Electronic Supplementary Information

Antibacterial Nitric Oxide- and Singlet Oxygen-Releasing Polystyrene Nanoparticles Responsive to Light and Temperature Triggers

Jiří Dolanský,[ab] Petr Henke,[a] Zuzana Malá,[c] Ludmila Žárská,[c] Pavel Kubát [d] and Jiří Mosinger[ab]*

[a]*Faculty of Science, Charles University, Hlavova 2030, 128 43 Prague 2, Czech Republic, E-mail address: mosinger@natur.cuni.cz.*

[b]*Institute of Inorganic Chemistry, v.v.i., Czech Academy of Sciences, 250 68 Řež, Czech Republic.*

[c]*Department of Medical Biophysics, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University in Olomouc, Czech Republic.*

[d]*J. Heyrovský Institute of Physical Chemistry, v.v.i., Czech Academy of Sciences, Dolejškova 3, 182 23 Prague 8, Czech Republic.*

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Tab. S1. Parameters of prepared NPs, the case analysis.

Parameter	Description	Calculation / Note	
P1	Mass of NPs per ml ($mg\ ml^{-1}$)	From the gravimetric measurement	1.9±0.2
P2	Mass of sulfonated nanofiber membrane (mg)	From the gravimetric measurement	127
P3	Volume of the stock dispersion (ml)	-	60
P4	Yield of NPs preparation	**P4** = (**P1**×**P3**)/**P2**	90 %
P5	Molar mass of the NPs ($g\ mol^{-1}$)	See ref. 26 in main text	6.8×10^{7}
P6	Avogadro's number (mol^{-1})	-	6.022×10^{23}
P7	Average radius of NPs (cm)	From DLS measuring	3.6×10^{-6} (**NOP**) 3.5×10^{-6} (**TMPyP/ NOP**) 3.4×10^{-6} (**ZnPc/ NOP**)
P8	Surface of 1 NP (cm^2)	**P8** = 4π(**P7**)^2	$1.5 – 1.6\times10^{-10}$
P9	Volume of 1 NP (cm^3)	**P9** = $4/3\pi$(**P7**)^3	$1.6 – 2.0\times10^{-16}$
P10	Number of NPs per ml (in the stock dispersion)	**P10** = (**P1**/(**P5**×1000))×**P6**	1.7×10^{13}
P11	Theoretical effective volume reached by $O_2(^1\Delta_g)$ in 1 ml of the stock dispersion (ml)	**P11** = **P10**×$4/3\pi(r_{ef})^3$ r_{ef} = **P7** + l_r Effective radius (r_{ef}) = average radius of NPs (**P7**) + effective range of $O_2(^1\Delta_g)$ (l_r = 20.5×10^{-6} cm)	0.99
P12	Average IEC of NPs ($mol\ ml^{-1}$)	Determined by titration	6.2×10^{-7}
P13	Average IEC of NPs ($mol\ g^{-1}$)		1.8×10^{-4}
P14	Estimated number of sulfo groups per NP	**P14** = (**P12**×**P6**)/**P10**	2.2×10^{4}
P15	Mass of photodonor (NOP) encapsulated in 1 NP (mg)	From the gravimetric measurement/**P10**	2.0×10^{-15}
P16	Mass of photosensitizer bounded on a surface of 1 NP (mg)	From the gravimetric measurement/**P10**	(TMPyP) 4.5×10^{-17} (ZnPc) 6.7×10^{-17}

