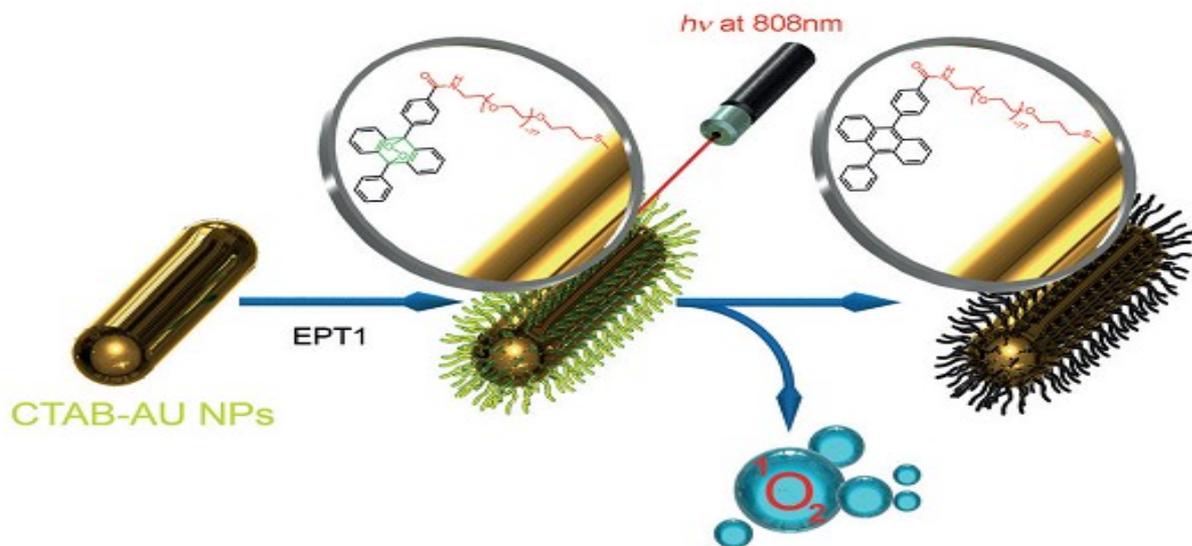
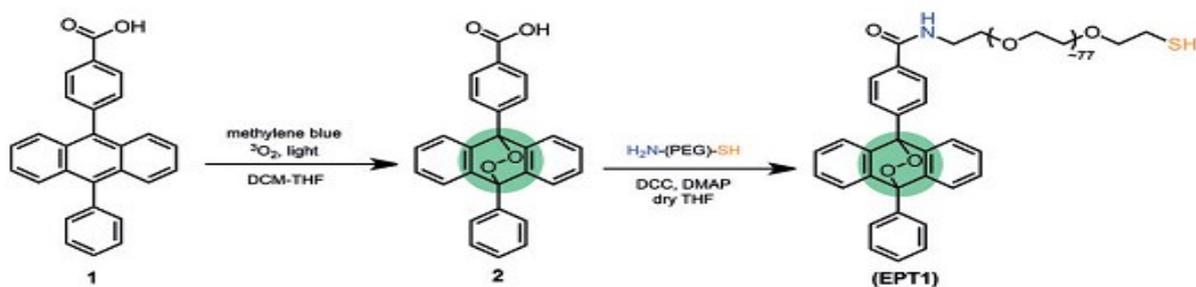


Remote-Controlled Release of Singlet Oxygen by the Plasmonic Heating of Endoperoxide-Modified Gold Nanorods: Towards a Paradigm Change in Photodynamic Therapy

