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Supporting information

Zinc phthalocyanine activated by conventional indoor light makes a highly efficient antimicrobial material from regular cellulose

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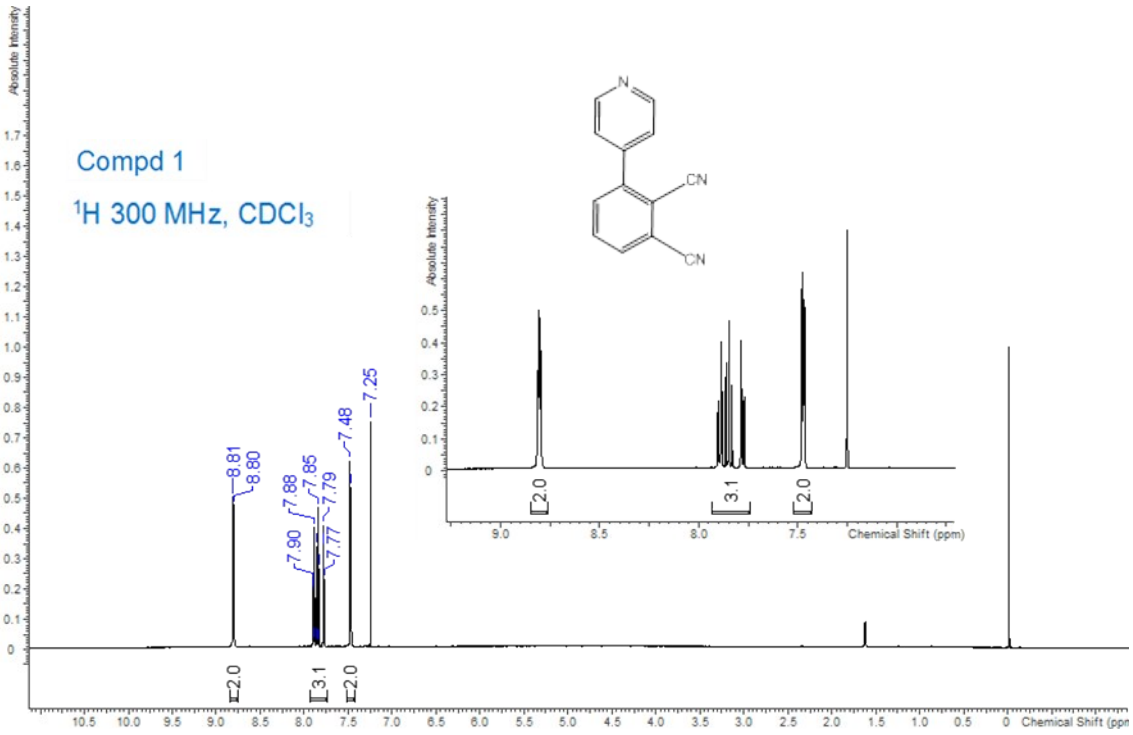
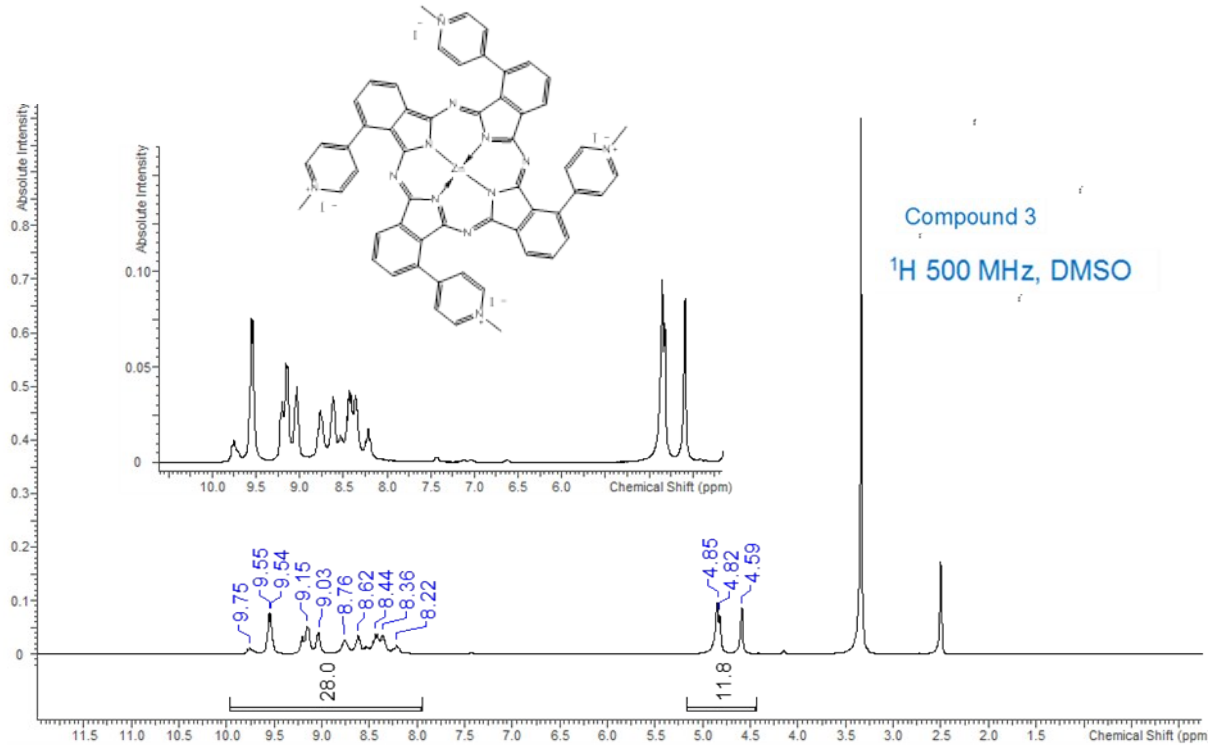
1. Synthetic methods and characterization data