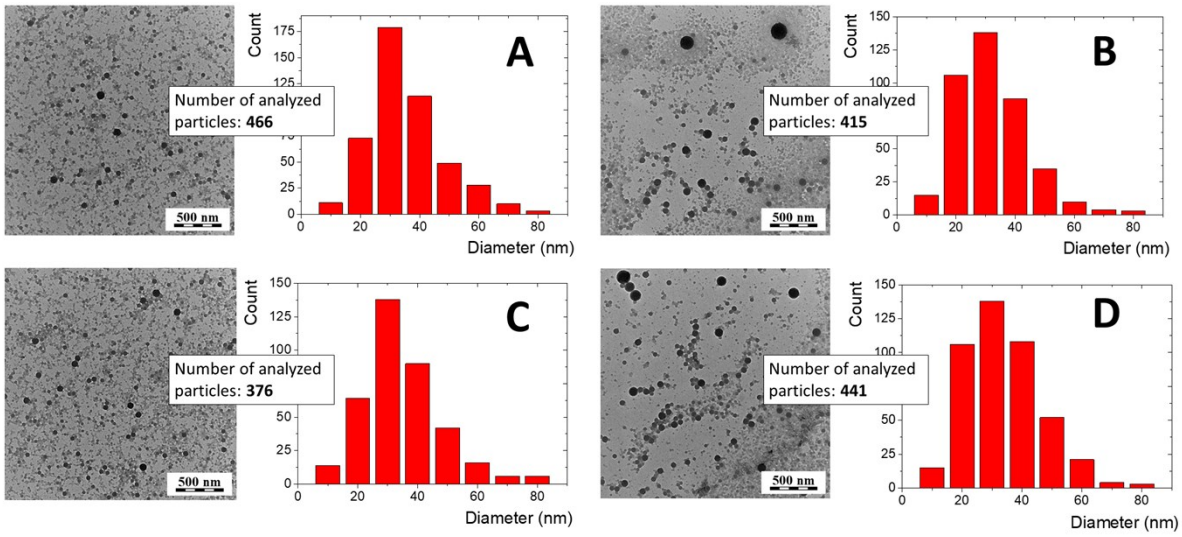


Fig. S1. TEM micrographs with the corresponding distribution of NPs diameters of sulphonated polystyrene NPs (panel A), **NOP-NPs** (panel B), **TMPyP/NOP-NPs** (panel C) and **ZnPc/NOP-NPs** (panel D) prepared by the nanoprecipitation method.

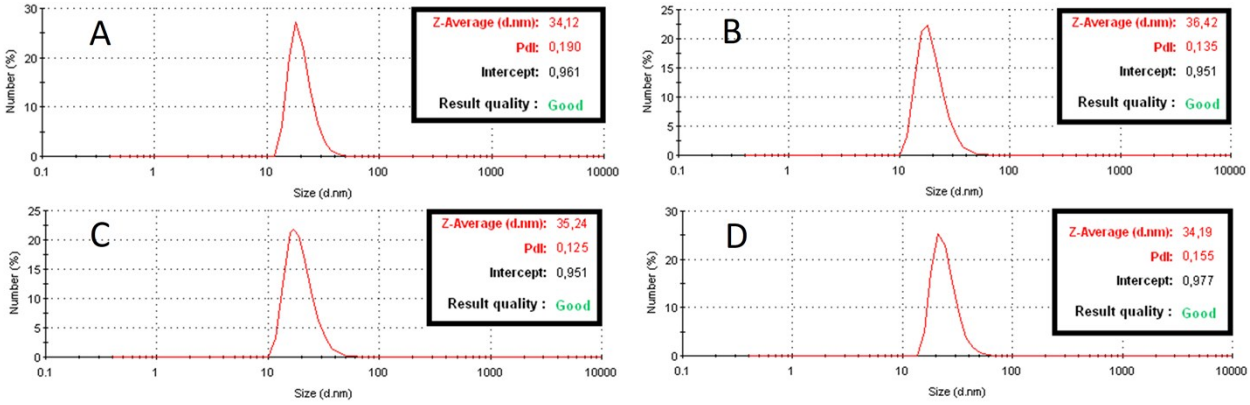
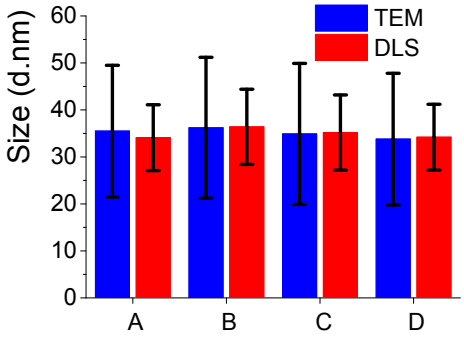


Fig. S2. DLS measurement of sulphonated polystyrene NPs (panel A), **NOP-NPs** (panel B), **TMPyP/NOP-NPs** (panel C) and **ZnPc/NOP-NPs** (panel D).

