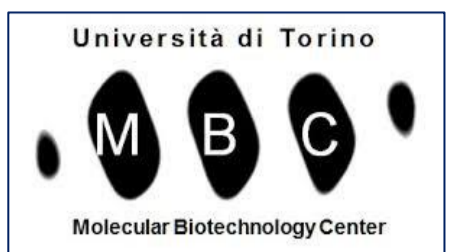


NEW NANOPHOTOSENSITIZERS WITH POTENTIAL APPLICATION IN ANTIMICROBIAL PDT BASED ON POLYMETHINE DYES INCORPORATED IN DIFFERENT NANOPARTICLES



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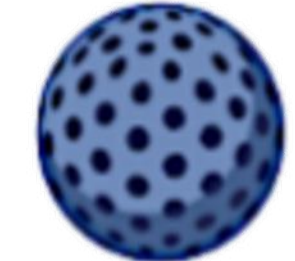
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INTRODUCTION

Recently, antimicrobial photodynamic therapy (aPDT) has emerged as promising strategy to improve antimicrobial treatment and face the antibiotic resistance to conventional therapy. Despite the high number of available photosensitizers (PSs), the search for ideal PSs with high selectivity and suitable photochemical properties is still in progress. Organic Near Infrared (NIR) polymethine dyes (Squaraines, SQs and Cyanines, Cys) has attracted considerable attention for a wide range of applications (PDT and theragnostic¹) although, their poor aqueous solubility and stability still limits their wide application. Their incorporation inside nanoparticles (NPs), could help to prevent the formation of dye aggregates, protect their photochemical properties, and play a key role in the infection treatment^{2,3}.

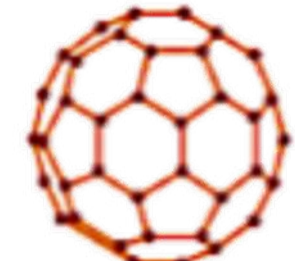
METHODS

MSN



Hexagonal MSNPs were prepared by the modified sol-gel Stober process through the hydrolysis of tetraethyl orthosilicate (TEOS) in ethanol medium with CTAB (1-hexadecyl) trimethylammonium bromide) as surfactant³. Dye loaded by adsorption after solubilization of MSNPs and dye in DCM (overnight under magnetic string at 80 °C).

ZIF-8



The room temperature sol-gel method was applied for the synthesis of ZIF-8 nano-MOF⁴, by using (Zn(NO₃)₂·6H₂O) and Hmim. Once obtained the ZIF-8, the dye solution was added and kept 12 h under constant magnetic stirring.

PLGA



Nanoprecipitation and single emulsion with probe sonication for empty and dye loaded NPs, according to the previous reported protocol⁵.

