– Electronic Supplementary Information (ESI) –

Automatable platform for genotoxicity testing of nanomaterials based on the fluorometric γ-H2AX assay reveals no genotoxicity of properly surface-shielded cadmium-based quantum dots

*D. Geißler[1], M. Wegmann[1,2], T. Jochum[3], V. Somma[2], M. Sowa[2], J. Scholz[2], E. Fröhlich[4], K. Hoffmann[1], J. Niehaus[3], D. Roggenbuck[2,5], and U. Resch-Genger[1]**

[1] Bundesanstalt für Materialforschung und -prüfung (BAM), Division 1.2 Biophotonics, Richard-Willstätter-Str. 11, 12489 Berlin, Germany

[2] MEDIPAN GmbH, Ludwig-Erhard-Ring 3, 15827 Dahlewitz, Germany

[3] Fraunhofer-Zentrum für Angewandte Nanotechnologie CAN, Grindelallee 117, 20146 Hamburg, Germany

[4] Medizinische Universität Graz, Zentrum für Medizinische Forschung (ZMF), Stiftingtalstrasse 24, 8010 Graz, Austria

[5] Institute of Biotechnology, Faculty Environment and Natural Sciences, Brandenburg University of Technology

*Correspondence and requests for materials should be addressed to U.R.-G. (email: ute.resch@bam.de)

Content

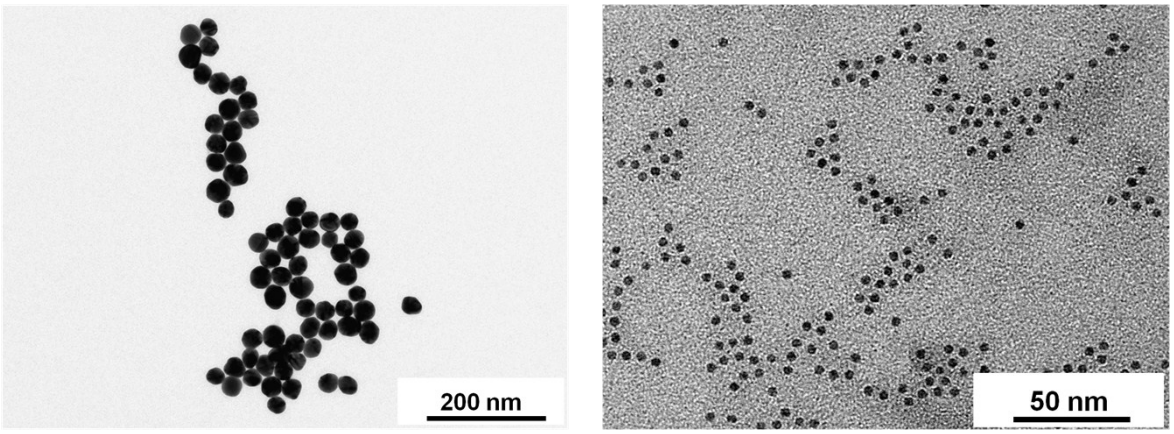


Figure S1. TEM images of the 30 nm Au-NP (***left***) and the 4 nm FeO_x-NP (***right***).

