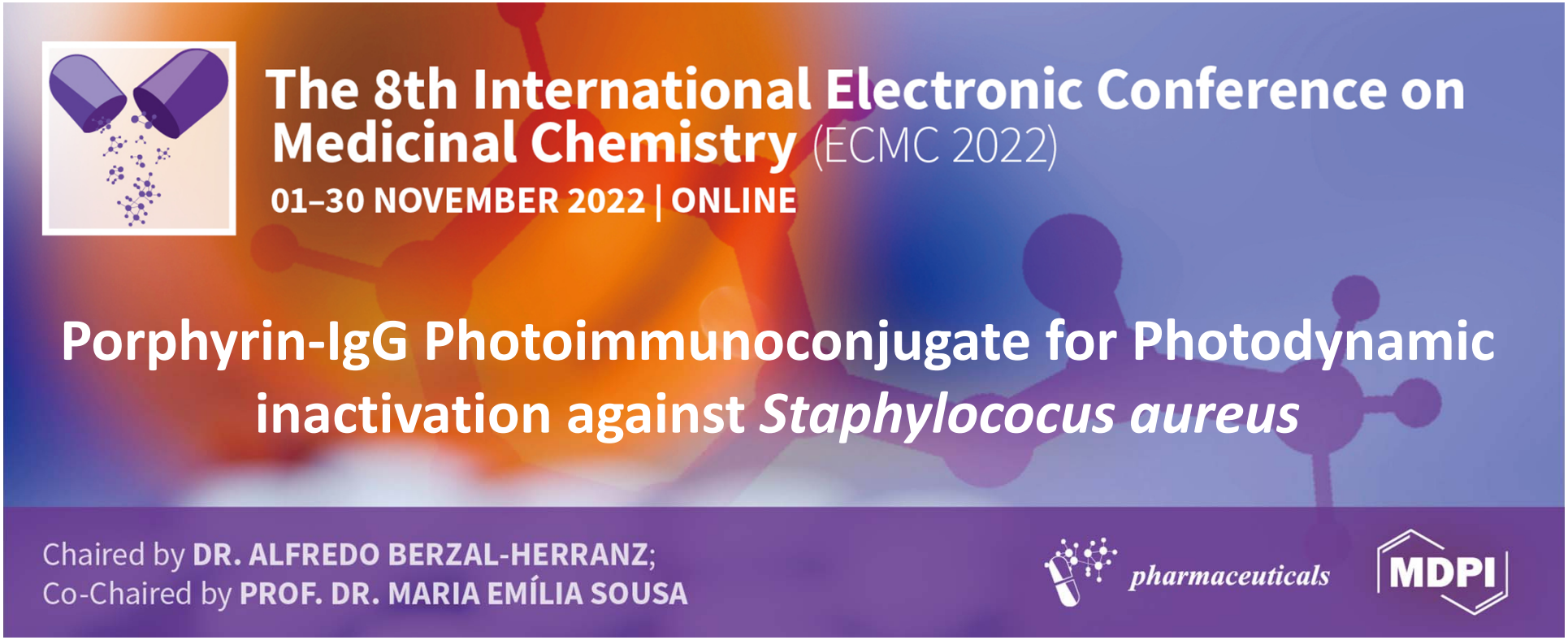


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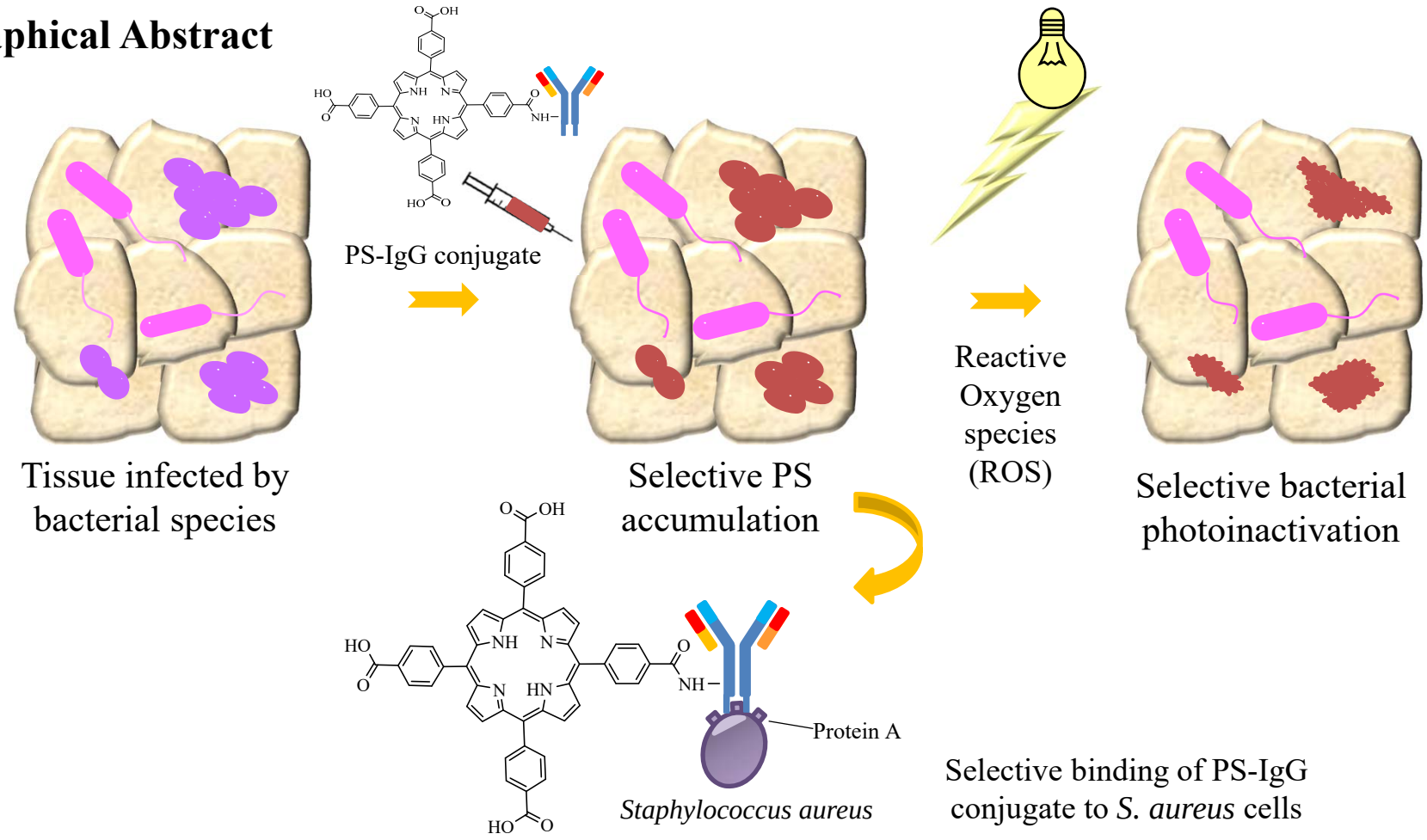
# Porphyrin-IgG Photoimmunoconjugate for Photodynamic inactivation against *Staphylococus aureus*

## Graphical Abstract



**Abstract:**
Photodynamic inactivation (PDI) is a therapeutic approach based on combined use of light, oxygen and a photosensitizing (PS) agent. These three components interact to generate reactive oxygen species, which are cytotoxic and irreversibly damage the vital components of microbial cells, leading to death. However, this methodology has not managed to be completely specific in its mode of action, since the photosensitizer can bind to both pathogenic and commensal microorganisms and even to host cells. Since subsequent irradiation of such cells could lead to their destruction, it is desirable to direct the photodynamic activity to the target cell. Therefore, the objective of this work was to direct the destruction of pathogenic microorganisms without affecting the normal flora. This could be achieved by binding the photosensitizing molecule to an antibody against the surface of the target organism. Therefore, a TCPP-IgG conjugate was synthesized using 4,4',4",4"'-(porphine-5,10,15,20-tetrayl)tetrakis(benzoic acid) (TCPP) and the antibody anti-protein A of *Staphylococcus aureus* (IgG). The UV-visible spectra of TCPP-IgG showed the typical Soret and Q bands characteristic of porphyrin derivatives and, additionally, a new band was observed, corresponding to the absorbance of the protein. However, the results indicated that the conjugation reaction affects the photochemical properties of fluorescent emission and the production of reactive oxygen species compared to TCPP free base. As a consequence, a lower cytotoxicity was observed in planktonic cells of *S. aureus*. PDI can become a promising therapeutic alternative, having as a strategy the specific control of bacterial death, for an efficient eradication.

