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Supporting Information

Photophysical efficiency-boost of aqueous aluminium phthalocyanine by hybrid formation with nano-clays

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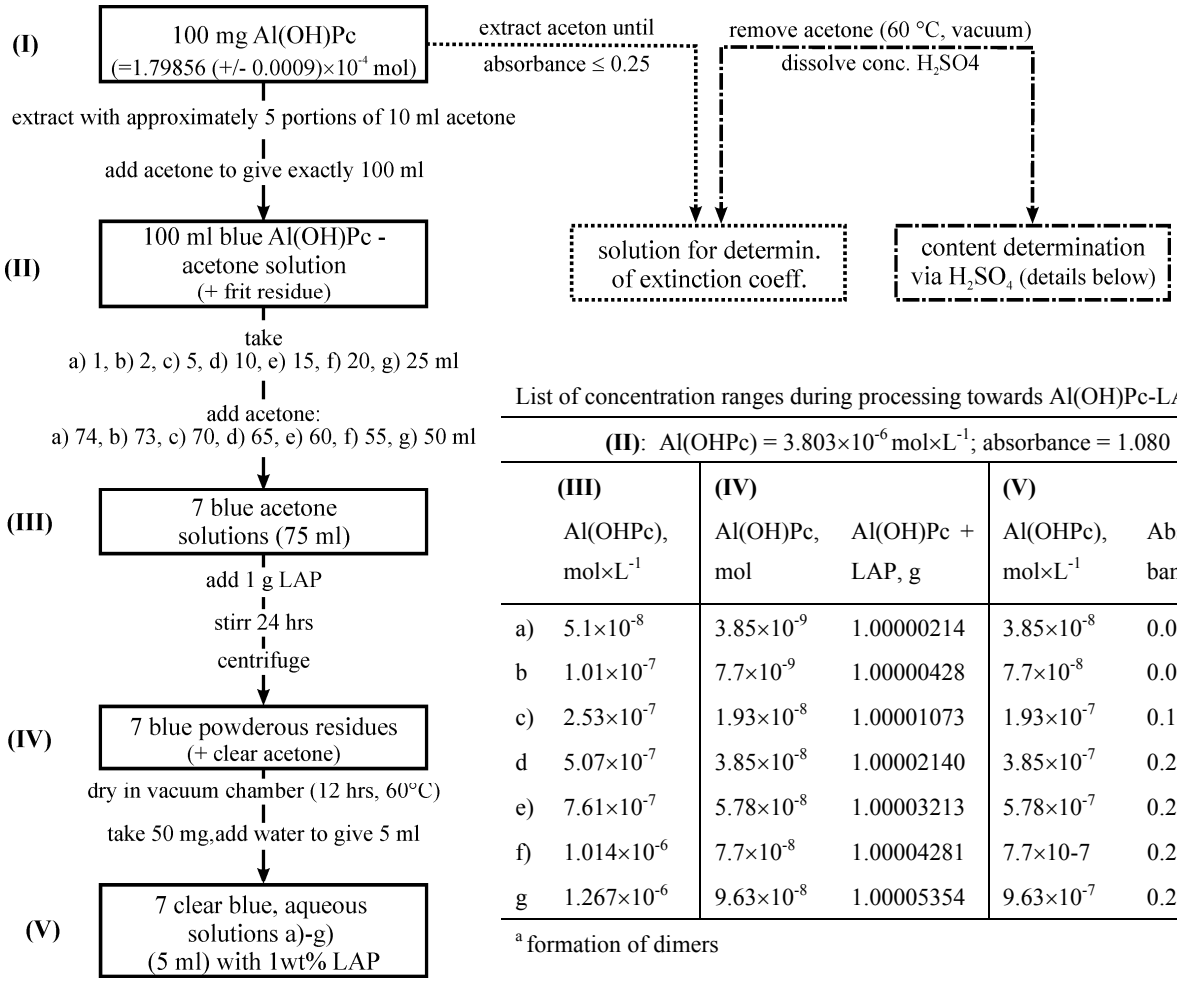
Schemes, tables and figures

1. Reagents and synthesis of Al(OH)Pc-laponite hybrids (Al(OH)Pc-LAP)

Reagents. Aluminium phthalocyanine hydroxide for the syntheses and Zinc 2,9,16,23-tetra-tert-butyl-29H,31H-phthalocyanine for analytical reference purposes as well as 2,4-Pentanedione (Hacac) and 1,1,1,5,5,5-Hexafluoro-2,4-pentanedione (Hhfa) acetylacetone from Sigma-Aldrich, octadecyl-methyldimethoxysilane from ABCR, acetone and sulfuric acid from Merck, Methylene Blue from Riedel de Haen (now Honeywell) and Laponite RD from Rockwood (now Altana) were used as purchased without further purification.