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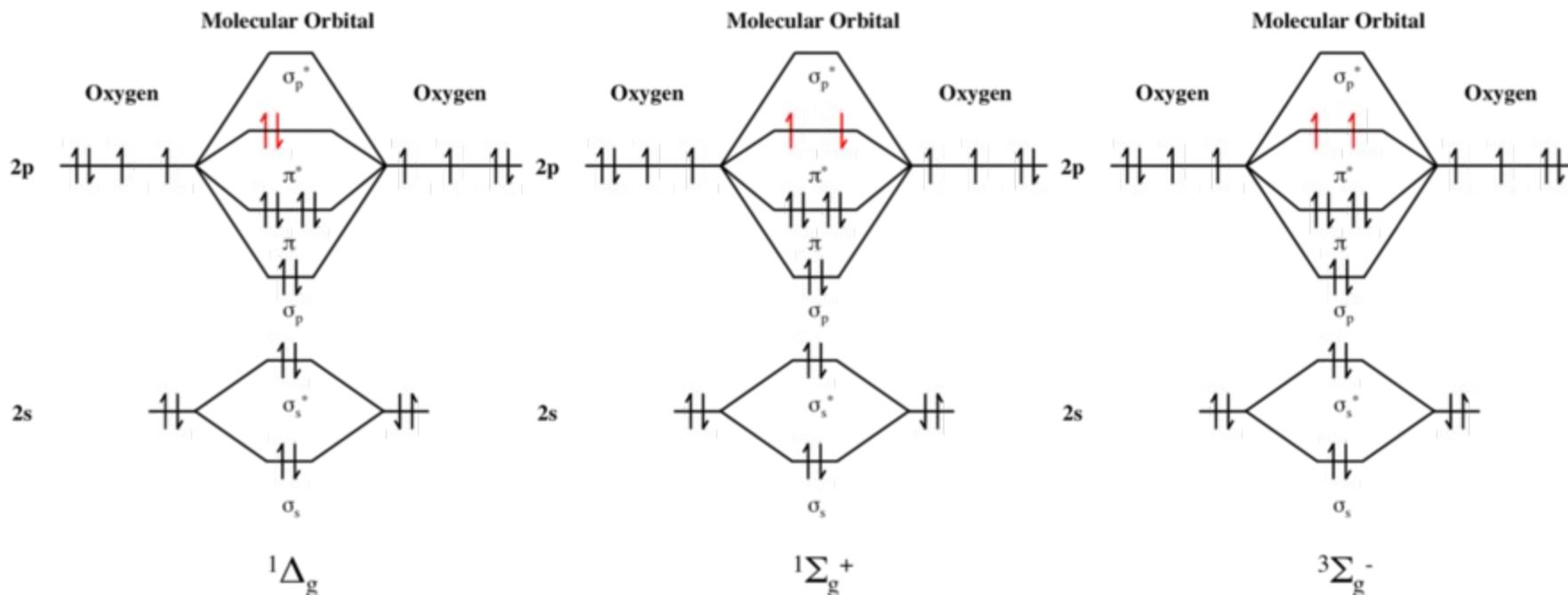
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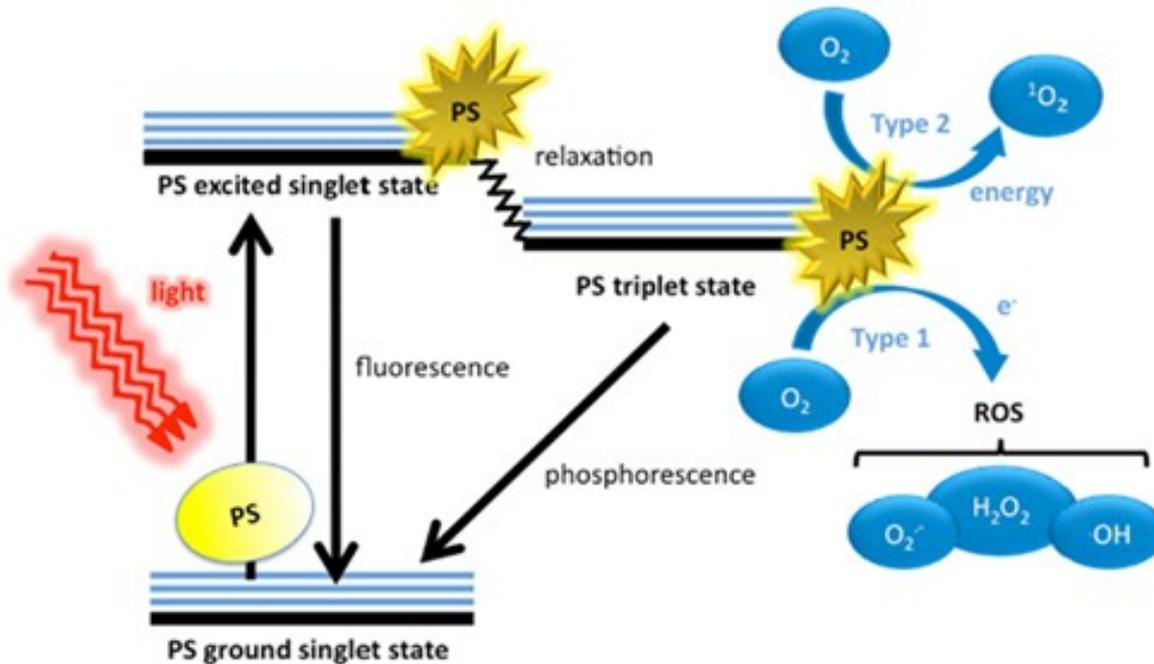
Singlet oxygen :

Molecular orbital theory predicts two low-lying excited singlet states of the molecule O_2 denoted by the molecular term symbols $^1\Delta_g$ and $^1\Sigma_g^-$. Singlet oxygen is far more reactive toward organic compounds. It is responsible for the photodegradation of many materials but can be put to constructive use in **Photodynamic therapy (PDT)** .



Photodynamic Therapy :

Photodynamic therapy (PDT) is a **treatment** that uses a drug, called a photosensitizer or photosensitizing agent, and a particular type of light. When photosensitizers are exposed to a specific wavelength of light, they produce a form of oxygen that kills nearby cells.