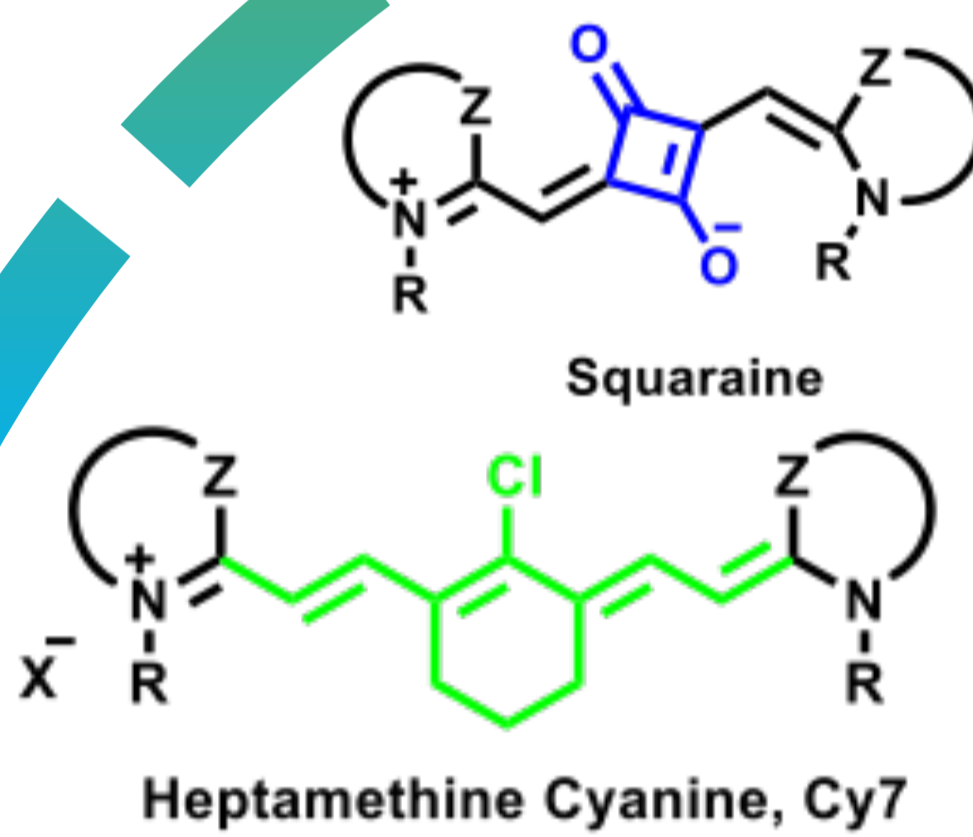
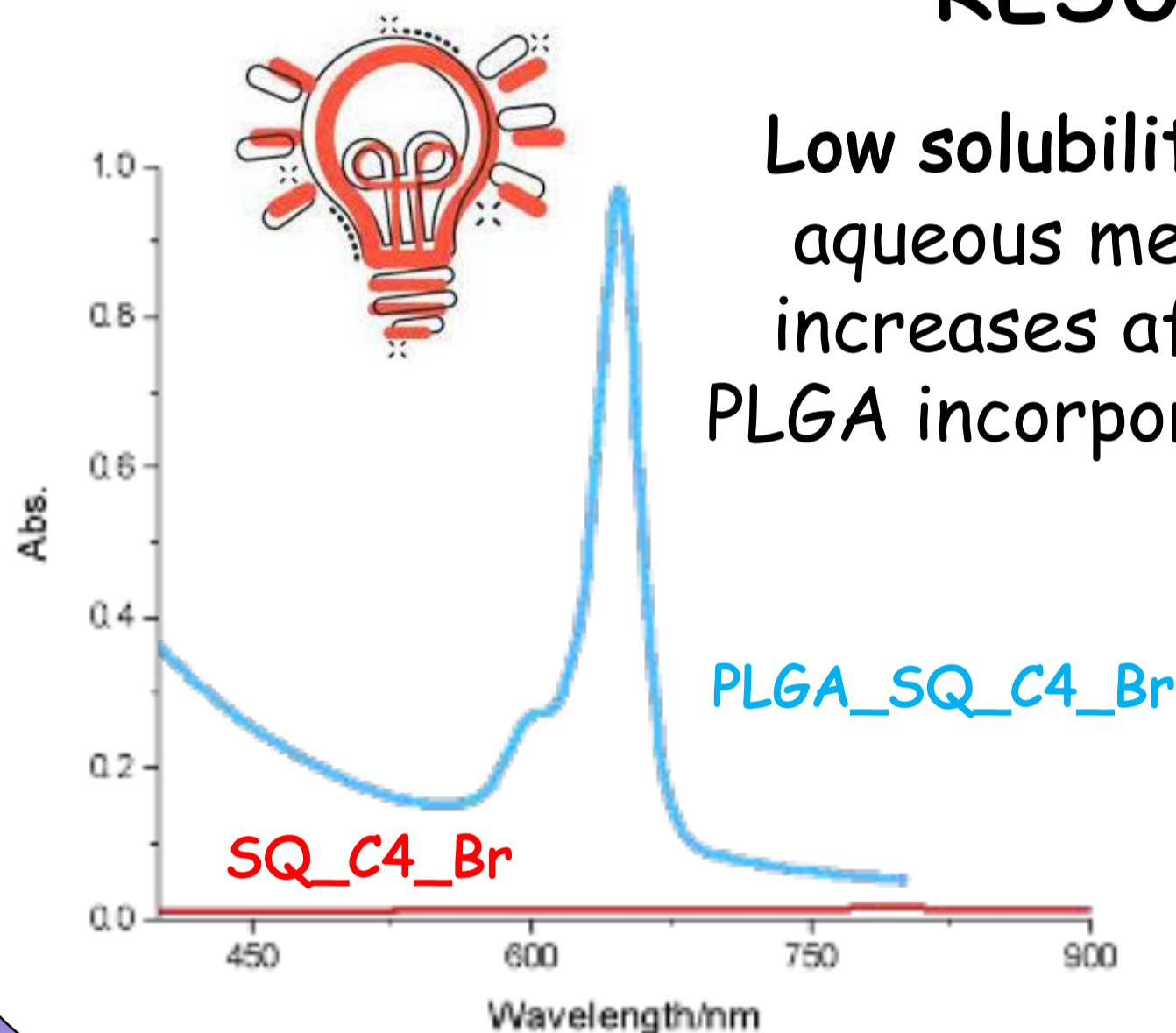
Ratio of PLA:PGA is 50:50

