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

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D. Paunescu,[a] C. A. Carlos,[a] M. Puddu,[a] F. Krumeich[a] and R. N. Grass[a]

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DNA is protected against UV-induced damage by encapsulation in a core-shell-shell particulate construct. The DNA is hermetically sealed in SiO_2 particles coated with TiO_2. The TiO_2 coating acts as a physical sunscreen and prevents high energy photons from damaging the nucleic acids. DNA can be recovered unharmed from the protection system with fluoride comprising buffers, and then directly analyzed using biochemical standard techniques (quantitative PCR, gel electrophoresis and Sanger sequencing). The coatings increase the DNA UV resistance by 42 times, which is equivalent to the increase in UV resistance obtained by bacteria during sporulation. The attenuation coefficient of the 20 nm titania layer is 1.8 10^6 cm^{-1} at 254 nm UV irradiation and optical attenuation is largely attributed to light scattering on the titania surface.

Introduction

In biomedical routines DNA is regarded as a relatively stable molecule, especially compared to other biopolymers such as RNA and proteins. DNA has also been proposed as a useful molecule for many non-biomedical applications, including catalysis,[1] nanodevice fabrication,[2] DNA computing,[3] data storage devices[4] and as an anthropogenic tracer molecule.[5] Yet the vulnerability of DNA towards light irradiation severely limits its use and therefore technological progress in many of these areas, especially if they involve exposure to daylight for extended time periods. UV radiation is the most harmful and mutagenic component of solar radiation.[6] UV-induced damage is usually described by direct and indirect mechanisms, depending on the wavelength of the UV.[7] The energy of a short-wavelength UV photon (UV-C <280 nm, UV-B 280-315 nm) is directly absorbed by DNA bases, which causes the most efficient damage. Photochemical reactions are more efficient within DNA at this wavelength, which is close to the absorption maximum of the pyrimidine and purine bases.8 UV-C radiation is, therefore, used for convenient germicidal treatment and is highly relevant in sterilization procedures in healthcare.9 DNA can also be damaged indirectly by UV-A exposure through the production of radical oxygen species (ROS).[10] The exposure of DNA to UV-A light is less efficient in inducing DNA damage, but can still affect it via indirect photosensitizing reactions generating a variety of reactive oxygen species (ROS). A number of sensitizers have been identified, which upon photolysis, transfer their excitation energy onto an adjacent dioxygen molecule, converting it to singlet oxygen while the photosensitizer molecule returns to its ground state.[11]

In nature, microbial spores developed an outstanding resistance to UV radiation, amongst other harsh environmental conditions. Dormant spores can survive for extremely long periods of time, largely because spore DNA is well protected against damage, e.g. viable Bacillus sphaericus spores were recovered from amber after an estimated 25 to 40 million years.[12]

Artificial UV protection is found in everyday products, such as sunscreens, cosmetics or paints. One of the most useful UV blocking materials is submicron titania particles, manufactured commercially at 4 million tons per year.[13] Titania is selected as both common crystalline forms anatase and rutile are semiconductors with a bandgap of 3.2 and 3.0 eV. This means they can efficiently absorb UV irradiation at wavelengths smaller than 388 and 414 nm.[14]

In order to circumvent the sensitivity of DNA to UV light we considered coating DNA molecules with a thin layer of titania. However, titania is also an excellent photocatalytic material, which generates aggressive radical oxygen species if irradiated in water with UV light. If UV-protection of DNA by titania is to be successful, the biopolymer must not be in direct contact with the photocatalyst. As a result of this argumentation we decided to use silica as an insulating layer resulting in the material design illustrated in Figure 1. To prepare the protection system, we first encapsulated DNA in silica particles according to previously established routines.[15] In a second step, a titania coating was obtained by reacting the encapsulates with titanium n-butoxide (TBOT) in ethanol and water at ambient temperature overnight.[16]

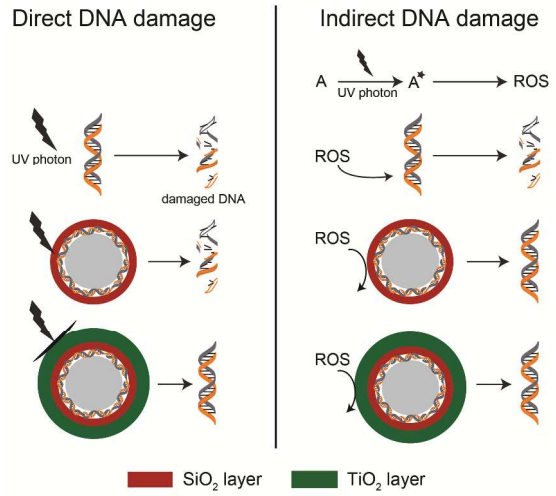

Figure 1. DNA is adsorbed on an ammonium functionalized silica bead (gray). Silica (red) is grown as an insulating layer via TEOS hydrolysis, titania (green) is grown from TBOT. In the encapsulate DNA is protected from direct and indirect UV damage. DNA can be released unharmed from the encapsulate by treating it with a mild fluoride etch.