The compounds 2,3-dicyanophenyl trifluoromethanesulfonate 4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl) pyridine were synthesized according to the literature procedure.[1, 2] The synthesis of pthalocyanine **2** and its cationic form **3** has been reported in our earlier paper.[3] An improved procedure for gram scale synthesis of 3-(pyridin-4-yl) phthalonitrile **1** is described below.

Improved Synthesis of 3-(pyridin-4-yl) phthalonitrile **1**:

Pyridine boronate ester (0.726 g, 3.78 mmol), triflate phthalonitrile (1.0 g, 3.78 mmol), $PdCl_2(dppf)\bullet DCM$ (0.092 g, 0.1134 mmol) and K_3PO_4 (2.4 g, 11.31 mmol) were dissolved in a 1:1 mixture of water and toluene (40 mL). The reaction mixture was stirred at room temperature and saturated by bubbling with argon for 15 minutes. After saturation, the mixture was heated with vigorous stirring at 90 °C for 2 h under argon. The reaction mixture was cooled to room temperature, extracted with $CHCl_3$, washed with brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to yield crude product. Re-precipitating of crude product from a 9:1 mixture of $CHCl_3$/ hexane yielded 0.603 g of brown solid as pure product 60 %. The analytical data (NMR, MS etc) was identical to the previously reported values.[3]


Compd 1
^{1}H 300 MHz, CDCl$_3$
8.81
8.80
7.90
7.88
7.85
7.79
7.77
7.48
7.25
2.0
3.1
2.0
CN
CN
N
Absolute Intensity
Chemical Shift (ppm)


Compound 3
^{1}H 500 MHz, DMSO
9.75
9.55
9.54
9.15
9.03
8.76
8.62
8.44
8.36
8.22
4.85
4.82
4.59
28.0
11.8
Absolute Intensity
Chemical Shift (ppm)

