Sunlight-triggered nanoparticle synergy: Teamwork of reactive oxygen species and nitric oxide released from mesoporous organosilica with advanced antibacterial activity.

Julia Gehring[†], *Bastian Trepka*[†], *Nele Klinkenberg*[†], *Hannah Bronner*[†], *David Schleheck*[‡], *Sebastian Polarz*[†]

Department of Chemistry[†] and Department of Biology[‡], University of Konstanz, D-78457 Germany

E-mail: sebastian.polarz@uni-konstanz.de

Supporting information

EXPERIMENTAL METHODS

Materials. Chemicals were received from Sigma-Aldrich and they were carefully purified and dried, prior to use, when applicable. Glass slides were purchased from Menzel; 15 x 15 mm. All reactions on the precursor state were performed under inert gas conditions using the Schlenk technique. The synthesis of 1,5-bis-tri(isopropoxysilyl)-benzene-3-thiol has been described previously. NMR-spectra were acquired on a Bruker Avance III 400 spectrometer using $CDCl_3$ as a solvent. Solid-state NMR spectra were recorded using a Bruker DRX 400 spectrometer. For sunlight irradiation a solar simulator (Abet Technologies; model 10500) was used and UV/VIS spectroscopic analysis was performed by Varian Carey 100 spectrometer. TEM measurements were performed using a Zeiss Libra 120 and for high resolution TEM measurements a JEOL lJEM2200FS was applied. Small-angle X-ray scattering (SAXS) measurements were conducted with a Bruker AXS Nanostar. N_2-physisorption measurements were recorded on a Micromeritics Tristar. FT-IR spectra were recorded by using a Perkin Elmer Spectrum 100 spectrometer using ATR unit. Cw-*EPR* measurements were performed on continuous wave (cw)-X-band EPR Miniscope spectrometer MS400 from magnettech at room temperature.

Synthesis of UKON-2j nanoparticles via the modified Stoeber method. A typical preparation of UKON-2j nanoparticles was as follows: 300 mg (0.57 mmol) 1,5-bis-tri(isopropoxysilyl)-benzene-3-thiol (3) were prehydrolyzed in 3.1 mL 2-propanol and 1.86 mL 0.1 M HCl at 60 °C for 16 h. Aging solution was prepared by adding 160 mg (0.44 mmol) CTAB and 116 mg (0.17 mmol) Brij-56 to 25 mL carbonate buffer pH 9.4 at 60 °C. Finally the prehydrolyzed silica source was transferred quickly into the buffered surfactant solution and then aged at 60 °C for 5 days under stirring. Template removal was performed by liquid extraction with 15 mL EtOH and 15 mL HCl conc. at 60 °C for 4 days.

Synthesis of 4-vinyl benzyl Rose Bengal. The 4-vinyl benzyl Rose Bengal was synthetized in a process described previously [57]. In the first step commercially available Rose Bengal sodium salt was dissolved in a mixture of deionized water and acetone. In the second step, 4,-vinyl benzyl chloride was added and the reaction mixture was heated up to 65 °. The precipitated product was centrifuged, washed with water and dried under vacuum. 4,-vinyl benzyl Rose Bengal could be obtained as a deep red solid and was characterized by ESI-MS (negative mode).

m/z: $C_{29}H_{11}Cl_4I_4O_5$ $[M-H]^-$ 1088.55

Synthesis of UKON-2jRB nanoparticles via click chemistry. 34 mg UKON-2j nanoparticles were suspended in 9 mL dimethylformamide. 3.1 mg of 2, 2-Dimethoxy-2-Phenylacetophenon and 258 mg (1.5 eq) 4- vinyl benzyl Rose Bengal were added followed by irradiation (150 W; mercury lamp) at 420 nm for 11 h. After completion of the reaction the as received UKON-2jRB nanoparticles were washed with diethylether, dimethylformamide and ethanol and dried on air.

Synthesis of UKON-2jNO nanoparticles. 35 mg (0.18 mmol) UKON-2j nanoparticles were suspended in 1.6 mL methanol at 0 °C. 0.8 mL HCL (5 M) and 0.8 mL $NaNO_2$ solution (192 mg dissolved in 6.4 mL deionized water) were added. The reaction mixture was stirred at 0 °C for 4 h then warmed up to room temperature. The suspension was centrifuged and washed 3 times with 10 mL methanol to remove excessive $NaNO_2$. UKON-2jNO nanoparticles were stored at 10 °C in the dark.

Reactive oxygen species (ROS) detection via uric acid degradation. The following procedure was performed for the pure UKON-2jRB and UKON-2jNO nanoparticles as well as for the mixtures of both nanoparticles described as UKON-2jRB^{x}NO^{1-x} nanoparticles. 2.1 mg of UKON-2jRB nanoparticles were suspended in 3 mL of a 1 mM uric acid solution followed by irradiation for a defined amount of time (2 min, 4 min, 6 min, 8 min, 16 min) via solar simulator (Abet

Technologies; model 10500). After centrifugation of the suspension the resulting uric acid concentration of the supernatant was analyzed via UV/VIS measurements by a Varian Cary 100 spectrometer.

NO detection by Griess reagent. To determine quantitatively the amount of NO released from the UKON-2jNO nanoparticles during light exposure (or heating) a modified Griess assay was performed. As the resulting Griess-azo dye strongly adsorbs on the particle surface and cannot be detected in the supernatant, the material has to be completely dissolved in 1 M NaOH in order to quantify the amount of azo dye. As the color changes in the basic media, first calibration measurements have to be realized and the extinction coefficient at 498 nm (pH= 14) was calculated (Fig. S-6a).

For UKON materials: 4 mg of UKON-2jNO nanoparticles (0.016 mmol) were suspended in 2.9 mL deionized water. 50 μL of 1 % sulphanilamide solution (in 5 % phosphoric acid) and 50 μL of 0.1 % naphthylethylenediamine dihydrochloride solution were added. The suspension was stirred during exposure to light (solar simulator Abet technologies; model 10500) or heating (40 °C). To detect the formed azo dye which strongly adsorbed on UKON-2jNO nanoparticles, the material was centrifuged and dissolved in 1 M NaOH solution. UV/VIS measurements were carried out by Varian Cary 100 spectrometer.

Ellmans test. To quantify accessible thiol functionalities of UKON-2j and UKON-2jRB nanoparticles we used a standard Ellmans assay: 3 mL of 10 mM 5, 5'-dithiobis-(2-nitrobenzoic acid) solution (in phosphate buffer pH= 8) were added to 0.0016 mmol UKON nanoparticles. After 30 min stirring at room temperature the suspension was centrifuged and the colored supernatant solution was analyzed by UV/VIS measurements (Varian Cary 100 spectrometer).

Disulfide bridge cleavage. In order to regenerate UKON-2jNO nanoparticles after NO release 1 eq of UKON-2jNO nanoparticles (after sunlight exposure for 24 h) were suspended in PBS buffer pH= 8 containing 1 eq 1,4-Dithioerythritol (Cleland reagent). The suspension was stirred over night at room temperature, centrifuged and extensively washed with destilled water.

Peroxynitrite detection with dihydrorhodamine 123 oxidation. Suspensions containing 0.1 mM DHR and 0.05 mM UKON nanoparticle materials in 3 mL 0.1 M sodiumphosphate buffer (pH= 7.4) were irradiated for 1 h with sunlight. After centrifugation the colored supernatant was analyzed via UV/VIS spectroscopy.

Electron Paramagnetic Resonance (EPR) Spectroscopy. Spin trapping measurements of nitric oxide radicals were performed on PMO nanoparticles (UKON-2j, UKON-2jNO; 13 mg) in 40 μl ethanol with 0.075 mmol *N-tert*-Butyl-α-phenylnitrone (PBN, Sigma). The suspension placed in a sealed quartz glass sample tube was irradiated with visible light (solar simulator Abet technologies; model 10500).