Experimental

DNA/SiO_2/TiO_2 particle synthesis

DNA-labeled SiO_2-particles were synthesized as described in previous studies.[15d, 15e] Briefly, silica particles (SiO_2-R-L2897, 142 nm, 50 mg ml^{-1}, micro particles GmbH) were functionalized with N-trimethoxylsilylpropyl-N,N,N-trimethylammonium (TMAPS, 50% in methanol, ABCR) to adsorb double-stranded DNA on the particle surface. A silica layer was formed on top of the DNA by adding TMAPS and tetraethoxysilane (TEOS, ≥99.0%, Aldrich). The synthesized DNA/SiO_2 particles were washed twice by sedimentation and redispersion in 500 µl ethanol. The titania nanocoating was obtained by mixing the particle solution with 10 µl of dH_2O and a solution of 2.5 µl of titanium(IV) butoxide (TBOT, 97%, Aldrich) in 500 µl ethanol. The reaction was stirred (900 rpm) at ambient temperature overnight and afterwards washed 3 times with ethanol and resuspended in 1 ml ethanol. Two kinds of particles with different incorporated DNA were synthesized for variable analysis methods. The one incorporated DNA was a double strand 113 bp amplicon (5'-ATT CAT GCG ACA GGG GTA AGA CCA TCA GTA GTA GGG ATA GTG CCA AAC CTC ACT CAC CAC TGC CAA TAA GGG GTC CTT ACC TGA AGA ATA AGT GTC AGC CAG TGT AAC CCG AT-3'; Microsynth AG), which primers were designed using the online Primer3 tool[17] and tested for uniqueness using the Basic Local Alignment Search Tool[18] (BLAST). The double-stranded DNA was prepared by annealing the DNA sequence with its complementary sequence. For agarose gel electrophoresis a standard DNA ladder (DNA ladder 1KB Plus, 1 µg $µl^{-1}$, Invitrogen) was incorporated into particles.

DNA recovery

Encapsulated dsDNA in SiO_2/TiO_2 particles (2.4 µg ml^{-1}) was recovered using a 1:100 diluted buffered oxide etch solution (BOE, 0.23 g of NH_4FHF (pure, Merck) and 0.19 g of NH_4F (puriss, Sigma-Aldrich) in 10 ml TE-buffer) prepared and safety handled as described in previous studies.[15e]

UV irradiation of samples

For comparison, free dsDNA (diluted $1:10^6$ with starting concentration of 600 µg ml^{-1}, determined by Qubit dsDNA HS assay, Invitrogen), in SiO_2 encapsulated dsDNA (2.4 µg ml^{-1} particle dispersion) and in SiO_2/TiO_2 encapsulated dsDNA (2.4 µg ml^{-1}) were irradiated by different UV sources for various times. Aqueous solutions of each sample including similar DNA concentrations were placed into quartz cells (170-2700 nm, thickness 10 mm, Starna). Following treatment 20 µl of diluted BOE (1:100) was added to 20 µl of each sample and directly analysed by quantitative real-time PCR (qPCR) (see ESI† for qPCR data).

UV-C exposure. Samples were treated with UV-C light from four low pressure mercury lamps (253.7 nm, 15 W, HNS 15 ORF, Osram). The UV-C lamps were set at a distance of 50 cm from the sample, resulting in a dose rate of approximately 5.2 W m^{-2}. The irradiation output was measured by a standard photodiode sensor (PD300-UV, 200-1100 nm, 3 mW-20 pW, Ophir Photonics). All experiments were performed in duplicates with exception of the 72 h treated DNA/SiO_2/TiO_2 particle sample. Additionally to qPCR analysis, gel-electrophoresis and Sanger sequencing of the samples were performed (detailed procedures are provided in ESI†).

Sunlight exposure. For exposure to solar radiation, the experiment was conducted with a solar simulator (Newport Sun Simulator, Class A 91195A-1000, AM1.5global, 1000 Wm^{-2}, simulating irradiation for a 37° tilted surface).