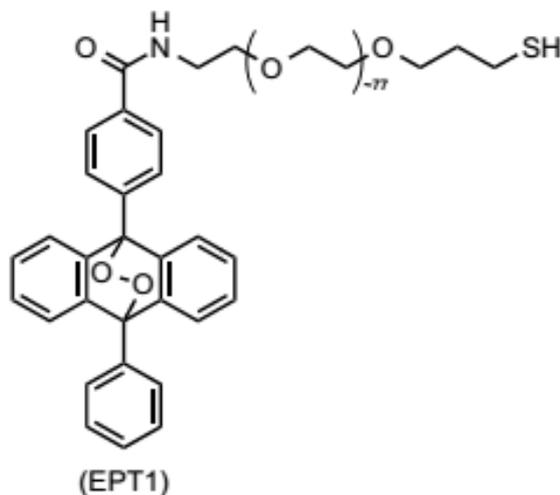


Introduction :

- ❖ A photosensitizer that can be excited by light in the visible, but more preferably in the red or near-IR region of the spectrum could sensitize ground state molecular oxygen and generate singlet oxygen.
- ❖ This process is inherently regioselective, as the singlet-oxygen generation will take place only in the region at which the light beam is directed.
- ❖ The photodynamic therapy (PDT) of cancer, in combination with the enhanced permeation and retention (EPR) effect which leads to sensitizer accumulation in tumors, has been considered a promising therapeutic approach for decades.
- ❖ However, some limitations are there-
 1. Even at the optimum wavelengths, the tissue penetration of light is very ineffective beyond the first few millimeters.
 2. Most tumors, specially aggressive metastatic tumors develop a hypoxic (*deficiency in the amount of oxygen reaching the tissues*) region that are highly resistant to chemotherapy and radiotherapy.
 3. In PDT normoxic oxygen concentrations are essential for effective singlet-oxygen generation. Furthermore, even in normal tissues, the PDT process itself decreases the cellular oxygen concentration

In this Paper :

- ✓ This work proposes that if singlet oxygen could be generated directly in the desired region by remotely controlled mechanisms, all of these issues would be successfully circumvented.
- ✓ When gold nanorods with tethered endoperoxides are irradiated at 808 nm, the endoperoxides undergo thermal cycloreversion, resulting in the generation of singlet oxygen.
- ✓ Here, it is demonstrated that the amount of singlet oxygen produced in this way is sufficient for triggering apoptosis in cell cultures.
- ✓ Plasmonic heating has been chosen to be the method of remote controlled heating.
- ✓ The half-lives of anthracene 9,10-endoperoxides are typically years at room temperature, but when heated, they decompose rapidly, mostly by cycloreversion. A thiol-terminated and water-soluble 9,10-disubstituted anthracene, EPT1 is synthesized as the endoperoxide source.



Chemical assessment of the decomposition or cycloreversion process

1. Upon heating in DMSO or HEPES buffer, the endoperoxide cleanly regenerates the parent anthracene, as confirmed by the typical electronic absorption spectrum of anthracene.
2. By using the selective singlet-oxygen trap 1,3-diphenylisobenzofuran (DPBF), we were able to detect singlet-oxygen formation (Figure 2d) during the thermal decomposition of the endoperoxide in DMSO, as the trap absorbance decreased with a concomitant increase in the intensities of the characteristic anthracene peaks.