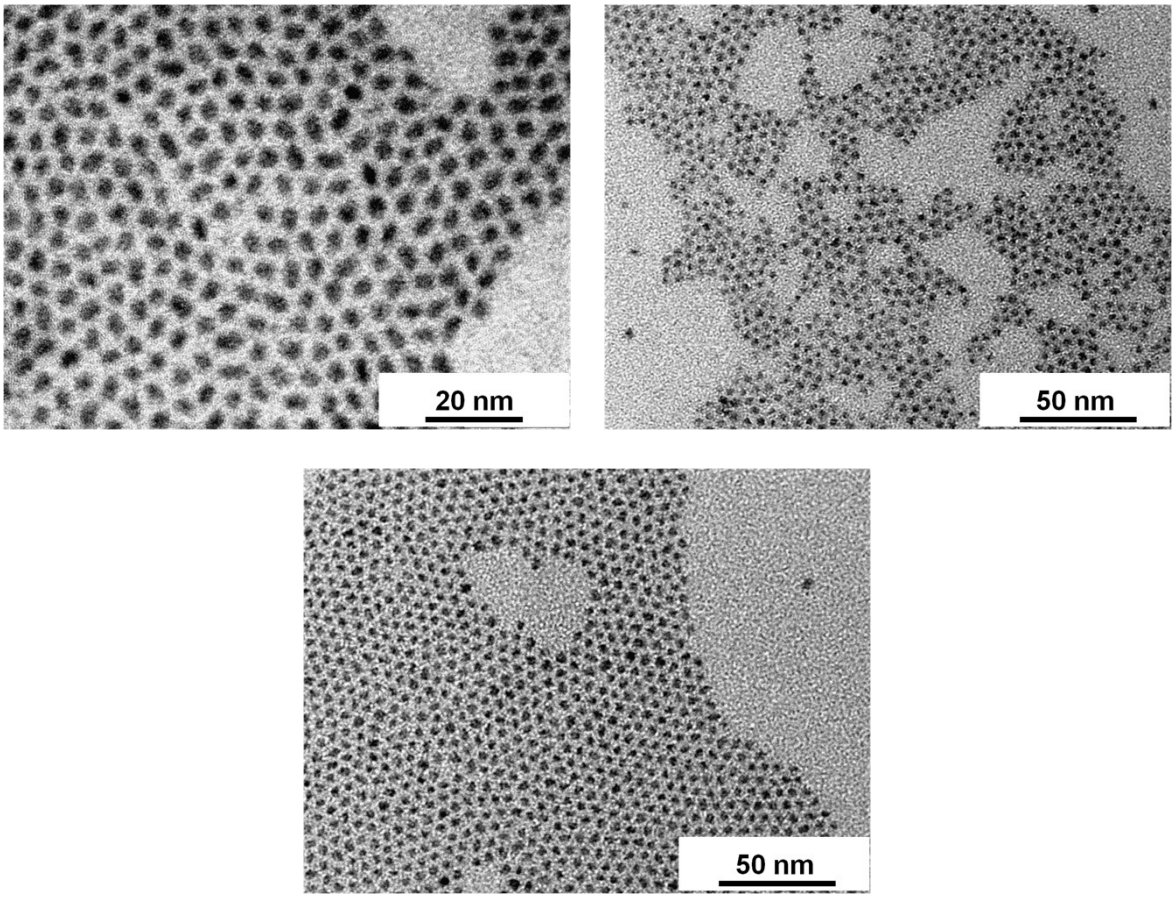


Figure S2. TEM images of the Cd-based quantum dots with different shell compositions: C-QD (CdSe core only, ***top left***), CS-QD (CdSe/CdS core/shell, ***top right***), and CSS-QD (CdSe/CdS/ZnS core/shell/shell, ***bottom***).

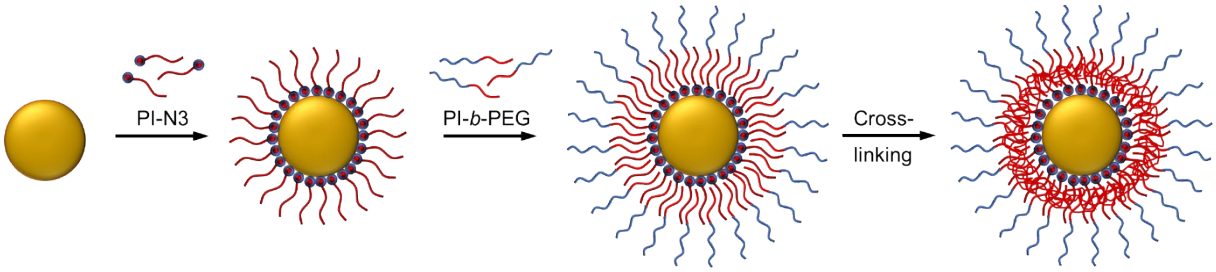


Figure S3 Micellar encapsulation of nanoparticles as applied for the FeO_x-NP and the different Cd-based QD. The native apolar surface ligands present after NP synthesis are partly exchanged with polyisoprene-diethylenetriamine (PI-N3), then polyisoprene-*b*-polyethylene glycol di-block copolymer (PI-*b*-PEG) is added, and the PI units are cross-linked using a radical initiator (AIBN) to yield a stable, water-soluble NP dispersion.