Results and discussion

Particle characterization

For the DNA protection system, DNA was first encapsulated in silica particles. To evaluate the completeness and tightness of the coating the particles were treated with highly aggressive heavy metal and hydrogen peroxide species, which induce the formation of ROS and are known to disintegrate DNA.[19] The DNA could be released from the particles by etching the silica with a mild fluoride etch and >90% of the originally present DNA could be recovered unharmed, proving a hermetic silica layer (see ESI† for ROS stability). In a second step, a titania coating was obtained by reacting the encapsulates with titanium n-butoxide (TBOT) in ethanol and water at ambient temperature overnight. As evidenced by energy dispersive X-ray spectroscopy (EDXS; Figure 2), infrared spectroscopy (IR, Figure S1 in ESI†) and UV-Vis spectroscopy (Figure 3), the original structures were effectively coated with a thin (ca. 20 nm) layer of titania. Detailed experimental proof of DNA presence in the layer by layer design is shown in the supporting information (Table S1 in ESI†).

The optical properties of the titania/silica encapsulates in water show a complete blocking of light at wavelengths smaller than ~320 nm, thereby shadowing the UV absorption peak of DNA at 260 nm (Figure 3).

While it is well known from computer chip manufacturing (silicon-wafer chemistry) that silica can be rapidly dissolved in buffered solutions of fluoride (e.g. buffered oxide etch = buffered ammonium fluoride, pH~4-5), the solubility of titania under these conditions has been less commonly exploited. The above prepared encapsulates could be dissolved rapidly (<1 min) in diluted (2.5 wt%F^-, pH=3.8) buffered fluoride solutions, releasing the DNA unharmed (see below).

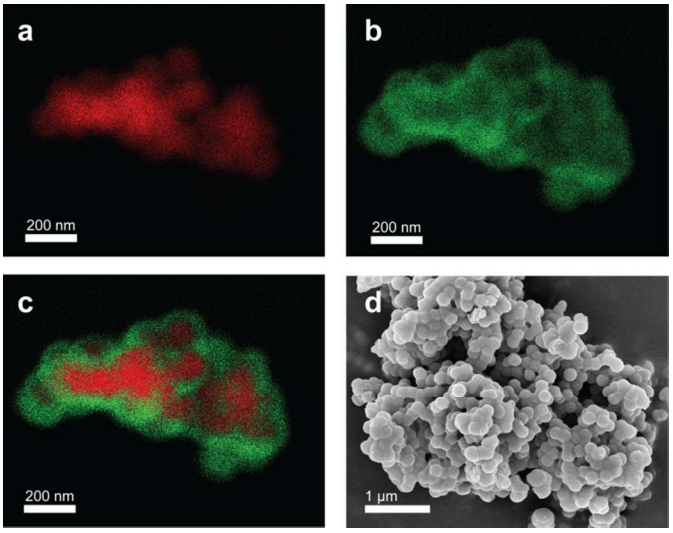

Figure 2. Electron microscopy images and elemental X-ray map-ping of TiO_2 coated DNA/SiO_2 encapsulates. Elemental X-ray mapping shows silicon (a) in red and titanium (b) in green. The mapping in (c) shows the coated nanostructure with SiO2 cores (Si shown in red) and outer layers of titania (Ti shown in green). (d) SEM image of TiO_2 coated nanoparticles.

UV shielding properties

Having obtained a method to encapsulate DNA with silica and titania and with the possibility of releasing the DNA from these encapsulates, we investigated the UV shielding properties of the coating layers. Solutions of unprotected DNA, SiO_2-protected DNA and SiO_2 protected DNA with the additional TiO_2 layer were prepared, ensuring similar DNA concentrations (mg ml^{-1} solution) in all cases. These solutions were exposed to 254 nm UV-C light at a dose rate of 5.2 W m^{-2} utilizing the sterilization unit of a standard laboratory biological-safety flow-bench. Following the UV-C treatment, the particles were dissolved in a diluted BOE solution. The induced DNA damage

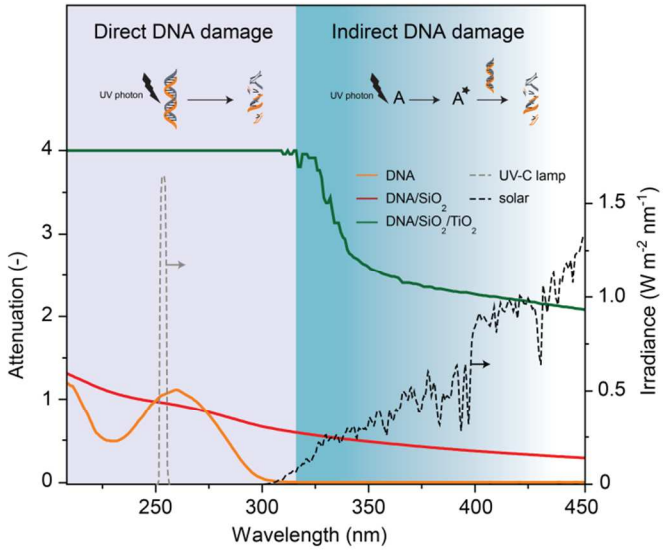

Figure 3. Comparison of UV-Vis absorption spectra of unprocessed DNA (7.1 µg ml^{-1} orange), encapsulated DNA/SiO_2 particles (0.4 mg ml^{-1} red) and encapsulated DNA/SiO_2/TiO_2 particles (0.4 mg ml^{-1} green). Emission spectra of the UV-C lamp (dashed grey, right axis) and a section of solar spectrum of direct AM 1.5 (ASTMG-173, dashed black, right axis).

