ZINC PHTHALOCYANINE-INITIATED POLY-L-GLUTAMATE-BASED NANOCARRIERS WITH THERANOSTIC PROPERTIES FOR GLIOBLASTOMA MULTIFORME TREATMENT

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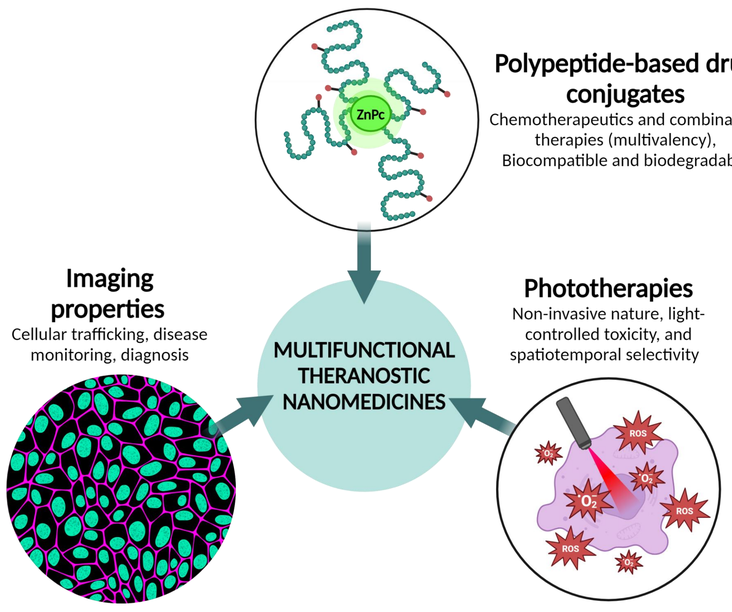
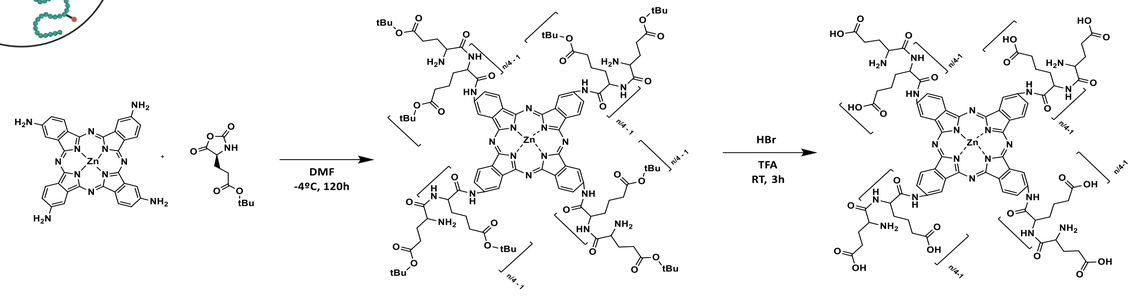
1 AIM OF THIS WORK

Glioblastoma multiforme (GBM) represents the most common class of malignant primary brain tumors in adults and one of the most aggressive forms of cancer overall, with poor prognosis and a survival rate of less than one year after diagnosis [1]. **Photodynamic therapy** represents a potentially efficient and non-invasive approach to GBM treatment. **Phthalocyanines** represent promising photosensitizing agents as components of **phototherapies** and **optical imaging systems**; however, they face limitations such as low water solubility and short in-vivo circulation times [2]. To address these challenges, this work aimed to reformulate **zinc phthalocyanine** (ZnPc) as a polypeptide-based polymer to improve water solubility, circulation times, and targeting [3]. We employed ZnPc as a core to synthesize a four-armed **poly-L-glutamate (PGA)-based polymer**. The study also explored the potential for drug conjugation to PGA - **combining chemotherapy and photodynamic therapy -** as a combination strategy for **efficient GBM treatment**.



