Supporting information

A search for enhanced photodynamic activity against *Staphylococcus aureus* planktonic cells and biofilms: the evaluation of phthalocyanine-nanodiamonds-Ag nanoparticle nanoconjugates

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Equipment

A Shimadzu UV-2250 spectrophotometer and a Varian Eclipse spectrofluorimeter were used to record all the ground state absorption spectra and the fluorescence excitation including emission in solution, respectively. Time correlation single photon counting (TCSPC) equipped with a Picoquant GmbH containing a LDH-P-670 diode laser with a 44 ps pulse width and 20 MHz rate repetition was used to determine fluorescence lifetimes for all complexes. Triplet state quantum yields were determined using a laser flash photolysis system consisting of an LP980 spectrometer with a PMT-LP detector and an ICCD camera (Andor DH320T-25F03). The signal from a PMT detector was recorded on a Tektronix TDS3012C digital storage oscilloscope. The excitation pulses were produced using a tunable laser system consisting of an Nd:YAG laser (355nm, 135mJ/ 4–6 ns) pumping an optical parametric oscillator (OPO, 30mJ/3–5 ns) with a wavelength range of 420–2300 nm (NT-342B,Ekspla). Triplet lifetimes were determined by exponential fitting of the kinetic curve using Origin Pro 8 software. Singlet oxygen determination was carried out in a general electric quartz line projector lamp combined with a 600 nm cut off filter along with a water filter. An additional interference filter (Intor, 670 nm having a bandwidth of 40 nm) was aligned before the sample. ^{1}H and ^{13}C

NMR measurements in deuterated DMSO were performed using a Bruker® AVANCE 600 MHz NMR spectrometer. A Bruker AutoFLEX III Smartbeam MALDI-TOF mass spectrometer was employed for the recording of mass spectra. Infrared spectroscopy was performed using a Bruker Alpha IR (100 FT-IR) spectrophotometer with universal attenuated total reflectance (ATR). A Zeiss Libra 120 TEM operating at 80 kV and an INCA PENTA FET coupled to the VAGA TESCAM using 20 kV accelerating voltage were used to record transmission electron microscopy (TEM) micrographs. Raman spectroscopy data were collected on a Bruker Vertex 70-Ram II Raman spectrometer equipped with a 1064 nm Nd:YAG laser and liquid nitrogen cooled germanium detector. A Metrhom Swiss 827 pH meter was used for pH measurements. HERMLE Z233M-2 centrifuge was used for the harvesting of the bacteria cells. PRO VSM-3 Labplus Vortex mixer was used for the homogenization of the bacteria suspension. A thermostatic Oven was used for incubation processes. The Optical density of the bacteria was determined using the LEDETECT 96. Scan® 500 automatic color colony counter was used to evaluate the colony forming units CFU/mL of the bacteria. Irradiation for PACT studies was conducted using Modulight (ML7710-680/690-RHO) with emissions at 670 nm.