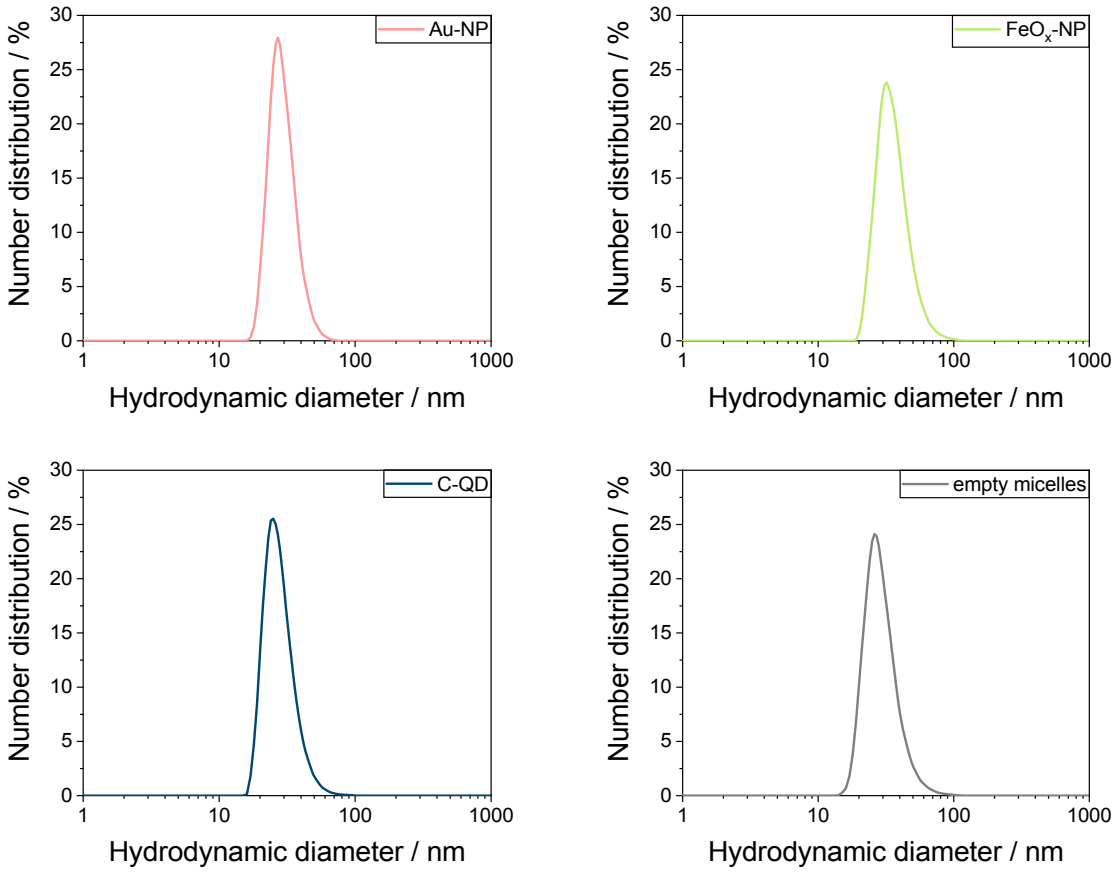


Figure S4. Representative number-weighted size distributions measured with DLS for the citrate-stabilized 30 nm Au-NP (***top left***), as well as the 4 nm FeO_x-NP (***top right***) and the CdSe core only QD (C-QD, ***bottom left***) after micellar encapsulation in comparison to the (empty) PI-*b*-PEG micelles (***bottom right***), demonstrating that the micellar encapsulated FeO_x-NP and Cd-based QD have the same size (ca. 30 nm) as the citrate-stabilized Au-NP.

Table S1. Total amount of protonable/deprotonable functional groups (FG) on the particle surface as measured using conductimetric acid/base titration.