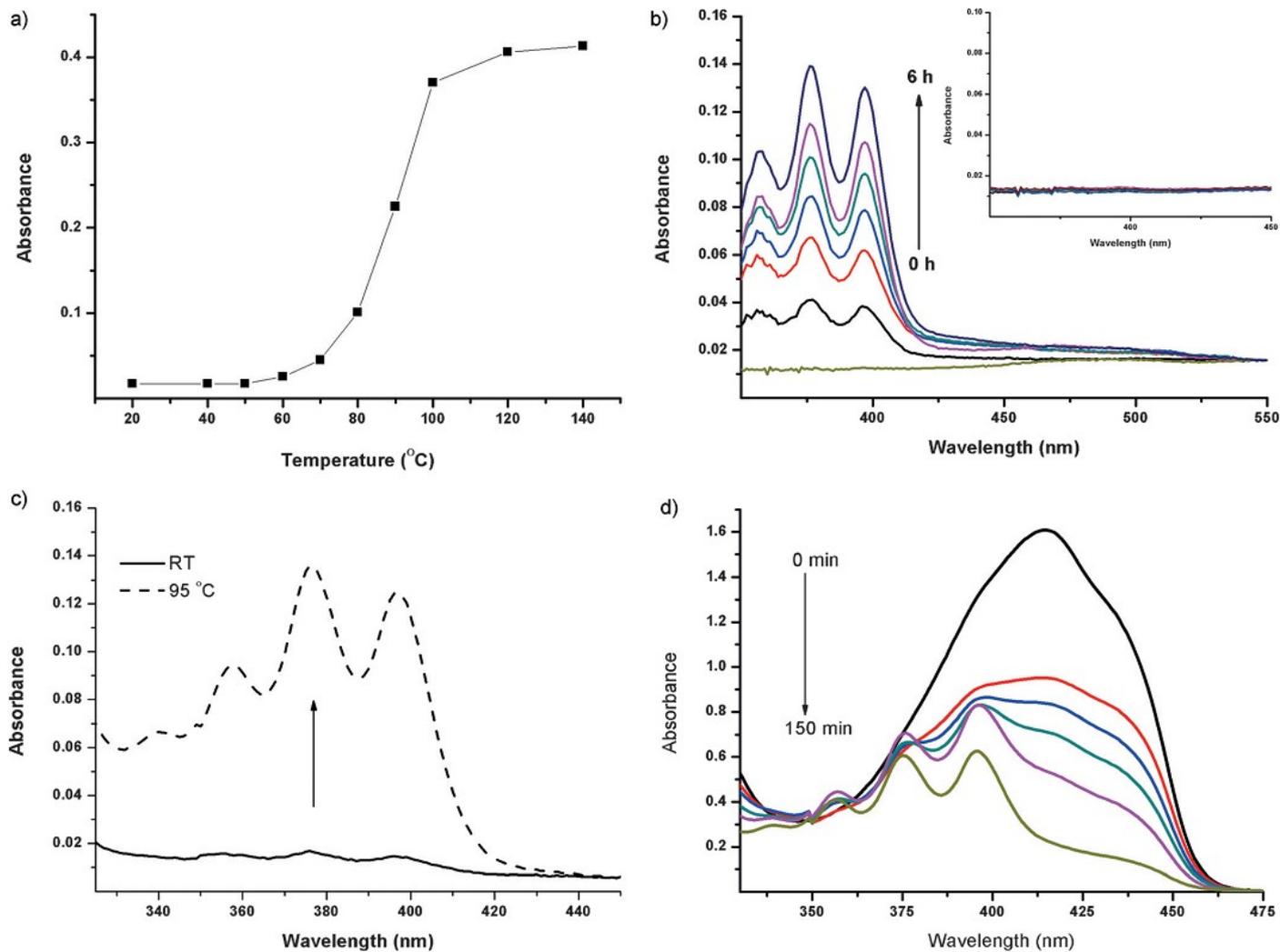
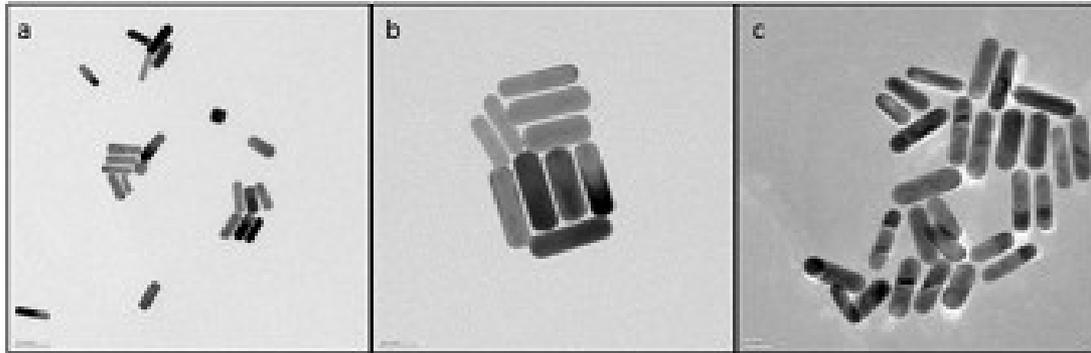


Figure 2. Thermal cycloreversion of EPT1. a) Absorbance at one of the anthracene peaks (404 nm) after heating EPT1 for 30 min at the indicated temperatures. b) Thermolysis of EPT1 in DMSO at 95 $^{\circ}\text{C}$ for 6 h. Inset: Absorbance of EPT1 in DMSO at 37 $^{\circ}\text{C}$. Samples were heated for 6 h. c) When EPT1 is heated in HEPES buffer (pH 7.2, 20 mM), the typical anthracene spectral signature is observed. d) Decrease in absorbance of DPBF in DMSO in the presence of EPT1 upon heating at 70 $^{\circ}\text{C}$ and reappearance of the parent anthracene absorption bands.

Characterization of gold nanorods :



- ✓ GNR: 40 nm, A.R = 4
- ✓ GNR-CTAB were treated with EPT1 in HEPES buffer.

