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Supporting Information

DNA Protection against Ultraviolet Irradiation by Encapsulation in a Multilayered SiO_2/TiO_2 Assembly

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Particle analysis

SEM/STEM-EDXS mapping

The sample was characterized using scanning electron microscopy (SEM; FEI NovaNanoSEM 450, 5 kV). The scanning transmission electron microscopy (STEM) investigations were performed on an aberration-corrected HD-2700CS (Hitachi) and operated at an acceleration potential of 200 kV. An energy-dispersive X-ray spectrometer (EDXS; Gemini system of EDAX) is used for elemental mappings (measuring time ca. 30 min).

FT-IR

The presence of the titania coating was confirmed by Fourier transform infrared spectrometry (FT-IR spectrometer Tensor 27, Bruker Optics, equipped with a diffuse reflectance accessory, DiffuseIR™, Pike Technologies) on sample milled with KBr (2 w/w %). A strong absorption band in the range of 900-500 cm^{-1} was observed, which is associated with the characteristic vibrational mode of TiO_2.[1]

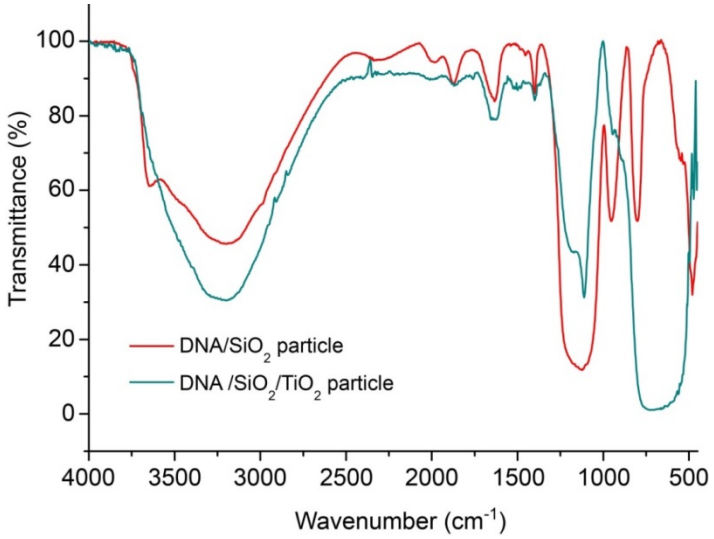


Figure S1. IR spectra of encapsulated DNA into SiO_2 and TiO_2 coated SiO_2 particles.

DNA analysis

Quantitative real-time qPCR

After the TiO_2 and SiO_2 dissolution with diluted BOE (1:100), the released dsDNA sequence was directly amplified using a standard qPCR protocol (Roche LightCycler 96). For the qPCR the following primers were used: 5'-ATT CAT GCG ACA GGG GTA AG-3' (forward primer) and 5'-ATC GGG TTA CAC TGG CTG AC-3' (reverse primer), (Eurofins MWG Operon).

Agarose gel electrophoresis

DNA Ladder: Volume of 300 µl of DNA ladder (6 µg/ml, determined by Qubit dsDNA HS assay) was treated with UV-C radiation for 1.5 h and analyzed by gel electrophoresis (E-Gel® Agarose Gel Electrophoresis, Invitrogen).

DNA Ladder/SiO_2/TiO_2: Volume of 300 µl of 5.7 mg/ ml particle dispersion was treated for 1.5 h and 100 µl of treated particles were centrifuged and dissolved in 100 µl BOE. The DNA solution was then purified (QIAquick PCR purification kit) and the obtained DNA analyzed by gel electrophoresis.

Sanger Sequencing

Protected DNA in SiO_2/TiO_2 particles and free DNA were treated with UV-C radiation for 1.5 h. Particle dispersion was dissolved in BOE, purified (QIAquick PCR purification kit) and sequenced. DNA was sequenced with the primer 5'-CAG GGG TAA GAC CAT CAG-3' (Microsynth AG).