Stabilizing agent: Pluronic F127 or PVA

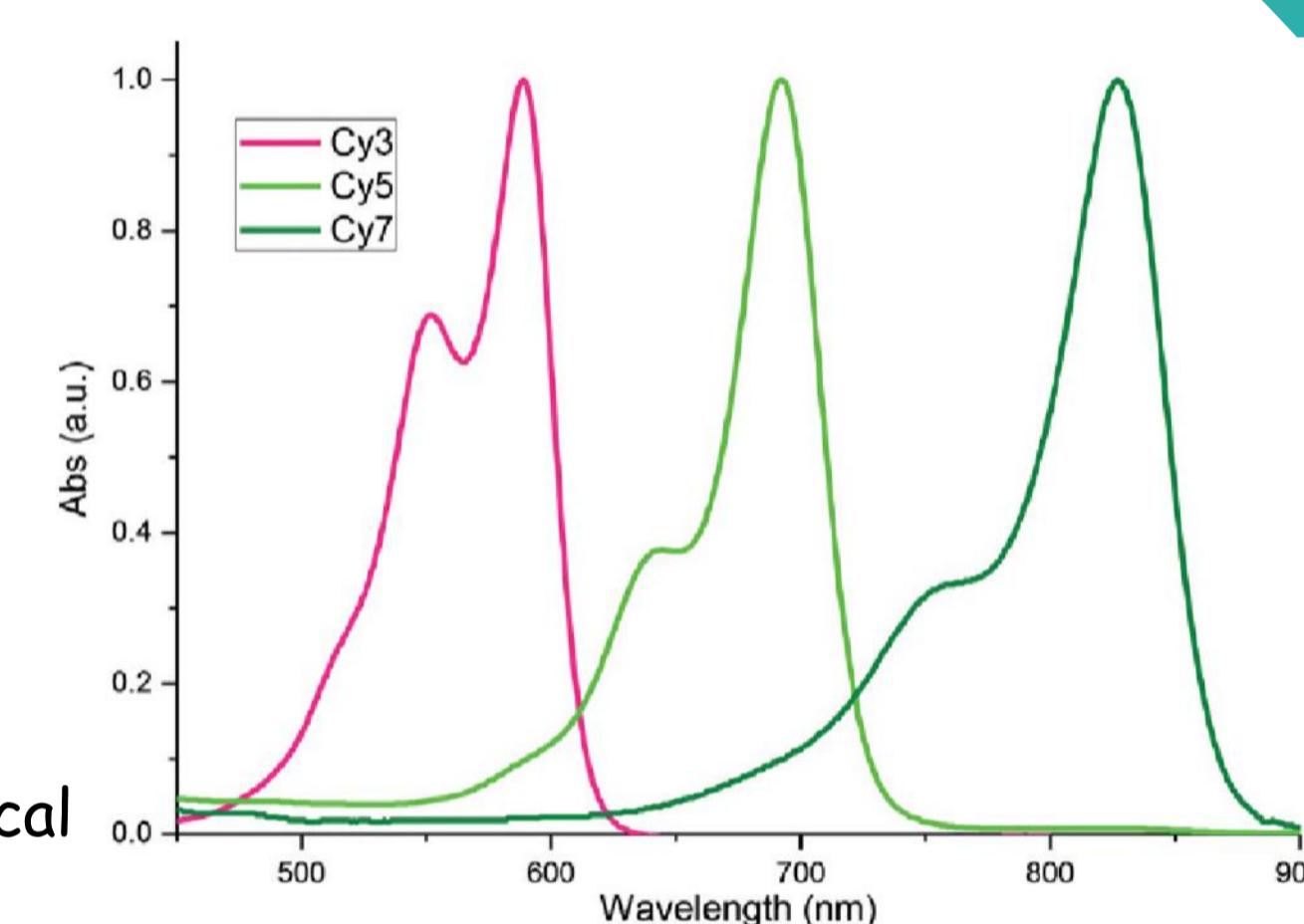
A DoE was applied to investigate how the different process parameters can affect the final PLGA size, yields and dye entrapment efficiency.