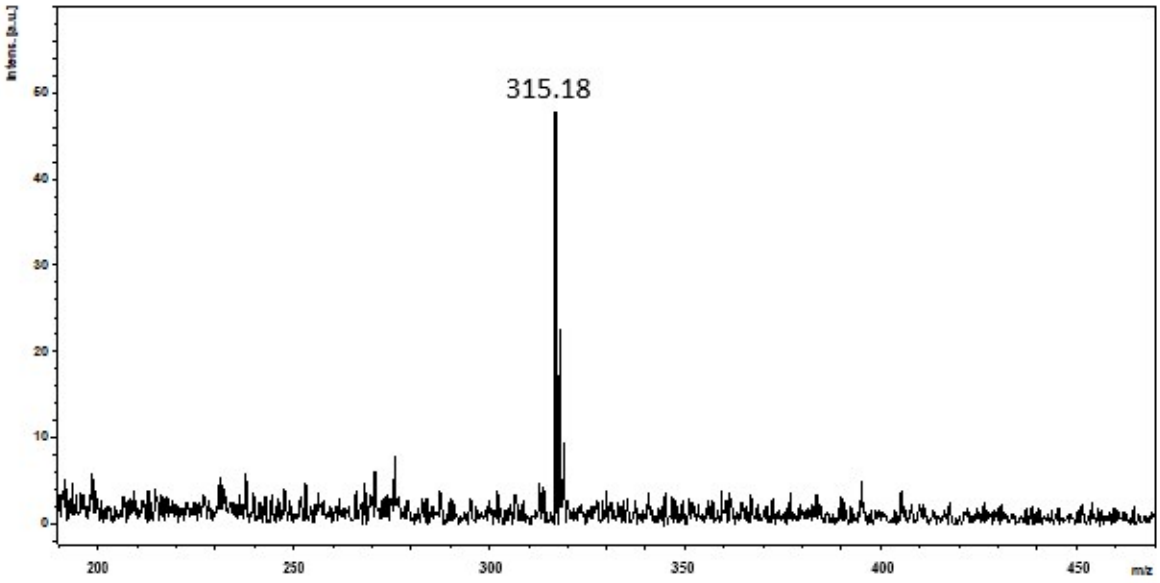


Fig. S1. Mass spectrum of compound **1**

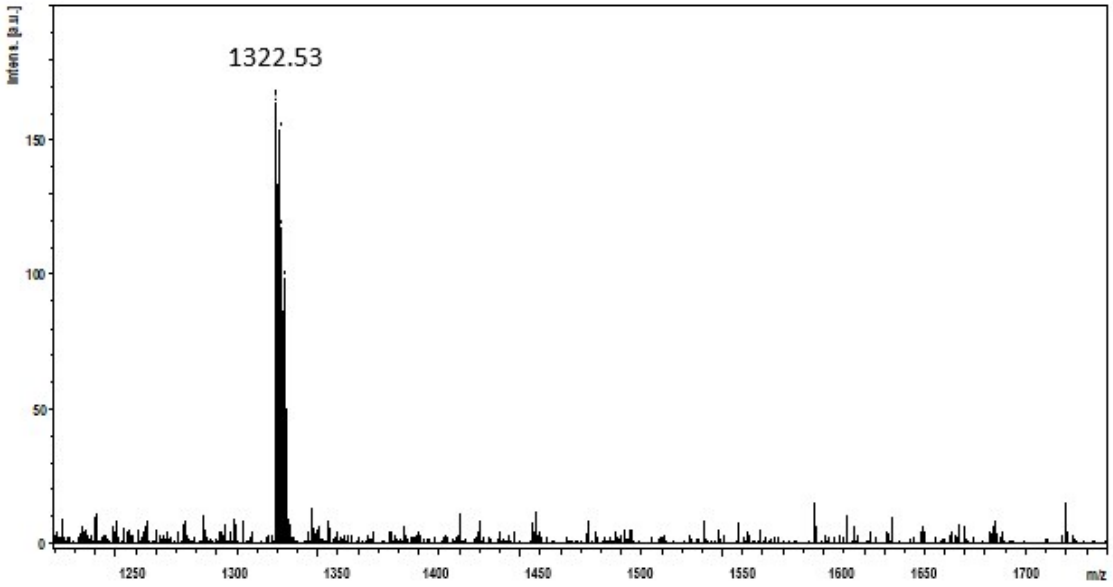


Fig. S2. Mass spectrum of complex **2**

