Semiconductor and Carbon Quantum Dots in Photodynamic Therapy

Christodoulis Panagiotis

University of Groningen

Supervisor: Dr. A. Salvati

1 April 2018

Abstract

Photodynamic Therapy (PTD) is an important tool in the fight against various diseases, including cancer, and thus it is essential for the efficacy of the technique to be optimized. The treatment occur upon irradiation of a photosensitizer close to environmental oxygen, resulting in the generation of reactive oxygen species which destroy the target cells in their vicinity. Quantum dots (QDs), with their special optical properties arising from their tiny size, possess considerable potential in improving PTD. Not only they can act as energy donors, extending the region of wavelength at which the treatment can operate, but they can also act themselves as photosensitizers (PS) generating singlet oxygen ($^1O_2$). In addition they can act as agents for two-photon excitation (TPE), enabling the use of irradiation of longer wavelengths which increases the depth at which PTD can operate. However, despite their promising potential, initials reports using semiconductor QDs showed that these systems are unable to bypass important drawbacks like toxicity and low $^1O_2$ generation. On the other hand carbon dots (CB), owing to their hydrophilic functional groups, possess the necessary biocompatibility and in vivo studies show that they exceed semiconductor QDs in performance. Among them graphene QDs (GQDs) show the most promising results combining biocompatibility and high $^1O_2$ generation via a unique mechanism termed multistate sensitization. Finally, even though this field is quite immature, QDs (and especially CDs) have been tested as double agents combining PTD with Photothermal Therapy (PTT) with positive in vivo results.

Contents

1. Introduction

Photodynamic therapy (PTD), is a very popular treatment used widely against diseases like acne, psoriasis, atherosclerosis or even cancer. Its working principle, as the name suggests, is based on the dynamic interaction of light, the body and the system used to trigger the therapy (photosensitizer). This interaction induces a series of reactions on the diseased area which lead to the death of microbial cells and the treatment of the disease. Variation of the technique have been used for a century now[1] and in the late years the use of nanotechnology has considerably expanded its potential. Among different nano-systems that have been applied to improve the efficiency of PTD, quantum dots (QDs) seem to be one of the most promising ones as they exhibit suitable optical properties that make them attractive for a variety of modern (bio)applications.

In the present study, after a brief introduction on PTD and the basic properties of QDs, the different ways that they can assist PTD will be described. Subsequently I will attempt to highlight the important breakthroughs that occurred along the years, with the purpose of proving the reader with a clear image of the paths that have been followed, or are to be followed, in the journey to maximize the efficiency of the photodynamic therapy using semiconductor and carbon (the most widely studied) quantum dots.

Before I attempt to address the aforementioned issues, I would like to say some words about the reasons that drove me to choose the present topic. Being a master student of Nanoscience myself, I have a strong interest on the unique properties that manifest themselves only when one escapes our macro (or even micro) world and looks down on the nano world. As it will become obvious through reading the present work, quantum dots owe their unique properties to "quantum confinement", a phenomenon that exclusively appears only in the nanoscale. Furthermore, I do have a special interest in clinical applications of science which, not only I find important, but I am also fascinated by the way that initially abstract theories finds applications in a field so complex like medicine, giving rise to new possibilities and expanding our current capabilities. Putting these two factors together, a topic related to the application of quantum dots in photodynamic therapy was an ideal one for me to conduct my Research paper on.

1.1 General principles of PTD

Treatment of a pathological area via PDT requires the presence of two things: a photosensitizer (PS) and molecules containing oxygen supplied by the surrounding molecular environment. Upon light irradiation, the PS is excited to a higher energy state and its interaction with the surrounding oxygen results in the generation of several reactive oxygen species (ROS) (chemically reactive species containing oxygen). In general, ROS occur naturally in biological processes. However, under environmental stress (irradiation or heat exposure), their numbers can increase dramatically and due to their reactive nature they interfere with the normal function of biomolecules which results in the damage and very often in the death of the

cell via apoptosis (programmed cell death triggered by damages coming from the extracellular environment). This effect is called oxidative stress.

The mechanism through which ROS are generated upon excitation of the PS has been well understood.[2] The excited PS, except of relaxing to its ground state via the emission of a photon (fluorescence), may in some cases undergo an intersystem crossing (ISC) from its excited single state to an excited triplet state. This process is possible when there is an overlap of an excited singlet energy level with an excited triplet energy level. The possibility of the excited triplet state to relax to the ground state via the emission of a photon (phosphorescence) is extremely low (due to the fact that the spin configuration needs also to change), thus increasing the life time of the triplet state (^{3}PS). The increased life time of the triplet state allows for different chemical reactions to occur between the PS and its environment. These reactions result in the generation of ROS via two different chemical pathways, which are depicted schematically in fig. 1A[2(modified)]:

Type I: $^3PS + substrate \rightarrow PS^+ + substrate^-$

$Substrate^- + {}^3O_2 \rightarrow substrate + ROS\ (OH^-, O_2^-, H_2O_2)$

Type II: $^3PS + {}^3O_2 \rightarrow {}^1PS + {}^1O_2$

In the first pathway, an electron is transferred from the PS to the substrate (which can be a cell membrane or another molecule) to create a radical anion. In a similar reaction a radical (uncharged species) could also be formed. The radical anion (or the radical) further reacts with oxygen to generate oxidized products (ROS) like hydroxyl radicals (OH^-), superoxides (O_2^-) or hydrogen peroxides (H_2O_2). Pathway II is possible because molecular oxygen is a triplet in its ground state ($^3O_2$), therefore an energy transfer interaction from the PS in its triplet state is spin – allowed. The ROS generated in this case is molecular oxygen in its excited state $^1O_2$ (a singlet state) (fig. 1B[3]). Singlet oxygen is extremely reactive due to the pairing of two electrons in one of the π_{2px}^* antibonding molecular orbitals, which leaves the other π_{2py}^* antibonding unfilled, and subject to attack from electron rich molecules. It has been shown[4] that usually type II mechanisms dominate during PTD, so most efforts have been concentrated on increasing the singlet oxygen quantum yield (ΦΔ) of the process.