RESULTS

Low solubility in aqueous media increases after PLGA incorporation

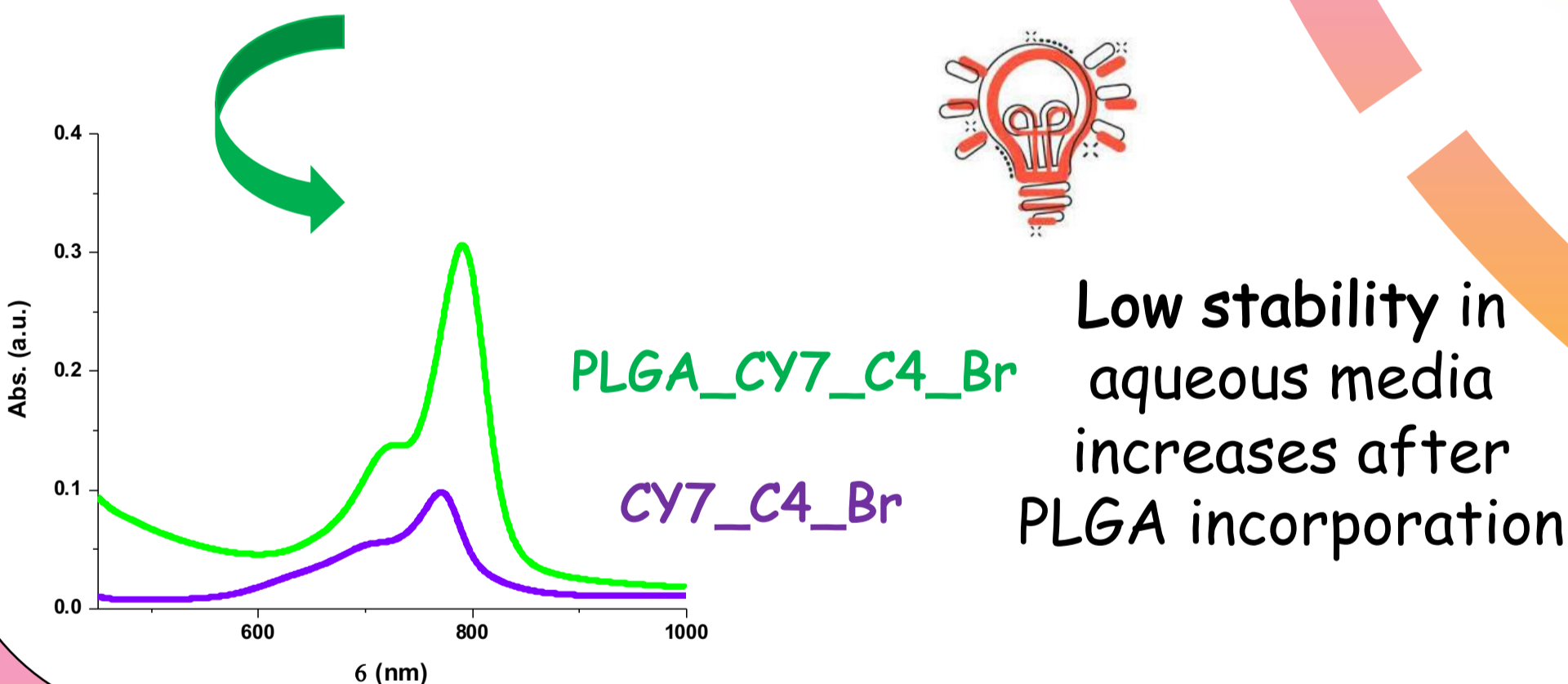
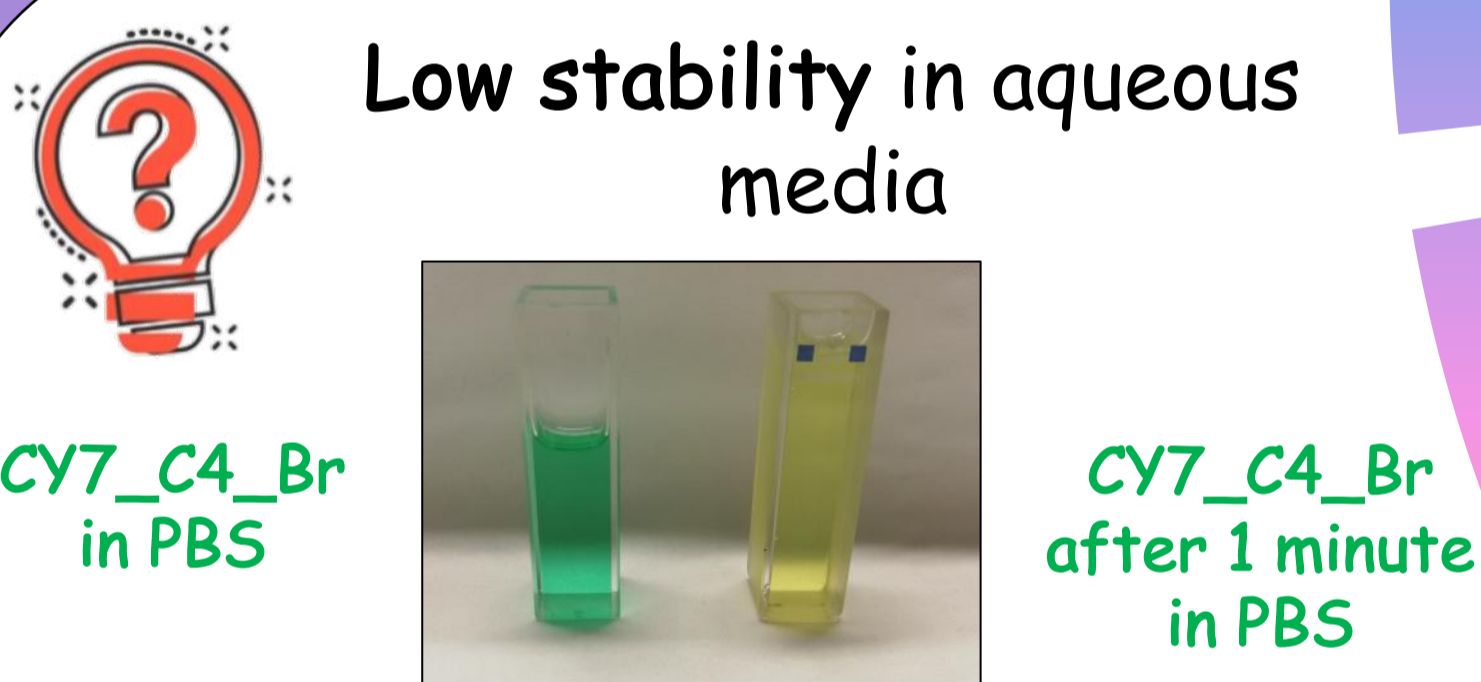