Table S1. Output data of a one sided unpaired two-sample t-test using Origin 8.6 on Phred quality data of sequencing data

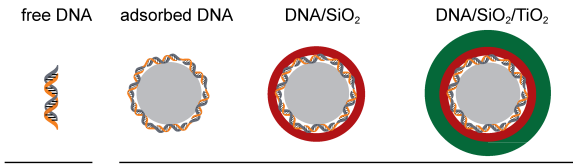
Descriptive Statistics		**N**	**Mean**	**SD**	**SEM**
DNA		17	33.1765	9.53438	2.31243
DNA in SiO_2/TiO_2 particles (UV-C treated)		17	44.8824	8.99918	2.18262
	Difference		-11.706		

t-Test Statistics	**t Statistic**	**DF**	**Prob>t**
Equal Variance Assumed	-3.68132	32	0.99958
Equal Variance NOT Assumed	-3.68132	31.89379	0.99957

DNA properties of final encapsulates

In order to characterize the core-shell particle system, the quality of the DNA encapsulation process was evaluated by investigating the DNA protection properties of the particles. To understand the geometric presence of DNA in the particles, the amount of DNA bound during the synthesis procedure was followed, together with the radical stability of the corresponding DNA (to discriminate coated/non-coated). Following the dissolution of the particles, the resulting DNA concentration was measured by using the Qubit dsDNA HS kit. Free DNA and DNA that was adsorbed on the functionalized SiO_2 particle surface were nearly completely destroyed after the radical treatment. The subsequent synthesis steps of silica layer growth and the additional TiO_2 nanocoating provided DNA stability against ROS (>90%). This designed stability assay of DNA shows, therefore, the successful encapsulation of DNA. The DNA shown in Table S1 consists of 113 bp.

Table S1. Evidence of DNA presence and stability in the core-shell particle systems

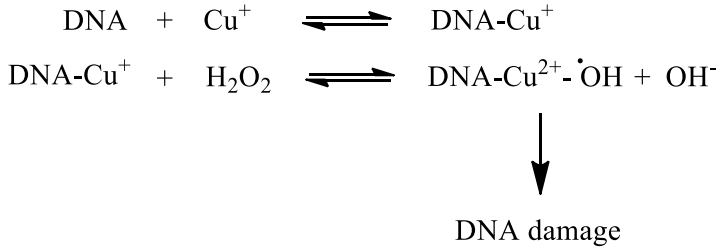


	µg/ml	µg DNA per mg particle		
DNA loading	3.1	4.7	2.2	2.0
ROS resistance (DNA loading after ROS treatment)	< 0.0005 *	< 0.0005 *	2.0	1.9
ROS stability (%)	< 0.1	< 0.1	91	95

star (*) indicates data below the detection limit (< 0.5 ng/ml)

Control experiment of radical treated free DNA

To show the generation of free radicals and the associated degradation of DNA within our designed assay, we also treated free DNA as a reference. It was treated with highly aggressive heavy metal and hydrogen peroxide containing solutions which induce the formation of reactive oxygen species (ROS). Under the conditions applied (230 µM $CuCl_2$, 6.6 mM H_2O_2, 1.3 mM ascorbic acid) the free DNA was destroyed completely (Figures S2), which proves the effectiveness of our radical stability test.



As a control experiment, Ethylenediaminetetraacetic acid (EDTA), complexing agent was added before the radical treatment. EDTA can generate a stable complex with Cu(I) and inhibits the redox process with H_2O_2 and Cu(I) and therefore the formation of free radicals.

Table. S2 Control experiment of radical treated free DNA

Sample	**DNA Concentration (µg/ml)**
Free DNA	3.1
Free DNA + EDTA + radical treatment	3.0
Free DNA + radical treatment	< 0.0005*

star (*) indicates data below detection limit (< 0.5 ng/ml)

Calculation of attenuation coefficients

The attenuation coefficient (β_λ) is the sum of the absorption coefficient (κ_λ) and the scattering coefficient (σ_λ).

$$\beta_\lambda = \kappa_\lambda + \sigma_\lambda$$

Attenuation coefficient of TiO_2/SiO_2/DNA particles measured by DNA damage

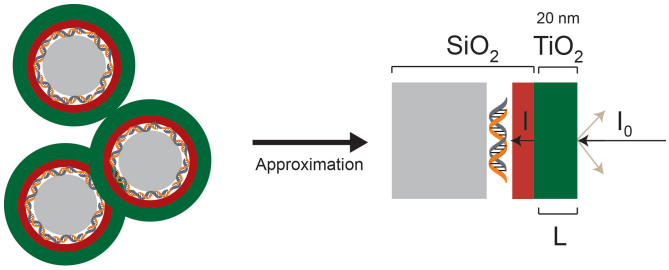


Figure S3. Approximation of light attenuation for DNA encapsulated TiO_2 coated particles

Calculation of the attenuation coefficient of the DNA/SiO_2/TiO_2 particles in UV light (254 nm), assuming a linear dependency of DNA damage of light intensity and a titania layer thickness of L = 20 *10^{-7}cm. DNA protected in TiO_2/SiO_2 was determined to be 42 times more resistant to UV-C light than the unprotected DNA.

$$\frac{I}{I_0} = \frac{1}{42} = e^{-\beta_{\lambda 254,DNA} \times L(cm^{-1})}$$

$$\boldsymbol{\beta_{254,DNA} = 1.8 \times 10^6 cm^{-1}}$$

Attenuation coefficient of DNA/SiO_2/TiO_2 particles in water by photometer

The attenuation coefficient can be obtained by conventional spectrophotometric measurements as absorbance readings.