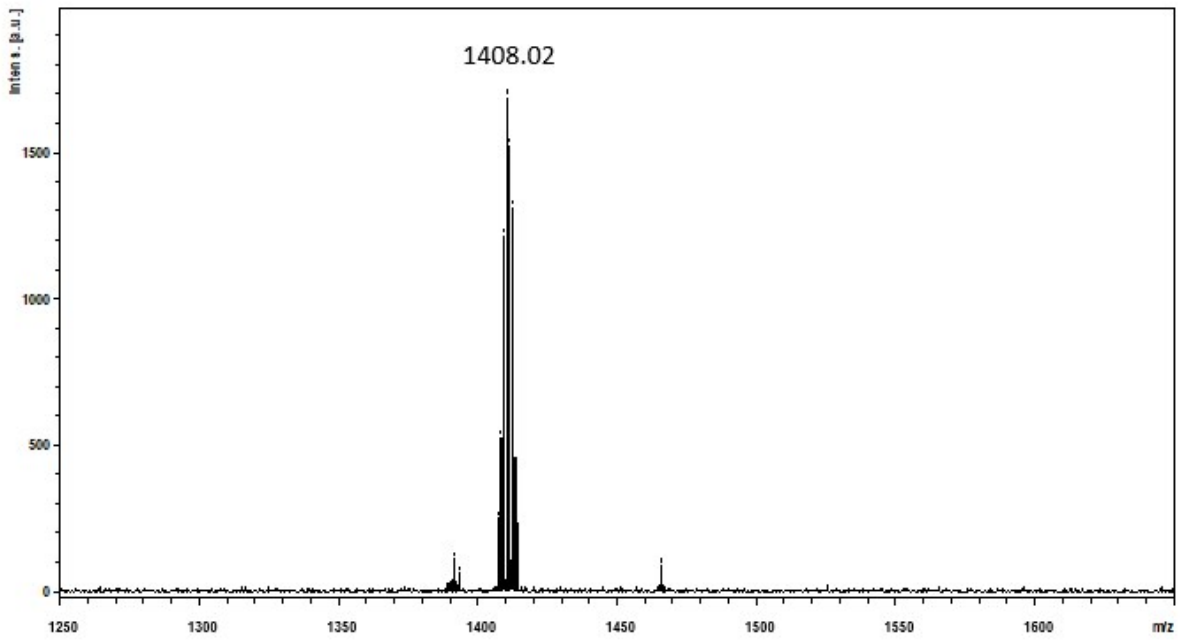


Fig. S3. Mass spectrum of complex **3**

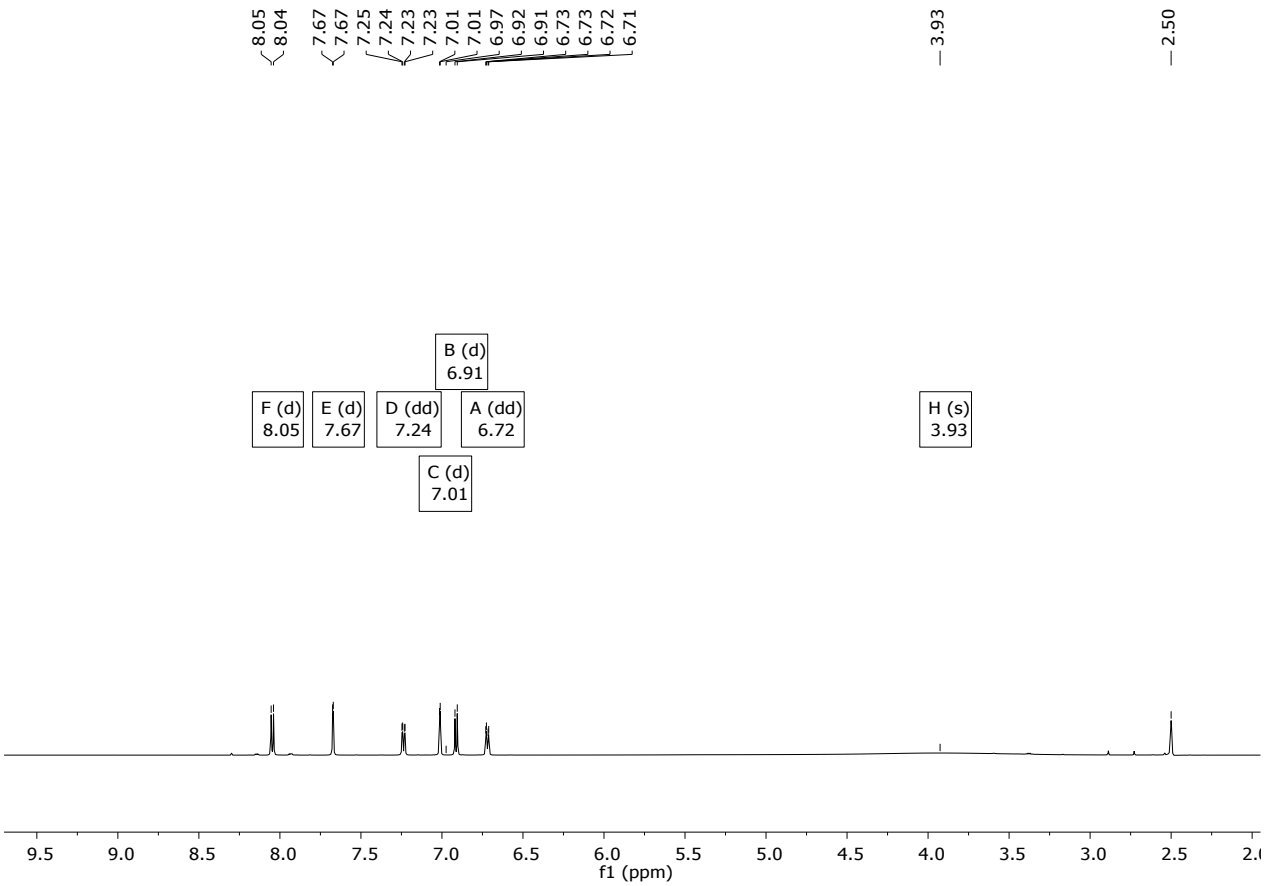