Particle	Surface ligands	FG	FG/NP
Au-NP	Citrate	-COOH	> 10,000
FeO_x-NP	PI-N3 / PI-*b*-PEG	$-NH_2$	220 ± 20
C-QD	PI-N3 / PI-*b*-PEG	$-NH_2$	170 ± 20
CS-QD	PI-N3 / PI-*b*-PEG	$-NH_2$	165 ± 10
CSS-QD	PI-N3 / PI-*b*-PEG	$-NH_2$	190 ± 10
Empty micelles	PI-N3 / PI-*b*-PEG	$-NH_2$	1100 ± 60

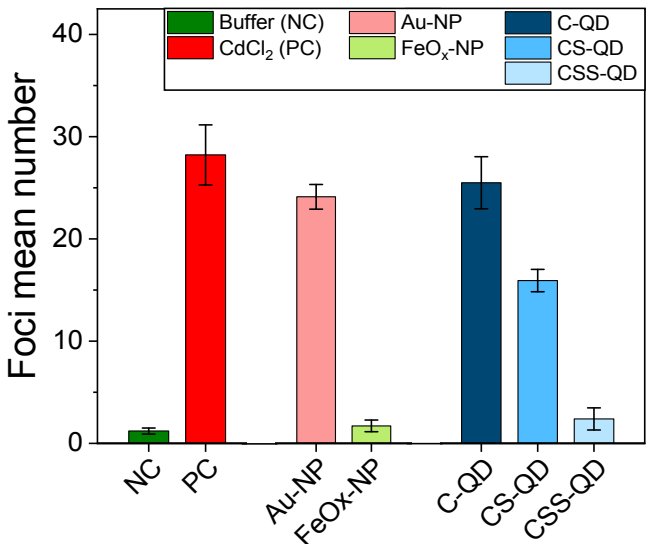
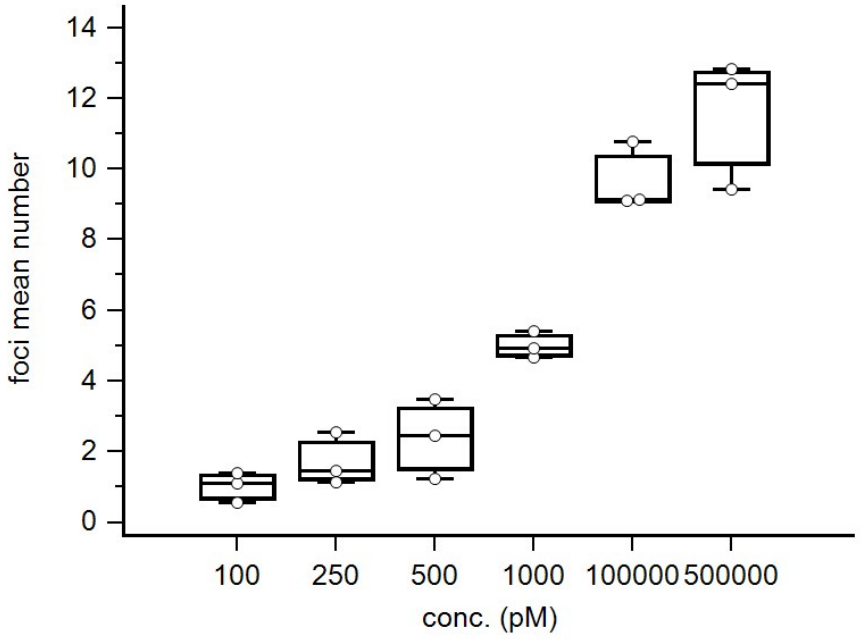


Figure S5. Comparison of the genotoxicity in Hep2 cells for the potential reference particles Au-NP (positive control) and FeO_x-NP (negative control) at a particle concentration of 250 pM, as well as the Cd-based quantum dots with different shell compositions (C-QD = CdSe core only QD, CS-QD = CdSe/CdS core/shell QD, CSS-QD = CdSe/CdS/ZnS core/shell/shell QD) at a particle concentration of 100 pM, in comparison to established toxicity standards (buffer as negative control and $CdCl_2$ as positive control). Error bars represent the standard deviation of three independent replicates.