Preparation. The concentrations and amounts of reactants as well as the intermediate steps to obtain the eventual aqueous hybrid solutions are best viewed in a flow diagram (Scheme 1).

Briefly, for the preparation of a stock solution, Al(OH)Pc (I) was extracted with acetone via filtration over G3 and G4 glass frits, yielding an intensely blue stock solution (II). The Al(OH)Pc concentration of this stock solution was determined in conc. H_2SO_4 as described below. From the stock solution, the amounts given in Scheme 1 were taken and diluted to give solutions (IIIa)-g), to which 1 g of laponite each was added and centrifuged to obtain the powderous hybrids (IV). After drying, 50 mg of the powders (IV) were redispersed to give 5 ml of clear aqueous solutions (V) a)-g). UV-Vis spectra of these are reproduced Fig. S2, excitation end emission spectra of V d) are additionally provided in Fig. 2 of the main text. The relevant concentrations and data for (II), (III), (IV) and (V) are also listed in the scheme.



List of concentration ranges during processing towards Al(OH)Pc-LAP

(II): Al(OHPc) = 3.803×10^{-6} mol×L^{-1}; absorbance = 1.080

	(III)	**(IV)**		**(V)**	
	Al(OHPc), mol×L^{-1}	Al(OH)Pc, mol	Al(OH)Pc + LAP, g	Al(OHPc), mol×L^{-1}	Absor-bance
a)	5.1×10^{-8}	3.85×10^{-9}	1.00000214	3.85×10^{-8}	0.098
b	1.01×10^{-7}	7.7×10^{-9}	1.00000428	7.7×10^{-8}	0.037
c)	2.53×10^{-7}	1.93×10^{-8}	1.00001073	1.93×10^{-7}	0.135
d	5.07×10^{-7}	3.85×10^{-8}	1.00002140	3.85×10^{-7}	0.270
e)	7.61×10^{-7}	5.78×10^{-8}	1.00003213	5.78×10^{-7}	0.214[a]
f)	1.014×10^{-6}	7.7×10^{-8}	1.00004281	7.7×10-7	0.236[a]
g	1.267×10^{-6}	9.63×10^{-8}	1.00005354	9.63×10^{-7}	0.263[a]

[a] formation of dimers

Scheme S1. Flow chart depicting the dissolution and seperation steps applied to obtain defined solutions of the Al(OH)Pc-laponite hybrids. The relevant concentrations at different stages are given in the integrated list, photographic images of powders (IV) and solutions (V) of the series are reproduced in Fig. S1.

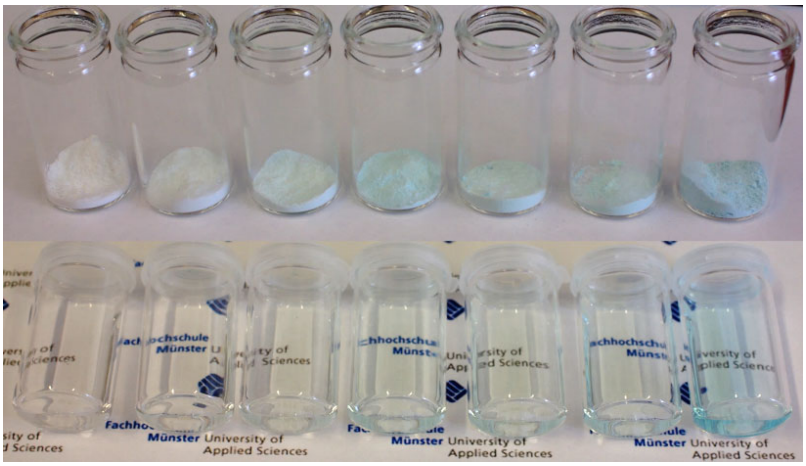


Fig. S1. Photographic images of the powders (IV) and eventual solutions (V) obtained from the series described in Scheme 1. Concentrations are increasing from left to right.

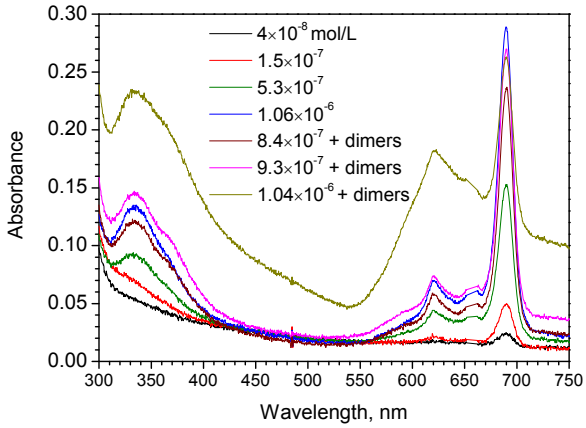


Fig. S2. Absorption spectra of Al(OH)Pc-laponite hybrids in aqueous solution. The concentrations given correspond to the data in scheme 1. All spectra are background corrected.