Cell viability assay by colony counting method. All glass materials used were autoclaved at 121 °C for 60 min to ensure sterility. First, 2 mg of UKON nanoparticle materials were suspended in 1 mL sterilized H_2O in glass tubes, and then 1 mL of *Pseudomonas aeruginosa* PAO1 cell suspension in nutrient medium (LB medium) was added to the nanoparticle suspension (final cell concentration, app. 1.5×10^7 CFU/mL). The suspensions were irradiated with light from a solar simulator (Abet technologies; model 10500), and at different time intervals, samples (10 μl) were taken and 1:10-serially diluted in sterilized water. For each dilution step, a 20-μl sample was drop-plated onto nutrient agar plates (LB), and the plates were incubated at 30 °C for 18 h in the dark. Finally, the numbers of colonies were counted in order to determine colony forming units (CFU),

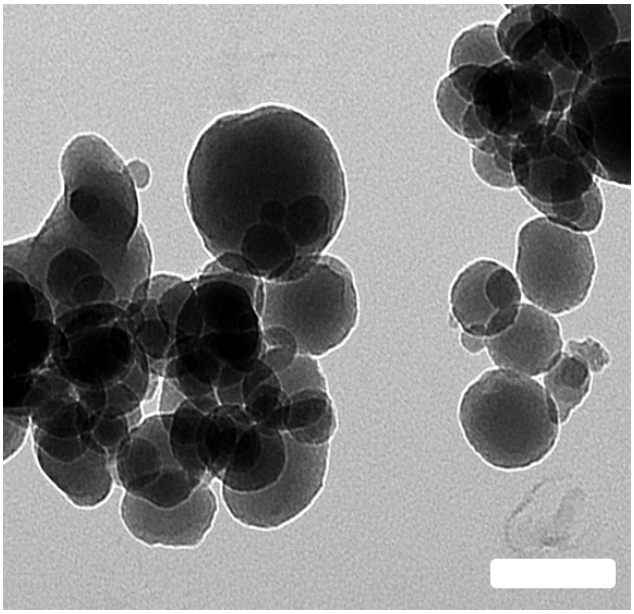
hence, the number of viable cells in the undiluted original sample. Each set of experiments was performed in triplicate.

Film preparation. Glass microscope cover slips (Menzel, 15 x 15 mm) were cleaned by immersion into piranha solution (H_2SO_4:H_2O_2; 2:1 v/v) for 1 h at room temperature, rinsed with distilled water, and dried at 80 °C for 4 h. Then, suspensions of the different UKON nanoparticles in ethanol were prepared (200 mg/ mL) and applied to the glass slides by doctor blading according to the following parameter: height 300 μm; speed 2.5·0.6 mm per sec; 4 cycles. The prepared films were dried at room temperature, and the film-covered glass slides stored at 2 °C in the dark.

Photoactivated disinfection assay. Prior to use, the UKON nanoparticle films on glass cover slips (see above) were sterilized by immersion in 80 % ethanol, and dried under sterile conditions in a laminar flow hood. Then, each film was soaked with a 20-µl portion of *Pseudomonas aeruginosa* PAO1 cell suspension (app. 5×10^9 CFU/mL). After sunlight irradiation for 1 h at room temperature and rinsing with 1 mL of sterile H_2O, the slides were placed on nutrient agar plates (LB medium) with the bacterial contamination side down for a further 30 min. Then the slides were removed and the agar dishes incubated for 18 h at 30 °C and evaluated for formation of a homogeneous bacterial lawn in dependence of the surface coating used.

Fig. S-1: Preparation of organosilica materials using compound (1) (((PrO^{i})$_3$Si)$_2$PhSH) as a precursor and adopting literature-known, modified Stöber methods.

(a) TEM image of UKON-2j material obtained by using only CTAB as structure directing agent.


(scale bar = 200 nm)

(b) TEM image of UKON-2j material obtained by using only Brij-56 as structure directing agent.

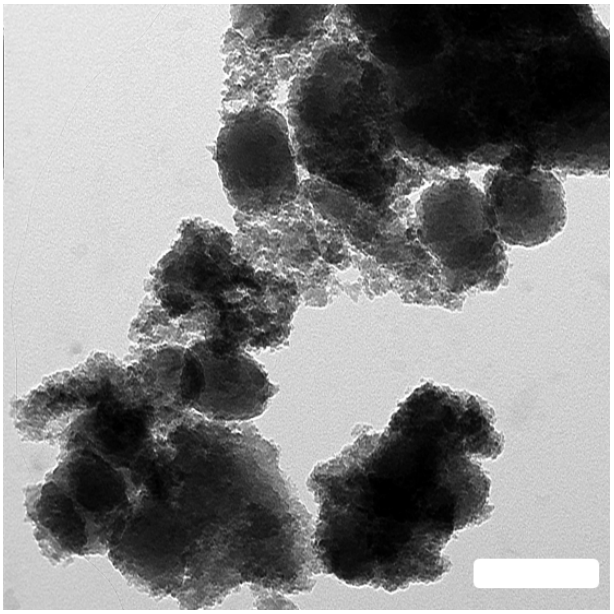
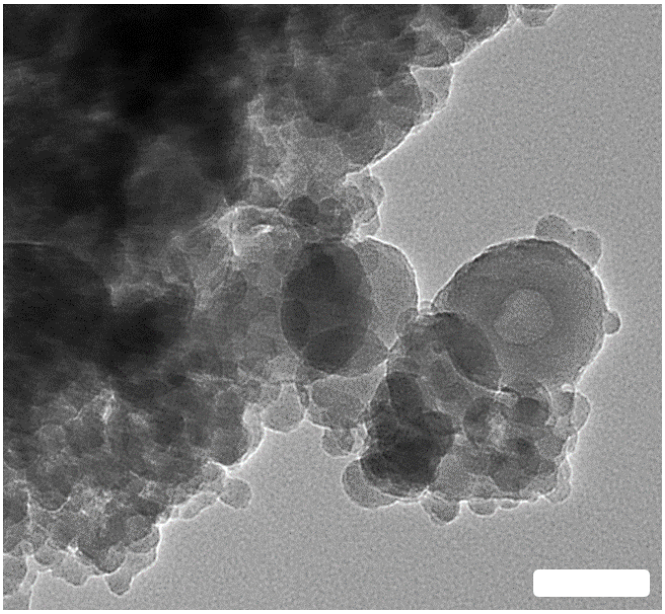

(scale bar = 200 nm)

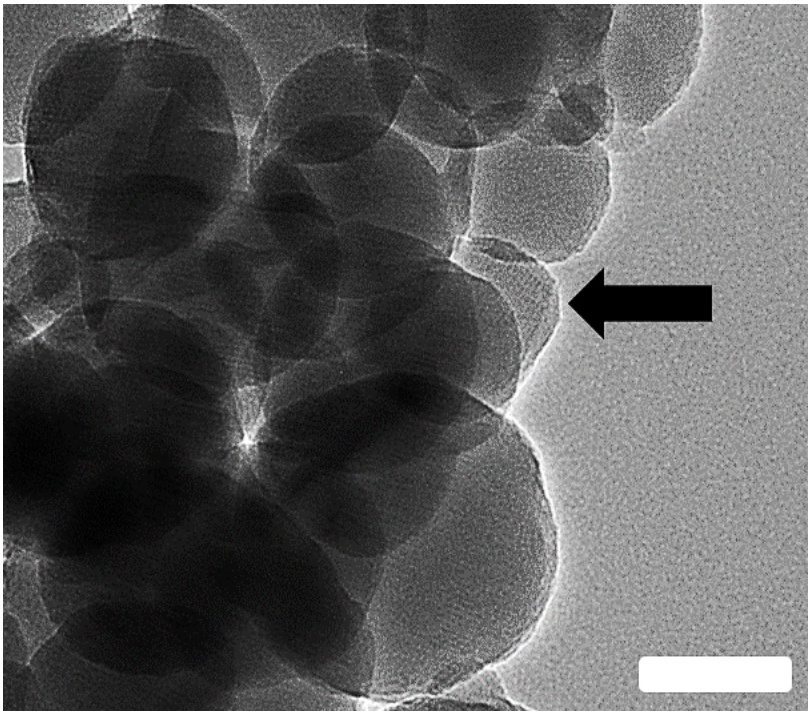
Fig. S-2: Two-step, two surfactant strategy; Variation of the CTAB: Brij-56 ratio.

(a) CTAB: Brij-56 = 1; TEM micrograph.



(scale bar = 50 nm)

(b) CTAB: Brij-56 = 15; TEM micrograph.