Fig. S4. ^{1}H NMR spectrum of compound **1**

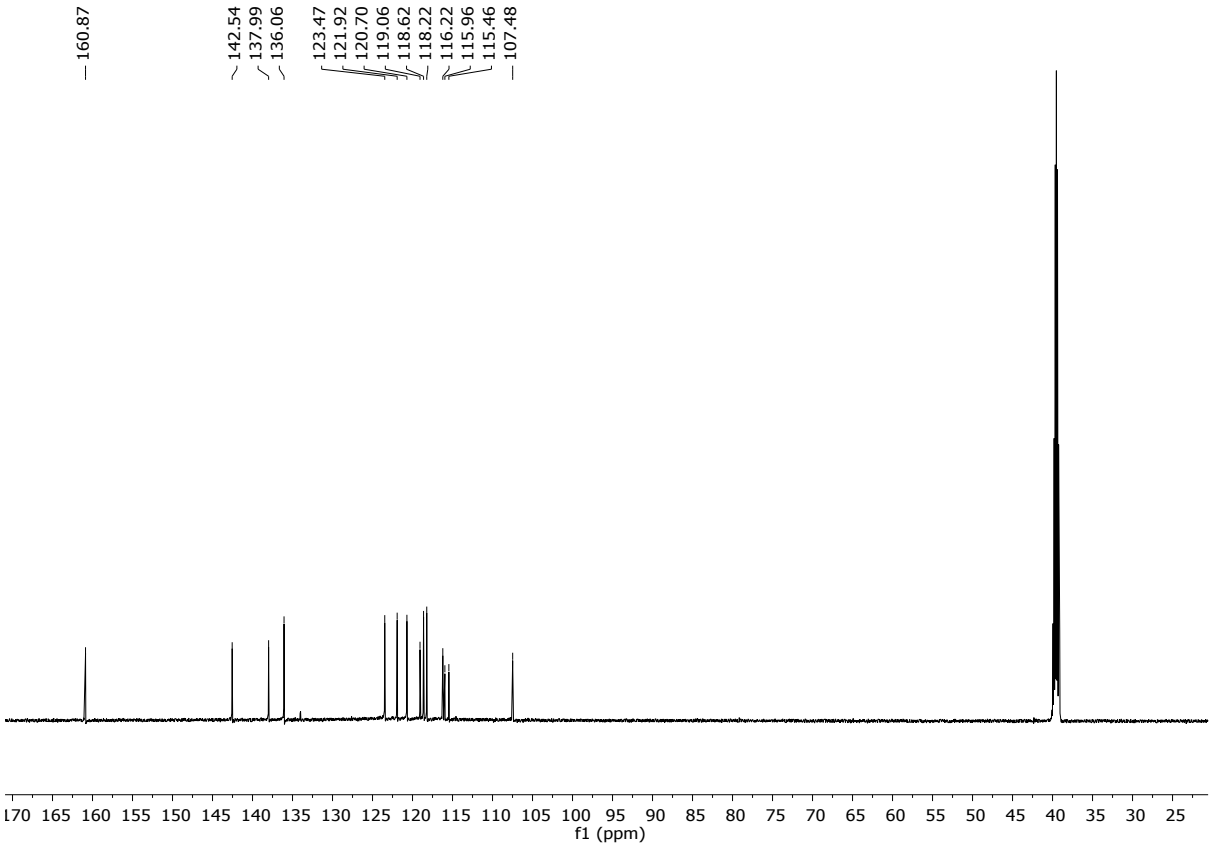


Fig. S5. ^{13}C NMR spectrum of compound **1**

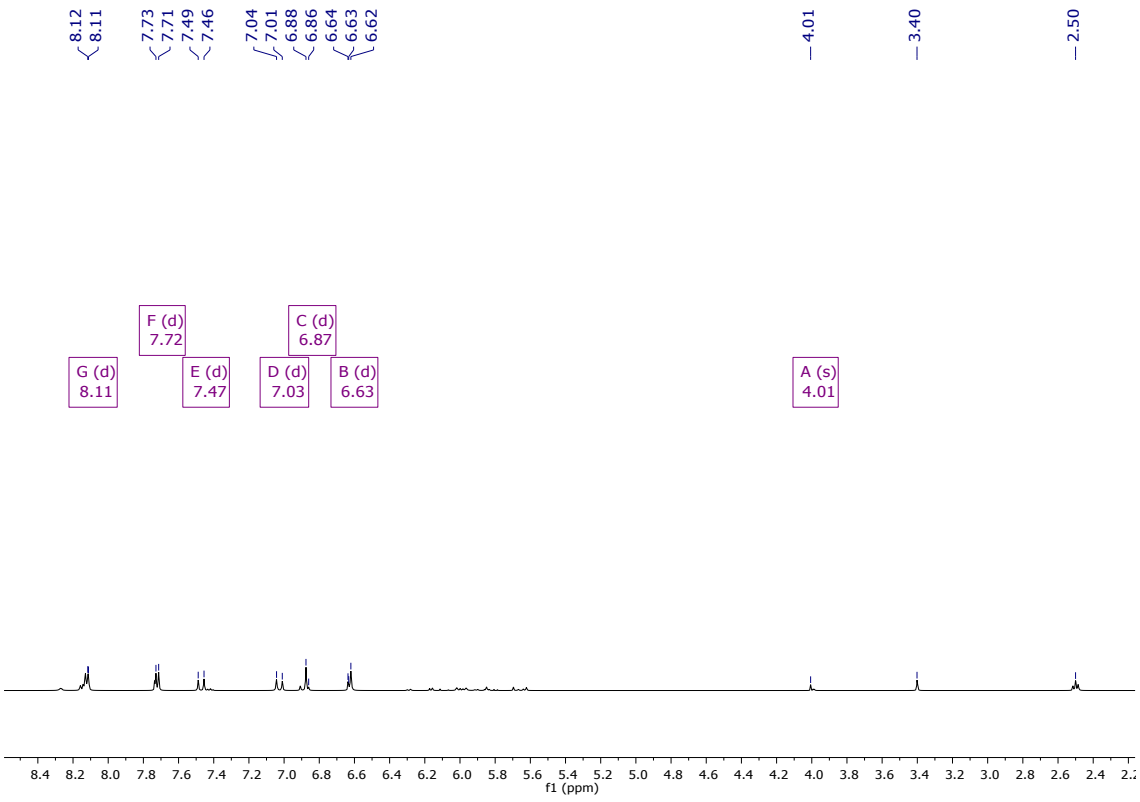