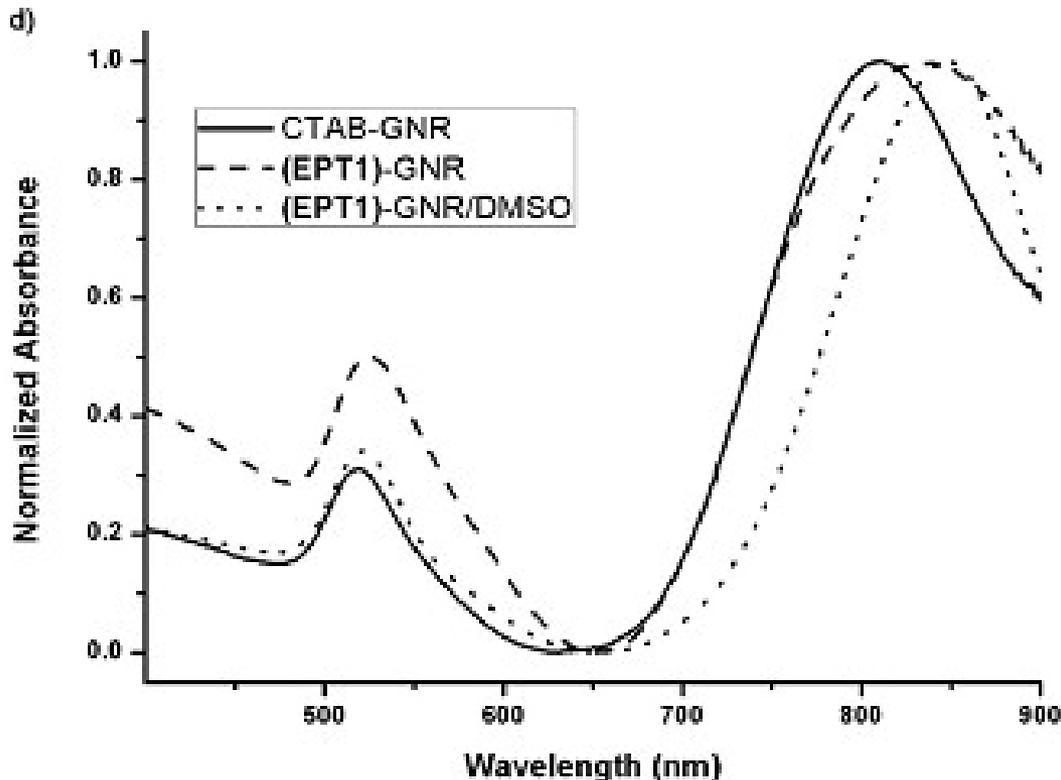


Figure 3. TEM images of the CTAB-GNR conjugates (a,b) and the EPT1-GNR conjugate (c). d) Normalized electronic absorption spectra of CTAB-GNR and EPT1-GNR in HEPES (pH 7.2, 20 mm) and of EPT1-GNR in DMSO.

