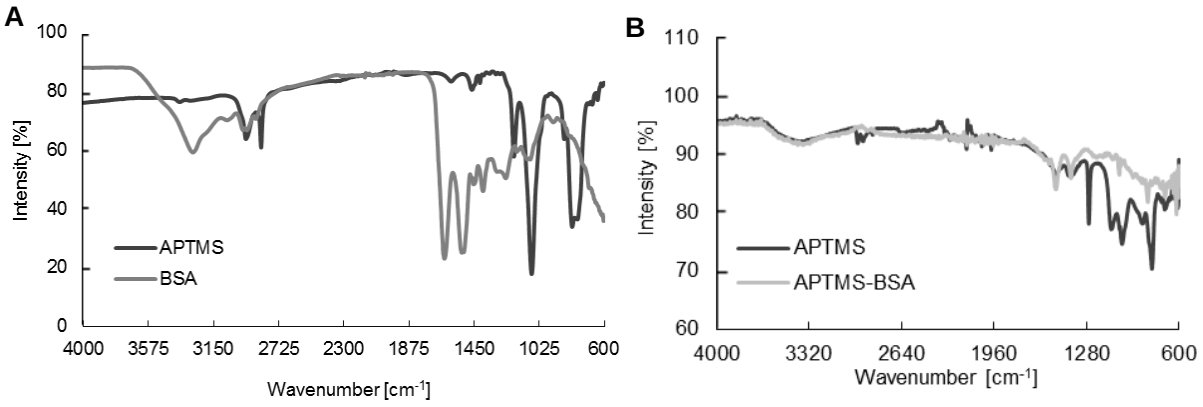

Figure S2. FTIR spectra of BSA and APTMS ligands (A) and ZnO NPs capped with APTMS and APTMS-BSA ligands (B).

S3. Size distribution and zeta-potential profiles of ZnO NP.

Size distribution and zeta-potential profiles of plain and capped ZnO NP dispersions were studied using image based Nanoparticle Tracking Analysis (NTA) and z-NTA on an NS500 instrument (NanoSight UK). Data collected in triplicates from individual batches of NP and analysed with NTA2.2 software, according to a method described in the Experimental section of the manuscript. Representative size and zeta-potential distribution profiles are shown.

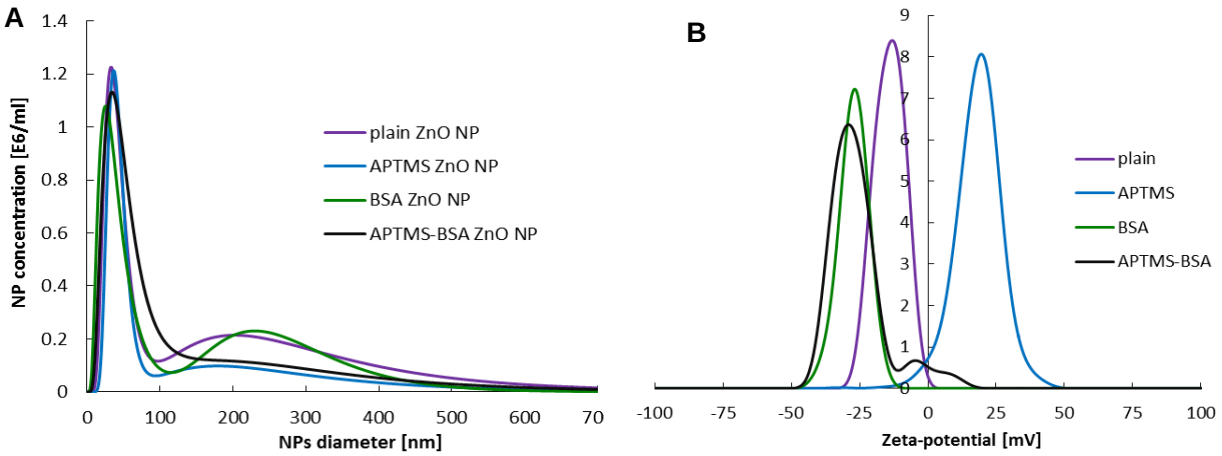

Figure S3. Representative size (A) and zeta-potential (B) distribution graphs of plain and capped ZnO NPs.

Capped and plain ZnO NP contain predominantly particles in the nanoscale, with an average diameter around 30nm, as detailed in Table1 of the manuscript. There are some additional agglomerates/aggregates present (also seen on TEM images), however, these form only small fraction off all particles in solution and are more evident in uncapped or weakly stabilised BSA NP, than in case of silan-protected APTMS and APTMS-BSA NP.

Zeta-potential distribution profiles of plain and capped NP (dispersed in pure Milli-Q water, pH 5.4) show clear surface chemistry dependent differences, with APTMS capped NP being positively charged, while BSA and APTMS-BSA NP negatively charged.