Determination of Al(OH)Pc concentration and extinction coefficients. As a first step, a calibration curve for Al(OH)Pc in concentrated H_2SO_4 was set up for concentrations ranging from 9×10^{-7} to 3.8×10^{-6} mol×L^{-1} (Fig. S3), yielding the extinction coefficient for Al(OH)Pc in H_2SO_4 at 804 nm as $\varepsilon = 2.35\times10^5$ L×mol^{-1}×cm^{-1} (± 1.4 %). In the employed method, the H_2SO_4 leads to the di- or tetra-protonated Pc, i.e. the metallo complex is destroyed, leaving H_4Pc^{2+} as the absorbing species.[1-3] The extinction coefficient found here agrees well with the 2.4×10^5 quoted for e.g. ZnPc in H_2SO_4.[1]

Next, the concentration of Al(OH)Pc was determined by extraction of 100 mg Al(OH)Pc through a G4 frit to give four extracts, which were diluted to a total volume of 25 ml. Care was taken that the absorbance of these solutions was < 0.25 in order to avoid the presence of dimers. Subsequently, the acetone was completely removed (vacuum drying chamber, 60 °C, overnight). The residues obtained were then dissolved in 25 ml of concentrated H_2SO_4 for the absorption spectrometric UV-Vis determination and measured immediately. The evaluation results against the calibration curve (Fig. S3) are depicted in Table S1.

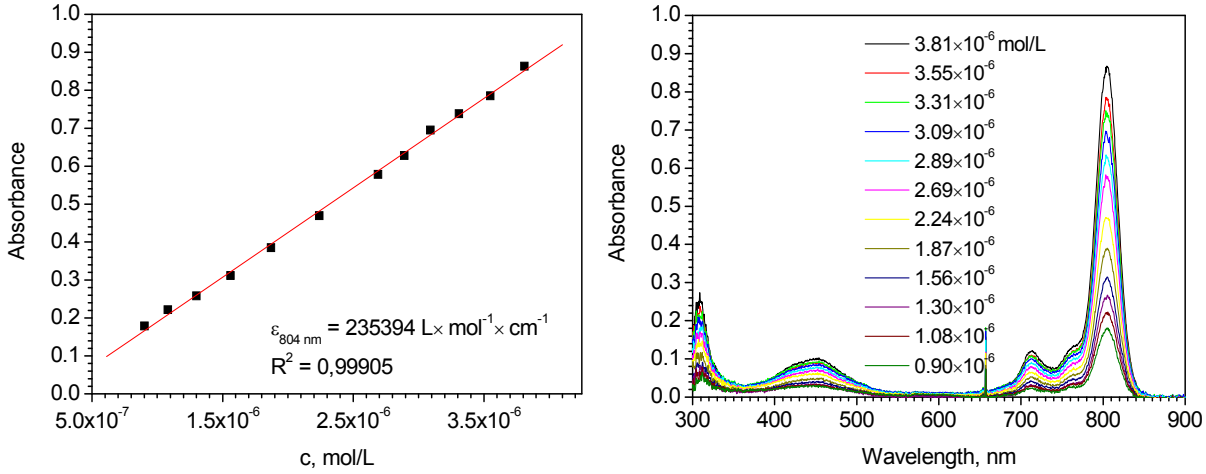


Fig. S3. Al(OH)Pc in conc. H_2SO_4. Left: Calibration curve for the photometric determination. Right: Absorption spectra of the samples used.

Table S1. Determination of the extinction coefficient of Al(OH)Pc in acetone.

Sample	Absorbance (Aceton)	Absorbance (H_2SO^4)	H_4Pc^{2+}, mol/L	ε, L×mol^{-1}×cm^{-1}
1	0,2316	0,1929	8,194×10^{-7}	284206
2	0,2351	0,1978	8,4029×10^{-7}	279784
3	0,1984	0,1603	6,8098×10^{-7}	291344
4	0,2371	0,1928	8,109×10^{-7}	292391