of thermal decomposition of nanorod tethered

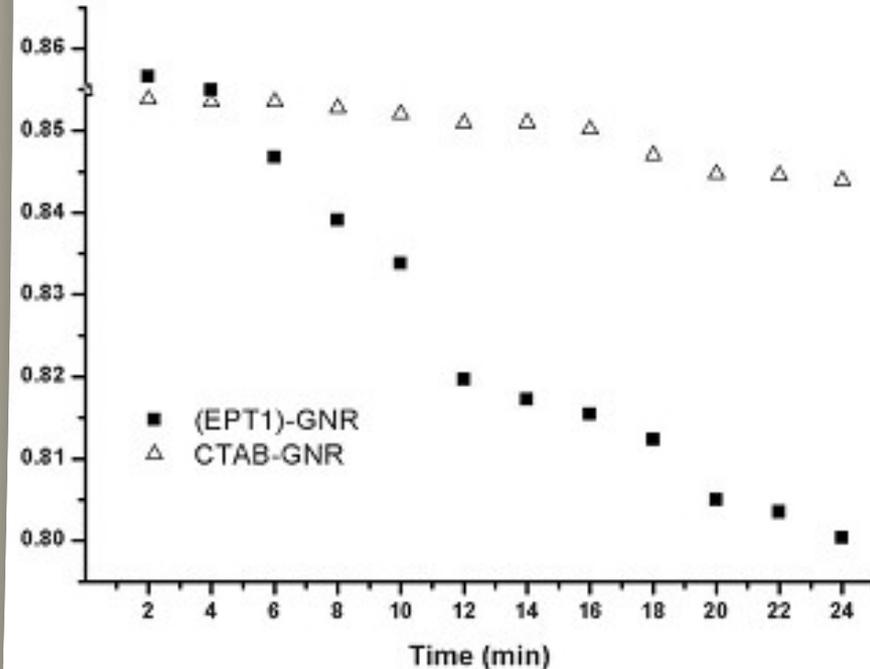
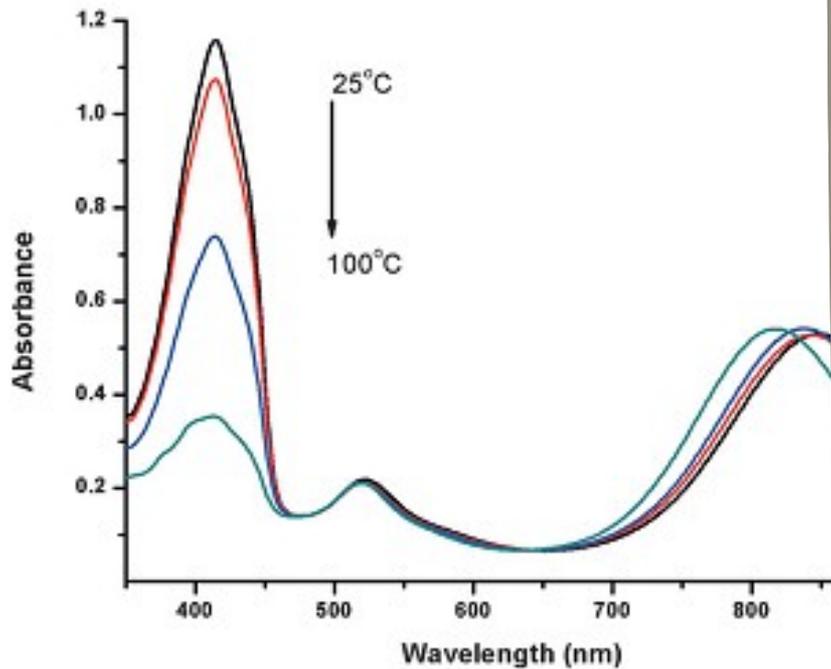
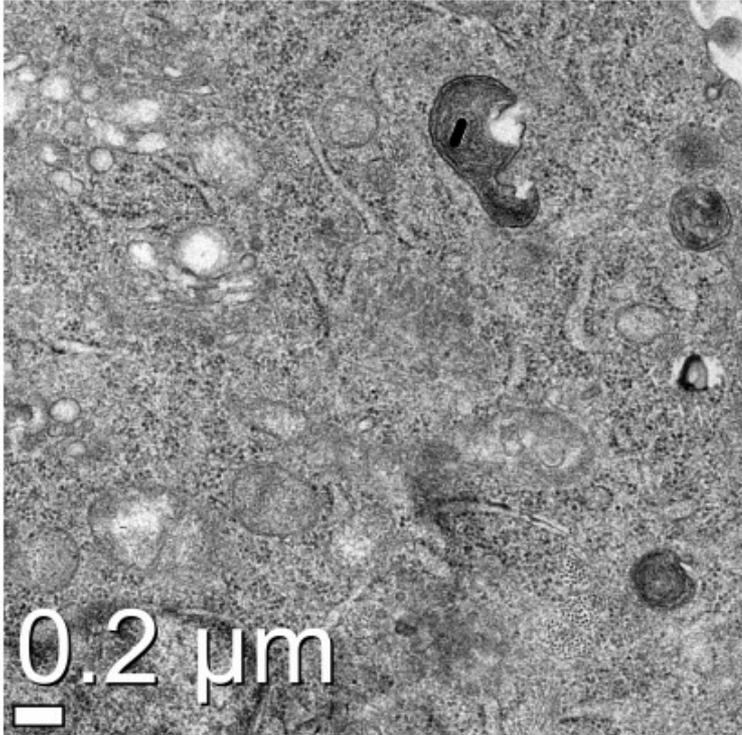


Figure 4. Decomposition and singlet oxygen generation of plasmonically heated EPT1-GNR conjugates. Left: Decrease in the absorbance at 414 nm of DPBF in DMSO in the presence of EPT1-GNR during heating in an oil bath. Right: Relative 1O_2 generation efficiency of EPT1-GNR and CTAB-GNR only in DMSO, detected by the absorbance decrease of DPBF at 414 nm with time. Excitation was at 830 nm (1.0 Wcm^{-2}). During the first 4 min, the samples were kept in the dark.

Viability of the GNR–endoperoxide agent as a remote-controlled source of singlet oxygen in cell cultures :



Cryo-TEM image of GNR-EPT1 incubated HeLa cells.

CONTROLS:

1. HeLa cells incubated with GNRs with a PEG-SH ligand for 24 h.
2. A singlet-oxygen-quencher azide (10 mM) was added to the cell media in one series.