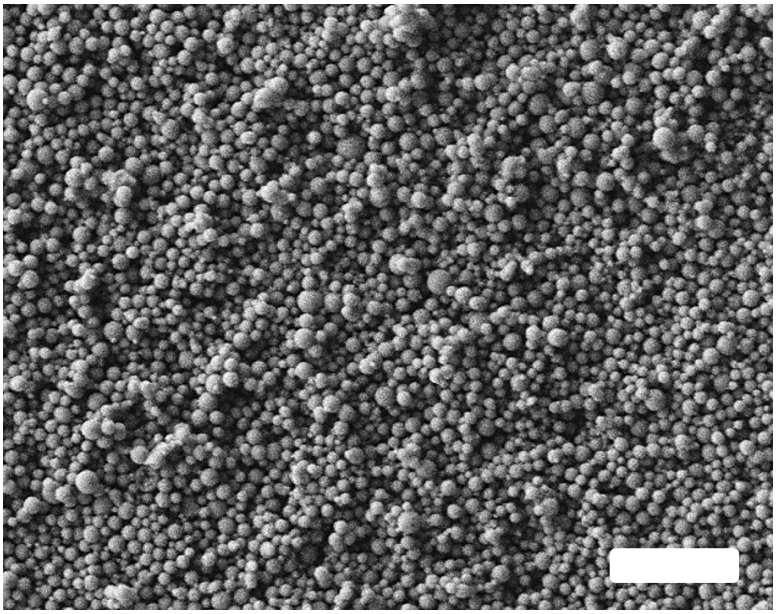


(scale bar= 50 nm)

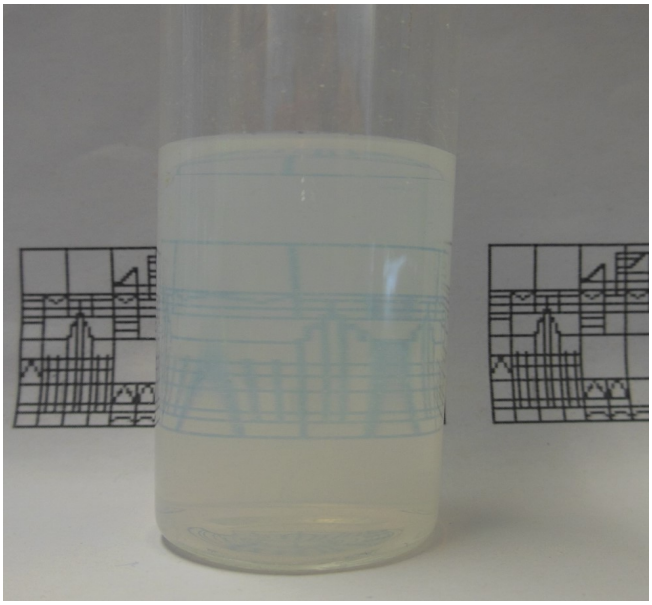
Arrow highlights well-structured domain.

Fig. S-3: Additional analytical data for nanoporous UKON-2j particles prepared via the modified Stoeber method.

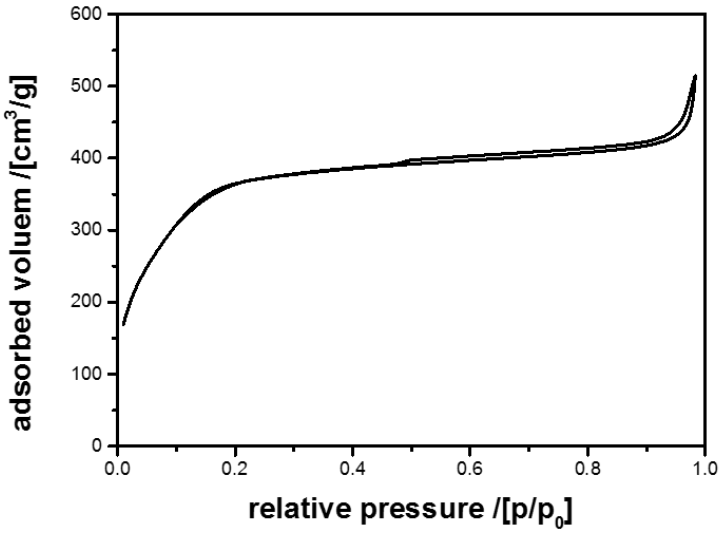
(a) SEM data.


(scale bar 1 μm)

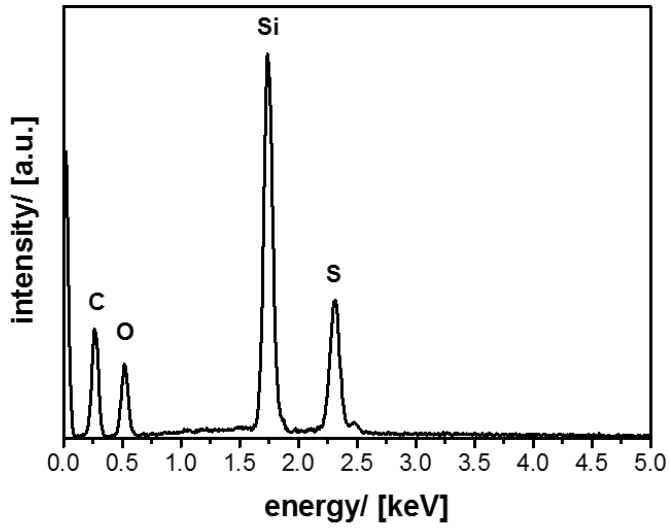
(b) Photography of a colloidal suspension of UKON-2j nanoparticles.



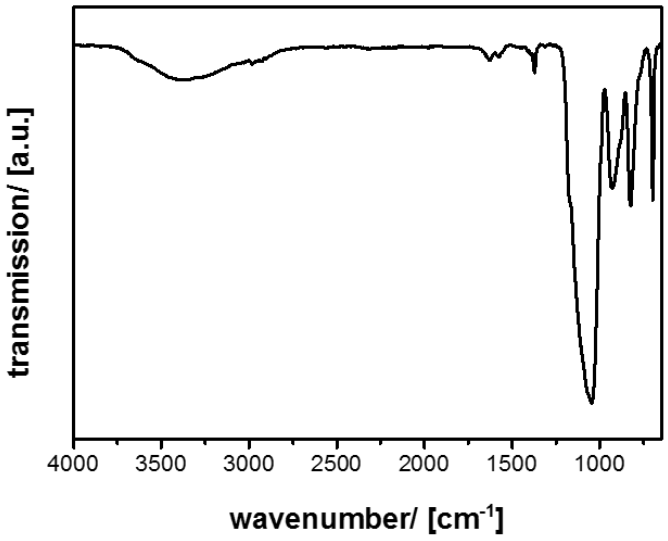
(c) Nitrogen physisorption data of UKON-2j nanoparticles.



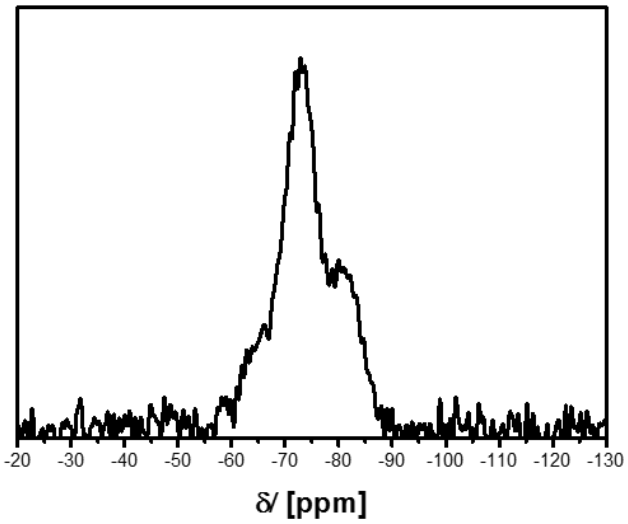
(d) EDX data.