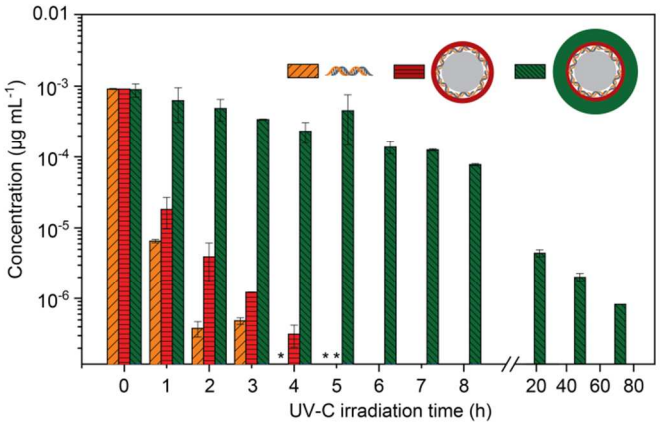

Figure 4. qPCR analysis of TiO_2 coated DNA/SiO_2 particles stability compared to that of DNA/SiO_2 particles and unprotected DNA. Dispersions of both particles (2.4 µg particles ml^{-1}) and free DNA amplicon (0.6 ng ml^{-1}) were exposed to UV-C light (254 nm). Orange bars represent free DNA, red bars DNA in silica particles and green bars DNA in titania coated silica particles; star (*) indicates data below the detection limit (< 10^{-7} µg ml^{-1}).

was monitored by quantitative polymerase chain reaction (qPCR) analysis (Figure 4), which could be undertaken without further purification steps when DNA concentrations were <10^{-3} µg ml^{-1} and a F^- concentration of the buffer was chosen as 0.025 wt%. As expected, 99.2 % of the unprotected DNA was destroyed after 1 h of UV-C irradiation and the silica layer only gave a marginal improvement. Only the DNA additionally protected by titania withstood the one hour irradiation experiment (71 % survived) and even after 72 hours of UV-C irradiation the DNA could still be amplified by qPCR.

Further evidence of DNA protection against UV-C irradiation was given by agarose gel electrophoresis and DNA analysis by Sanger sequencing. To prove efficient DNA protection by gel electrophoresis a commercial dsDNA ladder (100 to 12 000bp) was encapsulated with silica and titania and irradiated by UV-C light for 1.5 h. Figure 5a displays both the successful encapsulation of the DNA ladder as well as the effective protection of the encapsulated DNA from UV light at conditions in which the unprotected DNA ladder is no longer visible by gel electrophoresis.

In order to show that the DNA sequence was neither permutated by the encapsulation/de-encapsulation scheme, nor by UV-C irradiation, the encapsulated DNA amplicon utilized in the qPCR study was purified by drop-dialysis and analyzed by Sanger sequencing.[20] The sequencing chromatograms in Figure 5b display successful sequencing of the DNA after encapsulation, UV-irradiation and subsequent particle dissolution, neither of which was different from the original DNA in sequence nor had a lower sequencing Phred quality score (not statistically smaller from two sample t-test).

Additionally to UV-C induced DNA damage, DNA can also be damaged by higher wavelengths in the range of UV-B and UV-A as well as by direct sunlight irradiation. UV-A does not usually display a direct effect on biological systems, but a significant indirect effect is reported.[21] The indirect damage is based on the formation of free radicals, which interact with DNA causing damage, such as single- and doublestrand breaks and modified bases. The effect of this indirect DNA damage can be mimicked by treating the materials of interest with radical oxygen species generated chemically. As schematically illustrated in Figure 1, DNA encapsulated in SiO_2 and in