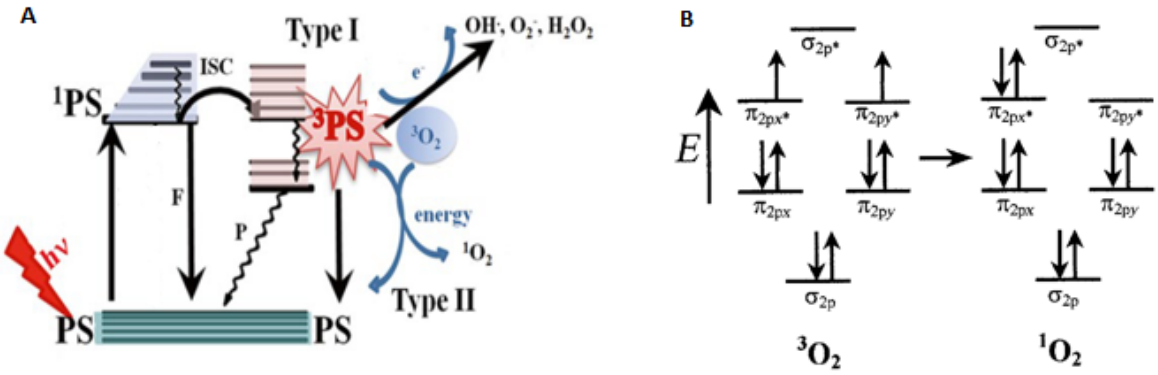

Fig. 1 A.[(reproduced from 2)] Schematic representation of different processes that occur during a PDT treatment including the two possible chemical pathways with which ROS are generated. The ROS generated during each process are also shown: OH^-, O_2^-, H_2O_2 in type one and $^1O_2$ in type II. **B.**[(reproduced from 3)] Spin configuration of Molecular Oxygen in its triplet ground state (left) and its singlet excited state (right). The unfilled π_{2py}^* antibonding molecular orbital makes the excited singlet state highly reactive.

In clinical applications of PTD the choice of the laser source depends on the location of the tumor (deep sited tumors require irradiation with longer wavelengths as will be explained later) and the light dose needed and the PS.[5] Laser and lamps have both been employed as light sources, and the superiority of the one towards the other has not been demonstrated. On the other hand, when

research on PTD comes into play, laser seems to have dominated over lamps.

1.2 Currently approved Photosensitizers

As with every existing treatment method, PTD's ultimate objective is to achieve optimum treatment combined with minimization of side effects. The photosensitizer is an essential component of the PTD system (as it the system that will receive the energy of the light and transfer it to the surrounding environment to produce ROS) and thus its performance greatly affects the efficiency of the technique. There exist a number of factor that distinguish the quality of a PS:

Basic requirements:

1. Maximized $^1O_2$ yield (ΦΔ): In order to kill the diseased cell efficiently. High yield of triplet formation (ΦT) is also desirable to excite efficiently the triplet ground state of molecular oxygen.
2. High extinction coefficient: Increased absorption assures improved the chances for ROS generation.
3. Low photo bleaching: so it can produce the required quantity of ROS before it is deactivated.
4. Water - solubility: Increasing the water – solubility of the PS, facilitates its transport inside the body and have a positive effect on its stability and biocompatibility.
5. Selectivity: The PS used should be able to reach its target efficiently to achieve improved efficiency and avoid undesirable effect on healthy areas.
6. Toxicity: An ideal PS should exhibit minimum toxicity under dark conditions and reveal its "cell killing" abilities only when irradiated with light of the desired wavelength.

Additional requirements:

1. Multifunctionality: So far, no known method is able to deal with certain diseases like cancer alone, thus the PS should allow the combination of different methods to achieve the optimum result.
2. Fluorescence: It is essential to be able to track the PS during the operation, thus fluorescent PS are preferred. In addition many dosimetry techniques that are used to evaluate the results depend on fluorescence. Of course the more energy is released as radiation, the less energy will be provided to generate ROS (because energy is released as radiation and is not transferred to the environment). Thus the right degree of fluorescence needs to be achieved in order for these two complementary processes to function synergistically.

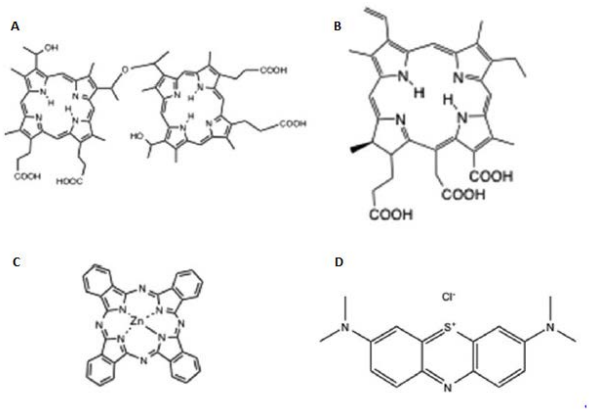

Fig. 2[modified from 6] Chemical structures of the most used photosensitizers in clinical trials: **A**: Photofrin **B**: Chlorin (c6) **C**: Zinc Phthalocyanine **D**: Methylene Blue

Many compounds can act as PS but very few have actually been clinically approved based on the basic requirements mentioned. Most currently available PS (already clinically applied) come from three main groups[7]: porphyrins, chlorines and dyes. Most of the molecules of these groups consists of tetrapyrrole structures. The PS of the first group are all derivatives of hematoporphyrin (Hp). These derivatives can be monomers, dimers or oligomers and the most suitable for a specific treatment depends on the wavelength range to which they absorb. These molecules are useful as they possess good absorbing properties and they also exhibit a small preference on diseased tissues rather on healthy ones. Depending on their structure they can absorb from 400 to 630 nm. Photofrin, which belongs to this group, is one of the first and the most used PS until today, and its structure can be seen in fig. 2A. The PS of the second group belong to the chlorin family. These

molecules absorb slightly deeper in the red region as compared to porphyrins (from 650 to 700 nm). The most used PS of this family is the mono-L-aspartyl chlorin e6 which is derived from naturally occurring chlorophyll. Its structure can be seen in fig. 2B. The third group of PS consists of dyes and most of them come from phthalocyanines and their derivatives. As these molecules are more hydrophobic than the previous categories, they require additional treatment to acquire the necessary water – solubility and they usually absorb in the red region 650 – 680 nm which allows for deeper penetration (as it will be explained later). It has also been observed that linking these dyes to metals increase the triplet quantum yield and their lifetime so metals as aluminum or zinc are often conjugated to these systems. Zinc phthalocyanine is one of the most widely used ones and its structure is depicted in fig.2C Synthetic dyes have also been used with methylene blue, (fig. 2D) being the most investigated one. The most frequently clinically used PS are listed in table 1, along with the wavelength of its maximum emission.

Table 1 Most frequently used clinically approved photosensitizers.

Group	Photosensitizer	Wavelength of maximum emission (nm)
Porphyrin	Photofrin	630
	ALA-induced ptotoporphyrin IX	635
Chlorin	Foscan	652
	Verteporfin	690
	Chlorin (e6)	660
	Monoaspartyl chlorin (e6)	660
Dyes	Zinc phthalocyanine	670
	Silicon Phthalocyanine	675
	Methylene Blue (synthetic)	660
	Rose Bengal (synthetic)	557

1.3 Recent advances

PS coming from the aforementioned categories have already been applied to clinical application of PTD with positive results. However they do suffer for some drawbacks that create limitation on the efficiency of the technique. To begin with, all clinically approved sensitizers do not exhibit sufficient solubility in aqueous solutions, which limits their transport properties and their ability to reach the target area. Furthermore, their hydrophobic nature causes the PS to form aggregates which greatly decreases their photo - activity[8]. In addition, the low penetration of light in the wavelengths that these PS absorb, is a major problem when the treatment of a deep sited tumor or other infected region in needed. A so called "phototherapeutic window" exists, expanding from 780 nm to 950 nm, below which light is attenuated by scattering in skin and other tissues and above which penetration is greatly inhibited by the absorption of water (which is ubiquitous in organic environment) due to its vibrational modes. Most clinically approved PS absorb in the visible region, which limits their application in superficial treatments (a few mm). Furthermore, even if a PS is fabricated such as that it can absorb in the therapeutic window, light of that range does not have enough energy to excite molecular oxygen to generate $^1O_2$.