2 RESULTS

ZnPc-PGA$_{20}$ SYNTHESIS AND CHARACTERIZATION

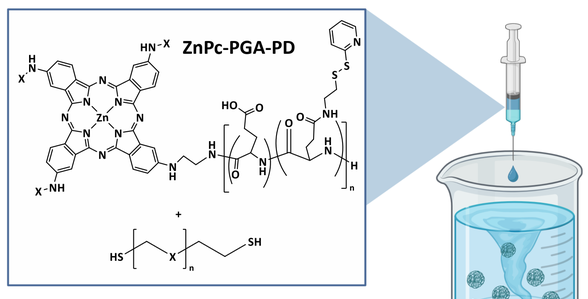


We synthesized PGA-modified ZnPc via **N-carboxy anhydride ring-opening polymerization** (NCA-ROP) [4-6] and **characterized resultant polymers using H-NMR, UV-Vis, and DLS**.

Theoretical ratio (ZnPC:PGA)	Experimental ratio (ZnPC:PGA)		Size (nm ± SD)[c]	Z potencial (mV ± SD)[c]
1:20	1:20.6[a]	1:11.2 ± 0.6[b]	52.5 ± 20.6	- 22.6 ± 1.9

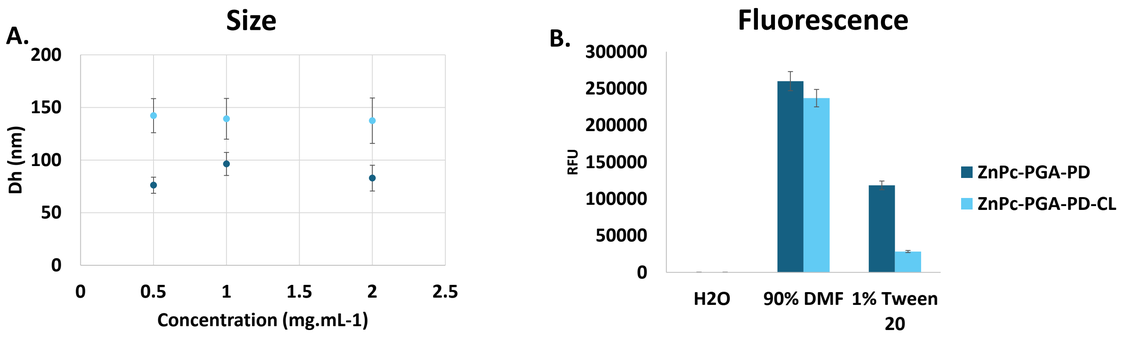
a. Determined by ^{1}H-NMR. b. Determined by UV-Vis. c. Determined by DLS

CROSSLINKED ZnPc-PGA$_{20}$ SYNTHESIS AND CHARACTERIZATION



We stabilized **ZnPc-PGA aggregates** in water through **disulfide crosslinking** to create higher-sized nanocarriers.

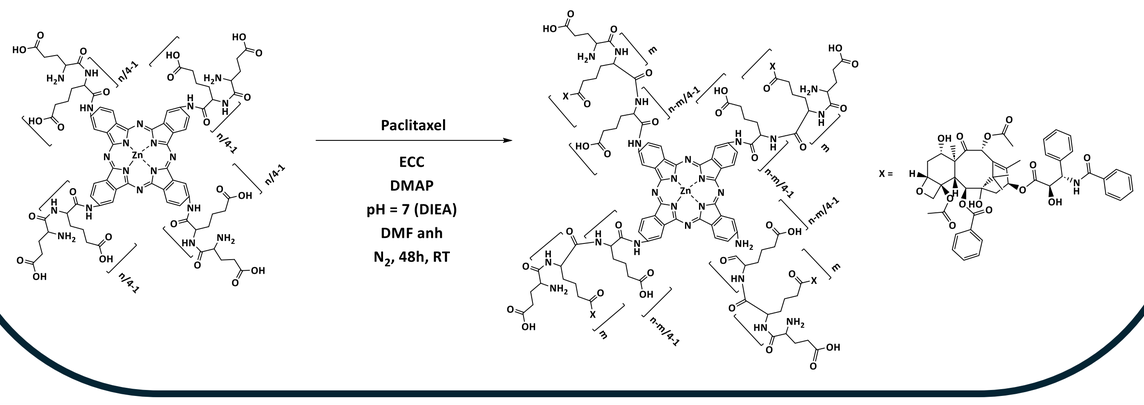
A. **DLS measurements** of non-stabilized pyridyl disulfide modified ZnPc-PGA (ZnPc-PGA-PD) and the crosslinked (ZnPc-PGA-PD-CL) at different concentrations in water.
B. **Fluorescence intensity** of ZnPc-PGA-PD and ZnPc-PGA-PD-CL in water, 90% DMF or a 1% Tween 20 solution.