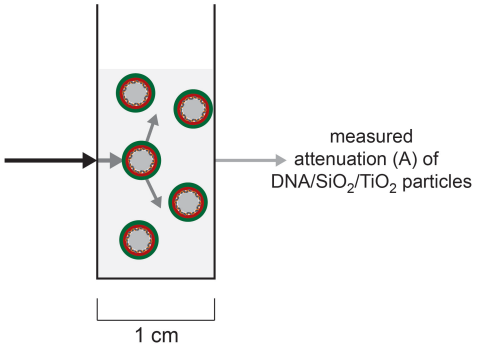


Figure S4. Spectrophotometric measurement of DNA/SiO_2/TiO_2 particles

The experimental value of attenuation from particle suspension was obtained by photometer (PM) (Nanodrop 2000C spectrometer, Thermo Scientific) with A_{254}= 0.01 and a cell path length of L=1 cm.

$$\beta_{254,PM}\ (cm^{-1}) = \frac{2.303\ A_{254}}{L\ (cm)} = 0.023 cm^{-1}$$

Accounting for the concentration of TiO_2 with c = 2 *10^{-7} g cm^{-3} and a density of ρ = 4.23 g cm^{-3}

$$\beta^*_{254,PM}(cm^2 g^{-1}) = \frac{\beta_{254,PM}\ (cm^{-1})}{c(g\ cm^{-3})} = 115\ 000\ cm^2 g^{-1}$$

$$\beta_{254,PM}(cm^{-1}) = \beta^*_{254,PM}(cm^2 g^{-1}) \times \rho_{TiO2}\ (g\ cm^{-3}) = 486\ 450\ cm^{-1}\ \approx \mathbf{0.5 * 10^6 cm^{-1}}$$

Control experiment of shadowing effect of TiO_2 nanoparticle

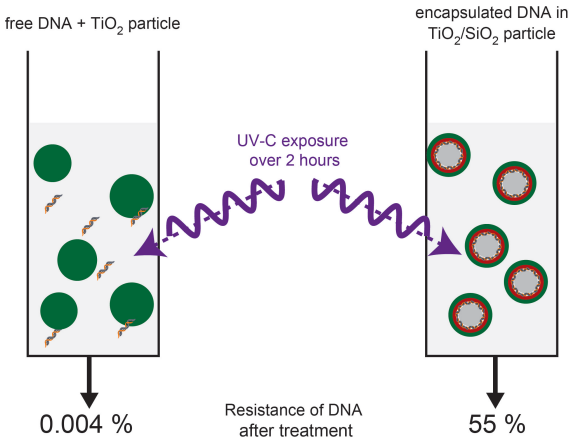


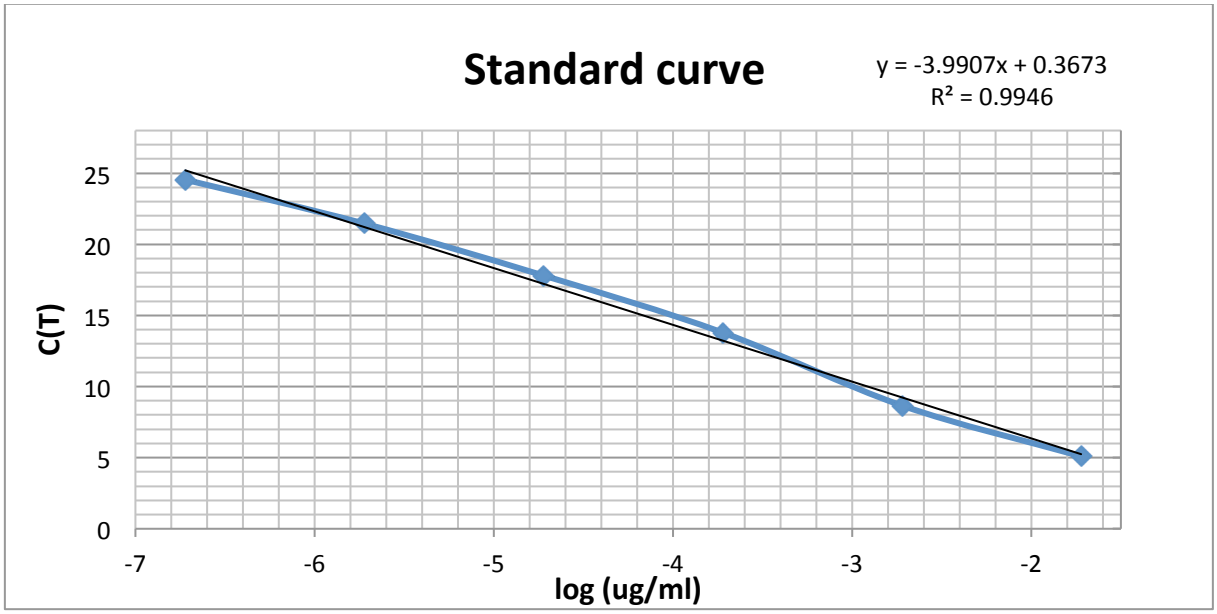
Figure S5. Analysis of shadowing effect by comparing TiO_2 particles mixed with free DNA to DNA protected in SiO_2/TiO_2 particles