Tab. S2. Comparison of average diameter of NPs by two independent methods.

	Average diameter (nm)	
Sample	**Determined by TEM**	**Determined by DLS**
Blank (A)	35.5±13 (from 466 particles)	34.1±5
NOP-NPs (B)	36.2±14 (from 415 particles)	36.4±6
TMPyP/NOP-NPs (C)	34.9±13 (from 376 particles)	35.2±6
ZnPc/NOP-NPs (D)	33.8±13 (from 441 particles)	34.2±5



Tab. S3. Maximum concentration of photoreleased NO from the aqueous dispersions (3 mL, ~ 1.7×10^{13} NPs mL^{-1}) of nanoparticles after 15 minutes irradiation with visible light.

Sample	**Concentration (nmol)**
NOP-NPs	381±19
TMPyP/NOP-NPs	324±16
ZnPc/NOP-NPs	288±14

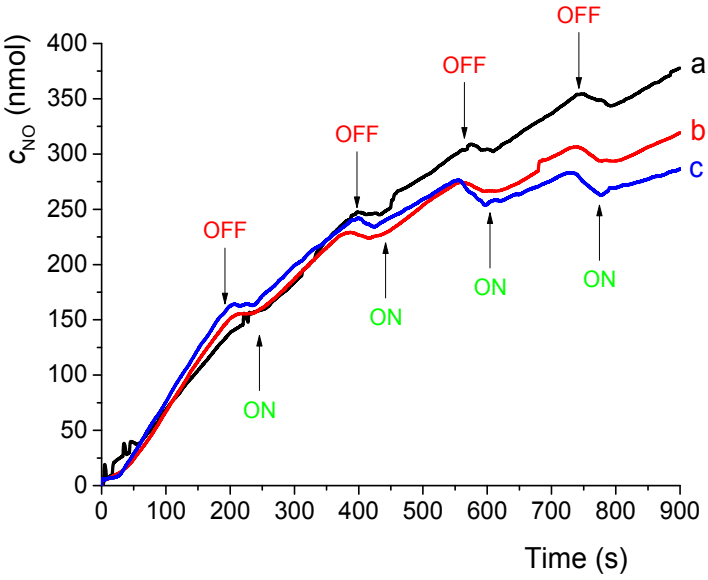


Fig. S3. Amperometric detection of NO photoreleased from aqueous dispersions of **NOP-NPs** (a), **ZnPc/NOP-NPs** (b) and **TMPyP/NOP-NPs** (c). The arrows indicate the switch-on/switch-off character of the NO release that was triggered by using a stabilized xenon lamp (500 W, Newport) with a long pass filter ($\lambda \geq 400$ nm, Newport).

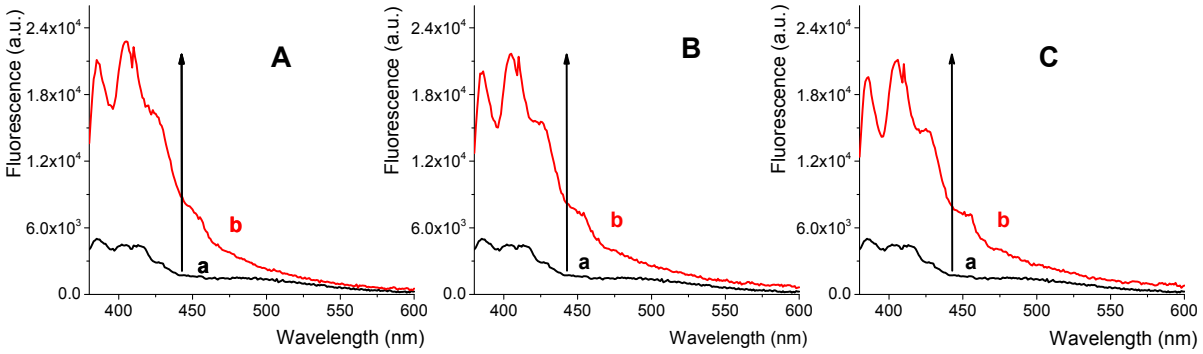


Fig. S4. Fluorescence detection by DAN test of the NO photoreleased from the dispersion of **NOP-NPs** (panel A), **TMPyP/NOP-NPs** (panel B), **ZnPc/NOP-NPs** (panel C)**.** The fluorescence (λ_{exc} = 365 nm) of 1.5 mL of 0.31 mM DAN in 0.62 M HCl with the nanoparticle dispersion (1.5 mL) before (a) and after (b) 20 min of irradiation (indicated by arrow) with visible light (Xe lamp $\lambda \geq 400$ nm).