Fortunately, the rapid evolution of technology (especially nanotechnology) the last two decades have equipped us with powerful tools that allow us to hope to bypass all the aforementioned limitations. More specifically, a special type of tiny nanocrystals, known as "quantum dots" (QDs) exhibit some remarkable properties that hold the potential to greatly expand the efficiency of PTD. First of all QDs can act themselves as a PS and due to their tunability on the absorption frequency they can function in different ranges of light frequency. Furthermore, when QDs are used in combination with a PS, they can deal with the aggregation problem by acting as a carrier delivering the PS on the desired area. In addition quantum dots do not have metastable states (they always relax back to their ground state) which means that all the energy that will be absorbed by the QD will eventually be reemitted. This released energy could possibly be transferred to other

species, provided that those absorb the wavelength of light emitted from QDs. Combined with the fact that their emission wavelength can by precisely tuned, it should be possible for this energy (at least theoretically), to be transferred in a PS in the proximity of the QD (by tuning the emission of the QD so it will overlap with the absorbance of the PS). Therefore, QDs (except of carriers) could also be used to indirectly excite The PS. Since QDs have a broad absorption spectrum, a large range of wavelengths can be used to excite the QD and subsequently the PS, even though the PS would normally exhibit zero absorbance in that range. Finally QDs provide an effective way to deal with the deep penetration problem through the mechanism of two-photon excitation which will be explained in detail.

2. Basic Principles of Quantum Dots and their application in PTD

In order to understand how QDs can expand our possibilities with PTD an introduction to their basic properties and nature is needed. The term "quantum dots" (QDs), refers to tiny, 0-D, nanoparticles with size less than 10 nm. Usually the term is referred to systems comprised of semiconductor nanocrystals in binary (less often tertiary) combinations. The combination take place in a core-shell relation usually notified as Core/Shell (e.g. CdTe/ZnS). In most cases the combinations are between elements of the II-VI or III-V groups of the periodic table. However every nanocrystal that exhibit similar properties due to their tiny size (like carbon quantum dots which are usually names as carbon dots) belong to the family of QDs. QDs are unique in the sense that their optical properties are size dependent, fact that that arises from the quantum confinement that manifest in that scale. Quantum confinement is an exclusively quantum effect (hence the name), that occur when tiny particles are trapped in a limited space in one, two or three dimensions. Similar to the famous quantum mechanical problem "particle in a box" the energies levels of the trapped particle cease to be continuous and they become discrete. As the energy levels of QDs (more specifically the energy levels that an electron of the QD can occupy) resemble that of individual molecules (discrete energy levels), very often they are referred as "artificial molecules". Their small size and quantum confined nature gives rise to a series of advantages that makes QDs attractive for a variety of applications, among them PTD.

First of all, due to their discrete energy levels, the absorption spectrum of QDs is quite broad, thus a large variety of light sources can be used. On the other hand, their emission is narrow, corresponding to their band gap (which depends on their size), and gives them their characteristic color. Therefore the wavelength of their emission can be easily tuned to the desired value according to the application. In additions to these advantages, they are also very resisting to photo bleaching[9]. Finally, due to their small sizes they possess high surface energy (large surface – volume ratio) which makes their surface quite active and subject to conjugation with different biomolecules (as well as PS). As we shall see all of these properties are beneficial for application in PTD.

There are two main approaches with which QDs can be applied to PTD: QDs in the presence of another PS and QD alone without the presence of another PS.

2.1 Quantum Dots in the presence of a PS

As mentioned above QDs can be used to indirectly excite the PS. Now the main question is: does the theoretically predicted transfer of energy actually take place and how effective it is? As Samia and al.[10] firstly demonstrated in 2003, energy is indeed transferred through the mechanism of Forster Resonance Energy Transfer (FRET). In their experiments a 5nm CdSe QD was linked to a silicon phthalocyanine photosensitizer (Pc4) through an alkyl amino group on the photosensitizer's axial substituent. The absorbance and emission spectra of the CdSe QD and the Pc4 photosensitizer are shown in fig.3A[10] and it seems that the necessary overlap (between the emission spectra of the donor and absorption spectra of the acceptor) needed for FRET in satisfied.

Judging from its absorption spectra, pc4 can directly be excited with wavelengths between 550 and 630 nm. However, an energy donor is definitely necessary if excitation at shorter wavelengths is needed. By showing that pc4 emits at 680 nm when excited with a wavelength of 488

nm, which would normally not be absorbed, the group proved that energy transfer from the QD to the PS is indeed possible. The FRET efficiency can be estimated by monitoring the decrease in emission intensity compared to the free QD and the value of 77% was calculated.

The importance of the discovery lies on the fact that, since quantum dots have broad absorption spectra and their emission can always be tuned, it is possible to exite the PS in any desired wavelength, regardless if the PS alone absorbs in that range or not. By the nature of the FRET mechanism, this flexibility is limited on any wavelength smaller than the ones that PS emits.

2.2 Quantum dots as photosensitizers

In the same publication, Samia et al. discovered that QDs can actually trigger the generation of $^1O_2$ without the presence of a PS. Singlet oxygen emits at 1270 nm[11], therefore $^1O_2$ should be detectable through by emission measurements. The emission peak at 1270 nm was indeed observed, proving that QDs alone can generate ROS. Therefore, except of QD-PS systems QD can also serve as PS themselves.

Two possible reactions between the QD and the surrounding oxygen, namely type I and type II, for the generation of ROS are described in literature (fig 3B).[12] (the symbol * represents the excited state)