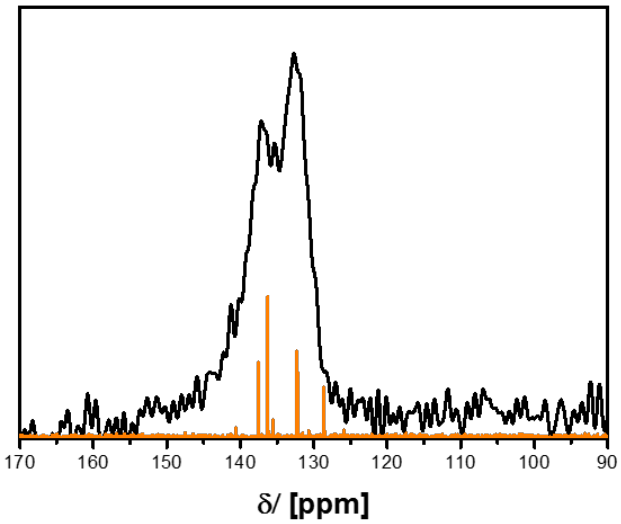
(e) FT-IR data.



(f) solid-state NMR: ^{29}Si.



(g) ^{13}C NMR data.

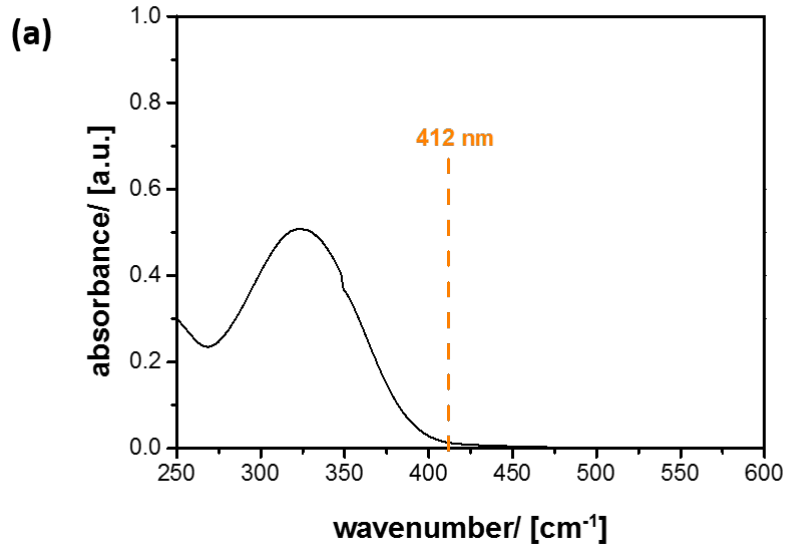


black: solid-state NMR data

orange: NMR data of 1,3-bis-tri(isopropoxysilyl)-thiophenol in $CDCl_3$

Fig. S-4: UV/VIS data of Ellmans test with UKON-2j nanoparticles.

(a) Signal of pure DTNB in buffer solution pH= 8 before particle addition.



Orange label indicates the wavelength of the upcoming TNB signal at 412 nm.

(b) Signal of resulting TNB in buffer solution pH= 8 after reaction with UKON-2j nanoparticles.

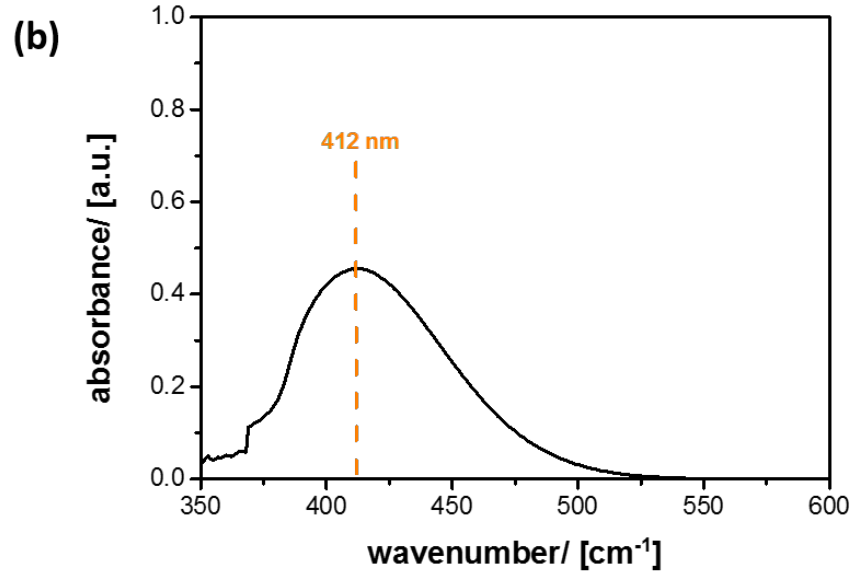
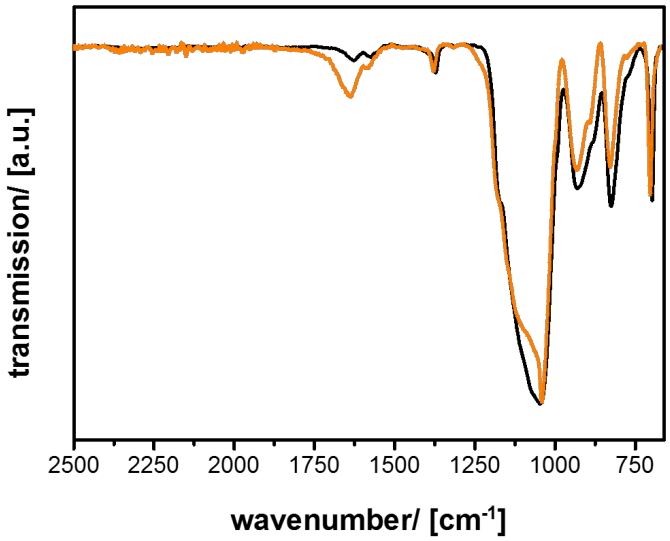


Fig. S-5: FT-IR investigation of the UKON-2jNO nanoparticles.

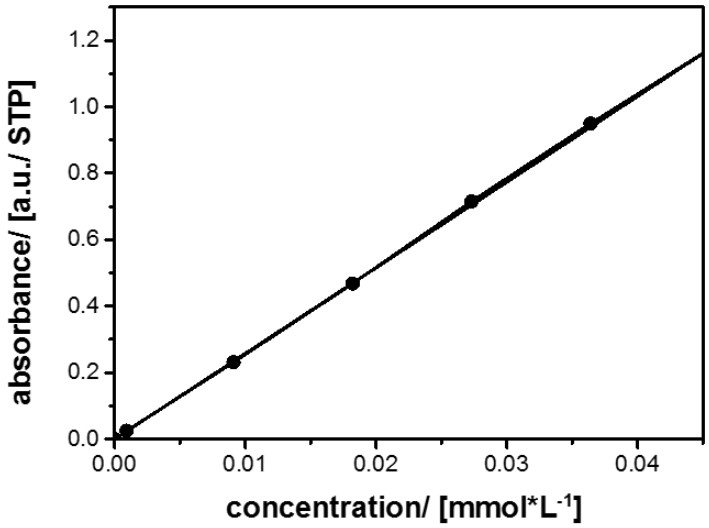


black curve= UKON-2j nanoparticles as a reference

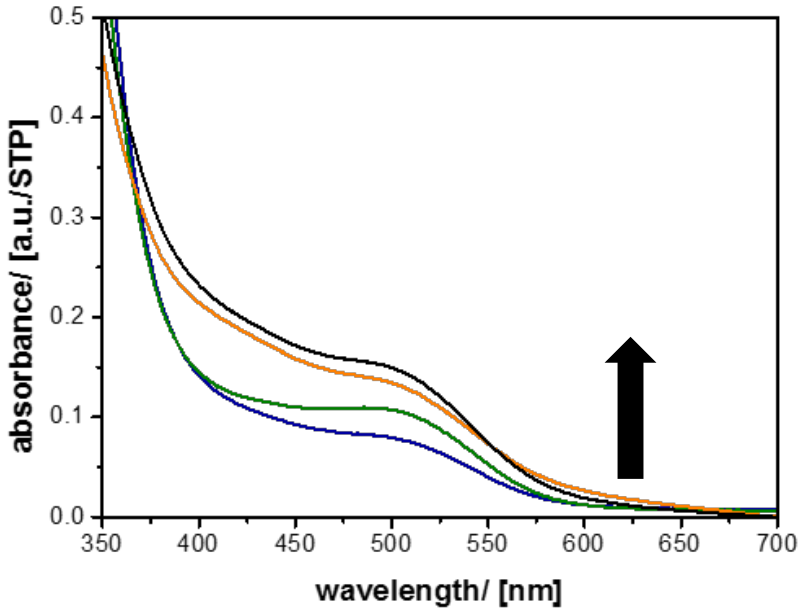
orange curve= UKON-2jNO nanoparticles

Fig. S-6: Griess assay for the detection of released NO of UKON-2jNO nanoparticles.

