
# PHOTODYNAMIC THERAPY REPORT 2016

## In Vivo Targeted Deep-Tissue PDT Based on Near-Infrared Triggered Upconversion Nanoconstruct

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*Department:*
Electrical and Computer Engineering
*Course Code:*
ELCE725
*Course Title:*
Special Topics for Biomedical Engineering

# Abstract

This report is concentrated on reviewing a paper on solving two major challenges of photodynamic therapy (PDT) and introducing several essential techniques and principles mentioned or used in this paper. The paper, published in 2012 on *ACS Nano,* illustrates a multifunctional nano-construct they built to cope with difficulties of light penetration over tissues, tumor-selectivity and solubility (in water) of photosensitizer. This research was the first to apply PDT in vivo targeted deep-tissue and could further motivate investigations of photosensitizer carrier and upconversion-based PDT system.

# Background

Photodynamic therapy (PDT) uses a drug and a special type of light. The light could be laser or another type of light. And the drug, or the photosensitizer, is a chemical that makes skin cells or other body cells sensitive to light. The drug can be injected into bloodstream or applied to skin as a cream. After absorption, the body cells will be exposed to light and cancer cells could be destroyed.

However, current PDT has two major limitations. The first is limited tissue penetration of excitation light. The light we used to excite the photosensitizer could only penetrate a few millimeters from tissue surface. The second is the difficulty in formatting PS in physiological mediums due to dissolubility in water and oil. Currently, the tumor selectivity is poor and it will minimize the efficiency of PDT in vivo, as well as increase the potential photo-toxicity to normal tissues.

# Techniques and Principles

### Photosensitizer of ZnPc

Photosensitizer is used in procedure of photoactivation, which causes the formation of singlet oxygen, peroxidative reactions that can cause damage and death. ZnPc is one of the most commonly used photosensitizer used in PDT. Advantages of choosing the chemical of ZnPc are that ZnPc itself is of low toxicity, until lighted of required wavelength. The wavelength of light to excite of ZnPc is 650nm. Fluorescence microscopy is usually used to determine the intracellular localization of ZnPc after 2- or 24-h incubation

## UCNPs: Up-conversion Nanoparticles

While Stokes fluorescence means re-emission of longer wavelength photons by a molecule that has absorbed photons of shorter wavelengths, up-conversion is the opposite of it. The upconversion is the phenomenon that absorption of two or more photons leads to emission of light at shorter wavelength than the excitation wavelength, in other words, from low frequency (energy) to high frequency (energy). Here in this research, the upconversion is applied to transfer the wavelength of 980nm near-infrared red (NIR) light to the wavelength of 660nm visible right light.

Nanoparticles, are particles that are in size of nano-scale, from 1 nm to 100nm. Here in this research, the core of the nano-construct is around 30nm and the total construct (with FASOC coated and photosensitizer of ZnPc loaded) is around 52nm.

The use of UCNPs can help to concur the difficulty of limited tissue penetration of excitation light. The nano construct can better help to deliver the photosensitizer of ZnPc to cells, and the NIR has better penetrability to tissues than visible red light. After penetrated, the NIR will transformed to visible red light (wavelength 660nm) and excite the ZnPc (excitation wavelength 650nm).

## FASOC: Folate -modified amphiphilic chitosan

In this research, FASOC is applied to coat the upconversion nanoparticle. The

chitosan has good characteristics that it is used to make the nanoconstruct soluble in water and can efficiently trap the ZnPc for FRET (Fluorescent resonance transfer)-mediated PDT. It also has good biocompatibility and is biodegradable, can be applied to most patients.

The folic acid (FA) is the other chemical that coats the UCNPs, to improve the selectivity of tumor cells. The folic acid can be efficiently affined to folate receptor (FR), which is overexpressed in diverse cancer cells. When normal cells turn into tumor cell, FA will be largely needed, thus, better for tumor cells in deep tissue to absorb our nanoconstruct in tumor cells and the harm to normal cells will be decreased. In addition, FR, the receptor of FA, can be recycled and regenerated. Thus, the utilization rate of FA is high in vivo, compared with passive targeting.

