Supporting Information for:

# Low Concentrations of a non-hydrolysable tetra-S-glycosylated porphyrin and low light induces apoptosis in human breast cancer cells via stress of the endoplasmic reticulum

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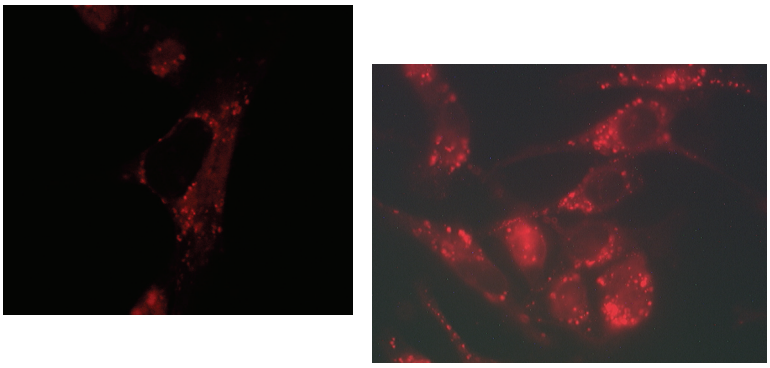

**A** **B**

**Figure SI-1.** Fluorescence image of human breast cancer MDA-MB-231 cells treated with A: 1 μM, and B: 10 μM P-Glu$_4$. Note there is apparently little P-Glu$_4$ in the cell nucleus.

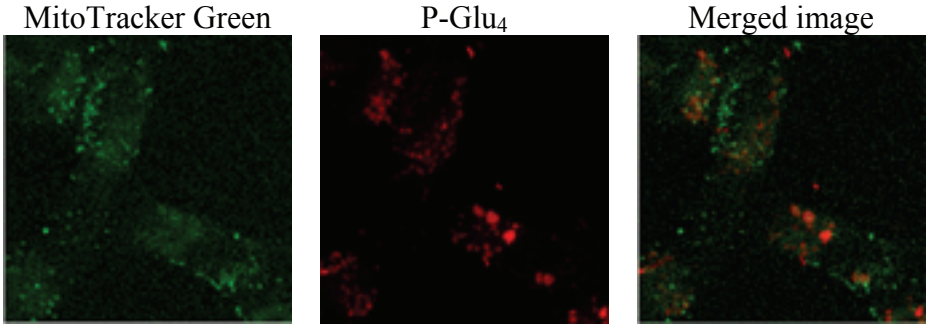


**Figure SI-2.** Some small amount of P-Glu$_4$ may be partially localized in mitochondria in human breast cancer MDA-MB-231 cells. Cells were incubated with 40 μM P-Glu$_4$ for 24 hours, treated with 40 nM MitoTracker Green, rinsed and fixed with 4% paraformaldehyde solution. Left: Mito-Tracker green, middle: P-Glu$_4$, right: overlapped image. Confocal fluorescence images were taken under identical conditions.