Type I: $QD^* + {}^3o_2 \rightarrow QD^{+*} + {}^3O_2^-$

$${}^3O_2^- + 2H_2O \rightarrow 2H_2O_2 + e^-$$

$$QD^{-*} + e^{-}\,{}^{e} \rightarrow QD^*$$

Type II: $QD^* + {}^3O_2 \rightarrow QD + {}^1O_2$

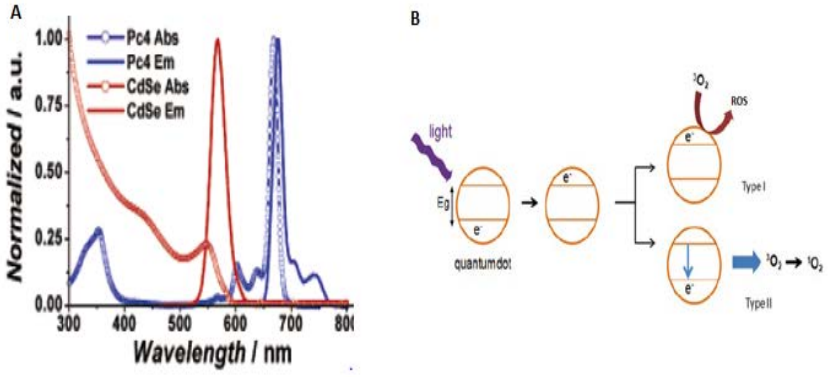

Fig. 3 A.[(reproduced from 10)] Absorbance and emission spectra of the CdSe QD and the Pc4 photosensitizer. The emission spectra was acquired using excitation wavelength of 488 nm where Pc4 exhibits zero absorbance. Nevertheless, Pc4 emitted at 680 nm, reveling that the energy was indeed transferred to the photosensitizer following the emission of the QD at 568 nm. **B.**[(reproduced from 12)] The 2 possible pathways with which ROS can be generated directly from QDs. Type I refers to the electron transport from the QD to the surroundings producing ROS (superoxides and hydrogen peroxides) . Type II refers to the energy transfer from the QD to molecular oxygen which results in the excitation of the later.

During type I reaction (very similar to the type I reaction between a PS and its environment), after the excitation of the quantum dot with a photon, an exciton (bound electron-hole pair) is created. This electron, except of relaxing by re-emitting a photon, can also tunnel to a trap state in the surface of the QD. In the close presence of oxygen, it is possible for this electron to be transferred to the oxygen molecule creating a superoxide ion O_2^-. In aqueous environment, the superoxide can react with water to produce hydrogen peroxide and a free electron which can neutralize the negatively charged quantum dot allowing the process to start all over again. During type II reaction, the electron-hole pair recombines and the energy released is transferred to surrounding oxygen which is then excited to its highly reactive singlet state.

The conclusion from these preliminary studies is clear: QDs hold the potential to be used both as PS themselves, or in conjugation with a second PS. QD bearing systems that have been widely studied for application in PTD, use QDs which belong to two main families, namely: semiconductor QDs and carbon QDs, and thus they form also the topic of discussion in the present review.

3. Families of Quantum dots applied in PTD.

Having described the basic principles of QDs and the way that they can be applied in PTD we are ready to begin our journey on the various systems of QDs that have been investigated since the first demonstration of their potential in 2003. To start with, systems containing semiconductor quantum dots will be described as all initial efforts concentrated in that direction. Subsequently carbon dots, which (as it will become apparent) appear to be superior to the semiconductor dots, will be analyzed. Finally, systems with QDs that were used for two-photon excitation (in order to access deeper areas) will be presented along with an explanation of the physics accompanying the two photon excitation.

3.1 Semiconductor QDs

Before discussing how efficiently QDs can help in PTD, some words about the side effects of these systems need to be said, as the first issue to be addressed when one tries to apply QDs is their toxicity. Although generation of toxic particles (RES) upon light irradiation which kill the infected cells is desired, it has been reported that QDs are also subject to other mechanisms that result in dark cytotoxicity (toxicity without the irradiation of light)[13]. Toxicity has been shown to be related to the release of ions which are highly reactive and interfere with the normal function of healthy tissues, altering their properties and in many cases resulting in permanent damage. The degree of toxicity depends on the accessibility of the core of the QD (where the ions originate) to the surrounding medium. Therefore the extra shell that is added for protection on the core, as well as the ligands attached to it, play an important role in the fight against cytotoxicity. The degradation of the QD can occur due to the diffusion of oxygen on the shell (producing cations of the protected layer) and subsequently on the core, resulting in its oxidation and the production of cations of the core. In acidic conditions (low pH), protonation of the ligands results in their detachment, which causes their aggregation and increases the accessibility to the core, thus enhancing cytotoxicity. The processed described are schematically depicted in fig.4[13] for the case of a CdSe core with a ZnS cell.

As one would easily expect, addressing of these toxic effect of QDs is of urgent importance in order for them to be applied in living organisms. Therefore, as it will become obvious by the different approaches that will be described, QDs always appear coated with an hydrophilic layer that will reduce their toxic effect, improve its solubility and transport properties and therefore enhance its biocompatibility. The main goal is to fabricate a system which will combine optimum biocompatibility, minimum cytotoxicity with maximum $^1O_2$ generation and subsequently high cytotoxicity upon irradiation. As it has already been mentioned QDs can function in the presence or absence of a PS, and thus these two approaches will be described separately. In both cases QDs can be used for two-photon excitation but this function will be analyzed alone.

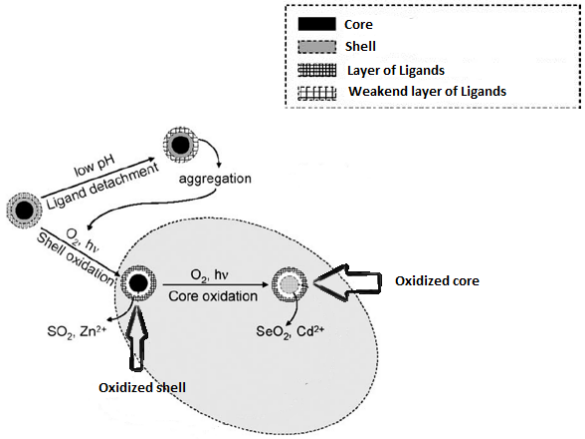

Fig 4.(reproduced from 13) Schematic representation of possible mechanism that result in the dark cytotoxicity of CdSe/ZnS QDs. Degradation of the QD can be caused by a) oxidation of the shell ZnS resulting in Zn^{2+} cations b) oxidation of the CdSe core resulting in Cd^{2+}. Ligand detachment in low ph conditions accelerate these processes due to the less steric inhabitance.

3.1.1 QDs in the presence of a PS

Following the first demonstration of $^1O_2$ generation by using QDs in PTD[10], various groups have tried to improve the ΦΔ by different conjugations and combinations. Initially Tsay et al.[14] (2007) fabricated a quite stable, water soluble product by coating CdS/ZnS QDs with peptide. Chlorin e6 was covalently linked to the peptide and used as PS. Even though a ΦΔ of 0.31 was indeed achieved in deuterium solution (still lower than the PS alole), using an excitation wavelength of 532 nm, the

FRET mechanism was weak and the absorption was dominated by the PS. Therefore the presence of the QD did not contribute as expected.

In the search of a better combination of QD and PS, Jiong Ma et al.[15] (2008) (synthesized water-soluble thiol-capped CdTe QDs conjugated with aluminium phtalocyanines (AlSPc's) as a PS. The system exhibited an effective FRET mechanism but the ΦΔ was calculated 0.15 in deuterium solution, lower than the value 0.36 that the PS AlSPc generated alone.