One-way analysis of variance

Data	foci1
Factor codes	conc
Sample size	18

Levene's test for equality of error variances

Levene statistic	3,027
DF 1	5
DF 2	12
Significance level	P = 0,054

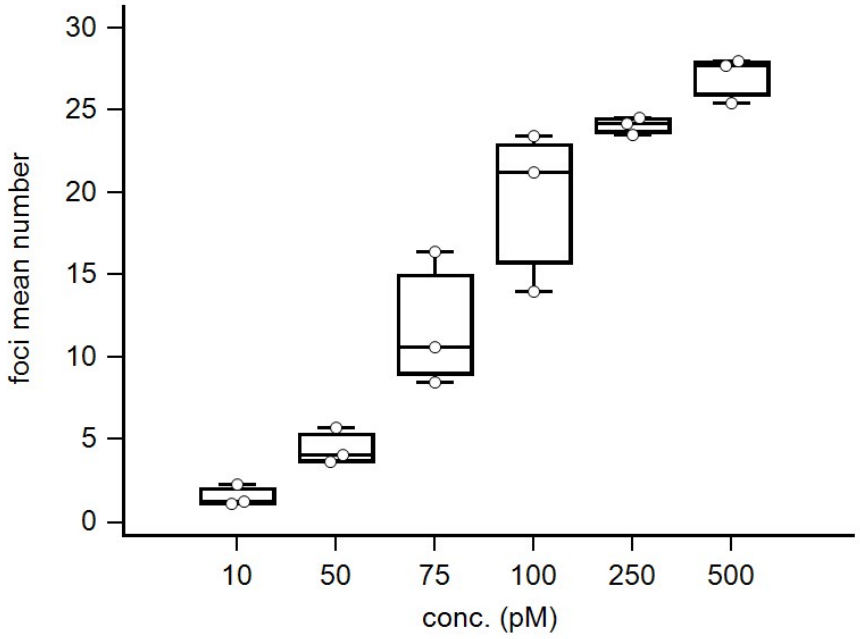
ANOVA

Source of variation	Sum of Squares	DF	Mean Square
Between groups (influence factor)	294,46	5	58,89
Within groups (other fluctuations)	13,10	12	1,09
Total	307,56	17	
F-ratio	53,93		
Significance level	P < 0,001		

Scheffé test for all pairwise comparisons

Factor	n	Mean	SD	Different (P<0,05) from factor nr
(1) 100	3	1,02	0,43	(4)(5)(6)
(2) 250	3	1,71	0,74	(5)(6)
(3) 500	3	2,38	1,13	(5)(6)
(4) 1000	3	5,01	0,37	(1)(5)(6)
(5) 100000	3	9,68	0,96	(1)(2)(3)(4)
(6) 500000	3	11,56	1,87	(1)(2)(3)(4)

Figure S6. Detailed statistical analysis of genotoxicity of FeO_x-NP (negative control).



One-way analysis of variance

Data	foci
Factor codes	conc
Sample size	18

Levene's test for equality of error variances

Levene statistic	5,163
DF 1	5
DF 2	12
Significance level	P = 0,009

ANOVA

Source of variation	Sum of Squares	DF	Mean Square
Between groups (influence factor)	1652,97	5	330,59
Within groups (other fluctuations)	89,98	12	7,50
Total	1742,95	17	
F-ratio	44,09		
Significance level	P < 0,001		

Scheffé test for all pairwise comparisons

Factor	n	Mean	SD	Different (P<0,05) from factor nr
(1) 10	3	1,53	0,62	(3)(4)(5)(6)
(2) 50	3	4,45	1,09	(4)(5)(6)
(3) 75	3	11,84	4,08	(1)(5)(6)
(4) 100	3	19,56	4,96	(1)(2)
(5) 250	3	24,11	0,51	(1)(2)(3)
(6) 500	3	27,04	1,38	(1)(2)(3)

Figure S7. Detailed statistical analysis of genotoxicity of Au-NP (positive control).