ε (average) = 286931 L×mol^{-1}×cm^{-1}

Diketonate modified Al(OH)Pc-laponite hybrids. Al(OH)Pc was extracted over a G3 glass frit using acetone, yielding a transparent blue solution. To this solution, Hacac (acetylacetone) or Hhfa (hexafluoracetylacetone) were added in approximately 10-fold excess with regard to Al(OH)Pc and the mixture refluxed for 24 h (Al(OH)Pc-contents were calculated to be 3.133×10^{-6} mol×L^{-1} and 2.465×10^{-6} mol×L^{-1}, respectively, from the absorption spectra of Al(OH)Pc-LAP, i.e. blanks without diketonate). Consecutively aqueous laponite was added to the dried samples to give a 1 wt% solution (Fig.2.) and the dispersion stirred at room temperature for an additional 24 h. Before putting them to use as singlet oxygen catalysts, the solutions were diluted to 3.52×10^{-7} mol×L^{-1}.

Al(OH)Pc-laponite hybrids with silane modified rims. For better dispersability in organic media, laponite was treated with octadecylmethyldimethoxysilane (ODMS). 0.5 g of laponite powder containing 3.94×10^{-7} mol of Al(OH)Pc were dried in vacuum for 24 hrs at 120 °C in a round-bottom flask. Under nitrogen, 20 mL of dried toluene and 0.3 mL of ODMS were added to give a dispersion, which was heated to 75 °C for 1 hr. A blue powder was obtained after centrifugation and drying for 24 hrs at 60 °C in vacuum.

2. Optical measurements

Emission and excitation spectra. Emission and excitation spectra of the solutions were measured in a 1 cm quartz cell in 90° mode at room temperature on an Acton spectrometer (monochromators of 300 mm focal length and gratings with 1200 grooves / mm, a 450 W Xe-lamp as light source, photomultiplier tube P2) equipped with optical fibres. For the measurements the excitation slits were set to 2 nm, emission slits to 0.5 nm. Furthermore, the integration time was set to 300 msec and the

detector was operated at a voltage of 900 V. The gratings for the monochromators were blazed at 500 nm in both, emission and excitation.

Emission spectra of $^1O_2$ in water itself could not be obtained due to too small intensities. Instead a hydrophobised powder (see silane rim modification above) was dispersed in $CDCl_3$ by sonification. The $^1O_2$ emission spectrum reproduced in Figure S4 was obtained under 688 nm excitation of the sensitiser using a FluoTime300 spectrometer from PicoQuant equipped with a 300 W Xe lamp, two emission monochromators (Czerny-Turner, a gratings blazed 1250 nm with 5.4 nm/mm dispersion and 600 grooves/mm), and Glan-Thompson polarizers for excitation.

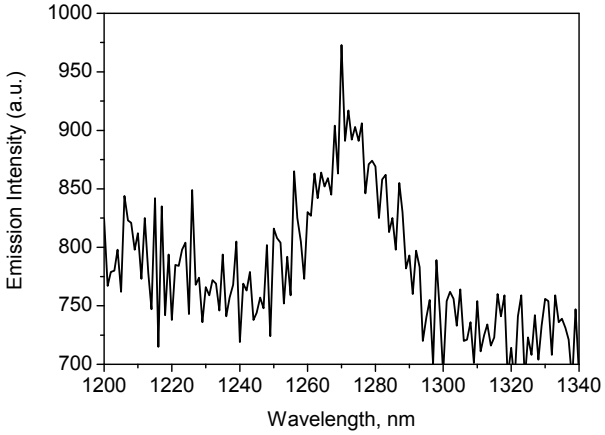


Fig. S4. Singlet oxygen emission spectrum in $CDCl_3$ obtained from sensitisation with Al(OH)Pc-LAP with ODMS grafted to the laponite rims.

Fluorescence quantum yields. Quantum yields Φ_F were determined relative to ZnPc(t-Bu)$_4$ dissolved in toluene, which is reported to have a quantum yield of 33 % or 37 %,[4, 5] by excitation at 620 nm; our calculations are based on the former value. The calculation was performed according to equation 1.[6]

$$\phi_F^S = \phi_F^R \times \frac{r_S}{r_R} \times \frac{I_S(1\text{-}10^{-A_R})}{I_R(1\text{-}10^{-A_S})} \times \left(\frac{n_S}{n_R}\right)^2 \qquad \textbf{eqn. 1}$$

(I = integral of the emission spectrum, A = absorbance of the solution at the excitation wavelength, $(n_S/n_R)^2$ = refractive index correction factor, R = reference, S = sample).

UV-Vis spectroscopy. To monitor whether monomeric or dimeric species were present, all samples were analysed by the means of absorption spectroscopy using an Ocean Optics HR 4000 CCD fibre spectrometer with a deuterium lamp and a 20 W halogen lamp as a light source. The integration time was set to 12 msec and an average of 50 measurements was chosen. As a reference the respective solvent has been chosen in any case. Furthermore, several measurements were crosschecked with an Analytic Jena Specord 200 Plus (double beam, 35 W Halogen).