An early in vitro test for the efficiency of QD-PS systems to kill cells was performed by Rakovich et al (2010).[16] The group synthesized CdTe QD which were mixed with PS of methylene blue (MB) solutions of different concentration. For the in vitro studies HepG2 and HeLa Cells were prepared. The results showed a greater $^1O_2$ production compared to the case of MB alone (this increase was observed by the comparison of their emission peaks in the characteristic wavelength of emission at 1270 nm) upon irradiation and growth cell studies confirmed a small increase in cytotoxicity in the presence of QDs. Even though the studies were conducted only in vitro and with limited efficiency, they revealed that QD-PS system can be indeed superior to bare PS in terms of cell killing efficiencies.

Trying to add specificity in their system, Morosini et al. (2011)[17] synthesized thiolglycol-capped CdTe QDs conjugated to folic acid. Folic acid is often used as a targeting ligand due to its affinity for cancer tissues. To evaluate the specificity of the complex, in vitro studies were conducted using KB cells (with overexpressed FR-a and HT-29 cells lacking FR-a. FR-a is a protein encoded with a gene that has a high affinity for folic acid. Their results show increased phototoxicity in the case of KB cells indicating the achieved specificity of the system. Phototoxic effects also seemed to be dependent on the concentration of the QDs.

Aiming to form a conjugate suitable for applications in living systems, which will survive the passage through the plasma membranes of the cell keeping its conjugate form and physical properties, Li et al. (2012)[18] electrostatically linked sulfonates aluminium phthalocyanines (AlPcSs) (as PS) with CdSe/ZnS and CdS/ZnS QDs (the PS was negatively charges and the QDs positively charged.). Their in vitro studies with human nasopharyngeal carcinoma cells (KB cells) revealed that the system easily penetrated into the cells. In addition the survival rate of the cells exhibited a significant decreased in the presence of the QD, compared to a control system without a QD, when irradiated with a 532 nm laser. Therefore the group demonstrated the possibilities of covalently linked systems in intracellular delivery.

In order to give more insight on the effect of the interaction between the PS and the QD, Rotomskis et al (2013)[19] followed a completely different approach forming a non-covalent complex of CdSe/ZnS QDs with amphiphilic chlorin C_{e6} as a PS. The QDs were coated with amphiphilic polymer (AMP) and polyethylene glycol (PEG) bearing amine groups. Spectroscopic measurements revealed that Ce_6 molecules immersed in the hydrophobic part of the coating and therefore the hydrophobic non-covalent interactions were verified. The PS was able to be excited indirectly with the FRET mechanism and $^1O_2$ was detected. Judging from the photoluminescence of SOSG (a detection reagent that is highly reactive for singlet oxygen), generation was greater in the complex compared to the PS. ($\Phi\Delta$ = 0.65 for C_{e6} alone). Furthermore in vitro studies with MiaPaca-2 Cells showed that the complex was indeed able to cause cell destruction however the intracellular environment caused a small instability on the system raising doubts about its application in a living organism.

An enlightening study revealing a main issue that should be addressed in order for QDs systems to be applied in vivo was conducted in 2015 by Viana et al.(2015)[20] The group prepared CdTe QDs coated with mercaptosuccinic acid and conjugated them with electrostatically with ZnP porphyrin (N-ethyl-2-pyridinium-2-yl) which acted as a PS and tested it in vitro against Candida albicans (a pathogenic yeast). According to their measurements, even though $^1O_2$ was generation was observed, there was a minimal effect on the C. albicans yeast. This unfortunate result was attributed to the low internalization of the QDs in the cells, despite their small size (2-3 nm). Furthermore, undesired

toxicity upon irradiation was observed in nearby fibroblasts (cells in connective tissues). Therefore this study made clear that modifications of greater quality, which will improve the cell uptake capabilities and increase the retention time in the cell, are needed in order for QDs to be eventually applied in vivo.

After a bit more than a decade, even though a slow progress was noticeable, no essential improvement had been achieved and QDs were far for being suitable for in vivo studies, much more to be applied in real applications. $^1O_2$ generation was still kept at low levels and the degree of biocompatibility of the designed systems had yet to reach the desired level. However, very recently Shen et al. (2017)[21] reported a very promising approach that achieved remarkable $^1O_2$ production with simultaneous imaging that was tested in vivo with great success. The QD-PS pair consisted of CdTe/ZnS QDs coated with anionic NAC ligand. (N-acetylcysteine) and a cationic porphyrin TMPyP (5,10,15,20-Tetrakis(1-methyl-4pyridinio)porphyrin tetra(p-toluenesulfonate)) bounded together through electrostatic interactions. The nature of the binding allowed the close proximity of the QDs and the PS which improved FRET efficiency. In addition TMPyP could chelate with Zn atoms of the shell of the QD preventing its TMPyP aggregation. To further enhance absorption and $^1O_2$ production the cationic R6G (a fluorescent dye of the rhodamin family) was also incorporated the system. R6G was selected because its emission spectra conveniently overlap with the absorption spectra of TMPyP and could provide a second energy transfer process. To enable simultaneous fluorescence, the hydrophobic NIR775 was added to the complex. To achieve the necessary biocompatibility the whole system was encapsulated in an amphiphilic phospholipid polymer doped with PEG 2000. Finally, to add tumor specificity in the system, folic acid (to target the folate receptor in tumors) was introduced in the surface of the polymer. The whole procedure of engineering modifications are depicted schematically in fig. 5A[21].

$^1O_2$ measurements in deuterium revealed the remarkable value of 0.91. This is the highest $^1O_2$ yield reported for inorganic systems. This increased efficiency was attributed to the success of incorporation the additional energy donor R6G which provided an additional energy transfer path to the PS. The dual transfer energy mechanism proposed is depicted in fig. 5B[21].

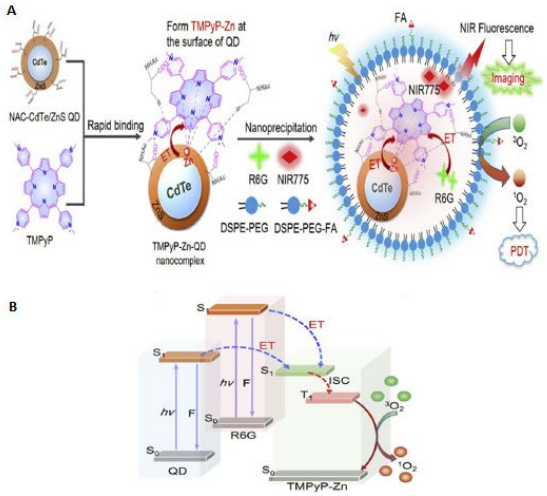

Fig. 5 (modified from [21]) **A.** Schematic representation of the engineering and PTD procedure for the system consisted of CdTe/ZnS QDs as energy donors and TMPyP as energy acceptors. The QD-PS system is hold together via electrostatic forces. R6G was added to the system hoping that the overlap of its emission spectra with the photosensitizer's absorption spectra will provide a second energy process. The fluorophore NIR775 was further incorporated to achieve simultaneous fluorescence. **B.** Schematic representation of the dual energy mechanism that led to the remarkable ΦΔ of 0.91. The additional energy donor R6G provided an extra energy path maximizing the energy that is transferred to the PS resulting in more efficient 1O2 production.