Perfect match with the suitable PDT window



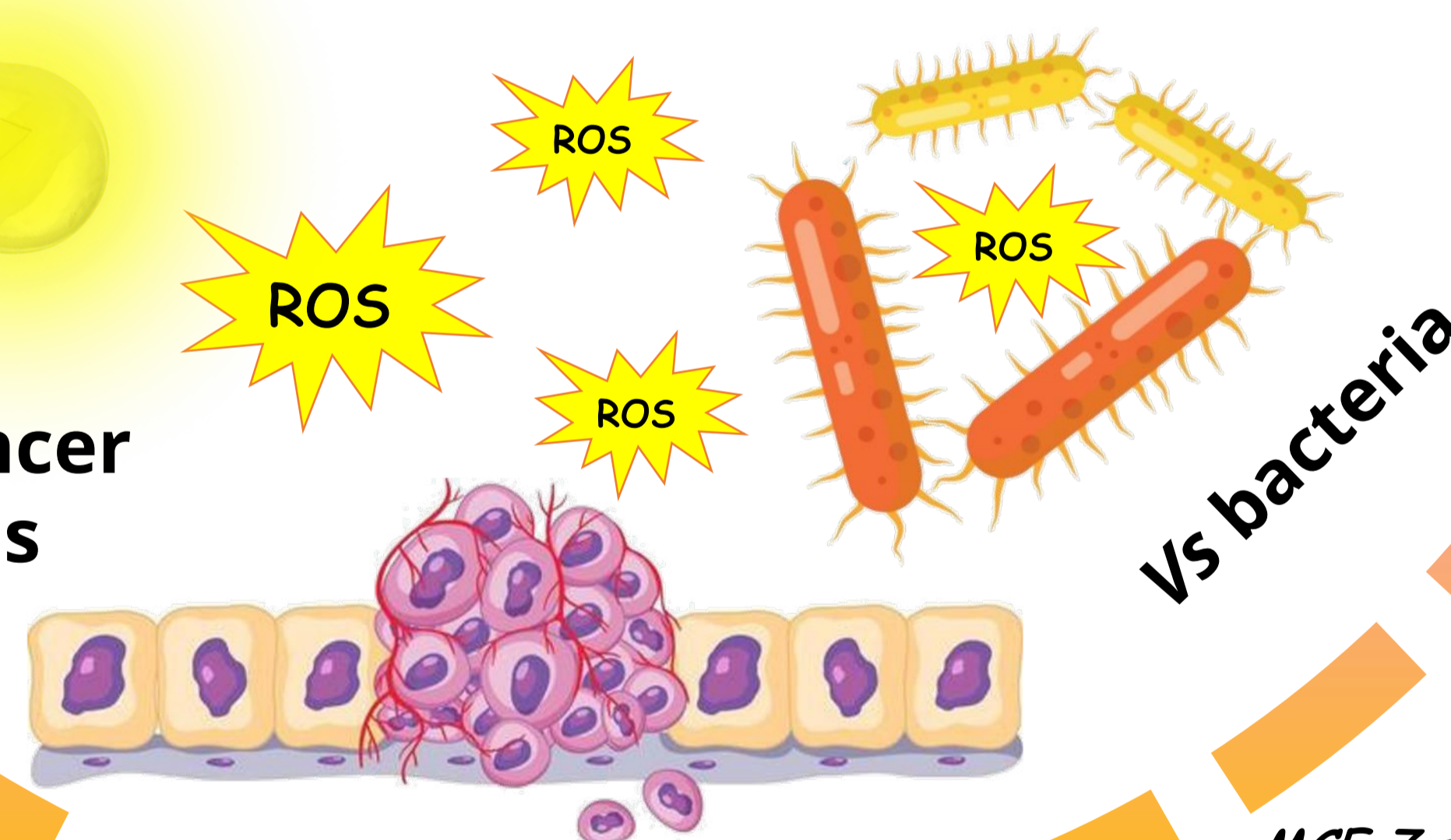
- ✓ Easy tuning of photophysical properties (towards NIR)
- ✓ Wide molecular structure diversity
- ✓ Low solubility in aqueous media
- ✓ Low stability in aqueous media