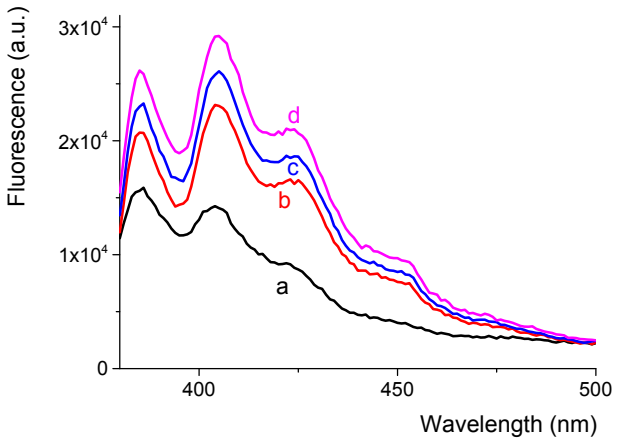


Fig. S5. Fluorescence detection by DAN test of NO photoreleased from the dispersion of **NOP-NPs** at different temperatures: 10 °C (a), 22 °C (b), 37 °C (c) and 45 °C (d). The NO photorelease was triggered by the irradiation with Xe lamp $\lambda \geq 400$ nm.

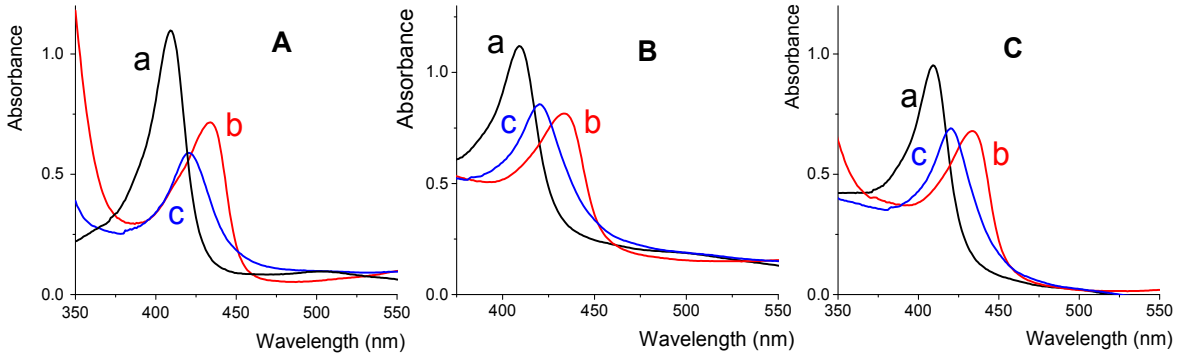


Fig. S6. The myoglobin test for detection of NO released from the **NOP-NPs** (panel A), **TMPyP/NOP-NPs** (panel B), **ZnPc/NOP-NPs** (panel C) after 20 min of irradiation by visible light. 3 mM of Mb(Fe^{III}) in 0.02 M phosphate buffer, pH = 7.0 (a), its reduced form, Mb(Fe^{II}), before (b) and after (c) binding of the released NO.