Regarding the cell penetrating abilities of the system, fluorescent in vitro studies using KB cells revealed that the system was successfully incorporated into the cell via a folate receptor-mediated endocytosis and was mainly distributed in lysosomes. In addition, these fluorescent studies revealed the targeting capabilities of the complex as fluorescence from folate-receptor deficient cell was very weak. Regarding the cell killing efficiency the in vitro studies show that cell collapse was achieved only 50 seconds of illumination. Encouraged by the remarkable success of their in vitro studies, they proceeded and tested their system in vivo by monitoring its effect on tumor bearing mice for a period of 10 days. They compared two cases: illumination with white light and without illumination. The volume of the tumor

injected by the QD complex and exposed to irradiation was significantly reduced verifying the efficiency of the treatment. On the other hand, there was no apparent effect in the case without illumination indicating the desired low dark cytotoxicity. Despite the remarkable success of their study, the group mentioned that the use of white light limits the application in superficial tumors and they proposed that optical fibers can be used in the future to deliver white light in deep-seated areas.

Even though this last successful report creates some hopes for the future, QDs seem to be unable to efficiently serve as energy donors to indirectly excite the PS. Despite that energy transfer is indeed observed, the transfer efficiency is very low which results in low ROS generation. Furthermore, as Viana at el.[20] demonstrated the toxicity of the QDs has not been completely eliminated and further investigation need to be conducted towards improved surface modifications. The lack of sufficient in vivo studies creates further doubts about the feasibility of the application of QDs in future clinical applications.

3.2.2 QDs in the absence of a PS

Besides the first report of a 5% $^1O_2$ yield by Samia et al.[10], following studies were unable to reproduce that result, thus normally most interest was concentrated on using the QDs in conjugation with an intermediate PS as described previously. However, after different combinations of QDs and PS have been tried, no safe conclusions can be made regarding their efficiency. In addition these QDs–PS systems require complicated preparations procedures. Therefore, in the late years there has been a slightly increased interest to use QDs as PS and directly produce $^1O_2$ species.

In a recent effort to revive the interest towards QDs as direct PS, Sonia et al.[22] tested the production of $^1O_2$ using water soluble CdSe and ZnSe QDs. Water solubility was achieved by covering the surface of the QDs with thiol groups which prevented their aggregation and uncontrollable growth in aqueous medium. For both systems $^1O_2$ generation depended strongly on the concentrations of QDs as well as the time of irradiation demonstrating that QDs alone hold indeed the potential to generate ROS. However no progress was made to limit the toxicity and increase the biocompatibility of the two systems.

Sun et al.[23] tried to go one step further and add target specificity to their system. The group synthesized CdTe/HA hybrid QDs by using HA (sodium hyaluronate) as stabilizing agent and target ligand simultaneously, aiming to take advantage of the specific binding of HA to CD44 receptors which are overexpressed in cancers. To test the specificity of the system in vitro, the inhibition rate caused by the QDs was measured in the case of HepG2 human hepatoma cells (model cancer cells) and BRL 3A rat liver cells (model normal cells). The inhibition rate was far greater in the case of the HepG2 cells indicating the potential for specific cytotoxicity on cancer cells rather than on normal cells.

Judging from these recent reports and from the low number of publications on using QDs alone, thy approach seems to be the most immature compared to the other approached on using QDs in PTD. Despite the stable product that Sun et al. reported, we still lack in vivo studies that will verify that QDs can be applied to living systems alone (without a PS).

3.2.3 Semiconductor QDs for two-photon excitation

As mentioned in previous sections, the poor penetrating abilities of visible light on which classis PS absorb created a limit on the depth accessible to treatment (a few mm). Also even if a PS was able to absorb in linger wavelengths, the energy of that light is not enough to excite molecular oxygen in its triplet state. Both of these restrictions could be dealt by means of a non-linear optical effect called Two Photon Excitation (TPE). QDs are advantageous compared to traditional PS due to their high two photon absorption cross section (TPAC)[xxiv.] Cross sections are usually reported in Goeppert-Mayer units (GM), where 1 GM = 10^{-50} cm^4 s / photon. As opposed to the linear absorption of one photon whose energy equals the band gap of the quantum dots, during TPE two photons (whose energy summed equals the band gap) are absorbed (fig. 6B)[24]. In the case that the QD is used in conjugation with a PS, this energy will

subsequently be transferred to the PS via the FRET mechanism and will result in the generation of singlet oxygen species ($^1O_2$) during the relaxation of the PS. The low energy photons, whose wavelength falls in the therapeutic window, are able to reach deep sited regions and their summed energy is sufficient to excite the PS and achieve $^1O_2$ production. Even though $^1O_2$ is the main ROS produced, other ROS like free radicals can also be generated. Applying the TPE in PTD, depths up to 2-3 cm have become accessible.

The first experimental demonstration that FRET can indeed occur after a two-photon excitation was conducted by Dayal et al.(2007)[25] Their system consisted of a CdSe QDS (average size 5 nm) covalently linked to silicon phthalocyanine 4 (Pc4). The absorption and photoluminescence spectra of the system are depicted in fig. 6A.

An excitation wavelength of 1100 nm was used, which falls quite far from the absorption regions of both the QD and PS. Nevertheless, photoluminescence was observed indicated that two-photon absorption indeed took place. Furthermore, judging by the considerable decrease of photoluminescence intensity of the QD-Pc4 system compared to the bare QD, it can safely be assumed that FRET also occurred.

Inspired by this first proof of energy transfer via FRET after a TPE, different groups tried to optimize the process[26], but these studies were conducted in solution and no actual evaluation was made on the production of ROS. It was only until 2011 when Qi et al.[27] fabricated the first biocompatible system which created observable amounts of $^1O_2$. For their experiments CdSe/ZnS quantum dots, protected by TOPO (tri-n-octylphosphine oxide) were encapsulated in amphiphilic micelles to improve water solubility and biocompatibility. The QD was conjugated to porphyrin which was used as PS. The system exhibited remarkable stability in water and physiological buffer showing a 100 % energy transfer under a TPE at 800 nm. The observed stability was due to the hydrophobic interactions between TOPO and the polymer hydrocarbon (porphyrin) which kept the system encapsulated and safe against hydrolysis. Finally the $^1O_2$ generation was found to be double compared to the porphyrin alone.

In the followed efforts, various groups reported biocompatible systems accompanied by production of ROS[28] [29] [30] [31]. However, the possibility of an in vivo application had yet to be verified until 2015, when Lemon at al.[32] reported a study that was tested in living mice, which unfortunately resulted in a negligible $^1O_2$ generation.