Singlet oxygen quantum yields. The determination the $^1O_2$ efficiencies were carried out using the same apparatus as for the emission and excitation spectra. Illumination and calculation were carried out in analogy to previously published procedures.[6] For the measurements, an excitation slit width of 1.5 nm and an emission slit width of 1 nm was used and the gratings of the monochromators were set to 1 (excitation; blazed for 300 nm) and 2 (emission; blazed for 500 nm). The samples were contained in 1 cm cuvettes placed on a black stencil with a 1cm^2 window, and irradiated through the bottom with a 400 W Halogen lamp using an OG 590 nm cut-off filter to select the red part of the spectrum, giving a measured irradiance of 8 mW×cm^{-2}. The spectrum thus used for irradiation is reproduced in Fig. S5.

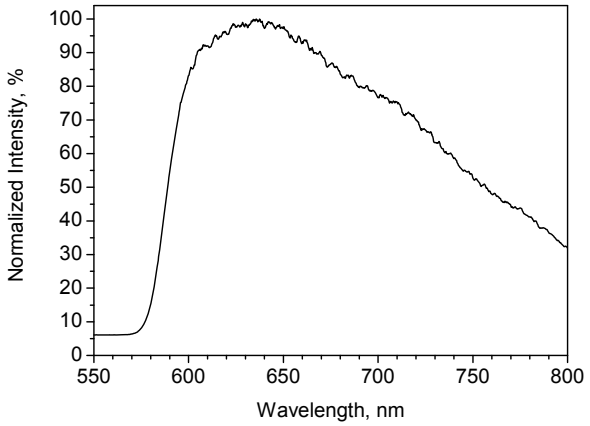


Fig. S5. Intensity distribution of the lamp used for irradiation of the samples for $^1O_2$ generation.

9,10-anthracenediyl-bis(methylene)dimalonic acid (ABMDMA, Fig. S6) was used to monitor the singlet oxygen quantum yield, as well as an aqueous solution of methylene blue as a reference. 0.459 mg of ABMDMA were dissolved in 10 mL of water. To dissolve ABMDMA completely, a drop of sodium hydroxide was added. This stock solution was wrapped in aluminium foil and kept refrigerated. All measurements were carried out in the dark assuring that the only illumination of the samples was provided from the light source. Prior to the measurements, 2 μL ABMDMA solution were added to 3 mL of every sample, avoiding incident light as much as possible. Subsequently, the depreciation of the fluorescence of ABMDMA under irradiation was recorded in 5 min intervals and measured. All samples were adjusted to an absorption of A = 0.1. As a reference, relative to which we evaluated our samples, a solution Methylene Blue in ethanol was used, which has a $^1O_2$ efficiency of 0.49 in ethanol under excitation at 632 nm.[7] Other authors report values very close at 0.5, for excitation at lower wavelengths as well. A comprehensive compilation covering "singlet oxygen yields from biologically relevant molecules", i.e. on other systems as well, up to 1999 has been given by Redmond and Gamlin.[8] Singlet oxygen quantum yields were calculated using equation 2.

$$\phi_{\Delta}^{S} = \phi_{\Delta}^{R} \times \frac{r_S}{r_R} \times \frac{\int_{\lambda_1}^{\lambda_2} I_0(\lambda)(1\text{-}10^{-A_R(\lambda)})d\lambda}{\int_{\lambda_1}^{\lambda_2} I_0(\lambda)(1\text{-}10^{-A_S(\lambda)})d\lambda} \qquad \textbf{eqn. 2}$$

(r = singlet oxygen generation rate from the slope of the monitor's bleaching vs. tome plot, λ_2-λ_1 is the irradiation wavelength interval, I_0 (λ) the incident spectral flow, which, being constant here, cancels out. Subscripts R and S indicate reference and sample.)

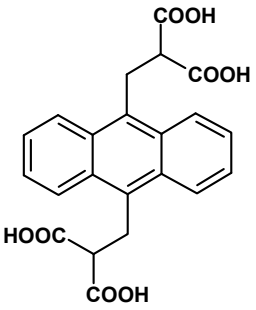


Fig. S6. Molecular structure of the fluorescent $^1O_2$ quencher (ABMDMA).