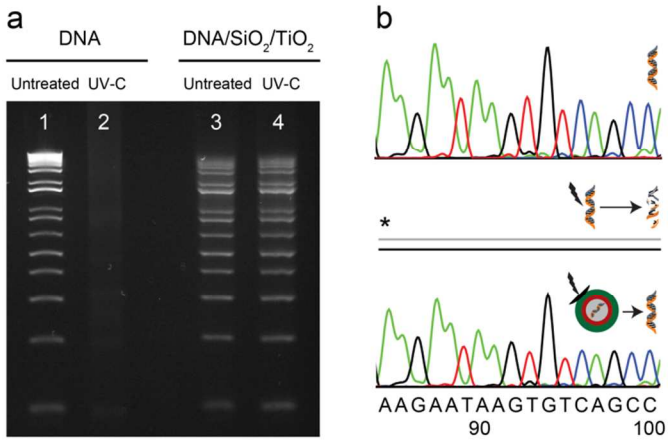

Figure 5. (a) Agarose gel electrophoresis of unprotected (lane 1 and 2) and protected (lane 3 and 4) dsDNA ladder. DNA encapsulated in SiO_2/TiO_2 particles (lane 4) survived the UV-C treatment, while unprotected DNA was completely destroyed (lane 2) under the same the conditions. (b) Sequencing chromatograms of base 92-111 of unprotected dsDNA sequence (1) and UV-C treated unprotected (2) and protected (3) DNA amplicon; (*) indicates data below the sequencing concentration.

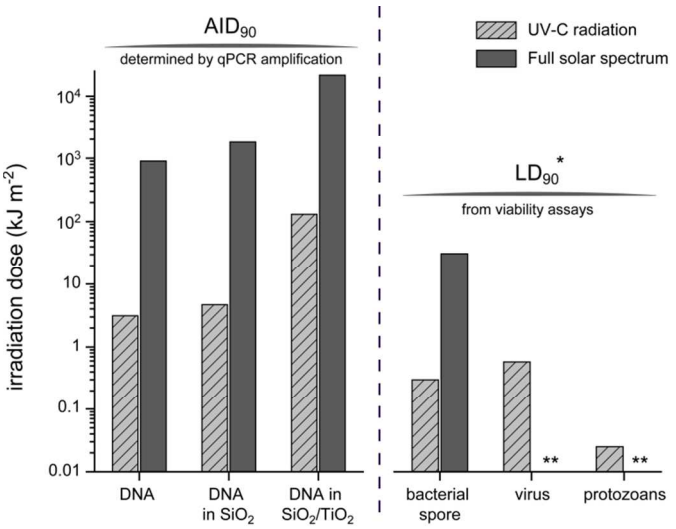

Figure 6. UV-C and solar resistance of DNA in SiO_2 and SiO_2/TiO_2 compared to spores, viruses and protozoans. AID_{90} and LD_{90} data is presented for UV-C (grey bars) and solar (black bars) irradiation. The definitions of AID_{90} and LD_{90} are given in the text. AID_{90} values were determined for UV-C exposure (254 nm) and for simulated sunlight irradiation at 1000 Wm^{-2} for up to 6 h. LD_{90} values are the dose radiation needed to kill 90% of the population and are given for dormant wild-type spores of Bacillus subtilis[24], viruses[25], and protozoans of Cryptosporidium[25]; star (*) presents values from literature and (**) indicates data not available.

SiO_2/TiO_2 particles is well protected against ROS (>90% DNA survived a harsh hydrogen peroxide/heavy cocktail, see Table S2).

Results for sunlight irradiation were generated using a sun simulator (1000 W m^{-2}, AM1.5global, simulating irradiation for a 37° tilted surface, see Figure 3) and to measure the damage we calculated the irradiation dose required to reduce the original DNA content by 90% (amplification inhibiting doses (AID_{90}) value, Figure 6). Under these conditions the silica layer, preventing indirect UV damage as shown above, was able to somewhat protect the DNA (2 fold increase in AID_{90}). A much more pronounced protection with a 24-fold increase in resistance to irradiation was achieved with the additional titania coating: a total irradiation dose to destroy 90% of the DNA of 22 * 10^3 kJ m^{-2} was required.

We obtain an increase of DNA stability against UV-induced damage by encapsulating DNA into SiO_2 and nanocoating the particles with titania. The DNA stability is shown by the AID_{90} value for UV-C and solar irradiation (Figure 6). The data nicely demonstrates the extreme vulnerability of DNA to UV-C irradiation compared to the same dose of sunlight irradiation. Additionally it shows that the protection effect of the titania coating is similar in both irradiation cases, with a slightly more pronounced effect in the UV (24 fold increase for sunlight, 42 fold for UV-C).

To put the data into perspective we compared the vulnerability of the protected DNA to the UV vulnerability of biological systems. We focused especially on bacterial spores, which are well known for their sunlight and UV tolerance (Figure 6). The key reason for their light tolerance is their unique UV photochemistry.[22] A variety of important factors for this elevated spore resistance to ionizing radiation have been presumed: (a) low core water content that potentially reduces the ability of c-radiation to generate damaging hydroxyl radicals. (b) UV-absorbing pigments located in the spore's outer layers, in particular the coats and outer membrane. These pigments can protect DNA against UV by absorbing the radiation before it reaches the nucleic acid in the spore core.[23] (c) Saturation of spore DNA with α/β-type small acid-soluble