**Keywords:** Photodynamic inactivation, antibody, conjugate, *S. aureus*

# Introduction

Photodynamic inactivation (PDI)

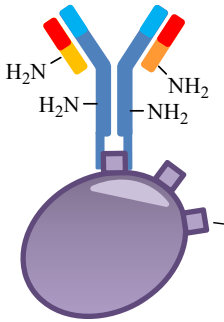
- Photosensitizer (PS)
- Light
- Oxygen

Reactive oxygen species (ROS) production

Inactivation of microorganisms

Conjugation with targeting structures for enhanced selectivity of PDI

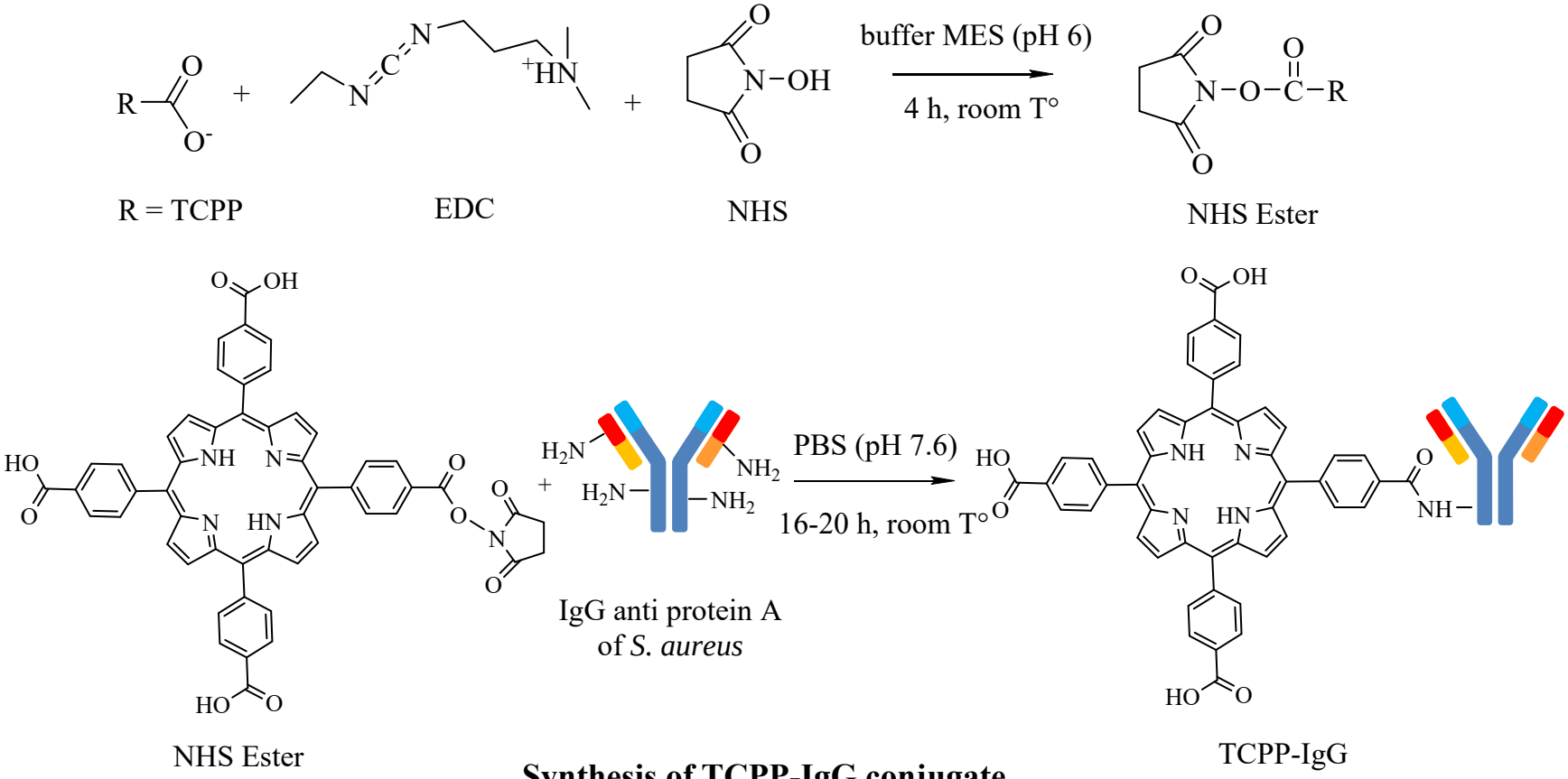
Antibodies




IgG anti protein A of *S. aureus*

*Staphylococcus aureus* expresses **protein A**, a cell wall-anchored immunoglobulin (Ig)-binding protein