3. Preliminary photodynamic antimicrobial chemotherapy (PACT) tests

Initial PACT results on *Kocuria palustris* obtained from the pure Al(OH)Pc-LAP hybrids revealed unsatisfyingly low killing efficiencies. These were due to a dislocation, i.e. lacking proximity of the laponite hybrids to the bacteria, as seen by microscopic inspection. On the microscope slides, islands of laponite precipitates, well isolated from the bacteria, had formed. We therefore chose to modify the rims of the laponite disks with 3-aminopropyldimethylethoxysilane (APES) prior to Al(OH)Pc loading, as has previously been described,[9] which we expected to increase the interaction with the peptidoglycane at the bacteria's surface. As determined with fluorescamine, the rim modification rate amounted to 21 %.[10] The microscopic images (Fig. 7) now unambiguously show that the bacteria co-locate with the laponite particles. Using rim-modified laponites carrying approximately 5×10^{-7} Mol×L^{-1} Al(OH)Pc (with 0.2 wt % LAP in total) the killing rates now approached 85 %.

As we suspected at this stage, yet higher killing rates might be hampered by too low a total amount of Al(OH)Pc (approximately "only" 10^5 Al(OH)Pc per bacterium, at roughly 10^9 bacteria /ml), we used higher concentrations of Al(OH)Pc beyond the µ-molar level (e.g. 5×10^{-5} Mol×L^{-1}), disregarding the fact that dimers would be present as well, the dimers now possibly serving as a reservoir for monomeric Al(OH)Pc.

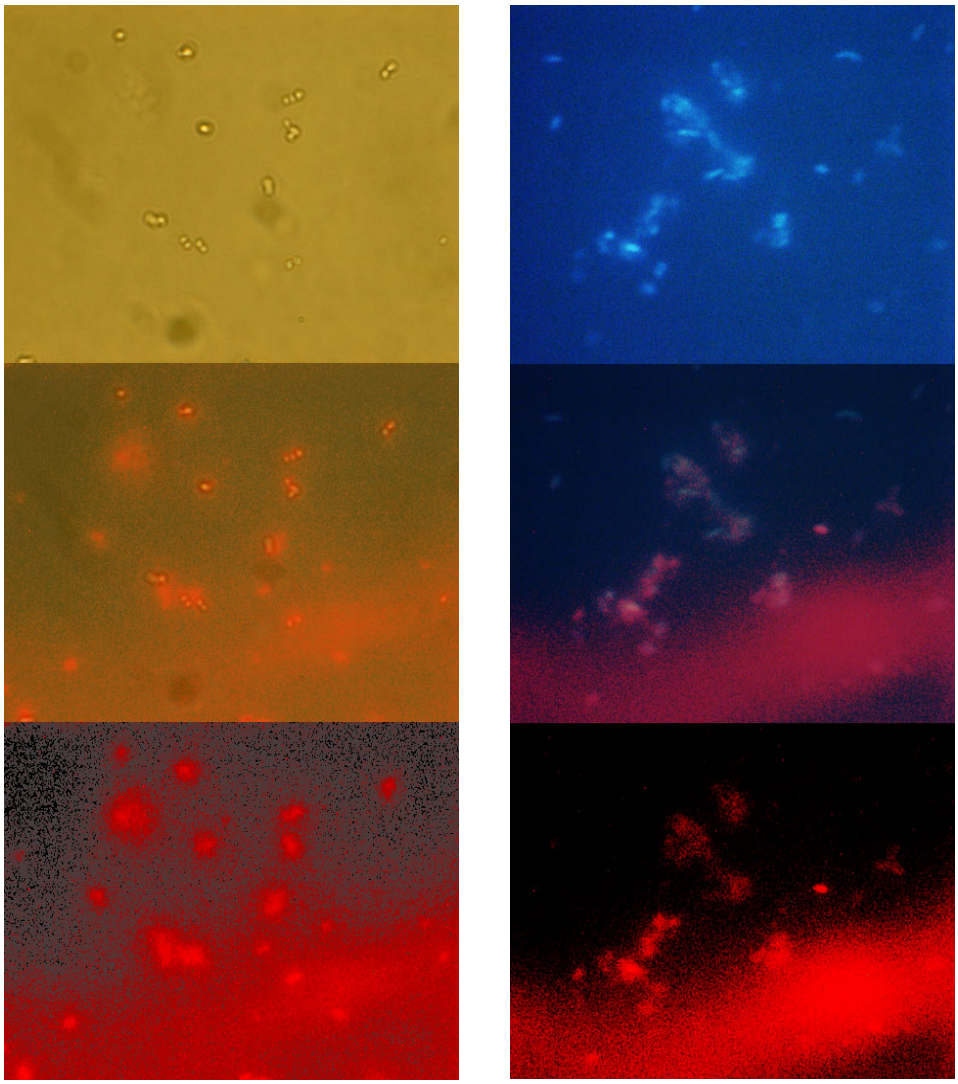


Fig. S7. Fluorescence microscopic images of *Kocuria palustris* (Gram-positive) and *Pseudomonas fluorescens* (Gram-negative) after irradiation (680 nm) in the presence of APES-modfied laponite rims. Left top transmission of *Kocuria palustris*, right top, fluorescence of *Pseudomonas fluorescens;* bottom pictures Al(OH)Pc under 380 nm excitation; middle, overlay.