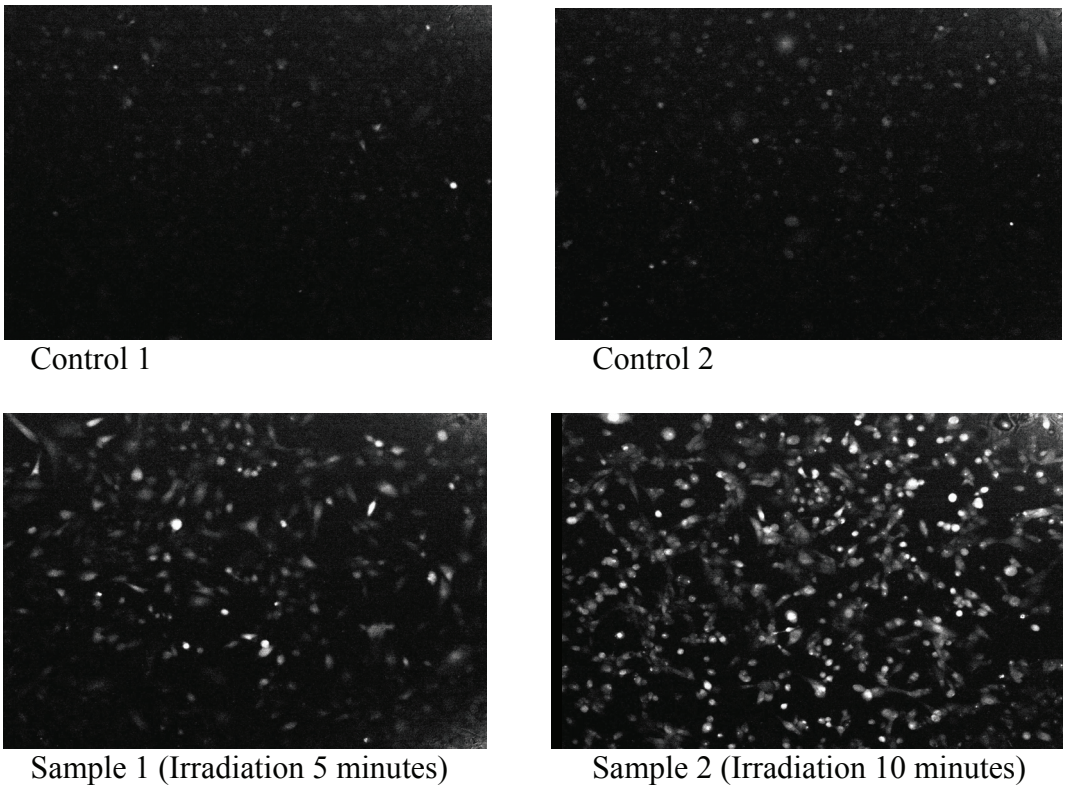


**Figure SI-3.** Release of calcium from the endoplasmic reticulum to the cytosol. Control 1: cells were incubated with 10 μM P-$Glu_4$ , Fluor-4 but not irradiated. Control 2: cells were incubated only with Fluor-4 and irradiated with 0.84 $mW/cm^2$ (2.52 $kJ/m^2$) white light. Sample 1: cells were incubated with 10 μM P-$Glu_4$ and Fluor-4 and irradiated with 0.84 $mW/cm^2$ (2.52 $kJ/m^2$) white light for 5 minutes. Sample 2: cells were incubated with 10 μM P-$Glu_4$, Fluor-4 and irradiated for 10 minutes under the same light conditions. Magnification is 20x.

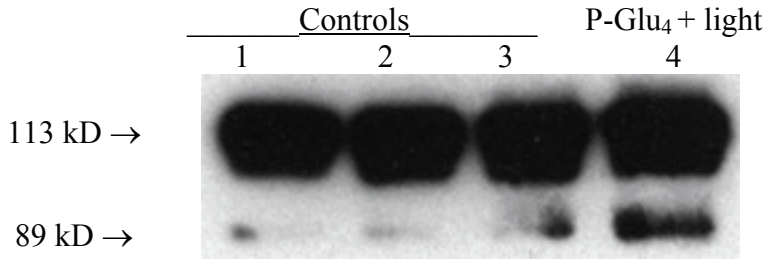


**Figure SI-4**: Detection of Poly-ADP-Ribose-Polymerase (PARP) cleavage in human breast cancer MDA-MB-231 cells as an indication of apoptosis. The cells were treated with 20 μM P-$Glu_4$ for 24 hours, irradiated with a 13W fluorescent light (0.27 mW $cm^{-2}$ for 10 minutes; 1.62 kJ $m^{-2}$), and 9 hours after irradiation, cells were collected and lysed. The supernatant of the lysate was applied to western blot to detect PARP cleavage. Lane 1: with no irradiation or P-$Glu_4$; Lane 2: with irradiation but no P-$Glu_4$; Lane 3: with P-$Glu_4$ but no irradiation; Lane 4: with P-$Glu_4$ and irradiation. Data from reference 23.

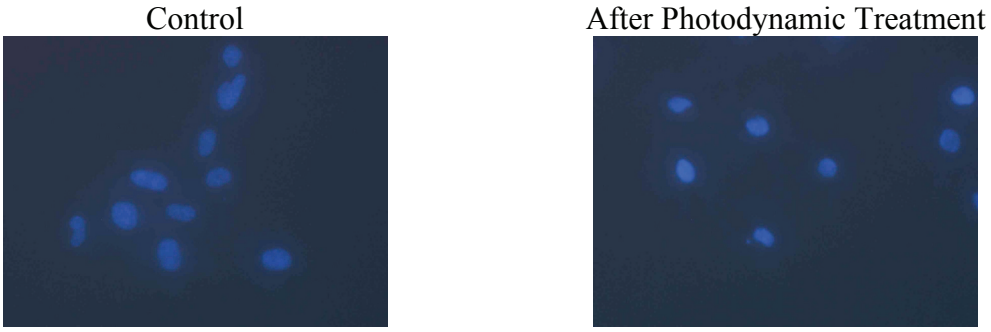


**Figure SI-5.** DAPI staining assays. 0 or 10 μM P-$Glu_4$ was incubated with human breast cancer MDA-MB-231 cells for 24 hours and irradiated for 5 min at 0.84 mW/$cm^2$ (2.52 kJ/$m^2$) under white light. 8 Hours after irradiation, cells were fixed with 4% paraformaldehyde and stained with 1 μg/ mL DAPI solution. Fluorescence images were taken under identical conditions