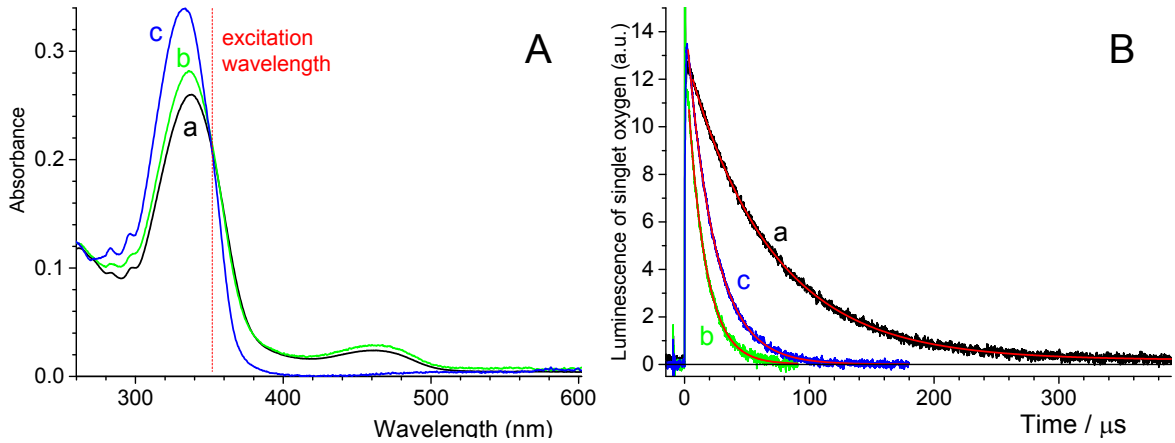


Fig. S7. Absorption spectra of NOP dissolved in acetonitrile (a), ethanol (b) and cyclohexane (c) (panel A) and corresponding luminescence of singlet oxygen after excitation by Nd YAG laser (355 nm) (panel B). Red lines are single exponential fits into experimental data, calculated lifetimes of singlet oxygen $\tau_\Delta \sim 67$ μs for acetonitrile, $\tau_\Delta \sim 14$ μs for ethanol, and $\tau_\Delta \sim 22$ μs for cyclohexane correspond with values published in literature.[1]

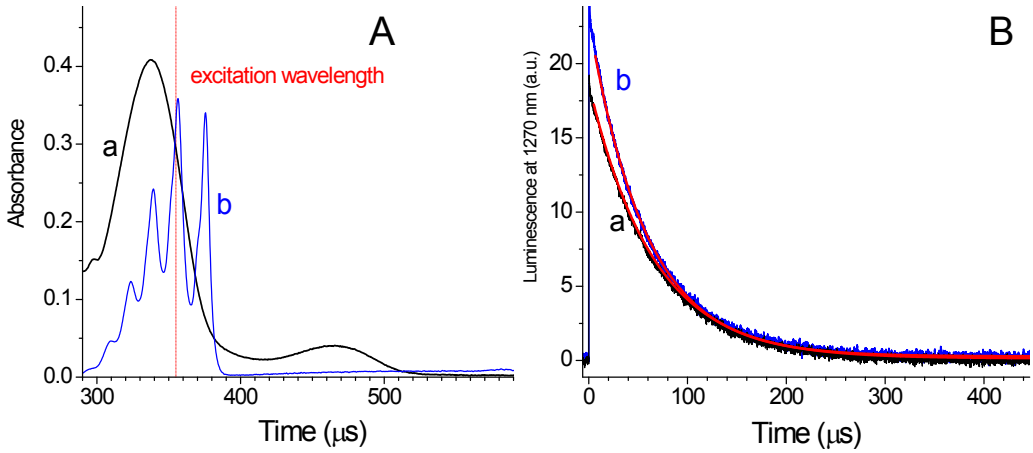


Fig. S8. Estimation of the quantum yield of singlet oxygen, Φ_Δ, in oxygen-saturated acetonitrile: UV/Vis spectra of NOP (a) and anthracene (b) in acetonitrile (panel A) and corresponding luminescence of singlet oxygen (panel B). $\Phi_\Delta \sim 0.56$ for NOP was calculated by comparison with anthracene standard ($\Phi_\Delta = 0.69$).[2] Note that NOP decomposes upon irradiation (Figs. S9 and S10).