A bacteria culture of *Staphylococcus aureus* (subspecies. *aureus* Rosenbach 1884, DSM 799) was thus grown overnight in CASO broth (Carl Roth), centrifuged for 5 min. at 4500 rpm and resuspended in the same volume of standard PBS (phosphate buffered saline). 2 ml bacteria suspensions each and Al(OH)PC-LAP-APES dispersion (or PBS for comparison) were mixed together and divided in two 50 ml sterile plastic (PP) tubes. The tubes were placed on a laboratory shaker (130 rpm). One tube was protected against ambient and parasitic light with aluminium foil, while the other tube was irradiated with a 680 nm LED with an optical energy output of 8 mW over 4.5 hours. Subsequently, the bacteria suspensions were diluted with buffer to 10^{-7} dilution and placed on the TSA (CASO) – agar plate (Oxoid). The droplet volume was 10 µl, for each dilution stage a threefold plating was done; the agar plates were incubated 20 h at 36 °C. 99.6 % of the bacteria could in this preliminary experiment be killed (see Fig. S7, S9).

Furthermore, another bacterial strain of *Kocuria palustris DG36*[11] (isolated from drinking water) was used to establish the findings above on Gram-positive bacteria. They were grown over night in CASO broth (Merck), centrifuged 10 Min at 2000 rpm (Heraeus Laborfuge 400) and resuspended in the same volume of 1 ml of the bacteria suspension and aq. Al(OH)PC-LAP-APES (0.2 wt%) dispersion (or PBS for comparison), each of which were mixed together and placed in sterile 15 ml glasses with a stirring bar. The glasses were placed on a magnetic stirrer (250 rpm). One tube was protected from parasitic light whereas the other tube was irradiated with a 680 nm LED (optical energy output 8 mW) within 4.5 hours. After that, the bacteria suspensions were diluted with buffer to a 10^{-6} dilution stage and plated (100 µl) on the LB – agar, made from lysogeny broth (Scienova) and agar-agar. The killing rate amounted to 98.6 % in these preliminary experiments (Fig. S8 and S10).

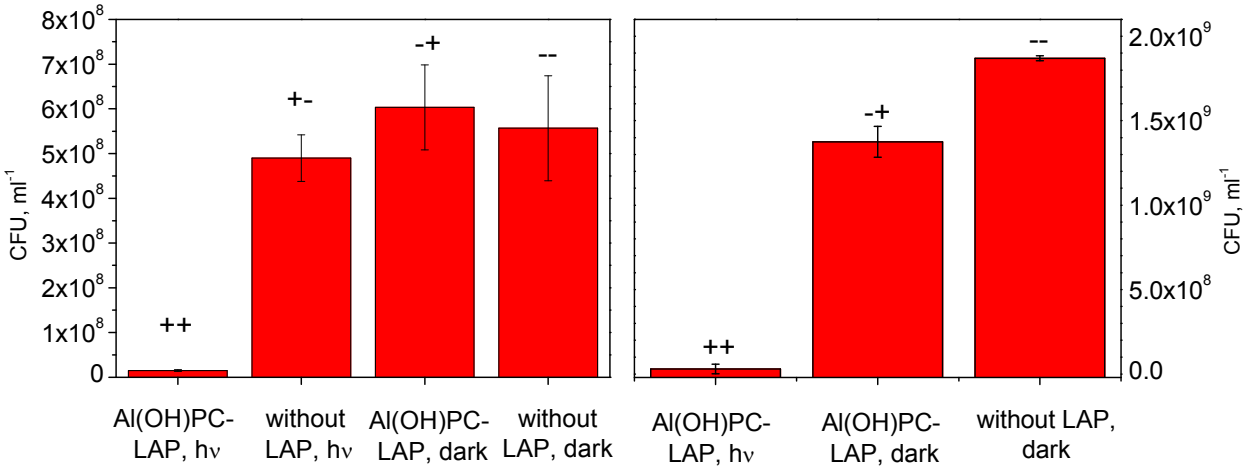


Fig. S8. Left: Number of colony forming units (CFUs) of *Staphylococcus aureus* 1884, (DSM 799) treated with Al(OH)Pc (Al(OH)Pc-LAP-APES). Right: *Kocuria palustris.*

Fig. S9. Example of CFU counting on exposures to 0 W/cm^2 (left) and 8 mW/cm^2 (right) of 680 nm radiation for *Staphylococcus aureus*.