# Results and discussion

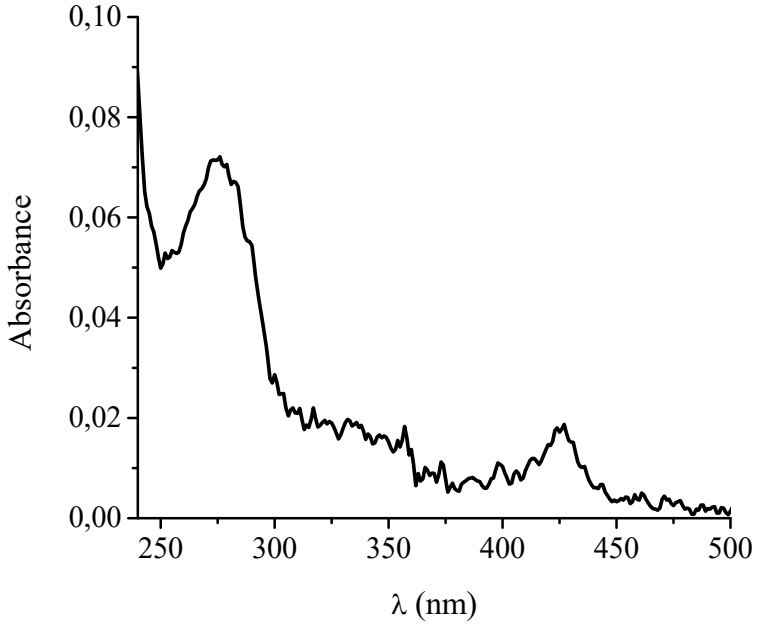



**Synthesis of TCPP-IgG conjugate**

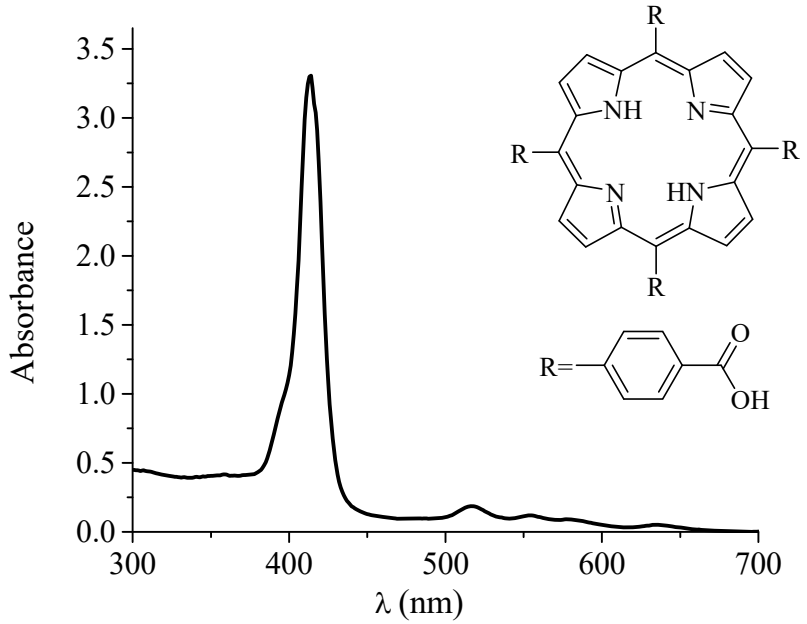
**TCPP**: 4,4',4",4"'-(porphine-5,10,15,20-tetrayl)tetrakis(benzoic acid), **EDC**: 1-ethyl-3-(3-dimethylaminopropyl)carboiimide hydrochloride, **NHS**: Sulfo-*N*-hydroxysucci nimide, **MES**: 2-(*N*-morpholino)ethanesulfonic acid.

# Results and discussion

| Porphyrin | Absorbance $\lambda_{max}$ (nm) | | | | |
|---|---|---|---|---|---|
| | Soret | Q1 | Q2 | Q3 | Q4 |
| TCPP | 413 | 515 | 554 | 586 | 634 |




UV-Vis spectra of IgG anti protein A monoclonal antibody (Sigma-Aldrich) in saline phosphate buffer (PBS).