our artificially designed assembly every DNA molecule is protected by a dense layer, similar to the outer coats found in spores. The silica shell acts as a hermetic diffusion barrier and protects equally well against very small molecules (ROS) and larger (bio)chemical reactants (e.g. nucleases). The additional titania layer absorbs and scatters UV light with an absorbance maximum in the region of UV-C and UV-B irradiation. It therefore blocks the high energy photons from penetrating through the particles to the DNA, similar to the UV-absorbing pigments in spores. Of course, our biomimetic protection system lacks the DNA repair systems, which are highly active in spores during outgrowth and can repair minor damages that occur during the dormant period.[12a] For Bacillus subtilis an increased UV tolerance of 5 to 50 times has been reported for its spores as compared to the corresponding growing cells.[24] The absolute irradiation cannot be directly compared between the microbes and our data as the microbe stability data is generated by viability assays (LD_{90} values) and we utilized qPCR to measure DNA damage (AID_{90} values). Nevertheless, the increase in UV tolerance of DNA protected by a 20 nm thick titania layer (24-42 fold) is directly comparable to the increase in UV tolerance these bacteria have by spore formation (up to 50 fold).

The UV protection of the thin titania layer can also be represented in terms of an attenuation coefficient. Assuming a titania layer thickness of 20 nm and a linear dependency of DNA damage of light intensity, the corresponding attenuation coefficient is $\beta_{254,DNA}$ = 1.8 10^6 cm^{-1} (see ESI† for calculation). This is more than an order of magnitude higher than the titania absorbance coefficient in the UV-C wavelength range reported in the literature (κ_{UV} = ~0.5 10^5 cm^{-1}).[26] However, it is well known that submicron titania particles utilized in white paints and sunscreen formulations create most of their optical extinction effect (>90 %) through light scattering and not light absorbance.[27] The values derived for the DNA protection indicate that also in the presented case light scattering on the

surface of the titania coated particles has the most pronounced effect on UV attenuation. To further validate this, we measured the attenuation coefficient of the DNA/SiO_2/TiO_2 particles in water using a photometer (PM) (see Figure S3). We then obtained an attenuation coefficient ($\beta_{254,PM}$ = 0.5 10^6 cm^{-1}) that largely exceeds the absorption coefficient of titania and that is close to the attenuation coefficient derived from the DNA damage data. A control experiment (see Figure S4) with non-encapsulated DNA in the presence of titania nanoparticles gave no UV protection and further confirms the necessity of the layered material design shown in Figure 1.

Conclusions

We engineered an approach for DNA protection against optical irradiation by incorporating DNA in a core-shell-shell assembly with a ~10 nm silica and a ~20 nm titania layer. While the silica layer protected the DNA molecules from radical oxygen species, the additional titania layer attenuated ~98 % of irradiated UV-C light, mostly by light scattering. Within the assembly DNA withstood (AID_{90}) UV-C irradiation exceeding 100 kJ m^{-2} and sunlight irradiation of over 10^4 kJ m^{-2}. An even higher UV resistance may be attainable by fine tuning the encapsulates for optimized light scattering, e.g. varying particle size. The concept developed here may be of further use for the encapsulation of other UV-sensitive (bio)molecules in titania.

Acknowledgements

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Notes

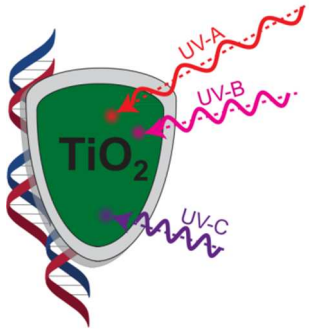
[a] Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, 8093, Switzerland. E-mail: robert.grass@chem.ethz.ch; Tel: +4144 633 63 34

†Electronic Supplementary Information (ESI) available: Particle characterization data, calculation of attenuation coefficients, control experiment of TiO_2 shadowing effect and complete exposure data set. See DOI: 10.1039/b000000x/