For analyzing a possible shadowing effect of TiO_2 particles, free dsDNA was mixed with synthesized TiO_2 particles. The TiO_2 particles were synthesized with the same procedure as the DNA/SiO_2/TiO_2 particle, just without the SiO_2/DNA particle as support (see section particle synthesis). For comparison, the free DNA with TiO_2 particle and encapsulated dsDNA in SiO_2/TiO_2 were treated with UV-C radiation for 2 h. Aqueous solutions of each sample including similar DNA concentrations were placed into quartz cells and exposed to UV-C light. Following the dissolution of the particles by BOE, DNA was analyzed by qPCR. Under the conditions applied the free DNA mixed with TiO_2 particles were destroyed after 2 h indicating no shadowing effect of titania.

Complete UV-C exposure data set and standard curve

DNA concentration was quantified using the amplicon at known concentrations (determined by Qubit dsDNA HS assay, Invitrogen) and preparing standards at $1:10^4$, $1:10^5$, $1:10^6$, $1:10^7$ and $1:10^8$ dilution.

Concentration (µg/ml)	Dilution	C(T)			Average C(T)	STDEV
6.00E-02	1:10^4	4.88	5.37	4.99	5.08	0.26
6.00E-03	1:10^5	9.09	8.49	8.4	8.66	0.38
6.00E-04	1.10^6	13.83	13.83	13.71	13.79	0.07
6.00E-05	1.10^7	17.84	17.75	17.81	17.80	0.05
6.00E-06	1.10^8	21.16	21.18	22.02	21.45	0.49
6.00E-07	1.10^9	24.53	24.5	24.58	24.54	0.04
6.00E-08	1.10^10	25.85	25.78	25.7	25.78	0.08



Samples	Time	C(T)	log(µg/ml)	µg/ml	incl. BOE dilution (µg/ml)	µg/ul	Average µg/ul	STDEV (µg/ul)
dsDNA	Ref	13.72	-3.35	4.51E-04	9.02E-04	9.02E-07	9.12E-07	1.49E-08
	Ref	13.68	-3.34	4.61E-04	9.23E-04	9.23E-07		
	15min	17.83	-4.38	4.21E-05	8.42E-05	8.42E-08		
	15min	18.05	-4.43	3.71E-05	7.41E-05	7.41E-08	8.00E-08	5.24E-09
	15min	17.88	-4.39	4.09E-05	8.18E-05	8.18E-08		
	30min	20.02	-4.92	1.19E-05	2.38E-05	2.38E-08	2.36E-08	8.28E-10