(a) UV/VIS spectra: calibration curve of different concentration of azo dye at pH= 14; absorption maxima at 498 nm; calculated extinction coefficient ***ε= 943.84 $M^{-1}cm^{-1}$***



(b) UV/VIS spectra of Griess reagent: NO release triggered by sunlight irradiation; arrow indicates the increase of Griess reagent



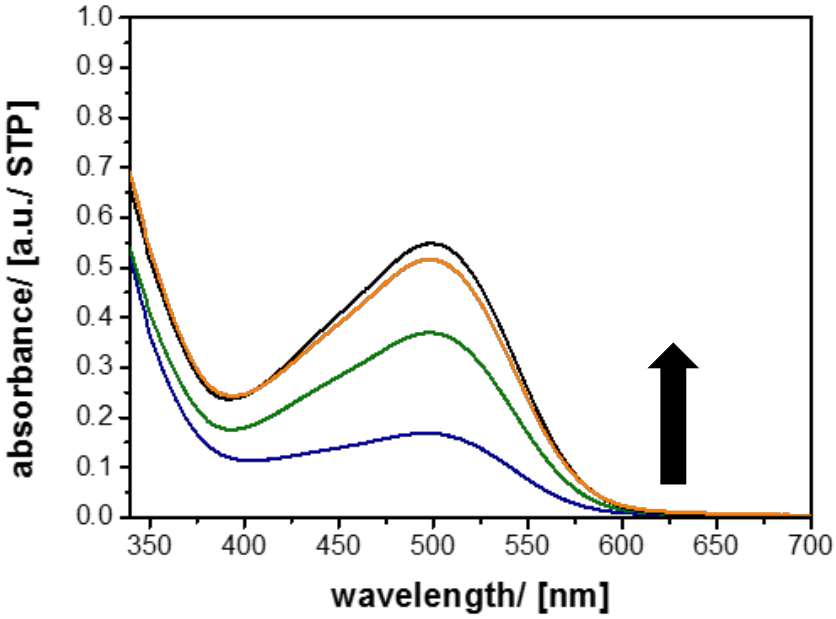
15 min: blue curve

60 min: green curve

120 min: orange curve

240 min: black line

(c) UV/VIS spectra of Griess reagent: NO release triggered by temperature; arrow indicates the increase of Griess reagent



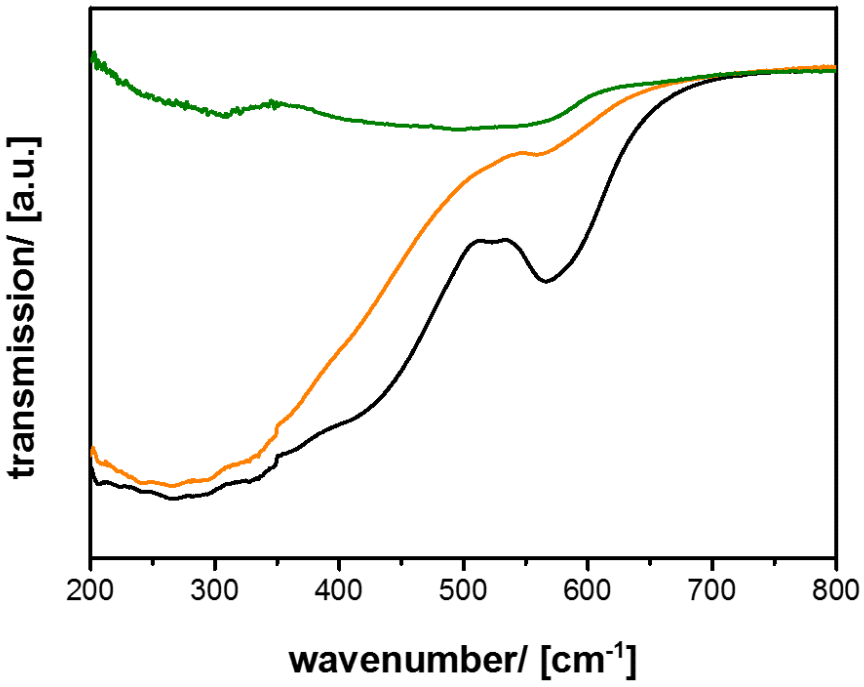
30 min: blue curve

60 min: green curve

180 min: orange curve

2 d: black curve

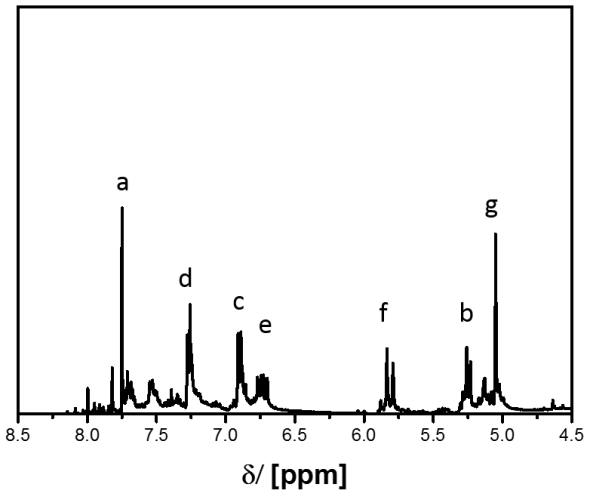
Fig. S-7: UV/VIS data of UKON-2jNO and UKON-2jSS (reflexion mode).

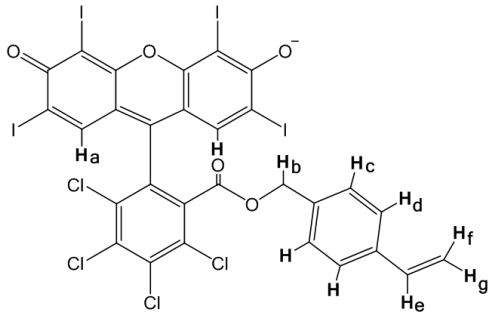


UKON-2j: green curve; UKON-2jNO: black curve; UKON-2jSS: orange curve

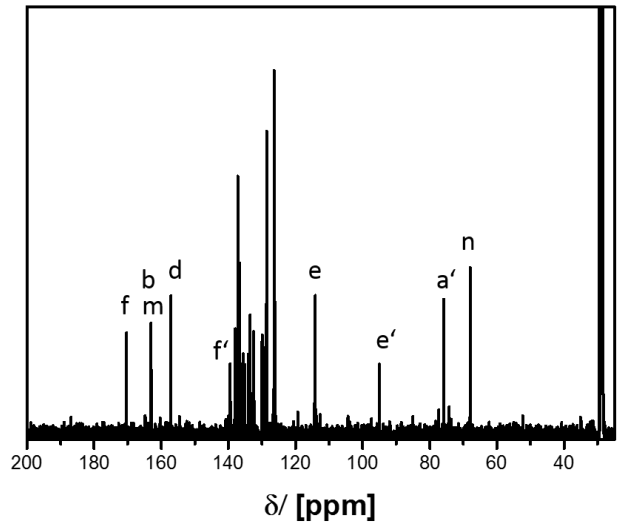
Fig. S-8: characterization of 4-vinyl benzyl Rose Bengal (VB-RB).