Fig. S6. ^{1}H NMR spectrum of complex **3** as an example

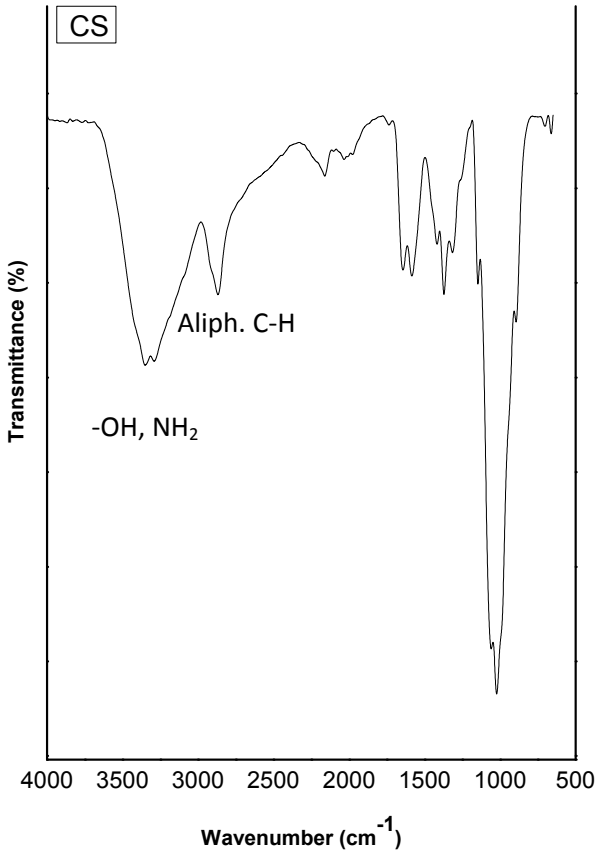


Fig. S7. FT-IR graph of chitosan (CS)