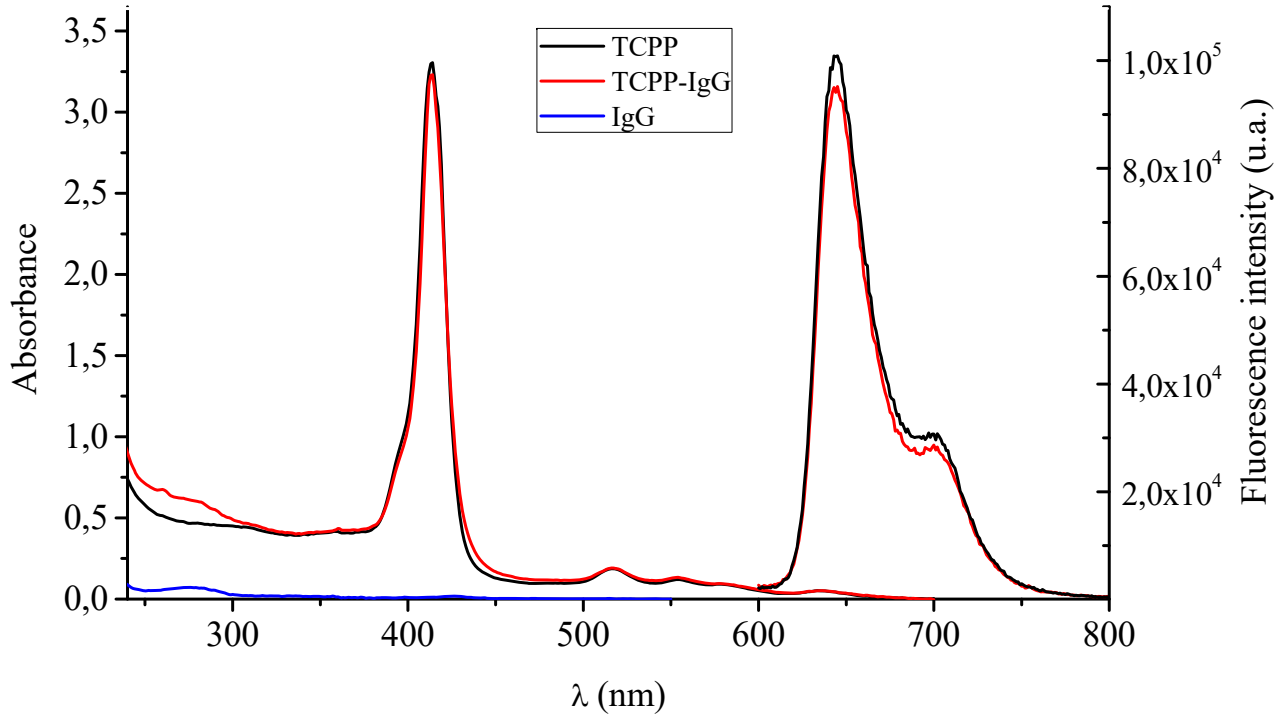



Molecular structure and UV-Vis spectra of TCPP in PBS.

# Results and discussion

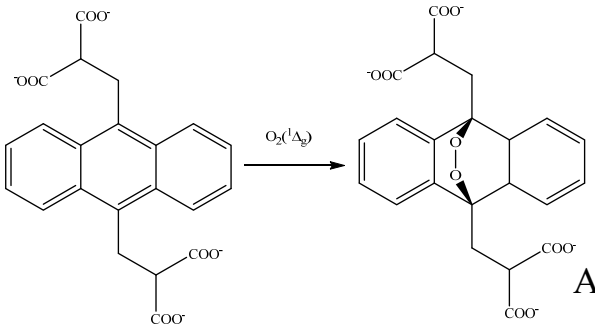
Fluorescence quantum yield ($\Phi_F$) of TCPP-IgG in PBS