## Incorporate strategy

To load PS of ZnPc to UCNPs, there are usually three methods, physical adsorption, covalent conjugation and physical encapsulation. Physical adsorption has low loading capacity and can cause premature release of PS from UCNP during the circulation in the blood. Covalent conjugation can avoid the leakage of ZnPc, however, it has limited drug-loading capacity, and thus, it is usually not used for in vivo PDT. The method applied in this research is physical encapsulation for the highest drug loading capacity (810 wt %) compared with the previous two methods.

NIR-to-visible UCNP-based FASOC-coated PDT nanoconstruct is built now. The remission of NIR causes two lights, one with wavelength of 540nm (for imaging) and the other with wavelength of 660nm(for therapy). First they inject the ZnPc loaded photosensitor into body cells and largely absorbed by tumor cells. Then use NIR light to excite the UCNPs and the remission visible red light will cause fluorescence resonance energy transfer (FRET) to the attached PS. Toxic will be generated (ROS) to kill surrounding cells.

# Result evaluation

This research did three types of evaluations, synthesis & characterization, in vitro test and in vivo test. Absorption spectra and PL intensity are used to test absorbance and concentration. Microscopy observation to compare tumor-targeted ZnPc delivery by the prepared nanoconstructs in cancer cells and toxicity ROS under different excitation lights in vitro incubation. Finally, fluorescence image on mice is used to track in vivo tumor-targeting of the nanoconstructs in different body tissues and two kinds of lights.

The result proves that this new structure can be applied for deep tissue treatment (due to NIR). Also, the coating makes the structure hydrophilic and higher selectivity of PSs that tumor cells will absorb more photosensitizer than normal healthy cells.

# Discussion

Previously, after presentation, my answer about the extra energy of the upconversion transform, the re-mission of shorter wavelength after the absorption of longer wavelength, is coming from the crystal lattice structure (searched “upconversion” in Wiki). The description is not accurate here. Actually, the extra energy is coming from the absorption of pump photons. The required additional energy being transferred from another laser-active ion undergoes nonradioactive de-excitation. Corresponding energy level diagram and analogy from everyday life is as shown in Fig.01.

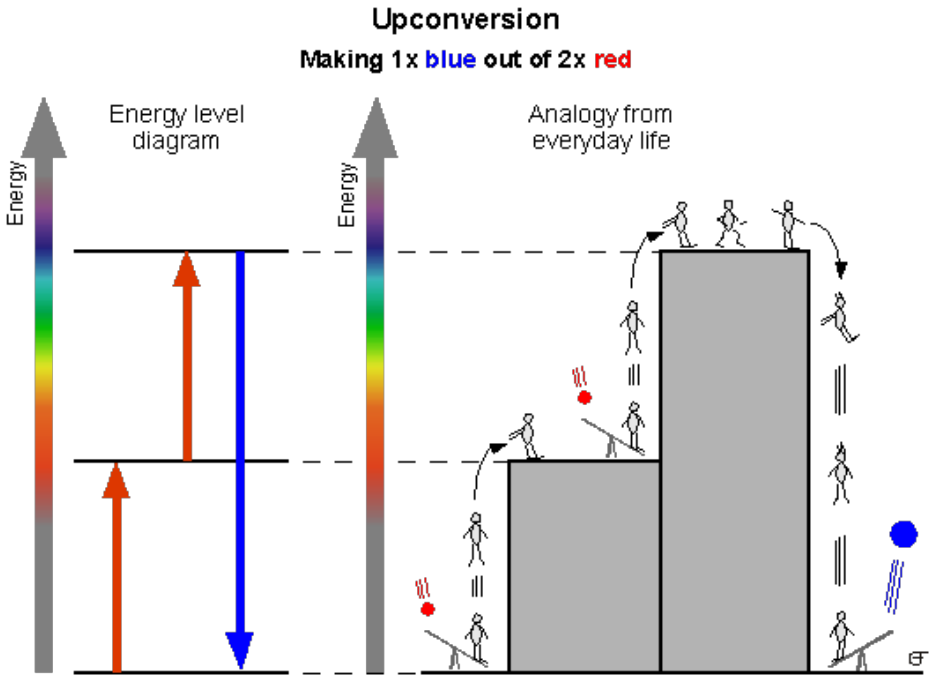


**Fig.01 average levels in cells expressing eNpHR3.0 and eNpHR2.0**

# References


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