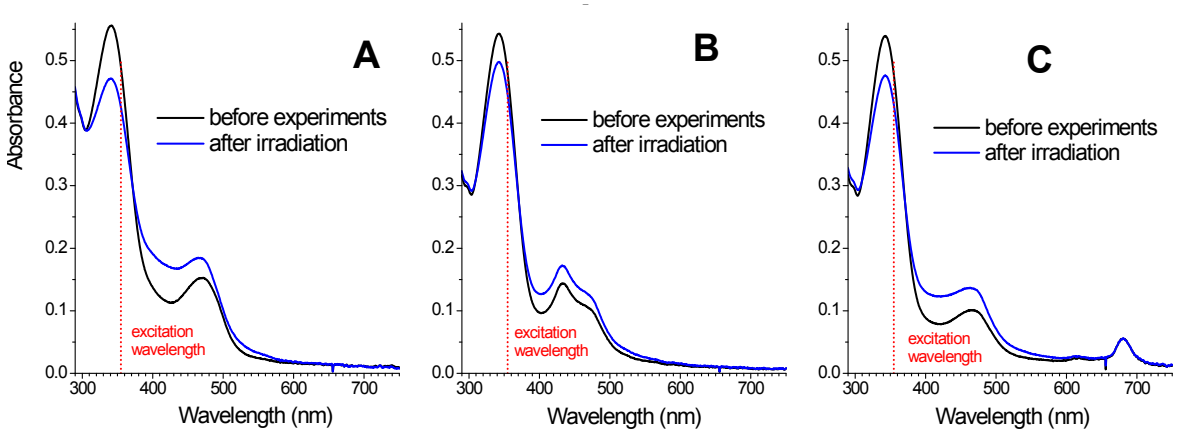


Fig. S9. Comparison of UV/Vis spectra of **NOP-NPs** (panel A), **TMPyP/NOP-NPs** (panel B), and **ZnPc/NOP-NPs** (panel C) dispersed in H_2O before measurements of singlet oxygen luminescence and after 2000 laser shots of Nd YAG laser.

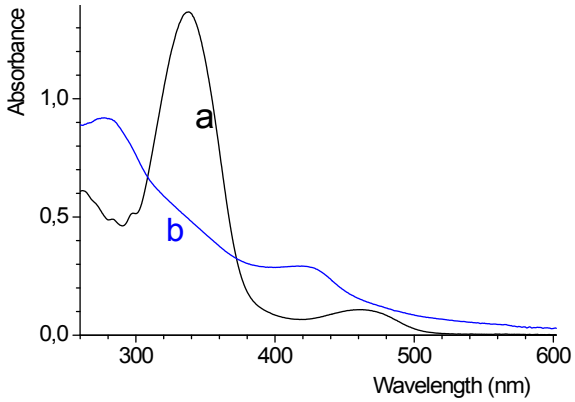


Fig. S10. Comparison of UV/Vis spectra of **NOP** in acetonitrile before measurements of singlet oxygen (a) and after 30000 laser shots of Nd YAG laser (b).