Low stability in aqueous media



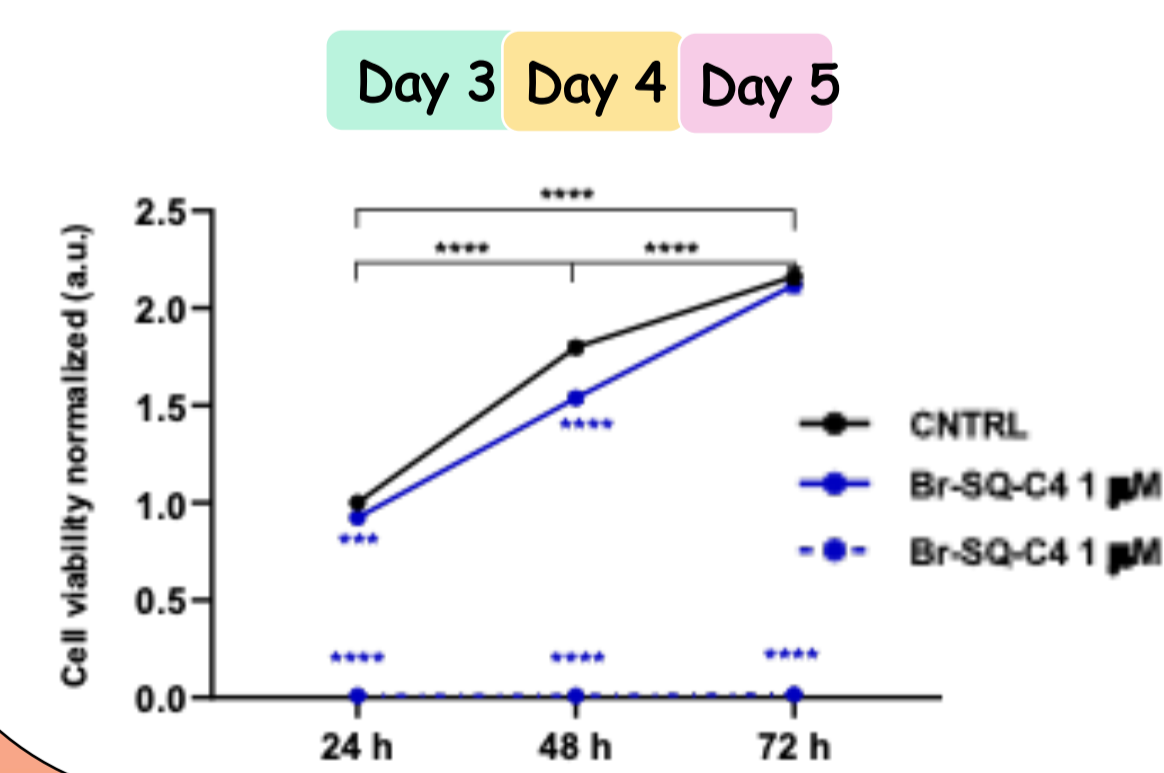
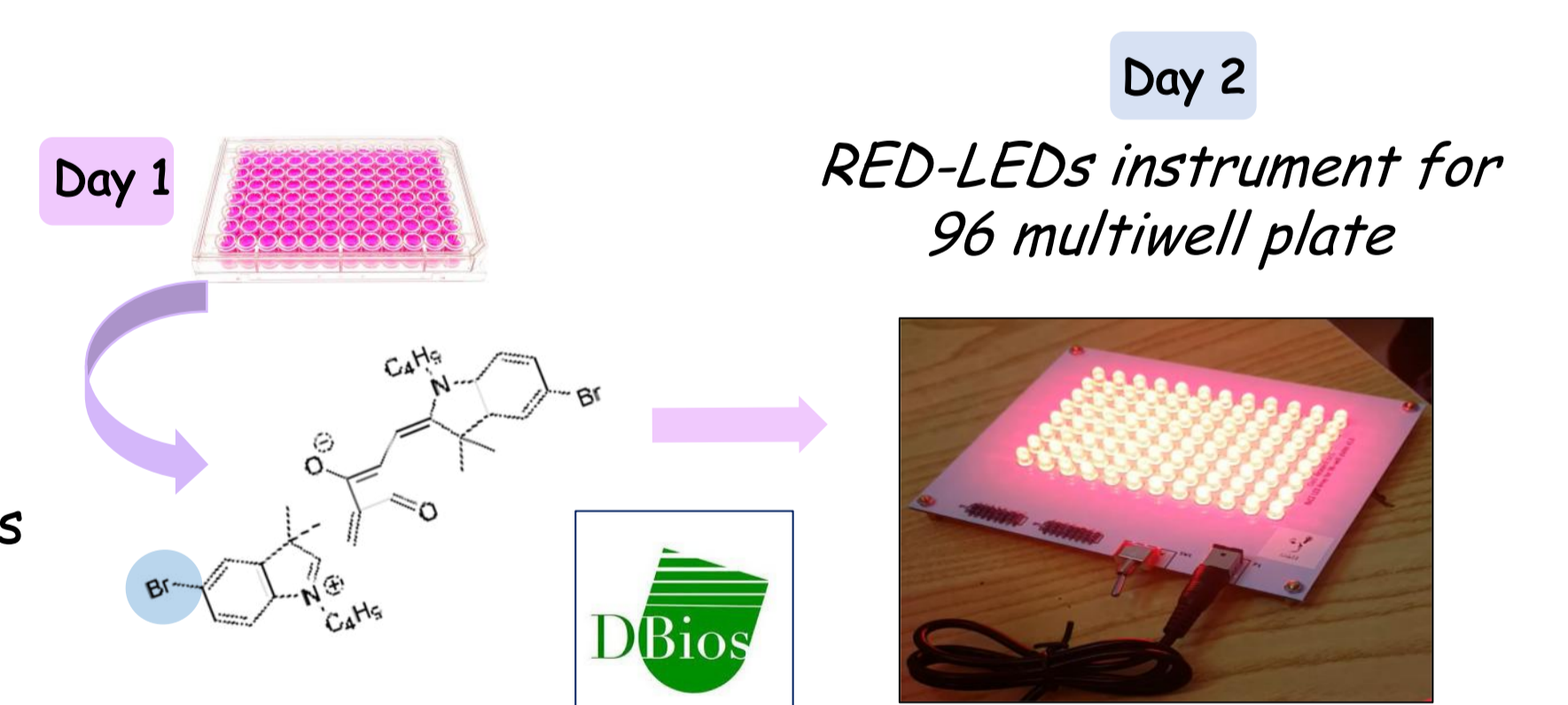
Low stability in aqueous media increases after PLGA incorporation