ZnPc-PGA$_{20}$-PTX SYNTHESIS AND CHARACTERIZATION

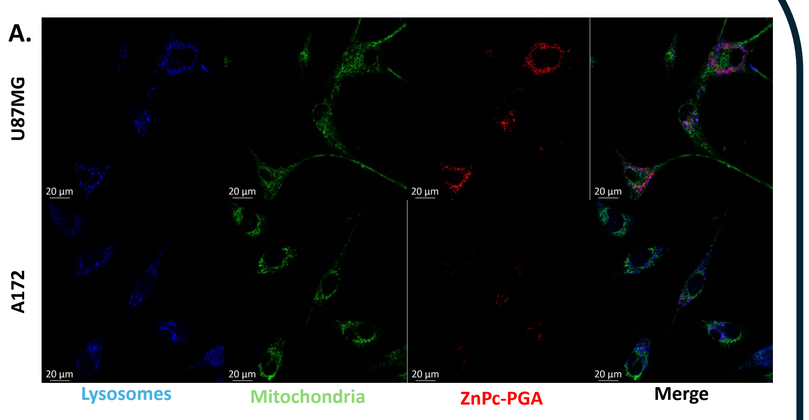
We aimed to explore the possibility of **drug conjugation** to the PGA sidechain by post-polymerization modification approaches to generate a **combination strategy**. We successfully conjugated **paclitaxel** to the polymer with 30%wt drug loading in weight.

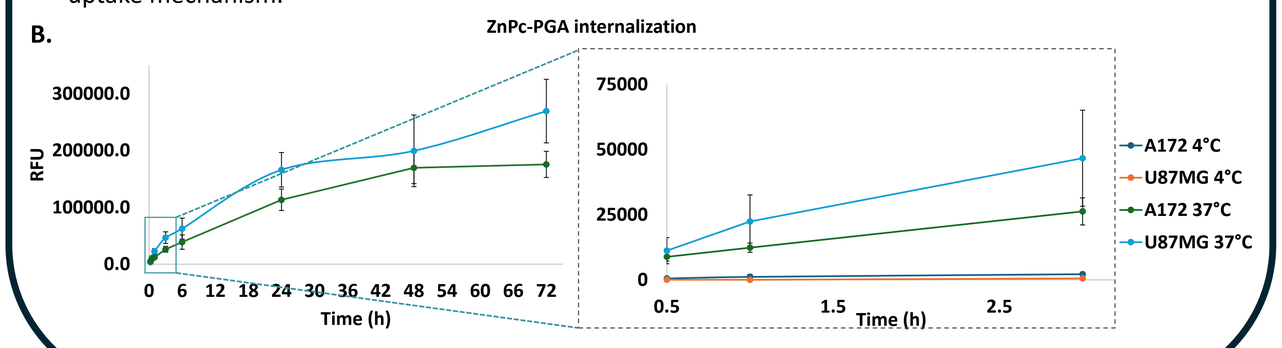
Total Drug Loading (TDL) (%wt)[a]	% Free drug to TDL (%wt)[b]	Size (nm ± SD)[c]	Z potential (mV ± SD)[c]
29.1 ± 1	0.02	36.6 ± 19.3	- 16 ± 2