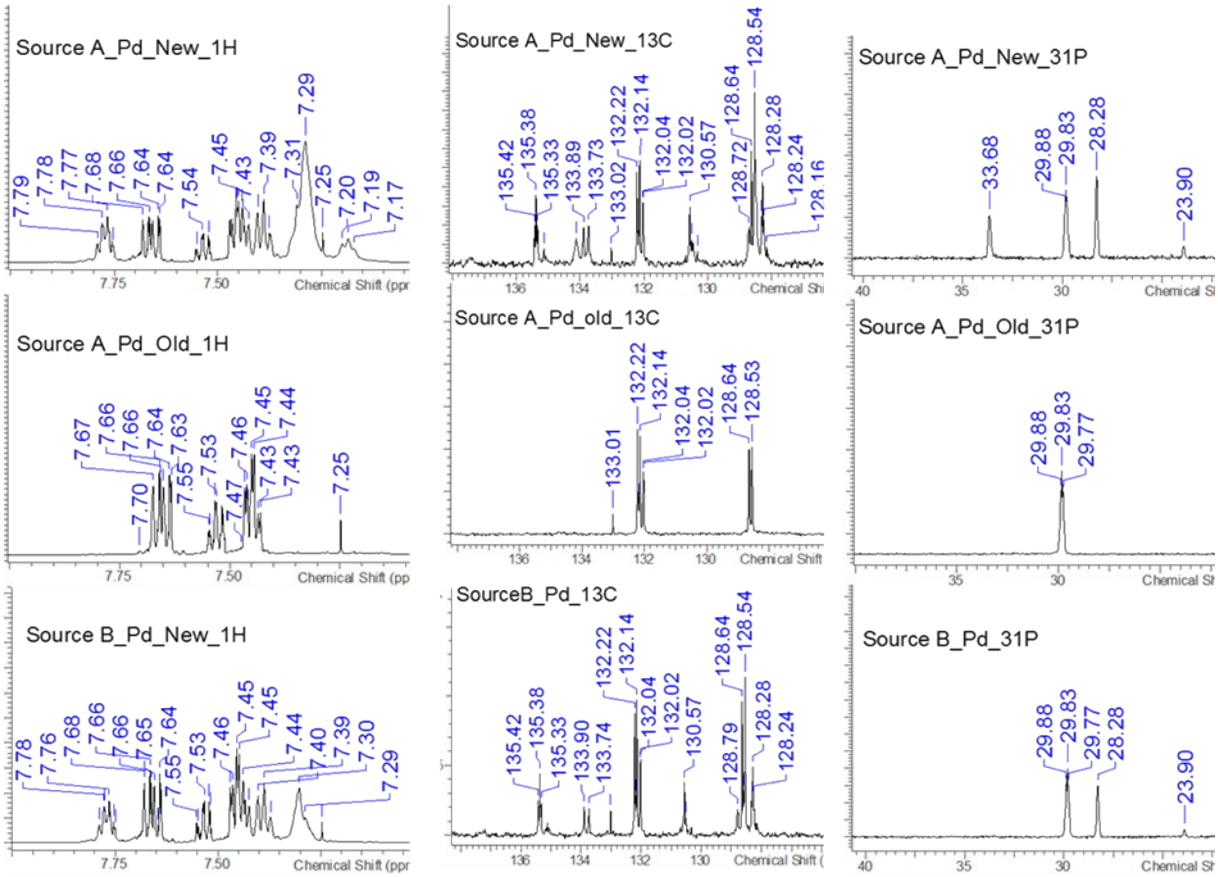
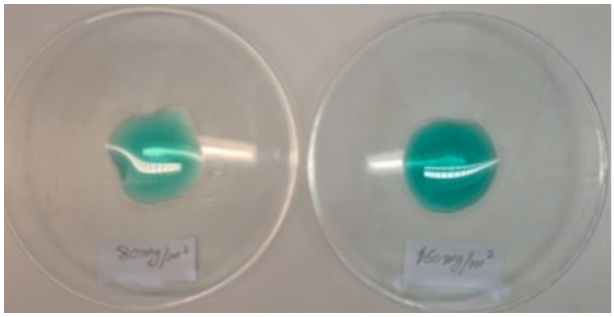