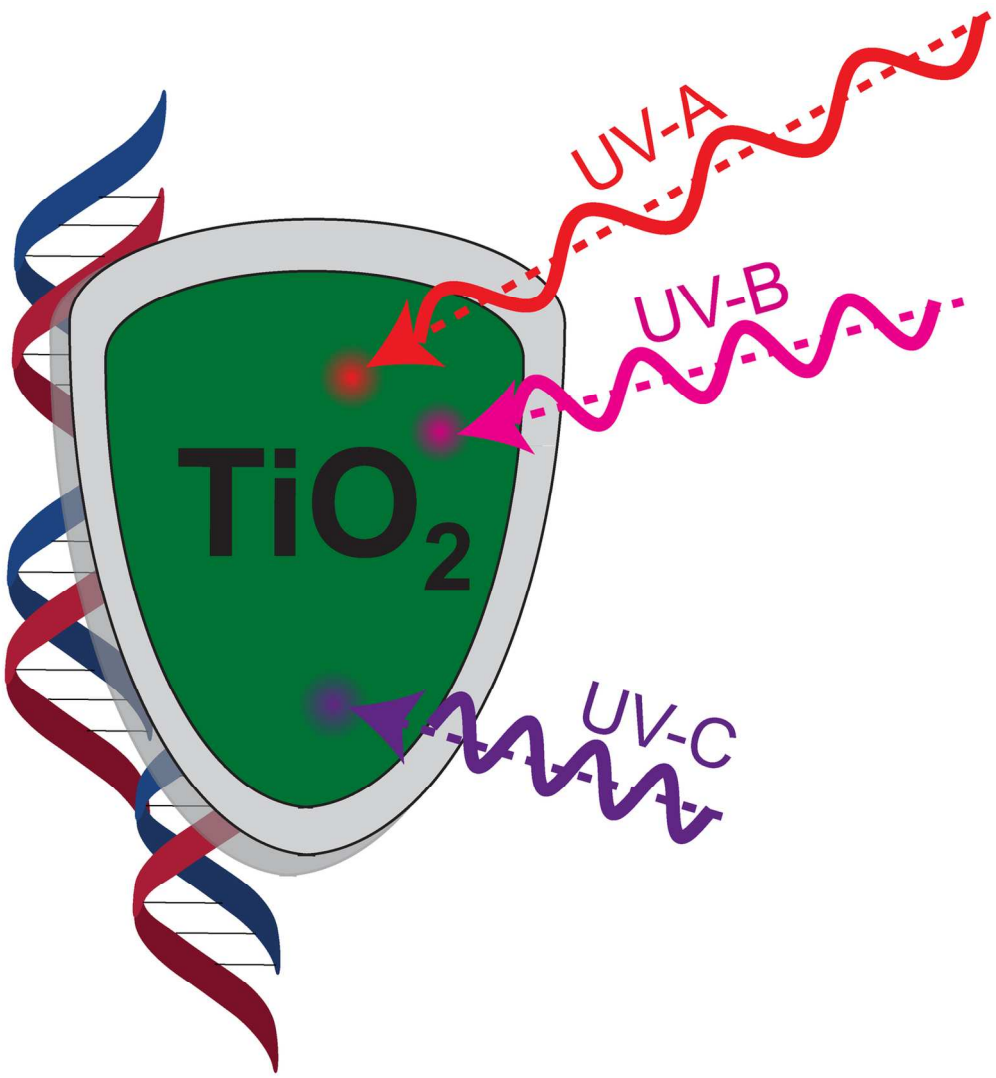
References

1. (a) R. R. Breaker, *Nat. Biotechnol.*, 1997, **15**, 427; (b) A. Peracchi, *ChemBioChem*, 2005, **6**, 1316.
2. (a) N. C. Seeman, *Nature*, 2003, **421**, 427; (b) C. A. Mirkin, R. L. Letsinger, R. C. Mucic and J. J. Storhoff, *Nature*, 1996, **382**, 607; (c) A. V. Pinheiro, D. Han, W. M. Shih and H. Yan, *Nat. Nanotechnol.*, 2011, **6**, 763; (d) F. C. Simmel and W. U. Dittmer, *Small*, 2005, **1**, 284; (e) N. L. Rosi and C. A. Mirkin, *Chem. Rev.*, 2005, **105**, 1547.
3. (a) J. Elbaz, O. Lioubashevski, F. A. Wang, F. Remacle, R. D. Levine and I. Willner, *Nat. Nanotechnol.*, 2010, **5**, 417; (b) Z. Ezziane, *Nanotechnology*, 2006, **17**, 27.
4. (a) G. M. Church, Y. Gao and S. Kosuri, *Science*, 2012, **337**, 1628; (b) N. Goldman, P. Bertone, S. Y. Chen, C. Dessimoz, E. M. LeProust, B. Sipos and E. Birney, *Nature*, 2013, **494**, 77.
5. (a) J. H. Choy, S. Y. Kwak, J. S. Park, Y. J. Jeong and J. Portier, *J. Am. Chem. Soc.*, 1999, **121**, 1399; (b) J. H. Choy, J. M. Oh, M. Park, K. M. Sohn and J. W. Kim, *Adv. Mater.*, 2004, **16**, 1181; (c) C. A. Mora, D. Paunescu, R. N. Grass and W. J. Stark, *Mol. Ecol. Resour.*, 2014, DOI: 10.1111/1755-0998.12299, DOI 10.1111/1755; (d) M. Puddu, D. Paunescu, W. J. Stark and R. N. Grass, *ACS Nano*, 2014, **8**, 2677.
6. T. Lindahl and R. D. Wood, *Science*, 1999, **286**, 1897.
7. Z. Kuluncsics, D. Perdiz, E. Brulay, B. Muel and E. Sage, *J. Photochem. Photobiol. B-Biol.*, 1999, **49**, 71.
8. J. L. Ravanat, T. Douki and J. Cadet, *J. Photochem. Photobiol. B-Biol.*, 2001, **63**, 88.
9. F. M. Collins, *Appl. Microbiol.*, 1971, **21**, 411.
10. (a) M. Ichihashi, M. Ueda, A. Budiyanto, T. Bito, M. Oka, M. Fukunaga, K. Tsuru and T. Horikawa, *Toxicology*, 2003, **189**, 21; (b) R. M. Tyrrell and S. M. Keyse, *J. Photochem. Photobiol. B-Biol.*, 1990, **4**, 349; (c) X. S. Zhang, B. S. Rosenstein, Y. Wang, M. Lebwohl and H. C. Wei, *Free Radic. Biol. Med.*, 1997, **23**, 980.
11. A. Hargreaves, F. A. Taiwo, O. Duggan, S. H. Kirk and S. I. Ahmad, *J. Photochem. Photobiol. B-Biol.*, 2007, **89**, 110.
12. (a) P. Setlow, *Trends Microbiol.*, 2007, **15**, 172; (b) R. J. Cano and M. K. Borucki, *Science*, 1995, **268**, 1060.