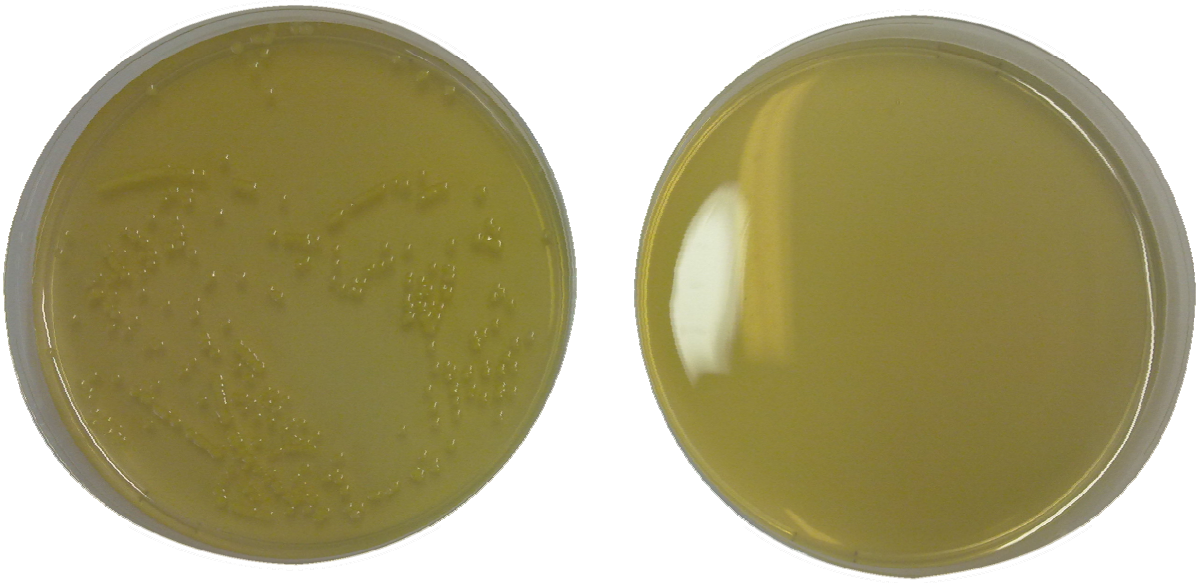


Fig. S10. Example of CFU counting on exposures to 0 W/cm^2 (left) and 8 mW/cm^2 (right) of 680 nm radiation for *Kocuria palustris.*

We have furthermore performed PACT experiments on Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), for which we have not found similar killing rates as yet, they typically amounted to around 60 % only. However, we believe that an adjusted laponite chemistry will lead to similarly encouraging results, which will provide an important tool in the defeat of pathogenic bacteria, *Pseudomonas aeruginosa* in particular.

4. References quoted in ESI

1 F. Ghani, J. Kristen and H. Riegler, *J. Chem. Eng. Data*, 2012, **57**, 439-449.
2 J. Ellis, A. H. Jackson, G. W. Kenner and J. Lee, *Tetrahedron Lett*., 1960, **1**, 23-27.
3 D. L. Ledson and M. V. Twigg, *Inorg. Chim. Acta*, 1975, **13**, 43-46.
4 D. A. Fernández, J. Awruch and L. E. Dicelio, *Photochem. Photobiol.*, 1996, **63**, 784-792.
5 D. S. Lawrence and D. G. Whitten, *Photochem. Photobiol.*, 1996, **64**, 923-935.
6 J. Voskuhl, U. Kauscher, M. Gruener, H. Frisch, B. Wibbeling, C. A. Strassert and B. J. Ravoo, *Soft Matter*, 2013, **9**, 2453-2457.
7 Y. Usui, M. Tsukada and H. Nakamura, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 379-384.
8 R. W. Redmond and J. N. Gamlin, *Photochem. Photobiol.*, 1999, **70**, 391-475.
9 P.A. Wheeler, J. Wang, J. Baker, L.J. Mathias, Chem. Mater., 2005, **17**, 3012-3018.
10 T. Felbeck, K. Hoffmann, M. M. Lezhnina, U. H. Kynast, and U. Resch-Genger, *J. Phys. Chem. C*, 2015, **119***,* 12978–12987.
11 G. Kovács, J. Burghardt, S. Pradella, P. Schumann, E. Stackebrandt and K. Màrialigeti, *Int. J. Syst. Evolut. Microbiol.*, 1999, **49**, 167-173.