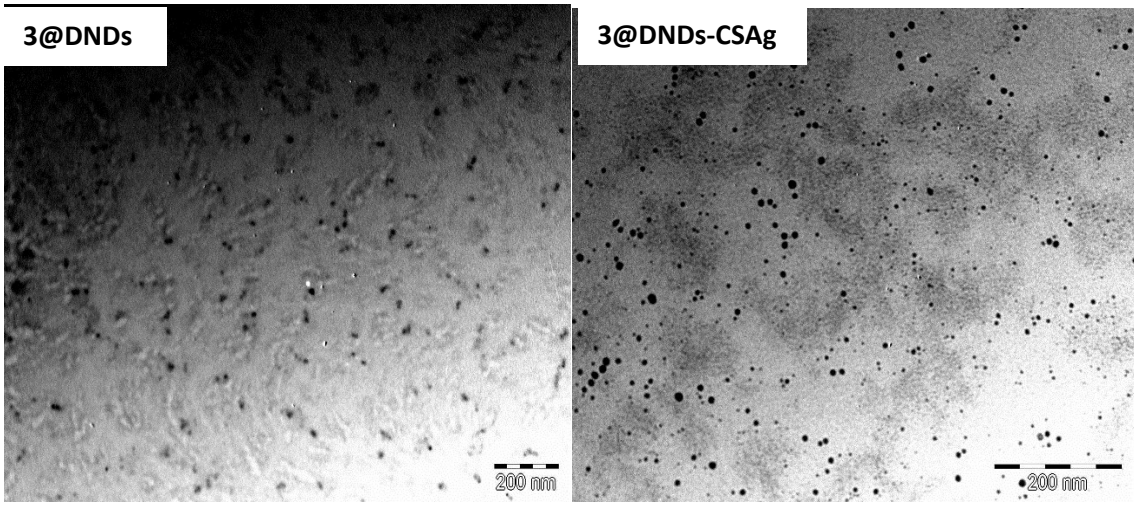


Fig. S8. TEM images of **3**@DNDs and **3**@DNDs-CSAg nanoassemblies showing the morphology and size increase upon conjugation.

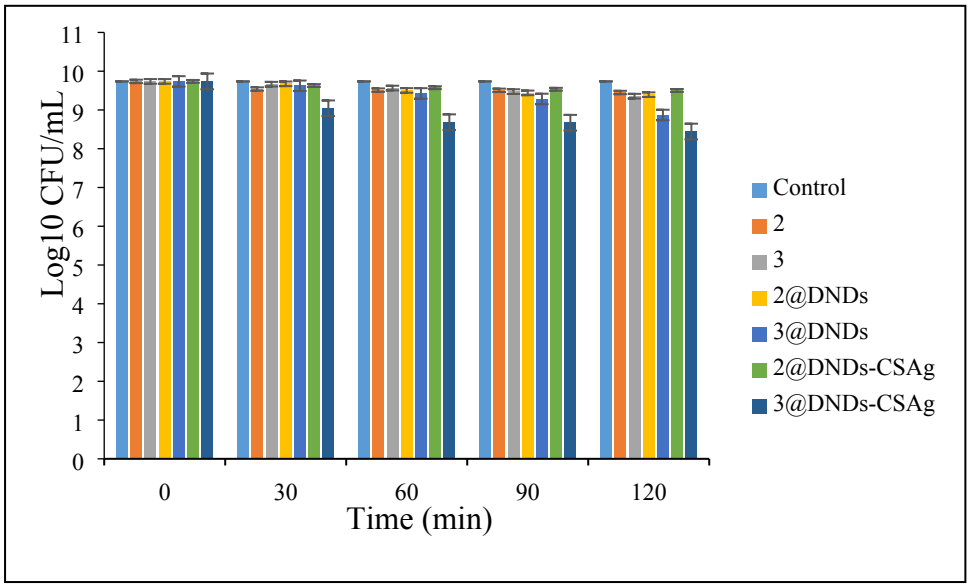


Fig. S9 Dark toxicity studies on *S. aureus* planktonic cells using Pcs alone and the Pcs@DNDs conjugates (Concentration of the Pcs and conjugates = 10 µg /mL. Data represent the mean ± SD. Treatment time = time left in the dark.