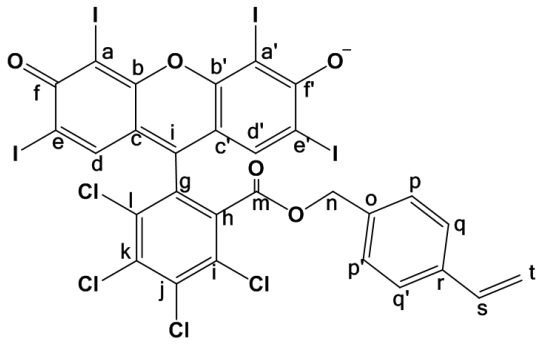
(a) ^{1}H NMR data of 4-vinyl benzyl Rose Bengal



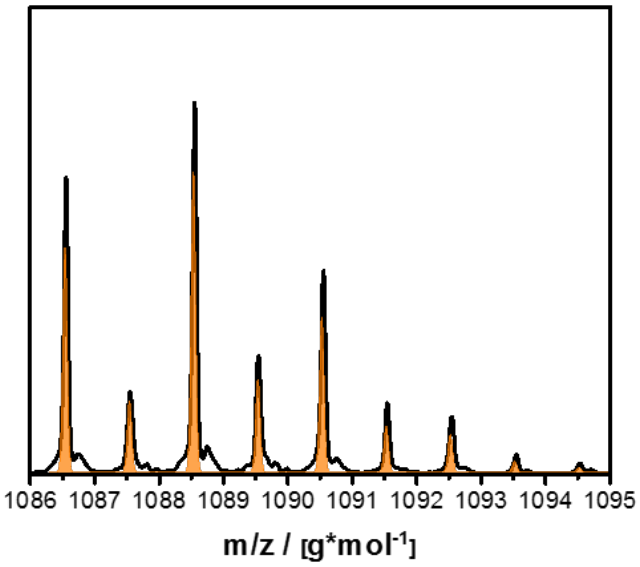


(b) ^{13}C NMR data of 4-vinyl benzyl Rose Bengal





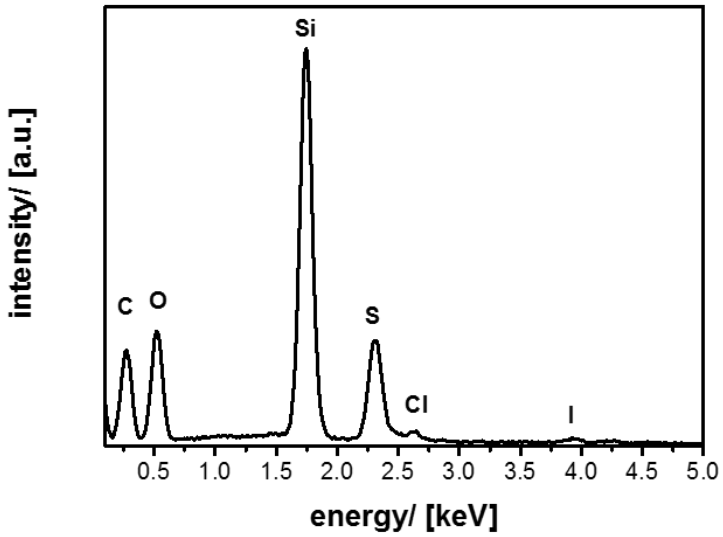
(c) ESI-MS data of 4-vinyl benzyl Rose Bengal measured in negative mode.



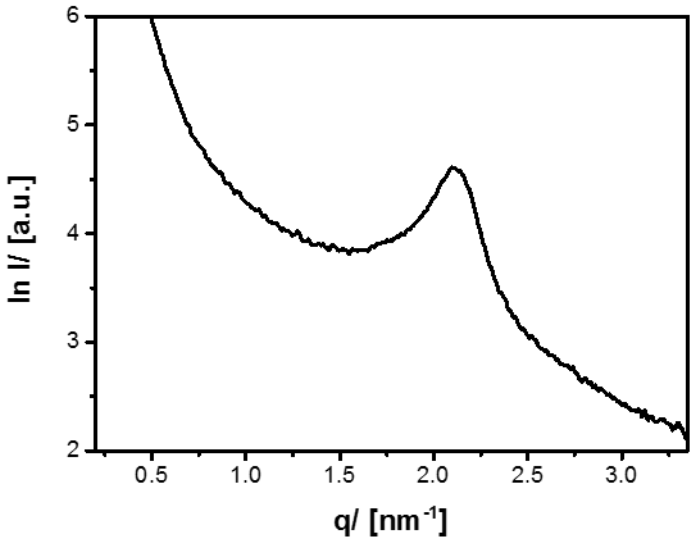
Experimental data (black curve) and simulated spectra for 4-vinyl benzyl Rose Bengal (orange areas).

Fig. S-9: characterization of the UKON-2jRB nanoparticles.

(a) EDX spectra of UKON-2jRB nanoparticles confirming the ratio of Si: S: Cl as 1: 0.4: 0.06



(b) SAXS data of RB hosting UKON-2jRB nanoparticles



(c) TEM image of UKON-2jRB nanoparticles; scale bar= 50 nm

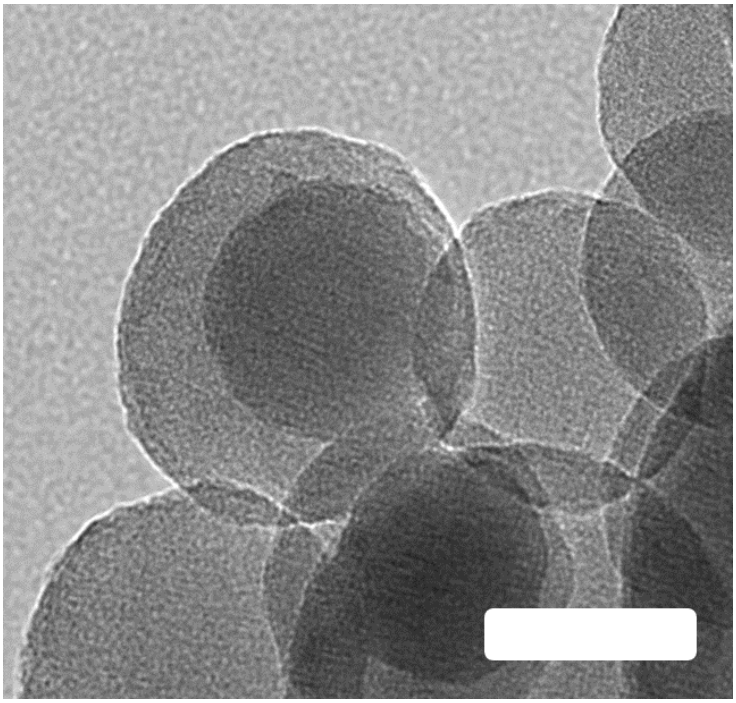
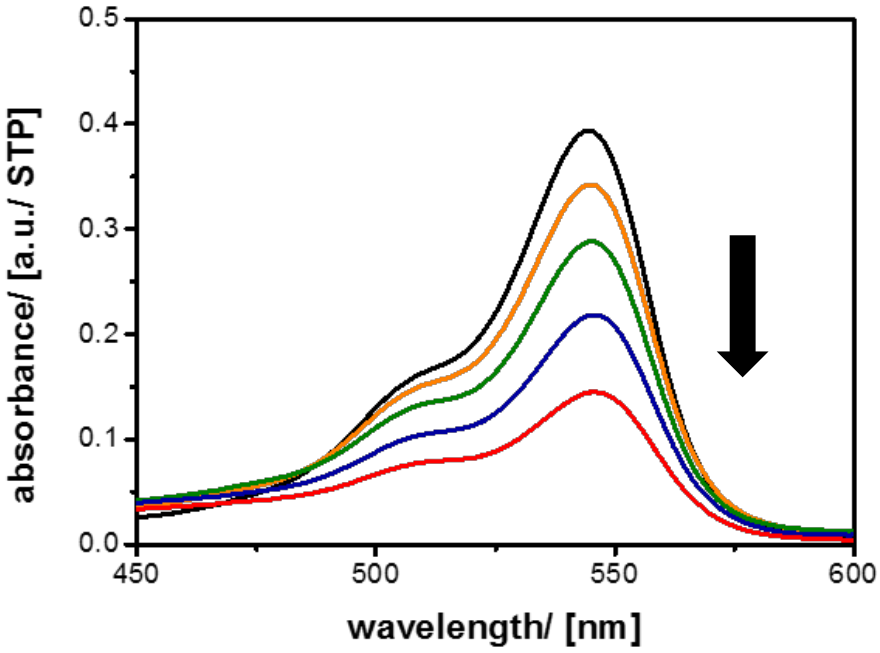


Fig. S-10: Photostability of VB-RB.

(a) UV/VIS spectra of free VB-Rose Bengal irradiated for different time periods.