**Comments**

*DAPI procedures*. The breast cancer cells were incubated with 10 μM P-Glu$_4$ for 24 hours, the growth medium exchanged to remove unbound porphyrin, and irradiated for 5 minutes at 0.84 mW cm$^{-2}$ (2.52 kJ m$^{-2}$); note that these conditions are significantly milder than those used to induce necrosis. Eight hours after irradiation, the cells were fixed with a 4% paraformaldehyde solution and stained with a 1 μg mL$^{-1}$ DAPI solution. Cells were incubated with DAPI (1 μg mL$^{-1}$ in PBS) for 5 minutes at room temperature and washed three times with PBS. The cover slips were mounted in Dako fluorescent mounting medium, put onto slides, air dried, and visualized using a Nikon Optiphot 2 fluorescence microscope where images were captured as high quality (>100kb) JPEG files.

*PARP* is a DNA repair enzyme whose expression is triggered by DNA-strand breaks. In cells undergoing apoptosis PARP is cleaved from a 113 kD peptide into 24 kD and 89 kD polypeptides. It appears plausible that cleavage of PARP facilitates the degradation of cellular DNA,[32] which is a hallmark of apoptosis. Our PARP assay results[23] also demonstrate that apoptosis was induced in MDA-MB-231 cells by photodynamic treatment with P-Glu$_4$.

The use of *white (broad band) light* in the present experiments may cause other natural chromophores in the cell to be involved in necrosis or apoptosis. The activation of metalloporphyrins is unlikely since the excited state is a few ps, but flavins and other species could be involved. [as pointed out by a reviewer see, for example, 'Mitochondrial Reactive Oxygen Species Generation and Calcium Increase Induced by Visible Light in Astrocytes'; Ann. N.Y. Acad. Sci. 1011: 45–56 (2004)]. However, this light intensity/power dependent and perhaps cell dependent. In the present case many dozens of control experiments with no P-Glu$_4$ show no difference between the percentages of necrotic or apoptotic cells in the dark versus in the light under the same conditions. Where the OD of the porphyrin is zero there cannot be any activation, yet irradiation over the entire Q band region is desirable because the transmittance of these photons through tissue, though small for some wavelengths, is not zero. (e.g. see: B. Chance, I. Luo, S. Nioka, D.C. Alsop, J.A. Detre, Optical investigations of physiology: a study of intrinsic and extrinsic biomedical contrast. *Philos. Trans. Royal Soc. London, B Biol. Sci.* 1997, **352**, 707-716.) The efficacy of the PDT agent depends on many factors beyond the optimal OD at wavelengths most transparent in tissues (such as triplet quantum yield).

*The entry and partition of photosensitizers* in cells is a complex issue that depends on nonspecific properties such as hydrophobicity and the specific subtituents. It is dependent on the type of cells and on the composition of the formulation (e.g. with albumin or serum with mTHPC). It may be that many compounds enter into cells via more than one pathway and partition into several cellular components. [See: J. Y. Chen, N. K. Mak, C. M. N. Yow, M. C. Fung, L. C. Chiu, W. N. Leung, N. H. Cheung, The Binding Characteristics and Intracellular Localization of Temoporfin (*m*THPC) in Myeloid Leukemia Cells: Phototoxicity and Mitochondrial Damage, *Photochem. Photobio.* 2000, **72**, 541-547.] We are investigating this for the glycosylated porphyrins.

**Trypan blue staining** is a quick assay to verify the observed morphological changes in PDT treated cells. Though this assay may both miss non viable cells by not staining, or count some that may survive, [See: P. R. Roper, B. Drewinko, Comparison of *in Vitro* Methods to Determine Drug-induced Cell Lethality *Cancer Research* 1976, **36**, 2182-2188] the purpose of this assay was to estimate the initial cytotoxicity as a starting point for the apoptosis studies. It is recognized that the survival is better determined by clonnogenic studies.