IMAGING PROPERTIES

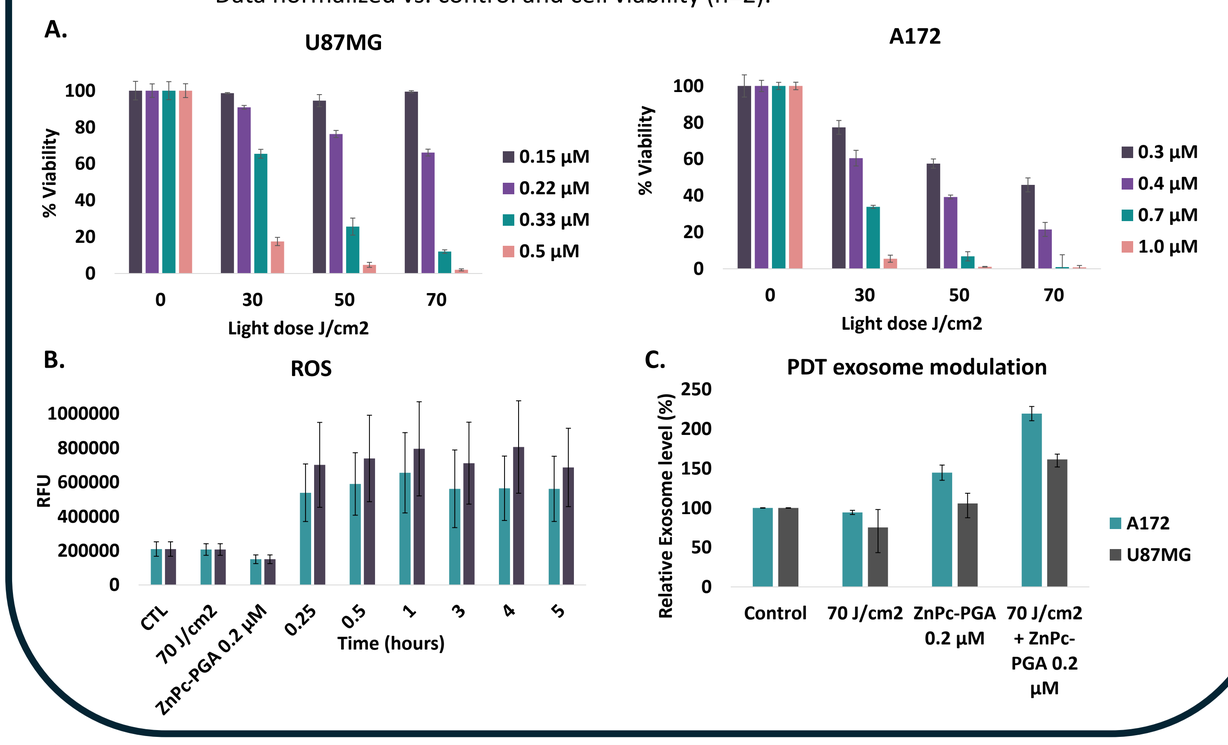
A. **Confocal fluorescence microscope images** of A172 and U87MG GBM cells incubated with ZnPc-PGA, and Lysotracker blue and Mitotracker green for lysosomal and mitochondrial staining.
B. **ZnPc-PGA cell uptake kinetics by flow cytometry** to determine a clear energy-dependent cell uptake mechanism.





PHOTODYNAMIC THERAPY

A. ZnPc-PGA prompts cell toxicity upon irradiation, but the polymer or irradiation does not induce toxicity. We studied cell toxicity by measuring **B. ROS concentration** over time using the DCFH probe in both cell lines and **C. exosome modulation** by Alpha Screen technology. Data normalized vs. control and cell viability (n=2).



3 CONCLUSIONS

We **successfully synthesized and characterized PGA-based polymers using zinc phthalocyanines as macroinitiators**. ZnPc-PGAs displayed solubility in water while maintaining their intrinsic spectroscopic characteristics. Cell studies demonstrated the **imaging properties** of ZnPc-PGAs by confocal microscopy and their applicability as photosensitizers for **photodynamic therapy in two GBM cell lines**. We observed the PDT effect on cells through an increase in ROS production and exosome release. We expect the results of this study to drive advances in GBM treatment forward.

4 REFERENCES

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5 ACKNOWLEDGMENTS

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