$\Phi_{F\ (TCPP\text{-}IgG)} = 0.14 \pm 0.02$

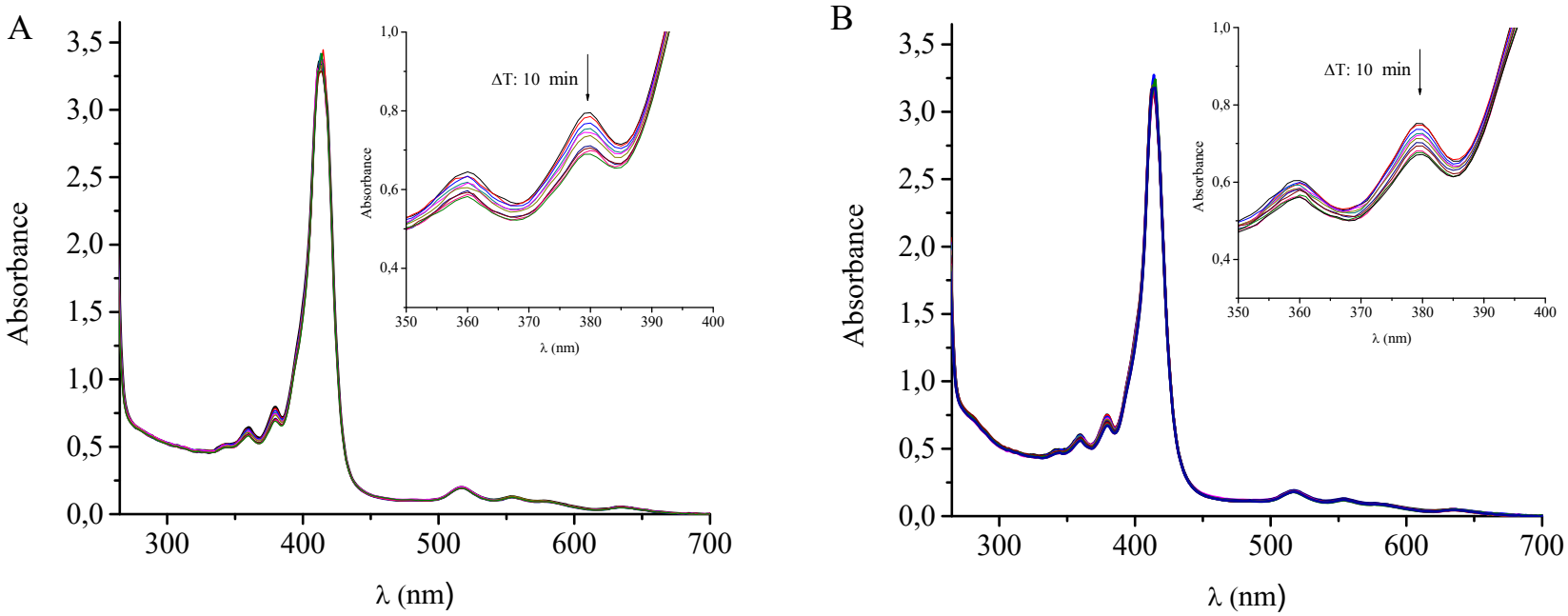



Uv-Vis and fluorescence emission spectra of TCPP, TCPP-IgG and IgG in PBS.

# Results and discussion




ABMM reaction with $O_2(^1\Delta_g)$

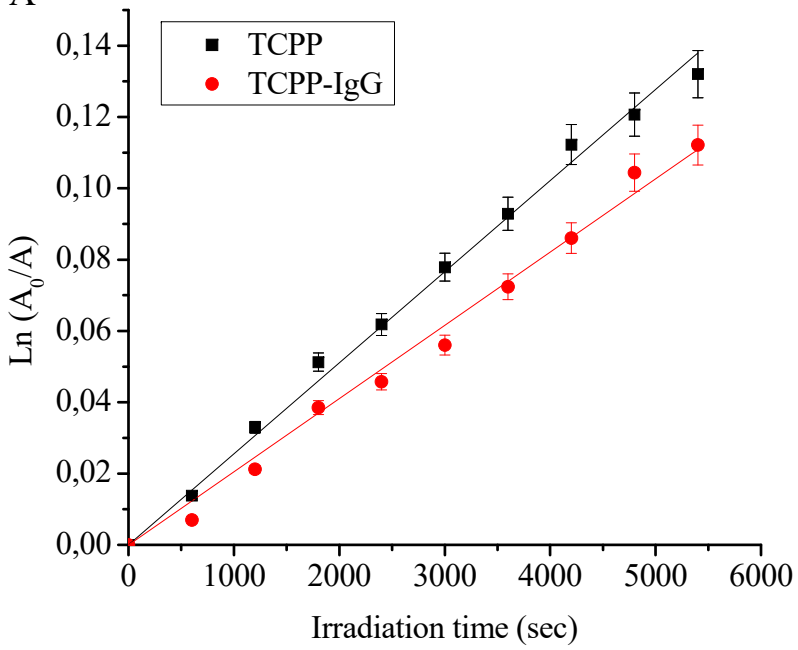



Photooxidation kinetics of ABMM photosensitized by TCPP (A) and TCPP-IgG (B). Insert: Spectral changes following the decrease at $\lambda_{max}$ = 379 nm.