	30min	19.98	-4.91	1.22E-05	2.43E-05	2.43E-08		
	30min	20.1	-4.94	1.14E-05	2.27E-05	2.27E-08		
	1h	22.33	-5.50	3.14E-06	6.27E-06	6.27E-09		
	1h	22.29	-5.49	3.21E-06	6.42E-06	6.42E-09	6.55E-09	3.62E-10
	1h	22.15	-5.46	3.48E-06	6.96E-06	6.96E-09		
	2h	26.86	-6.64	2.30E-07	4.60E-07	4.60E-10		
	2h	27.09	-6.70	2.01E-07	4.03E-07	4.03E-10	3.80E-10	9.36E-11
	2h	27.74	-6.86	1.38E-07	2.77E-07	2.77E-10		
	3h	26.9	-6.65	2.25E-07	4.49E-07	4.49E-10	4.84E-10	4.93E-11
	3h	26.65	-6.59	2.59E-07	5.19E-07	5.19E-10		
	4h	29.5	-7.30	5.01E-08	1.00E-07	1.00E-10		
	4h	30.06	-7.44	3.63E-08	7.25E-08	7.25E-11	7.34E-11	2.64E-11
	4h	30.8	-7.63	2.37E-08	4.73E-08	4.73E-11		
	Ref	13.70	-3.34	4.56E-04	9.12E-04	9.12E-07	9.12E-07	0.00E+00
	15min	19.32	-4.75	1.78E-05	3.56E-05	3.56E-08	1.47E-07	1.58E-07
	15min	15.88	-3.89	1.30E-04	2.59E-04	2.59E-07		
	30min	18.9	-4.64	2.27E-05	4.54E-05	4.54E-08	7.92E-08	4.78E-08
	30min	17.32	-4.25	5.65E-05	1.13E-04	1.13E-07		
dsDNA in SiO_2	1h	19.98	-4.91	1.22E-05	2.43E-05	2.43E-08	1.83E-08	8.60E-09
	1h	21.18	-5.22	6.09E-06	1.22E-05	1.22E-08		
	2h	22.57	-5.56	2.73E-06	5.46E-06	5.46E-09	3.91E-09	2.19E-09
	2h	24.02	-5.93	1.18E-06	2.37E-06	2.37E-09		
	3h	25.18	-6.22	6.06E-07	1.21E-06	1.21E-09	1.22E-09	9.94E-12
	3h	25.16	-6.21	6.13E-07	1.23E-06	1.23E-09		
	4h	28	-6.92	1.19E-07	2.38E-07	2.38E-10	3.15E-10	1.08E-10
	4h	27.14	-6.71	1.96E-07	3.91E-07	3.91E-10		
	Ref	13.50	-3.29	5.12E-04	1.02E-03	1.02E-06	8.91E-07	1.88E-07
	Ref	14.02	-3.42	3.79E-04	7.58E-04	7.58E-07		
	15min	15.38	-3.76	1.73E-04	3.46E-04	3.46E-07	1.35E-06	
	15min	12.06	-2.93	1.17E-03	2.35E-03	2.35E-06		
	30min	13.10	-3.19	6.45E-04	1.29E-03	1.29E-06	1.20E-06	1.22E-07
	30min	13.35	-3.25	5.58E-04	1.12E-03	1.12E-06		
	1h	13.81	-3.37	4.28E-04	8.56E-04	8.56E-07	6.29E-07	3.21E-07
dsDNA in SiO_2/TiO_2	1h	15.12	-3.70	2.01E-04	4.02E-04	4.02E-07		
	2h	14.41	-3.52	3.03E-04	6.06E-04	6.06E-07	4.88E-07	1.66E-07
	2h	15.26	-3.73	1.85E-04	3.71E-04	3.71E-07		
	3h	15.43	-3.77	1.68E-04	3.36E-04	3.36E-07	3.41E-07	6.96E-09
	3h	15.38	-3.76	1.73E-04	3.46E-04	3.46E-07		
	4h	15.71	-3.84	1.43E-04	2.86E-04	2.86E-07	2.33E-07	7.48E-08
	4h	16.51	-4.05	9.01E-05	1.80E-04	1.80E-07		
	5h	16.03	-3.92	1.19E-04	2.38E-04	2.38E-07	4.53E-07	3.04E-07
	5h	14.24	-3.48	3.34E-04	6.68E-04	6.68E-07		

6h	16.75	-4.11	7.85E-05	1.57E-04	1.57E-07	1.38E-07	2.73E-08
6h	17.24	-4.23	5.92E-05	1.18E-04	1.18E-07		
7h	17.10	-4.19	6.41E-05	1.28E-04	1.28E-07	1.25E-07	4.09E-09
7h	17.18	-4.21	6.12E-05	1.22E-04	1.22E-07		
8h	17.93	-4.40	3.97E-05	7.95E-05	7.95E-08	7.77E-08	2.53E-09
8h	18.01	-4.42	3.79E-05	7.59E-05	7.59E-08		
18h	22.96	-5.66	2.18E-06	4.36E-06	4.36E-09	4.36E-09	0.00E+00
24h	23.09	-5.69	2.02E-06	4.05E-06	4.05E-09	4.42E-09	5.21E-10
24h	22.8	-5.62	2.39E-06	4.78E-06	4.78E-09		
48h	24.46	-6.04	9.18E-07	1.84E-06	1.84E-09	2.02E-09	2.63E-10
48h	24.14	-5.96	1.10E-06	2.21E-06	2.21E-09		
72h	25.84	-6.38	4.14E-07	8.28E-07	8.28E-10	8.28E-10	0.00E+00

References

1. P. K. Khanna, N. Singh and S. Charan, *Mater. Lett.*, 2007, **61**, 4725.