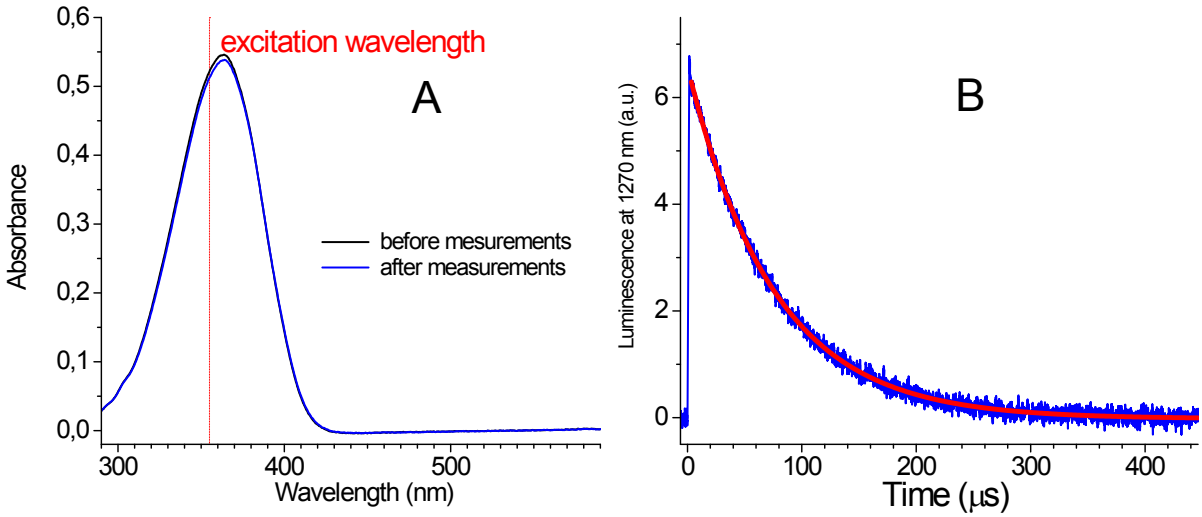


Fig. S11. Comparison of UV/Vis spectra of *p*-nitroaniline in acetonitrile before measurements of singlet oxygen and after 2000 laser shots of Nd YAG laser (panel A), and corresponding luminescence of singlet oxygen (panel B). Red line represents single exponential fit into experimental data.

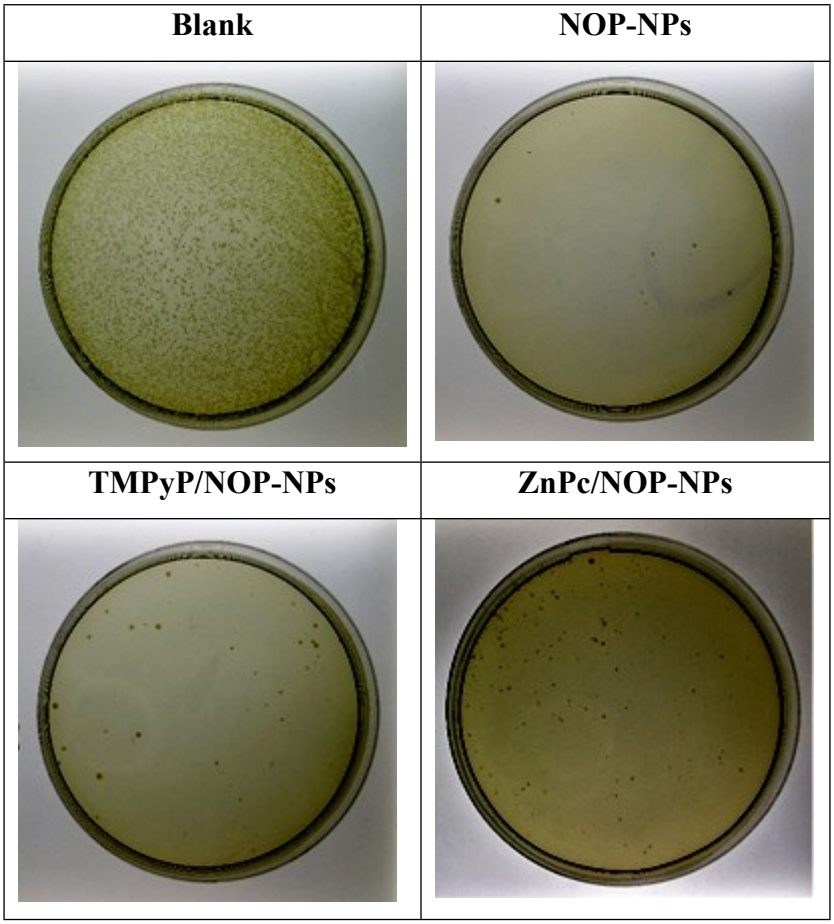


Fig. S12. Photographs of bacterial colonies grown on agar plates from antibacterial tests performed on *Escherichia Coli* after 10 minutes irradiation of bacteria suspension in the presence of given nanoparticle suspensions.

References

1. Wilkinson, F.; Helman, W. P.; Ross, A. B., Rate Constants for the Decay and Reactions of the Lowest Electronically Excited Singlet State of Molecular Oxygen in Solution. An Expanded and Revised Compilation. J. Phys. Chem. Reference Data 1995, 24 (2), 663-677.

2. Tanaka, F.; Furuta, T.; Okamoto, M.; Hirayama, S., Inverse correlation between efficiency of singlet oxygen production and rate constant for oxygen quenching in the S1 state of anthracene derivatives. Phys. Chem. Chem. Phys. 2004, 6 (6), 1219-1226.