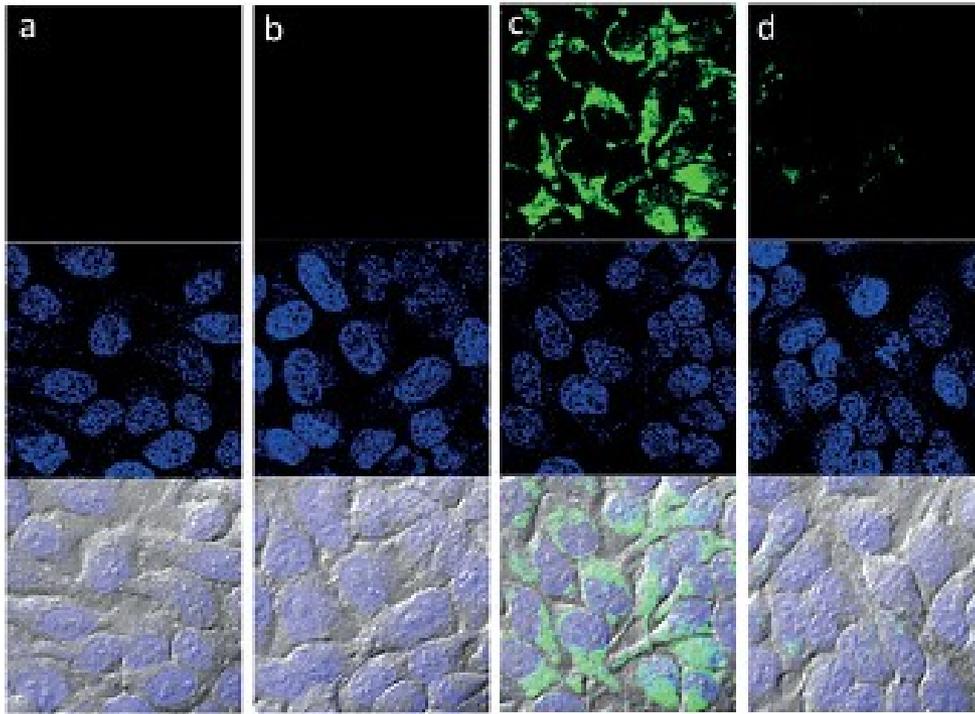
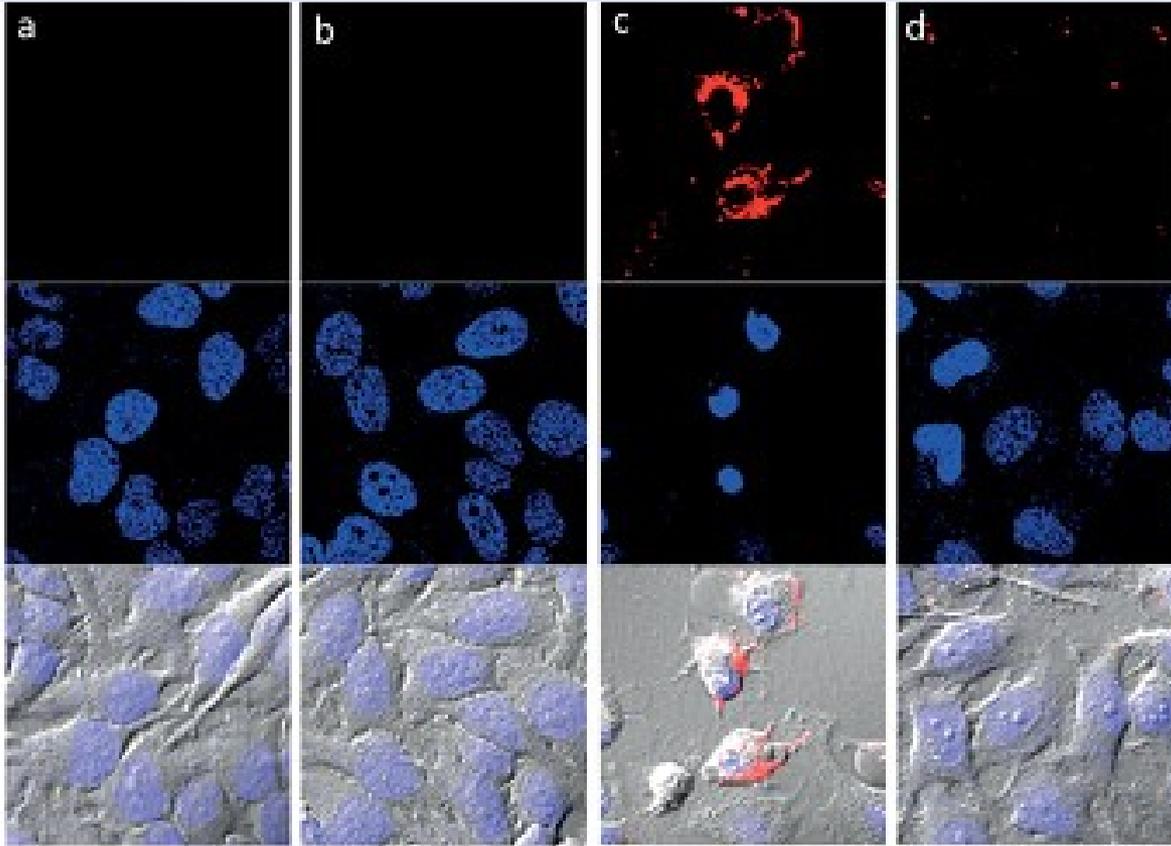


Figure 5. ROS generation by EPT1–GNR in cell cultures. HeLa cells were incubated with no additive (a), GNR–PEG (10 pM; b), EPT1–GNR (10 pM; c), or EPT1–GNR (10 pM) and NaN₃ (10 mM; d). All cells were irradiated with a 808 nm laser (2.0 Wcm², 10 min) and incubated with an ROS sensor (Cyto-ID[®] oxidative-stress detection kit) for 30 min. The cell images were acquired by confocal microscopy. Top: ROS sensor; middle: DAPI; bottom: merged with DIC.