0 min: black curve

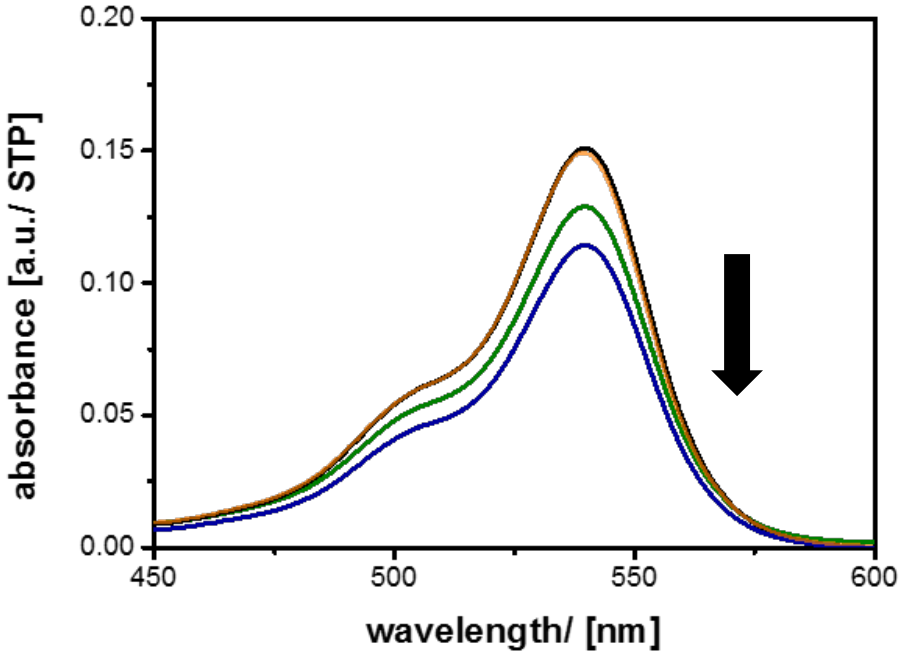
2 min: orange curve

4 min: green curve

6 min: blue curve

12 min: red curve

(b) UV/VIS spectra of immobilized Rose Bengal (UKON-2jRB) irradiated for different time periods.



0 min: black curve

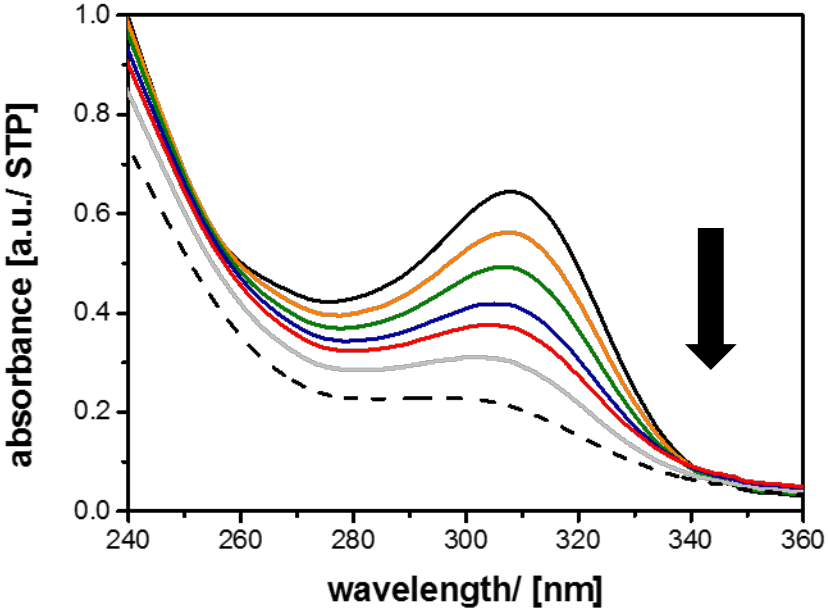
15 min: orange curve

30 min: green curve

60 min: blue curve

Fig. S-11: uric acid degradation by different materials: evaluation of biocidal activity.

(a) UV/VIS spectra of uric acid treated with free VB-Rose Bengal and sunlight exposure for different time periods.



1 min: black curve

2 min: orange curve

3 min: green curve

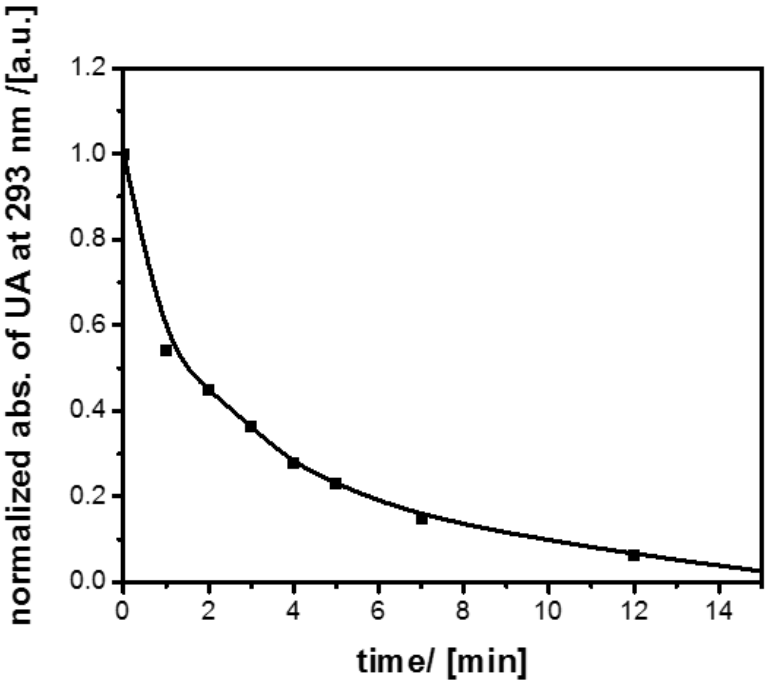
4 min: blue curve

5 min: red curve

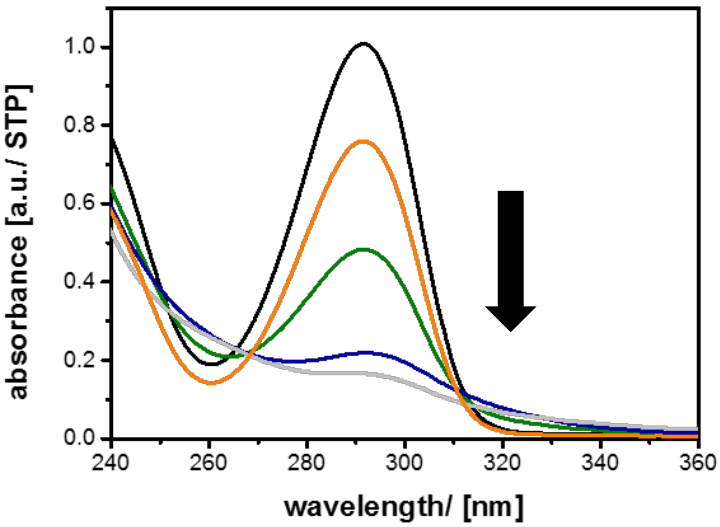
7 min: grey curve

12 min: black dots

(b) Decay curves for the absorption of UA at 293 nm in presence of free Rose Bengal



(c) UV/VIS spectra of uric acid treated with UKON-2jRB nanoparticles and light exposure for different time periods; arrow indicates the decrease of uric acid signal



0 min: black curve

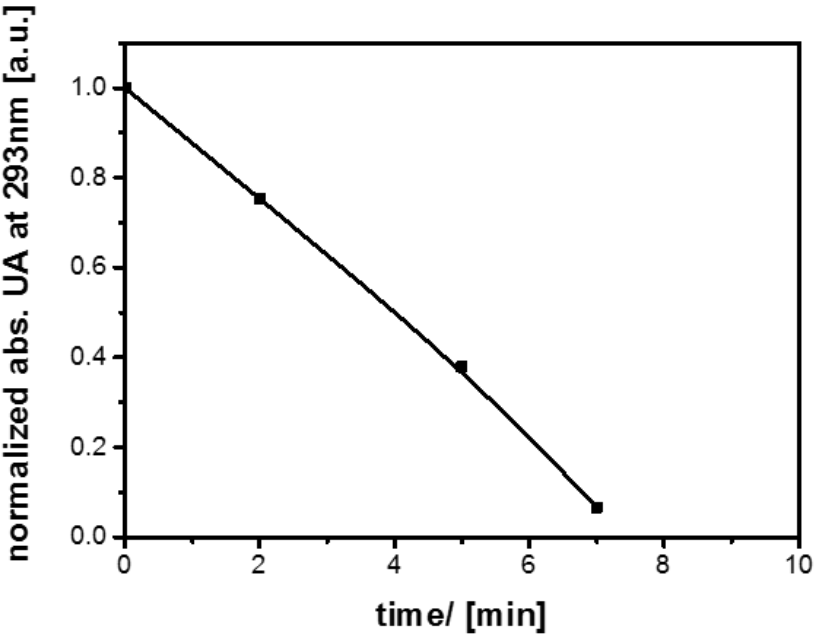
2 min: orange curve

5 min: green curve

7 min: blue curve

10 min: grey curve

(d) Decay curves for the absorption of UA at 293 nm in presence of UKON-2jRB nanoparticles



(e) Time- dependent uric acid degradation (initiated by sunlight exposure) using different UKON-2jRB^{x}NO^{1-x} nanoparticles (x= 0.75, 0.5, 0.25) monitored via UV/VISspectroscopy.