13. C. O. Robichaud, A. E. Uyar, M. R. Darby, L. G. Zucker and M. R. Wiesner, *Environ. Sci. Technol.*, 2009, **43**, 4227.
14. U. Diebold, *Surf. Sci. Rep.*, 2003, **48**, 53.
15. (a) E. Auyeung, R. J. Macfarlane, C. H. J. Choi, J. I. Cutler and C. A. Mirkin, *Adv. Mater.*, 2012, **24**, 5181; (b) C. Jin, L. Han and S. Che, *Angew. Chem. Int. Ed.*, 2009, **48**, 9268; (c) M. Numata, K. Sugiyasu, T. Hasegawa and S. Shinkai, *Angew. Chem. Int. Ed.*, 2004, **43**, 3279; (d) D. Paunescu, R. Fuhrer and R. N. Grass, *Angew. Chem. Int. Ed.*, 2013, **52**, 4269; (e) D. Paunescu, M. Puddu, J. O. B. Soellner, P. R. Stoessel and R. N. Grass, *Nat. Protoc.*, 2013, **8**, 2440; (f) B. Liu, L. Han and S. A. Che, *Angew. Chem. Int. Ed.*, 2012, **51**, 923; (g) B. Liu, Y. Yao and S. Che, *Angew. Chem. Int. Ed.*, 2013, **52**, 14186.
16. (a) A. Hanprasopwattana, S. Srinivasan, A. G. Sault and A. K. Datye, *Langmuir*, 1996, **12**, 3173; (b) X. A. Fu and S. Qutubuddin, *Colloid Surf. A-Physicochem. Eng. Asp.*, 2001, **178**, 151.
17. T. Koressaar and M. Remm, *Bioinformatics*, 2007, **23**, 1289.
18. S. F. Altschul, W. Gish, W. Miller, E. W. Myers and D. J. Lipman, *J. Mol. Biol.*, 1990, **215**, 403.
19. O. I. Aruoma, B. Halliwell, E. Gajewski and M. Dizdaroglu, *Biochem. J.*, 1991, **273**, 601.
20. F. Sanger, S. Nicklen and A. R. Coulson, *Proc. Natl. Acad. Sci. U. S. A*, 1977, **74**, 5463.
21. R. P. Sinha and D. P. Hader, *Photochem. Photobiol. Sci.*, 2002, **1**, 225.
22. P. Setlow, *J. Appl. Microbiol.*, 2006, **101**, 514.
23. W. L. Nicholson, A. C. Schuerger and P. Setlow, *Mutat. Res.-Fundam. Mol. Mech. Mutagen.*, 2005, **571**, 249.
24. P. Setlow, *Environ. Mol. Mutagen.*, 2001, **38**, 97.
25. USEPA, *Ultraviolet disinfection guidance manual for the final long term 2 enhanced surface water treatment rule*, US Environmental Protection Agency Washington, DC, 2006.
26. M. I. Cabrera, O. M. Alfano and A. E. Cassano, *J. Phys. Chem.*, 1996, **100**, 20043.
27. E. S. Thiele and R. H. French, *J. Am. Ceram. Soc.*, 1998, **81**, 469.

Graphical Abstract



DNA Protection Against Ultraviolet Irradiation by Encapsulation in a Multilayered SiO_2/TiO_2 Assembly. The here presented method allows to protect DNA against UV-induced damage by encapsulating it in a core-shell-shell particulate construct.



139x149mm (300 x 300 DPI)