**ABMM**: Tetrasodium 2,2′-(anthracene-9,10-diyl)bis(methylmalonate).

# Results and discussion

A

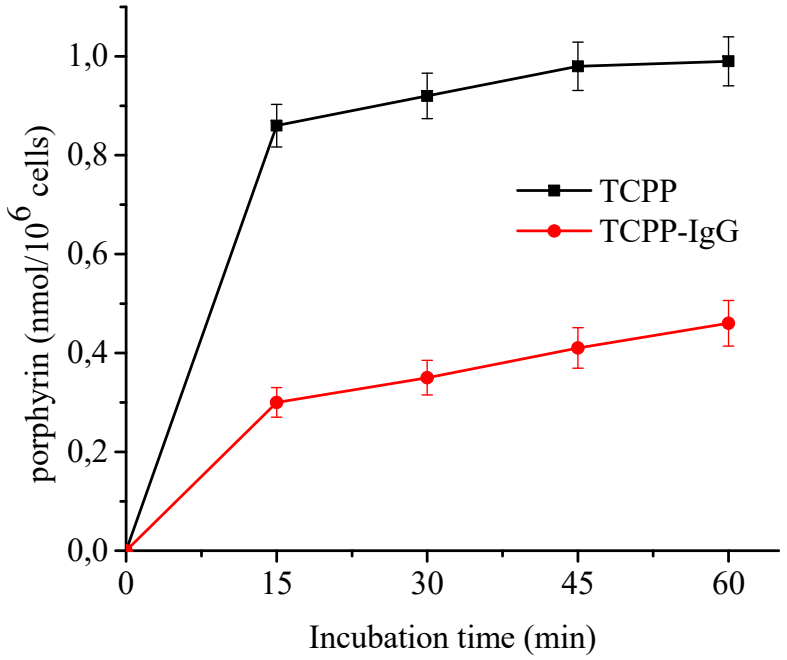



B

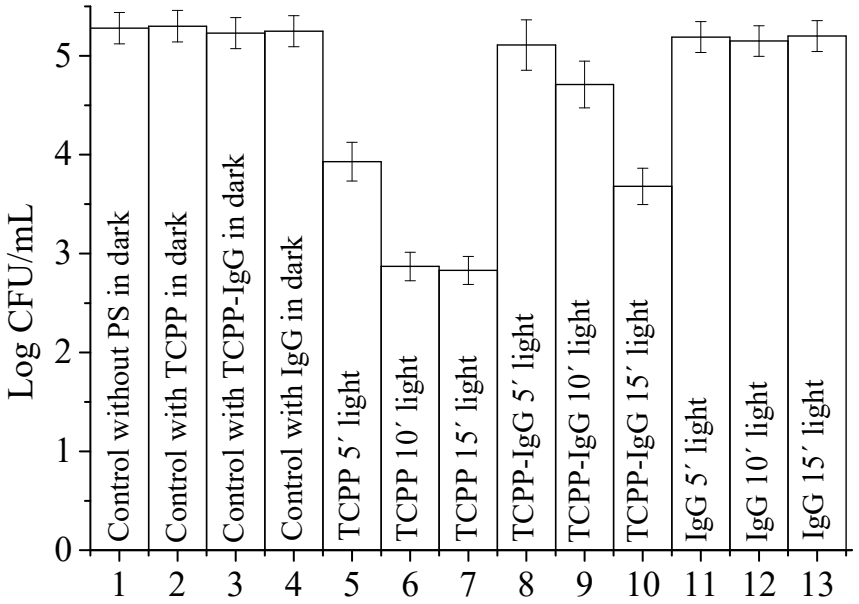
| PS | $k_{obs}^{ABMM}$ ($s^{-1}$) | ΦΔ |
|---|---|---|
| TCPP | $2.50 \times 10^{-5}$ | 0.53±0.06 |
| TCPP-IgG | $2.05 \times 10^{-5}$ | 0.43±0.06 |

A) First-order plot for the photooxidation of ABMM in PBS. B) Kinetic parameters for ABMM photooxidation reaction ($k_{obs}$ABMM) and quantum yields of singlet oxygen production ($\Phi_{\Delta}$).