(a) UKON-2jRB$^{0.75}$NO$^{0.25}$ after t= 0, 2, 4, 6, 8, 10 min

(b) UKON-2jRB$^{0.5}$NO$^{0.5}$ after t= 0, 2, 4, 6 min

(c) UKON-2jRB$^{0.25}$NO$^{0.75}$ after t= 0, 2, 4 min.

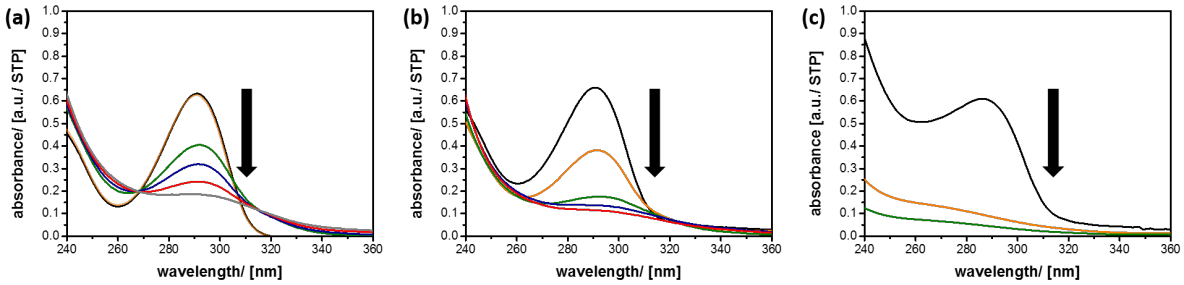


Fig. S-12: cell viability assay presented as log-reduction plot.

Black curve: control experiment

Orange curve: UKON-2jRB$^{0.25}$NO$^{0.75}$

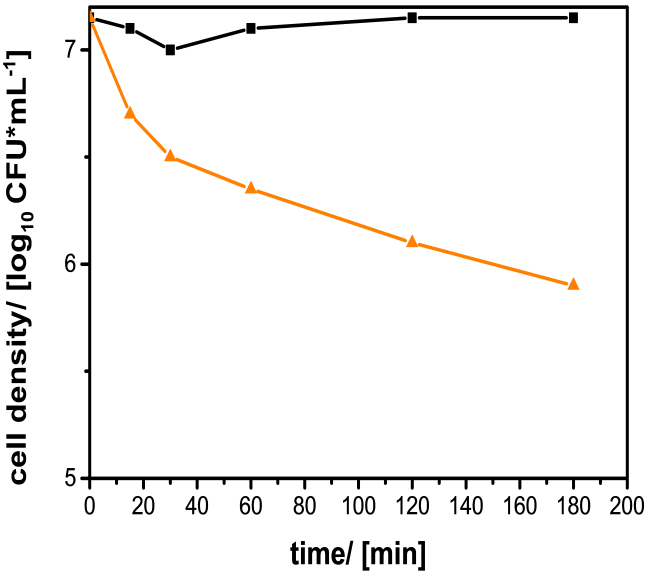
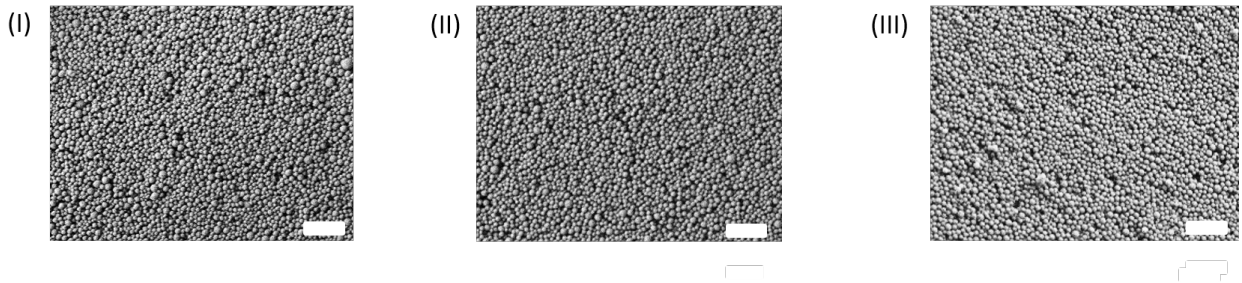


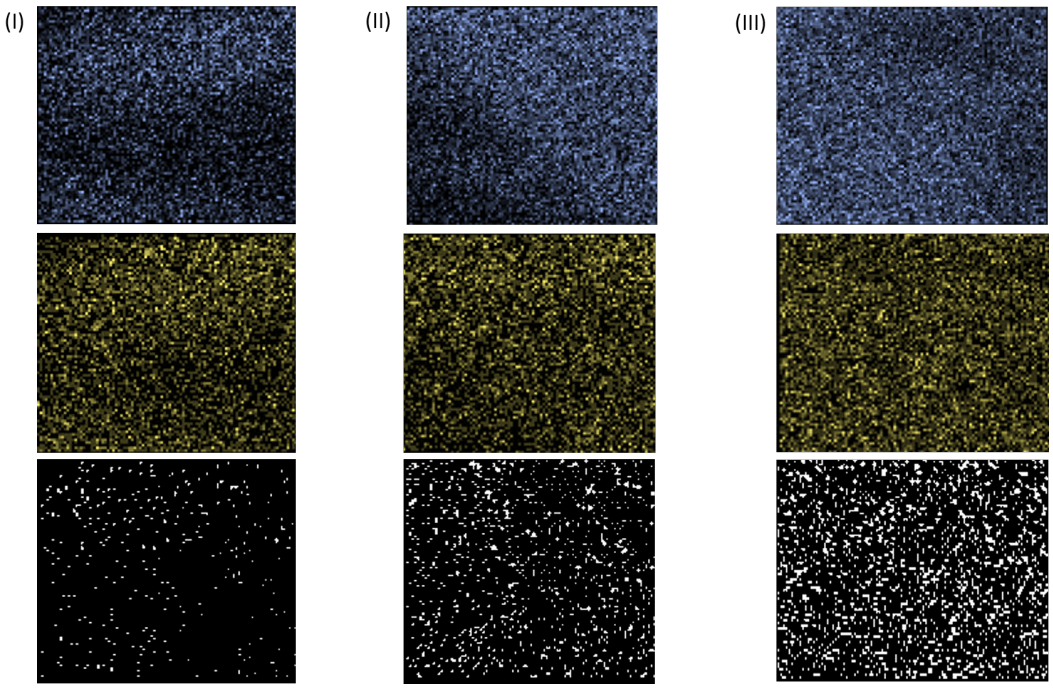
Fig. S-13: nanoparticle films obtained via doctor-blading.

(a) SEM image of (I) UKON-2jRB$^{0.25}$NO$^{0.75}$, (II) UKON-2jR$^{0.5}$NO^{075} and (III) UKON-2jRB$^{0.75}$NO$^{0.25}$nanoparticle film.



scale bar=1 μm

(b) EDX mapping of (I) UKON-2jRB$^{0.25}$NO$^{0.75}$, (II) UKON-2jRB^{025}NO^{075}and (III) UKON-2jRB$^{0.75}$NO$^{0.25}$nanoparticle film.



Blue: silica content, Yellow: sulfur content, White: iodine content