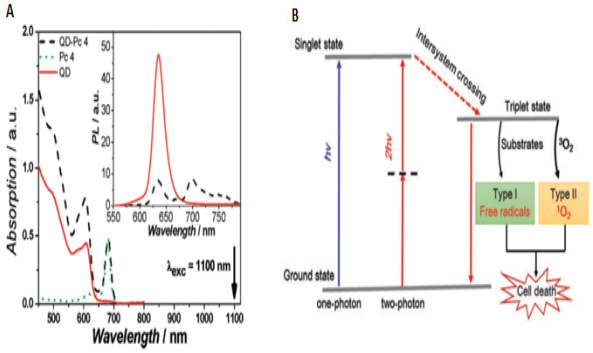

Fig. 6 (modified from 25) **A.** Absorption spectra of the QD (red line), Pc 4 (dashed green line) and the QD-Pc 4 conjugate. In the inset the PL spectra of the bare QD 9 (red line) and the QD-Pc 4 conjugate (dashed line) are depicted. The spectra were obtained using an excitation wavelength of 1100 nm. The large decrease of photoluminescence intensity of the QD-Pc4 system compared to the bare QD indicates the effective energy transfer via the FRET mechanism. **B.** Schematic representation of the generation of (mainly) singlet oxygen species during PTD using TPE. As opposed to the linear absorption of one photon (blue color) whose energy equals the band gap of the quantum dots, during TPE (red color) two photons (whose energy summed equals the band gap) are absorbed. The diagram refers to the case where a QD directly produce ROS, but the mechanism can be generalized to include the case where ROS are produced indirectly with the help of a PS.

Overall, looking at the various studies that have been conducted in a period of around 15 years the applications of semiconductor QDs in PTD seems to suffer from severe limitations that make the idea of applying them in living systems a distant dream. A lot of studies still need to be conducted in order to improve the low $^1O_2$ generation, improve the biocompatibility of the systems, decrease the side effects on surrounding tissues and increase their cytotoxicity. The recent promising study by Shen et al. allows us to keep some hopes for complexes involving semiconductor quantum dots but, not surprisingly, these systems are not in the center of attention lately as they are shaded by more promising systems like carbon dots.

3.2 Carbon Dots (CDs)

3.2.1 Carbon Dots in general

As mentioned in the previous chapter, modifications in the semiconductor QDs improves their biocompatibility and reduces their toxicity but unfortunately they do not eliminate it. However, there is a special category of organic dots, namely "carbon dots" (CDs), which combined the desirable effects arriving from their nano-size (<10 nm) (e.g. , fluorescence, tunability of emission wavelength) with increased solubility and biocompatibility and limited toxicity.

CDs are structures consisted of sp^2 and sp^3 hybridized carbons, functionalized with oxygen bearing groups which allows for surface modification possibilities. Even though the photoluminescence mechanism of CDs does not differ from the one of QDs, their functional groups play an additional role in the modification of their surface energy level, band gap and subsequently their fluorescent properties[33]. Therefore appropriate functionalization can expand their already extraordinary tuning possibilities. Regarding their biocompatibility, it arises from their functional groups (usually carboxylic acids) which equip them with excellent water solubility. Finally, there exist a wide range of possible, cheap synthetic methods through CDs can be produced.[34] All these advantages make CDs superior to QDs for their application in PTD and not surprisingly, the scientific interest has shifted towards their side the recent years.

The first effort on using CDs in PTD have been conducted in 2012[35] and already in 2014 Choi et al.[36] reported a very promising biocompatible, theranostic system with targeting properties. In their experiments, fluorescent CDs were functionalized with folic acid (FA), which is a known ligand with affinity for the folate receptors overexpressed in tumors. The surface of the CDs was further passivated with poly(ethylene glycol) diamine (PEG) to increase its biocompatibility. In addition to the "protective" role of PEG, there have been reports[37] that passivating the surface of QDs with PEG improves their fluorescence by decreasing the effective hole-trapping after generation of an electron-hole pair with illumination. This improved fluorescence turned the system into a theranostic platform for simultaneous therapy and imaging. Zinc phthalocyanine (ZnPc) was furthermore loaded on the CDs to function as PS. The system was first rested in vitro using HeLa cells and it was compared with a similar system but without the targeting ligand. According to their results, the cell death was much higher in the presence of the targeting ligand indicating the specificity of the complex. Encouraging from these successful they proceeded and tested their system in vivo against tumors in mice. The tumors were irradiated with light of 660 nm for twenty minutes and by monitoring the tumor behavior for a period of 10 days, they verified the remarkable suppression of its growth, which proved the efficiency and specificity of the system.

Investigating different parameters that affect the efficiency of CDs in PTD, Yang et al.[38] focused of optimizing the FRET mechanism by increasing the energy of the donor, by minimizing the CD-PS distance and by increasing the PS concentration. To achieve the first they double doped the CDs with Mg/N, according to results of one of their previous studies[39], which suggested that this kind of doping enhances the photoluminescence of the system. To minimize the CD-PS distance they passivated the surface of the CDs with 1,2-ethanediamine (EDA). EDA was selected due to its small size which allowed for the minimization of the distance. With chlorin e6 (Ce6) as a PS, the FRET efficiency was calculated as high as 84% and in vitro studies with HepG2 Cells revealed enhanced cell killing efficiency. Therefore, the group showed that further improvement of the efficiency of PTD is possible by changing parameters which are related to the energy transfer via FRET.

An innovative study using CDs was conducted by Zheng et al. (2016), who fabricated a system that effectively deals with the hypoxia problem in tumors. The term hypoxia refers to low oxygen conditions and it is very common in tumors. As PTD requires the presence of oxygen for effective treatment, hypoxia created serious problem in the procedure. To add to that, the consumption of oxygen that takes place during PTD can deteriorate the already low oxygen conditions creating serious

side effects for the patient. An effective way to deal with the hypoxia problem would be to construct a nano-platform that except of its usual role in PTD would also cause water splitting providing the necessary oxygen. Among various water splitting materials carbon nitride (C_3N_4) seems the most suitable one due to its conveniently positioned energy levels, but unfortunately it is not effective in the red region of light which they needed to operate. Therefore the group prepared red absorbing CDs and mixed them with C_3N_4 composited via a ball-milling process to enhance red light absorption. In addition photoporphyrin PpIX (linked with RGD (Arg-Gly-Asp) via PEG) bounded to C_3N_4 via π-π stacking. RGD was added to achieve active RGD targeting. (RGD is a common peptide sequence, found in proteins, that is used in target therapy as it mediates cell attachment). This system, under the illumination of a 630 nm light was able to simultaneously produce $^1O_2$ via PTD and O_2 via water splitting, as demonstrated with in vitro studies. Finally, in vivo studies with 4T1-tumor-bearing mice revealed satisfying cell killing efficiency and accumulation in tumor within four hours after injection. The overcoming of the hypoxia was verified by observing the decrease of CA9 (an enzyme overexpressed in hypoxic tumors) and HIF-a (a protein that promotes blood vessel formation that degrades in normal oxygen conditions).