Vs cancer cells



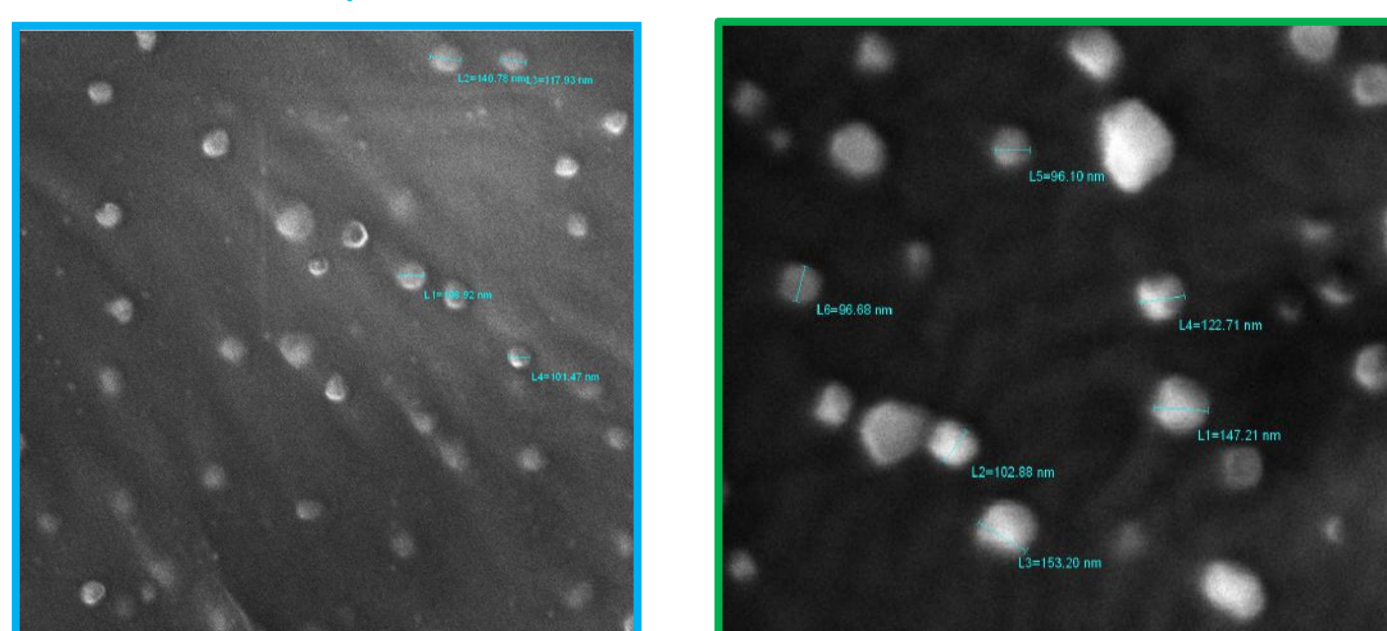
Vs bacteria

Radiation 15', LED 640 nm, 15 mW/cm², evaluation of cell viability at 48 e 96h with MTS assay