Figure: NMR spectra of different batches of $Pd(PPh_3)_4$ catalyst used in the study, showing different ratio of phosphine and phosphine oxide contents

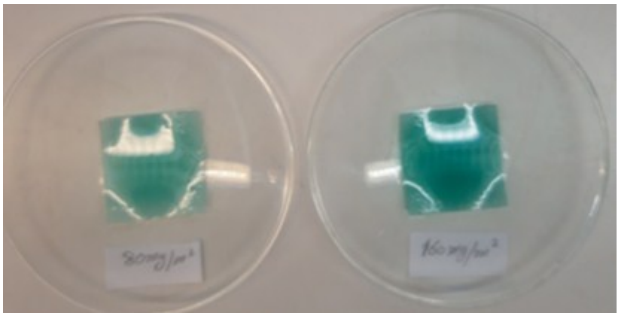
2. Antimicrobial studies

2.1. PACT paper samples

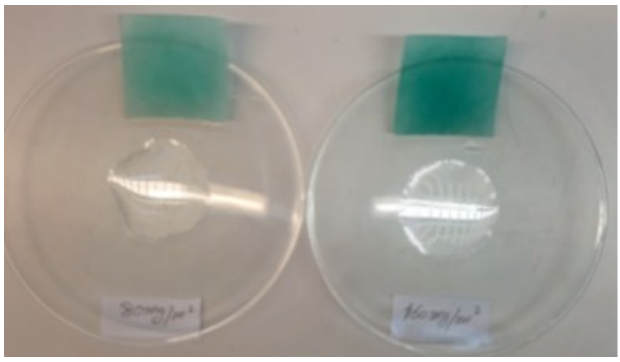
Paper samples containing 80 and 160 mg/m^2 of tetracation phthalocyanine **3** were prepared as illustrated below:



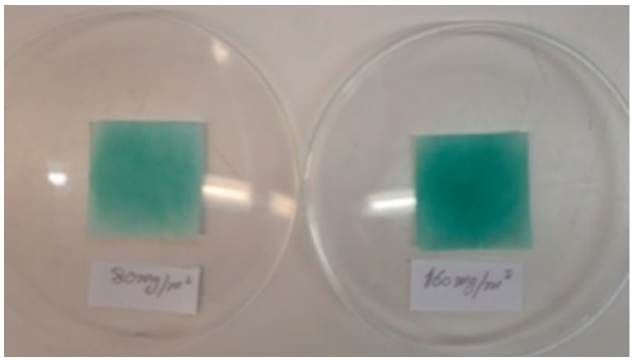
Aq. dye solution



Impregnated paper



Paper after dye absorption



PACT paper ready

2.2. Illumination source

Diode lamp OSRAM Star PAR16 80 W 575 lm GU10 has been used to generate 270 lux emittance.

Illumination power density has been measured with TKA-PKM(09) apparatus that allows to monitor luminous emittance (E, lux) in visible spectrum range (380-760 nm), percent flicker (PF, %) and luminance (L, cd/m^2) with overlapping method of extended self-luminous objects (self-luminous object with an extended spatial distribution) in visible range of the spectrum 380-760 nm.

2.3. Microorganisms and growth conditions

Clinical isolates of *Staphylococcus aureus* strain 88 (MRSA), *Enteroccus faecalis* 583 (vancomycin-resistant) and *Candida albicans* 604M, resistant to fluconazole, have been used for the study. Microorganisms were kept at -75°C in casein-soymeal-peptone broth with 10-15% of glycerol. Before experiment a bacterial strain was activated from cryoconservation by seeding it on CASO Agar medium («Sifin», Germany) and incubated at (35±2) ° C within 18-20 hours. *Candida albicans* 604M was seeded on Sabouraud Dextrose Agar medium (Becton Dickinson, Germany) and incubated at 35±2° C within 48 hours.

2.4. Cell culture preparation

Overnight bacterial strains were seeded on Mueller Hinton Broth («Sifin», Germany) and adjusted to have 0.5 McFarland standard turbidity. Microorganism suspension turbidity was determined by using densitometer DEN-1 (Densitometer (suspension turbidity detector) BioSan, Riga, Latvia).