Confocal microscopy :

- The control compound GNR–PEG did not generate oxidative stress in the cell cultures when irradiated at a fluence rate of 2.0 W cm⁻² (Figure 5).
- EPT1–GNR irradiation caused fluorescence emission in the cytosol, lighting up the reactive oxygen sensor.
- Inhibition by the added azide (Figure 5d) identified the reactive oxygen species as singlet oxygen.

Cells undergoing apoptosis were identified with fluorescently labeled Annexin V



- Only the irradiation of cell cultures containing EPT1–GNR resulted in the fluorescence labeling of the outer leaf of the cell membranes, an indication of apoptosis.
- The addition of azide resulted in a very large suppression of cell death.

Figure 6. Apoptosis induced by EPT1–GNR in cell cultures. HeLa cells incubated for 16 h, and stained with Annexin V and DAPI for 20 min. The cell images were acquired by confocal microscopy. Top: Annexin V; middle: DAPI; bottom: merged with DIC.

MTT assays of cell viability

The **MTT assay** is a colorimetric **assay** for assessing cell metabolic activity. NAD(P)H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present.

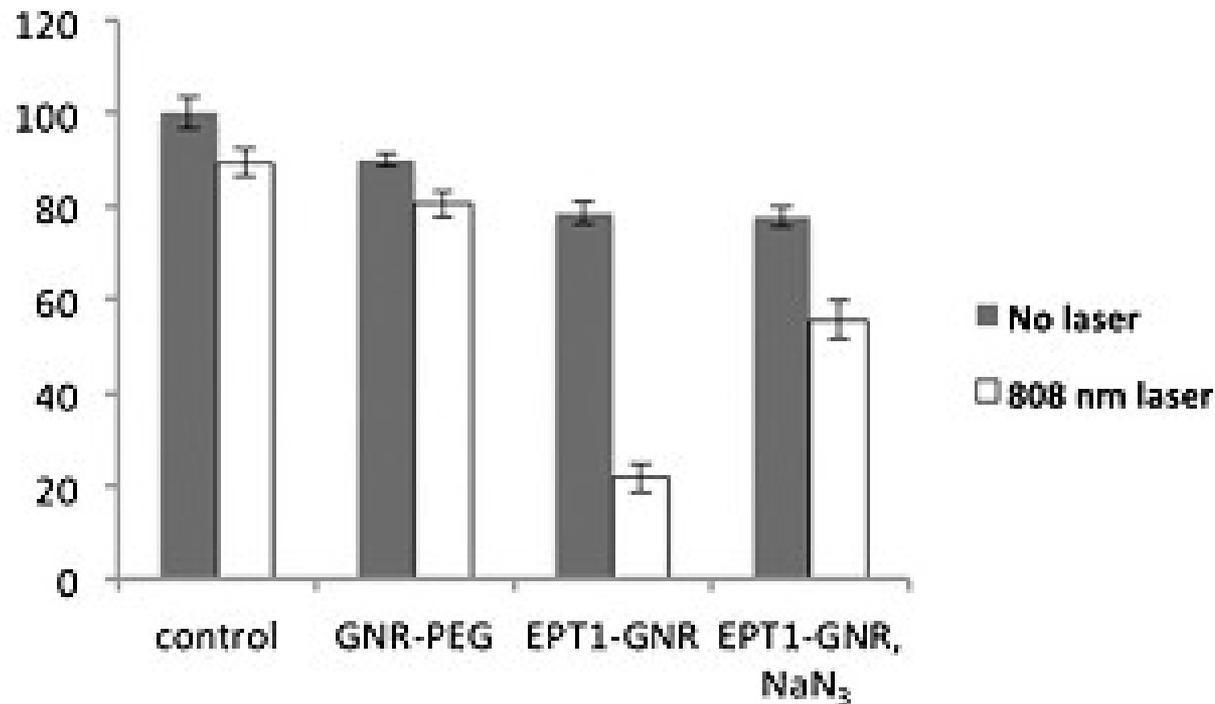


Figure 7. HeLa cells were incubated with 10 pm of GNR-PEG or EPT1-GNR for 24 h, washed with DPBS, and irradiated with a 808 nm laser (2.0 W/cm², 10 min). After 24 h, the cell viability was determined by an MTT test.

Conclusion

- Chemically generated singlet oxygen can induce apoptosis and lead to the death of cancer cells in cell cultures.
- Even a small quantity of singlet oxygen is capable of creating a significant apoptotic response through amplification mechanisms. Thus the fact that endoperoxides are stoichiometric agents does not present a problem.
- The chemical generation of singlet oxygen by the controlled cycloreversion of endoperoxides has the potential to alleviate the problems of tumor hypoxia (both induced and chronic) and light penetration associated with the photodynamic therapy of cancer.
- Having its roots in PDT, EPT may deliver all of the promises of PDT without its inherent limitations.

• *Questions ???*

Future Direction

- I am studying reaction between Ag44 cluster and MBA functionalized gold nanorod. If we choose a cluster with luminescence or that becomes luminescent on reacting with gold nanorods, we can think of similar type of application of it in cells.



Thank you