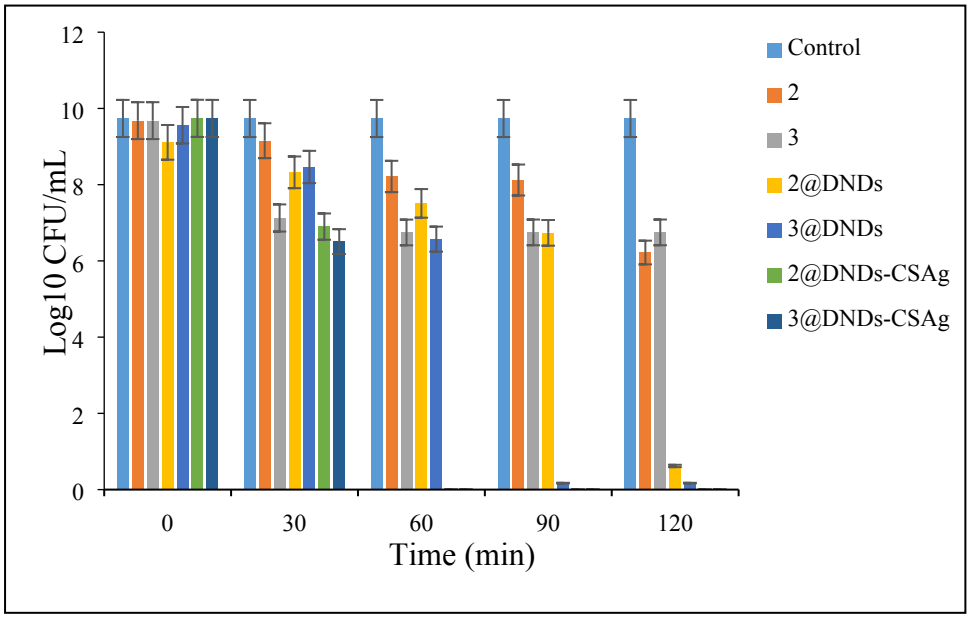


Fig. S10 Photoinhibition studies on S. aureus planktonic cells using Pcs alone and the Pcs@DNDs conjugates (irradiation at 670 nm). Concentration of the Pcs and conjugates = 10 µg /mL. Data represent the mean ± SD (standard deviation).