Microorganisms were incubated further at 35±2° C to obtain 1 McF optical turbidity. Then, they were precipitated by centrifugation and washed with phosphate buffer saline (PBS). Cell densities were adjusted with PBS to reach 0.5 McF that equals to ca. 1.5×10^8 CFU/ml (cells/ml).

To perform experiment with *Candida albicans* 604M, individual colonies were collected from Sabouraud agarose medium, suspended in PBS, pH 7.2-7.6 (Eco service, Russia), precipitated by centrifugation and washed twice with PBS. Cell density was adjusted in PBS solution to reach 3 McF turbidity that equals to 3×10^7 CFU/ml.

2.5. Experiment conditions

Each 6 mm disk of the paper impregnated with zinc phthalocyanine **3** has been placed into Petri dish (40 mm diameter) and covered with 25 µl of cell suspension of each microorganism suspended in PBS. Calculated infection dose equals to ca. $3.7x10^6$ (lg 6.5) for bacterial strains and ca. $7.5x10^5$ (lg 5.87) for *Candida albicans*.

Experiment included following experimental variants:

1. Control measurements at 4000 lux illumination;

2. Dark toxicity control measurements;

3. Photoinactivation of microorganisms at 270 lux illumination;

4. Photoinactivation of microorganisms at 4000 lux illumination.

Illumination of the samples was performed within either 30 or 60 minutes. Distance between illumination source and PACT paper disk was kept constant to be 14 cm to get 4000 lux. Office light has been utilized to obtain luminous emittance of 270 lux. Black background below Petri dish has been implemented to avoid the diffusely-reflecting effect on the results of the experiments

A 2-3 lux illumination has been provided in the end of all experimental variants. Series of dilutions was prepared having already the paper disk inside the Petri dish. Ten-fold dilutions have been already performed in vials. An aliquote of 0.1 ml of corresponding dilution has been introduced into Petri dish of 90 mm in diameter. All Petri dishes, including first one with the paper disk, were filled with selective media: for *S. aureus* – BD™ Mannitol Salt Agar (BD, France), for *Enterococcus faecalis* – BBL™ Enterococcosel ™ Agar (BD, France), for *Candida albicans* - BBL Sabouraud Dextrose Agar with Chloramphenicol (BD, France), and were left to harden the medium. They were incubated within 24-96 hours at 35±2° C.

Each experimental variant has been reproduced at different days in triplicates.

Microorganisms maximal CFU values have been achieved after 72 hours of incubation for bacterial strains and after 96 hours for *Candida*. Further increase in CFU values hasn't been observed. Each experimental variant has been analyzed in terms of CFU average values in Petri dishes, recalculated in respect to dilution and converted into $\log_{10}$ values ($\log_{10}$ CFU/ml).

2.6. Statistics analysis.

SPPS (SPSS: An IBM Company, USA) has been employed for statistics analysis. Figures have been plotted by using OriginPro 2018 software (OriginLab Corp., USA).

Briefly, testing for normality distribution for repeated measurements or for intergroup comparison in terms of $\log_{10}$ CFU/ml values has been performed by using Shapiro-Wilk`s test. Average mean (M) and standard deviation (SD) values has been used to analyze the data with normal distribution. ANOVA dispersion analysis with a posteriori tests for multiple comparison by using Tukey criterion for repeated measurements has been utilized to compare

the values of $\log_{10}$ CFU/ml in respect to both luminous emittance and exposure time. Testing of statistical hypotheses has performed by suing critical significance level of 95%, i.e. difference was statistically significant when $p < 0.05$. Figures represent average mean values, mediana, and standard mean square error.

Evaluation of the difference between controls and experimental groups has been carried out by using Mann-Whitney U-test when distribution of the values was different from normal. Hence, mediana values have been used for graphical presentation of the data.