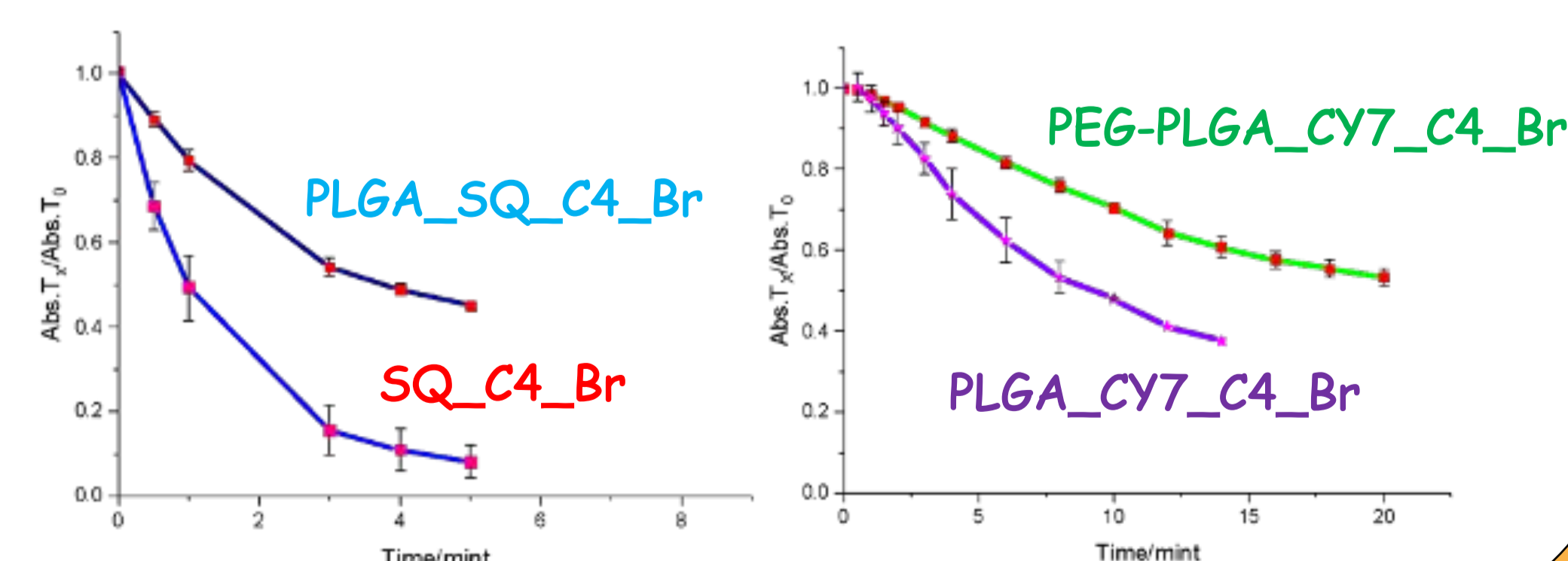
Good *in vitro* photodynamic activity at the maximum non-cytotoxic concentration (1 μM)
The reduction of cell proliferation is due to the production of ROS

PLGA_SQ_C4_Br PLGA_CY7_C4_Br



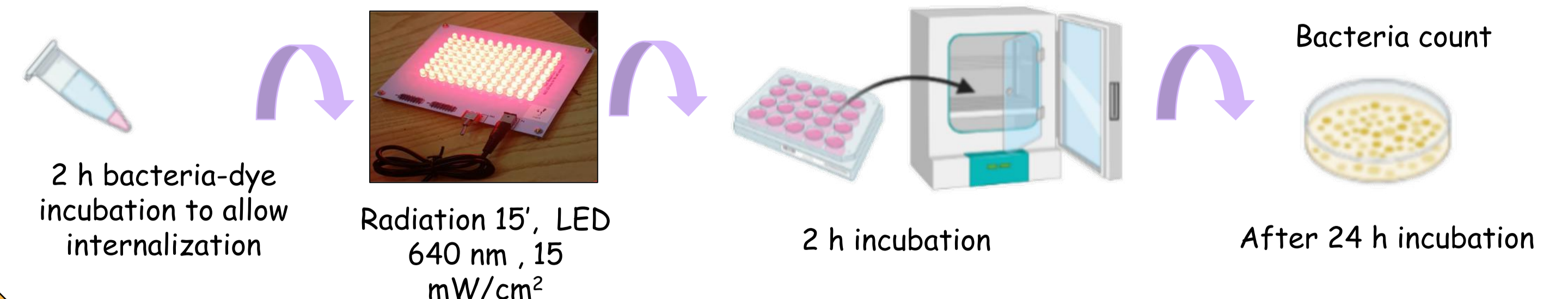
NTA (Nano tracing analysis)			
	Empty PLGA	PLGA_CY7_C4_Br	PLGA_SQ_C4_Br
Size (nm)	134-149	134 - 153	180- 154
ζ	-35	-25	-25
(EE%)	-	75	64

Good ROS production after the NPs incorporation



Antimicrobial PDT

Staphylococcus Aureus



CONCLUSIONS

- ✓ Empty PLGA NPs with a ratio of PLA to PGA 50:50 and Pluronic as stabilizing agent showed smaller size and PDI compared to PVA as stabilizing agent. Following the DoE suggestion, DCM has been selected as solvent, leading to a higher EE%.
- ✓ The encapsulation of cyanine and squaraine dyes in the PLGA NPs are confirmed by the presence of hypsochromic shifts in the salvatochromism analysis.
- ✓ The water solubility of squaraine encapsulated in PLGA NPs was enhanced, as well as the water stability of the cyanine encapsulated in PLGA NPs was enhanced. The incorporation into nanoparticles could be considered a good strategies to overcome the drawbacks related to the dyes.
- ✓ SQ_C4_Br showed an interesting *in vitro* PD activity vs cancer cells. Preliminary results on aPDT seems to confirm a good *in vitro* PD activity vs S. Aureus. This study is still in progress.

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