S4. Relationship between the impedance measurements and cell number of HepG2 cells using the RT-CES system.

HepG2 cell were seeded directly onto the gold electrode array of the standard format E-Plate (Roche Diagnostics) at densities ranging from 50,000 – 400,000 cells per cm^2. The cell were allowed to attached and acclimatise for 24 hours with impedance measurements taken every hour.

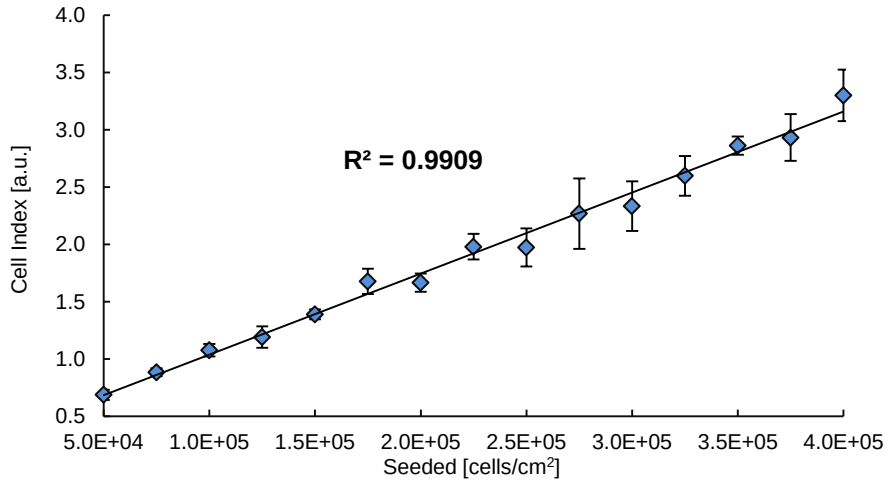

Figure S4. Graph showing the correlation between impedance based cell index measurements and cell number 24h after seeding (mean±stdev, n=3).

S5. The effect of capping ligands and TiO_2 NPs of HepG2 determined with RT-CES.

HepG2 were seeded onto an E-Plate at 20,000 cells per well and allowed to acclimatise for 24 hours. The cells were then exposed to APTM and BSA capping ligands or TiO_2 NP capped with the same ligand combination as used for ZnO NP in the manuscript and impedance measurements were taken every hour.

Neither the presence of ligands nor the non-toxic capped TiO_2 NP (added at substantially higher concentrations) compromised the biological status of HepG2 (**Fig. S6**), with no significant reduction in cell number during the test period. This indicates that the toxicity profiles seen for ZnO NP are not related to a toxic contaminant (like enodotoxins), but are linked to a chemical composition and particulate forms of ZnO core.

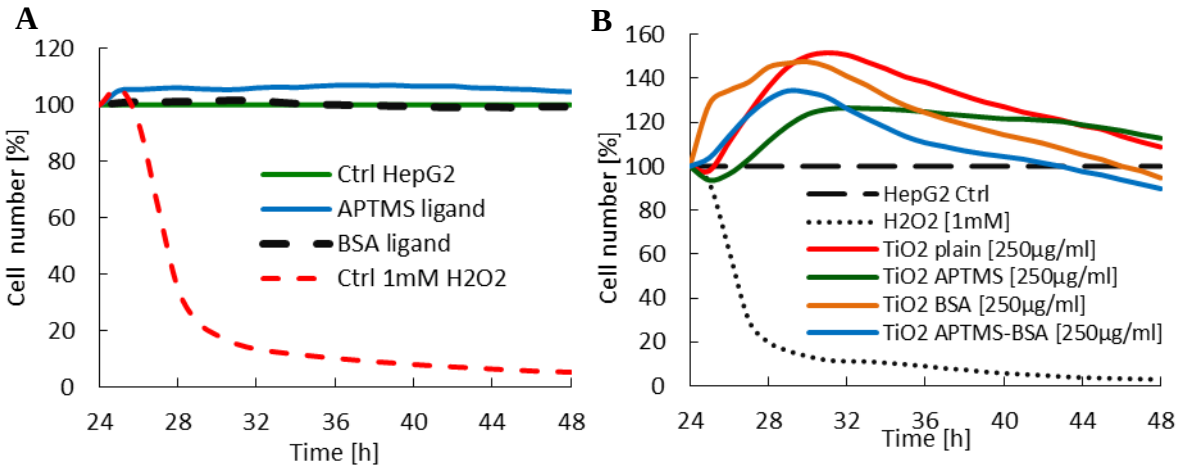

Figure S5. Impedance curves showing the toxicity of the capping ligands (A) and capped TiO_2 NP (B) in HepG2.