# Results and discussion

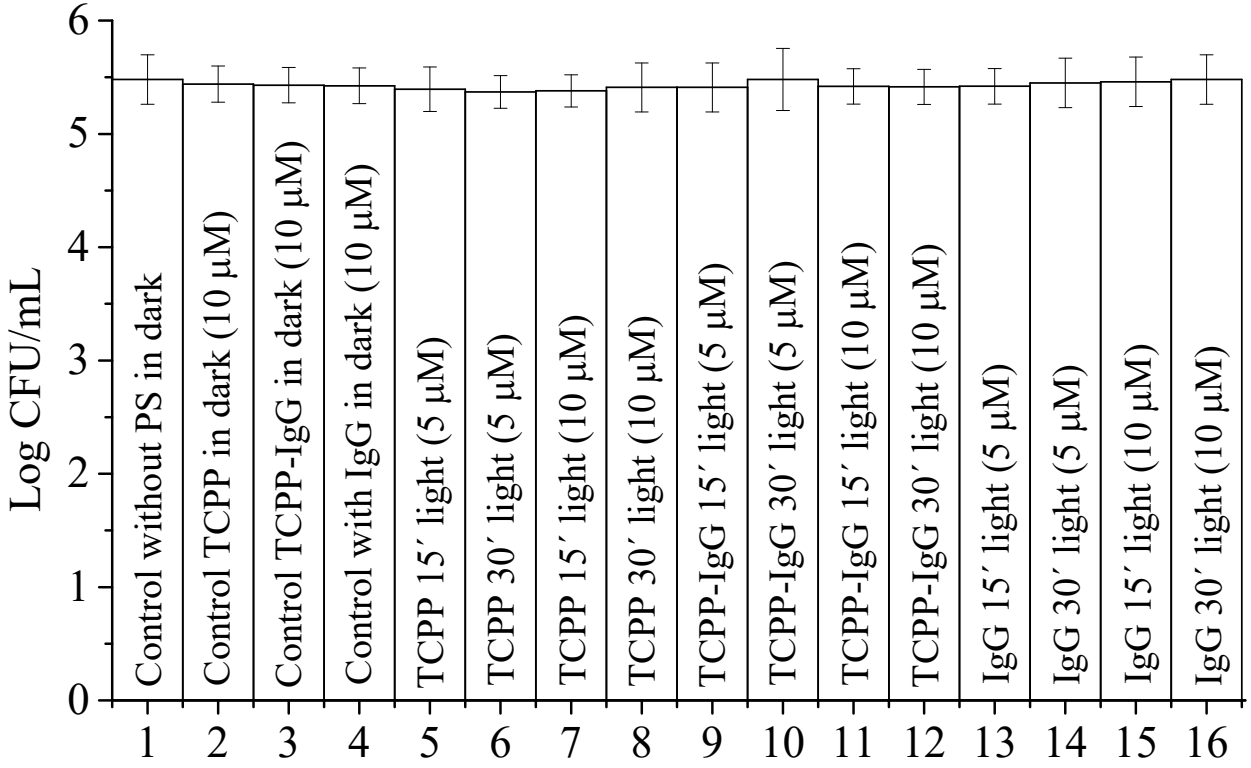



Amount of PS recovered from *S. aureus* cells incubated with 1 μM of PS at 37ºC in dark for different times.




PDI of *S. aureus* incubated with 1 μM TCPP, TCPP-IgG and IgG for 15 min at 37 °C in dark and exposed to visible light for different times.

# Results and discussion




PDI of *E. coli* incubated with 5 and 10 μM TCPP, TCPP-IgG and IgG for 15 min at 37 °C in dark and exposed to visible light for different times.

# Conclusions

- ✓ A new porphyrin-IgG photoimmunoconjugate was synthetized and photochemically characterized. It was synthesized from a porphyrin with four carboxylic acid groups (TCPP) and an IgG-type monoclonal antibody against protein A of *S. aureus*.
- ✓ Spectroscopic studies of UV-visible absorption indicate that the conjugation did not produce modification of the optical properties of the conjugate and evidenced the presence of the antibody in the conjugate by the appearance of a broad band in the UV region.
- ✓ Fluorescence studies show that both the TCPP porphyrin and the conjugate exhibit fluorescence emission with $\Phi_F$ ~0.14. This demonstrates that PS-Ac conjugation does not affect the ability to fluoresce.
- ✓ The photodynamic activity of these PSs was analyzed by decomposition of the ABMM substrate. Both PSs, generate $^1O_2$, with quantum yields of ~0.4-0.6.
- ✓ Comparative studies of the binding of TCPP and TCPP-IgG in *S. aureus* demonstrated better binding of free porphyrin. Despite the high specificity of IgG, the Fc portion of IgG appears to be less available due to PS binding.
- ✓ PDI studies on *S. aureus* planktonic cells indicate lower efficacy for TCPP-IgG compared to TCPP. On the other hand, no inactivation effect was observed using TCPP nor TCPP-IgG on *E. coli*, despite the high concentrations and light exposure times used.
- ✓ PDI can become a promising therapeutic alternative, as a strategy the specific control of bacterial death, for an efficient eradication. However, greater efforts are required to find PS that can enhance this therapy.

## Acknowledgments