2.7. Control experiments with *S.auresus 88*

Data analysis of *S. aureus* 88 cell viability under experimental conditions by using descriptive statistics method resulted in mean values (in lg10 CFU/ml, min 5.6, max 6.8, M 6.2, SD 0.27), whilst the compared averages had normal distribution (at $\alpha=0.05$ according to Shapiro–Wilk test p value>0.4; Levene's homogeneity of variables p>0.76). Hence, significant reduction in *S. aureus* 88 CFU/ml under different experimental conditions hasn't been observed (p<0,001). Figure S1 shows the $\log_{10}$ CFU/ml values distribution for different experimental groups.

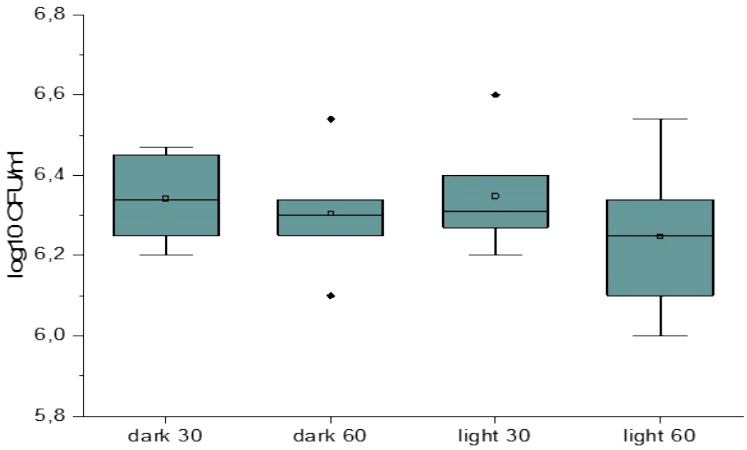


Figure S1. Viability values of *S. aureus* 88 suspension, applied on 80 mg/cm^2 PACT paper, under different conditions: dark conditions within 30 and 60 minutes (dark 30 and dark 60, respectively) in respect to control paper sample without **3** but upon 4000 lux illumination within 30 and 60 minutes (light 30 and light 60).

All control experiments showed almost the same values for viability of *S. aureus* 88 (in $\log_{10}$ CFU/ml) (Fig. S2)

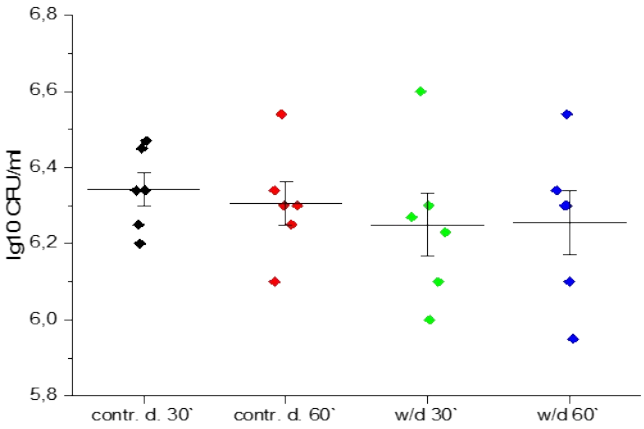


Figure S2. Viability of control *S. aureus* 88 suspensions in the dark when applied on top of either the PACT paper (contr.d.) or blank sample paper (w/d) within 30 or 60 minutes. Mean values, averages and relative standard deviations are depicted. Max and min values for combined sample are 5.95-6.6 log10 CFU/ml, respectively ($p<0.001$).

There was no significant difference between $\log_{10}$ CFU/ml values for *S. aureus* in blank paper controls performed under illumination within 30 and 60 minutes ($p= 0.787$). Further, all controls values have been combined into one mean value.

3. References

1. J. Ranta; T. Kumpulainen; H. Lemmetyinen; A. Efimov *J. Org. Chem.* 2010, **75,** 5178-5194.
2. T. Maki; K. Ishihara; H. Yamamoto, *Org. Lett*. 2005, **7**, 5043-5046.
3. L. George; A. Mueller; B. Roeder; V. Santala; A. Efimov, *Dyes Pigm*. 2017, **147**, 334-342.