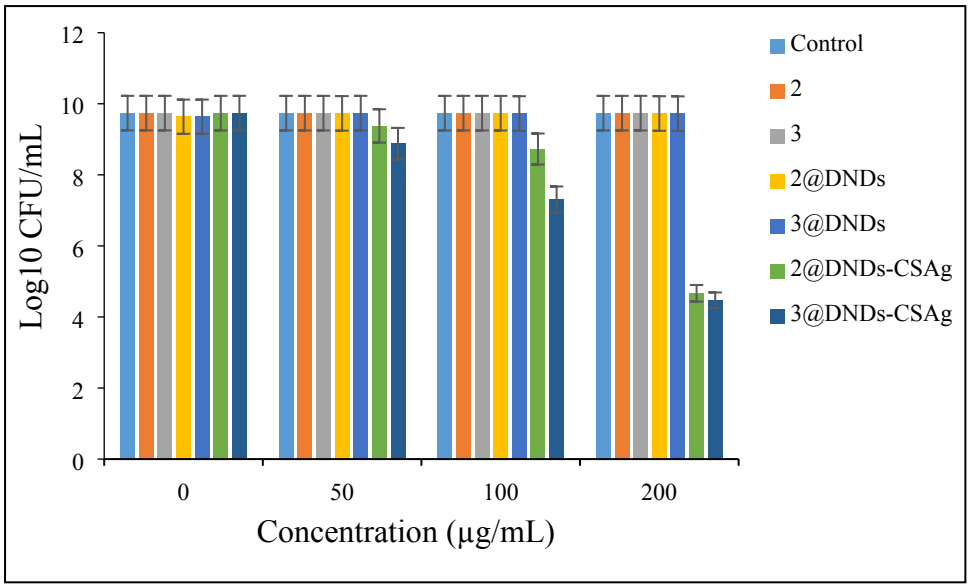
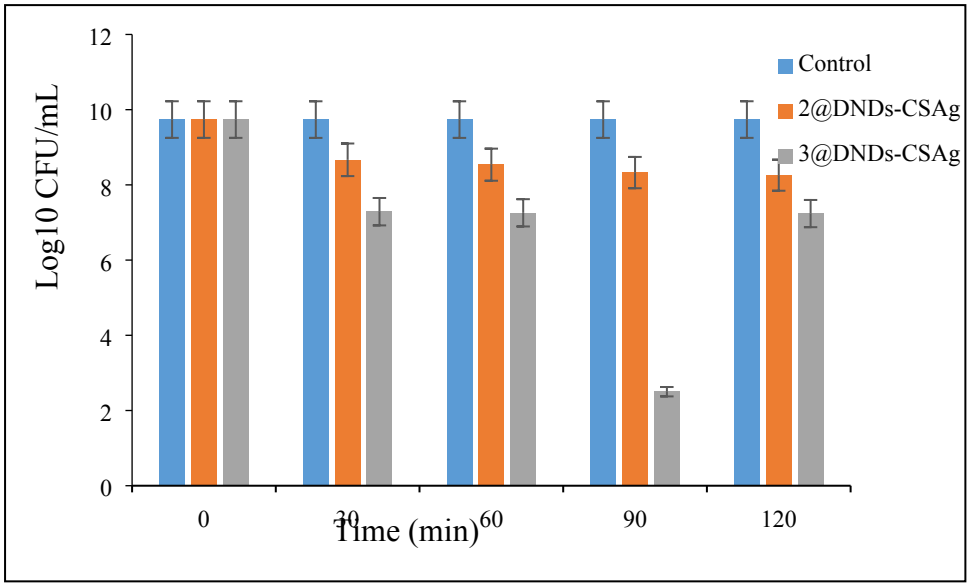


Fig. S11 Bacteria survival graph of photodynamic antimicrobial chemotherapy on S. aureus biofilm after 30 min of irradiation at concentration of 50, 100 and 200 µg/mL. Data represent the mean ± SD.

A



B

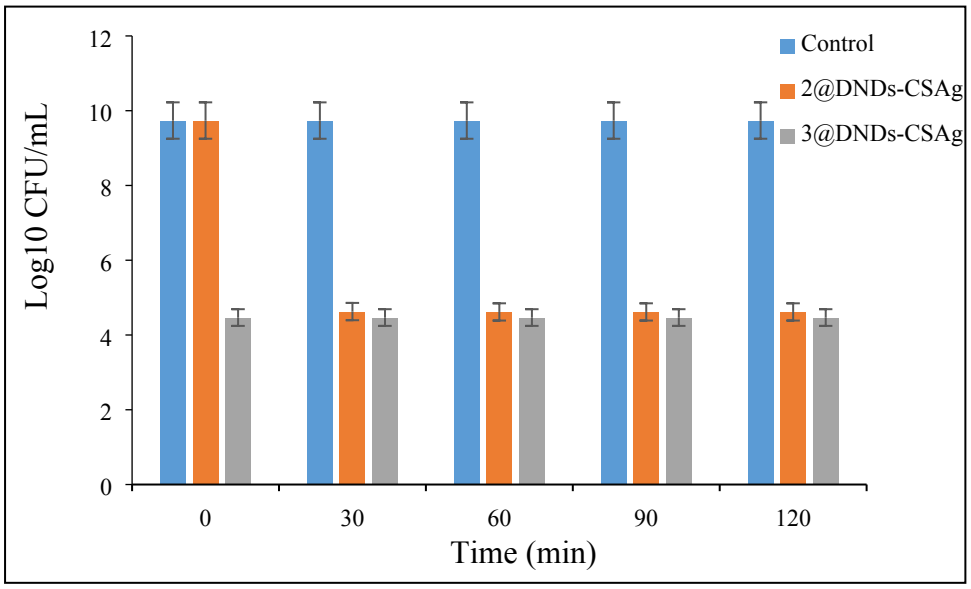


Fig. S12. Survival graph of photodynamic antimicrobial chemotherapy on S. aureus biofilm (**A**) for 2@DNDs-CSAg and 3@DNDs-CSAg at 100 µg/mL and (**B**) 200 µg/mL after 120 min irradiation. Data represent the mean ± SD.