Recently an alternative approach was published by Li et al.(2017)[40] who instead of linking PS with the CDs they incorporated the PS (TPP: mono-hydroxyl phenyl triphenylporphyrin) during the synthesis process. Chitosan was also added as control and the product was synthesized via hydrothermal synthesis (a synthesis method during which crystals are formed from high temperatures aqueous solution in high vapor pressures). In vivo studies using H22 tumor-bearing mice showed apparent decreasing of size in a period of 13 days. In addition by monitoring the weight change of the mice they verified that no severe side effects were caused. Therefore, the group introduced a new way to produce CDs systems for PTD, through rational design of the precursors, which equips the CDs with intrinsic photodynamic properties.

In general, as opposed to their semiconductor counterparts, CDs have been proved to be very promising in their role as energy donors in the presence of a PS. On the other hand, their intrinsic optical properties are not enough for the direct generation of ROS. In overall, CDs seem to be able to effectively combine the desirable effects of quantum confinement with the increased biocompatibility arising from their organic nature (serving as energy donors). Systems utilizing CDs have already been tested in vivo in multiple labs and their effective cytotoxicity upon illumination has been verified.

3.2.2. Carbon dots in TPE

The first effort of using CDs in PTD under two-photon excitation took place on 2013 by Fowley et al.[41] with already remarkable results. The group prepared a system consisting of CDs (coated with amine functionalized PEG which created binding sites for carboxylic acid functionalized molecules) covalently linked with photoporphyrin IX (containing carboxylic acid functionality) which acted as the PS. The CDs, whose maximum emission was at 430 nm well overlapping with the absorbance spectrum of the porphyrins were excited with light of wavelength equal to 800 nm and efficient FRET was observed. In their in vitro studies the viability of HeLa cells was reduced by 82% after irradiation in the presence of the CDs-PS complex. Finally they tested the system in vivo after inducing toumors in C3H/HeN mice using RIF-1 mouse tumor model. The size of the tumors showed an evident shrinkage, 4 days after a two-photon irradiation at 800 nm. The main achievement of this initial successful attempt, was to prove that CDs, superior to the usual semiconductor QDs in terms of biocompatibility, can be also used for TPE.

In a similar approach, Wang et al. (2014)[42] synthesized CDs via an hydrothermal method and 5,10,15,20-tetrakis(1-methyl-4-pyridinio) orphyrins (TMPyP) was covalently attached to it. As in the previous report, the aim of this conjugate was to take advantage of the convenient overlap between the maximum emission of the Cs at 415 nm with the absorbance spectra of TMPyP, hoping for an efficient energy transfer via FRET. TMPyP, due to its positive charge (four positive charges on its four

benzene rings), is superior to other traditional PS (like ALPcS or Ce6) in terms of cellular uptake (because the membrane of the cell is usually negatively charged). On the other hand, it is inferior to them due to its low absorbance at the red region of light limits its use in in vivo application where deep penetration is needed. Thus combining TMPyP (TPAC of 110 GM) with CDs (TPAC of 15000 GM at 700 nm, two orders higher than TMPyP ALONE) would overcome this limitation and would create a biocompatible and efficient TPE system. In their in vitro studies, the system was tested on HeLa cells. The CDs alone had no effect to the cells, but when the the CDs-TMPyP complex was irradiated with light of 700 nm, most of the cells were killed after a 45 min irradiation revealing that energy was efficiently transferred via FRET and sufficient $^1O_2$ was produced.

Despite these first promising results CDs for TPE was not tested in vivo until 2017 when Zhang et al.[43] formed nanohybrids of CDs and fluorescent ruthenium (Ru) and tested them in a model organism of zebrafish embryos, however without success. Even though the system provided remarkable contrast for deep imaging under TPE, no obvious toxicity was observed on the zebrafish embryos, creating doubts about the ability of CDs to be used as direct photosensitizers under TPE.

An in the case with one-photon excitation, direct generation of ROS using CDs seems to be very weak in the case of TPE. As energy donors, CDs have been utilized in some promising reports (even in vivo), allowing for hopes for further improvements in the future. However, as not much work has been done towards that direction and the TPE with CDs is a quite immature field (although more mature that TPE with semiconductor QDS).

3.2.3 Graphene QDs

There would not be such a strong interest for carbon based quantum dots, if It was not for a certain family that stands out among them: graphene QDs (GQDs). The first effort of using QGDs in PTD was in 2012[44] but it was in 2014 when Jiechao Ge at al.,[45] using a graphene quantum dot (GQD), reported an extraordinary $^1O_2$ yield of 1.3, which led to the massive increasing of the interest towards GQDs. This extraordinary result was attributed to a new $^1O_2$ generation mechanism that take place with GQDs, which was given the name multistate sensitization (MSS) (explained later).

The group prepared GQDs (from 2 to 6 nm) via the hydrothermal method using polythiophene derivatives as the carbon source. The absorption and emission spectra of the GQDs dispersed in water, after excitation with light of wavelength equal to 500 nm revealed a broad absorption in the visible range as desired and an emission peak at 680 nm (deep red). The Stokes shift was as large as 205 nm, indicating that the measurements were not affected by the re-absorption of their emission (since the wavelength of the emitted light was far from the region where the system absorbed). To evaluate the photostability of the system they compared it with the conventional red emitting semiconductor QD CdTe and the classic PS photoporphyrin IX (PpIX). The three samples were continuously irradiated with a 500-W xenon lamp and their absorbance in a period of 80 min was measured. The normalized absorption degradation of the three systems is depicted in fig. 7A.Even though the CdTe and PpIX systems exhibited obvious degradation, no obvious decline was observed for the GQSs implying their desirable photostability. Moreover, using the electron spin resonance (ESR) technique it was verified, that no other ROS besides singlet oxygen $^1O_2$ were generated. By comparing the emission peaks of $^1O_2$ at 1280 nm with the corresponding emission of $^1O_2$ of Rose Bengal (RB) PS,(fig. 7B) the ΦΔ was calculated as high as 1.34 (ΦΔ of RB is equal to 0.76[46]). The system was also tested initially in vitro and subsequently in vivo. In the in vitro studies, the cell viability of HeLa cells exhibited a strong dependence on the concentration of GQDs, demonstrating their ability to efficiently kill infected cells. In addition, no effect was observed in dark conditions indicating the low toxicity and good biocompatibility of the GQDs. In the in vivo studies, GQDs were injected in mice bearing breast cancer and were irradiated twice, on the first and seventh day, for 10 min using white light of 400-800 nm. The tumor began to decompose after 9 days and was completely destroyed after 17 days. No tumor regrowth was observed over 50 days. Finally by monitoring the weight change of the

mice over the days, no obvious side effects were observed indicating the low in vivo toxicity of the QGDs.

In addition to the remarkable results regarding the photostability, biocompatibility and efficiency of the GQDs, the same group illustrated a new $^1O_2$ generation mechanism to which the extraordinary result of ΦΔ=1.3 was attributed. In the well-known mechanism of $^1O_2$ generation, energy is transferred from the excited triplet state of the PS (T1) to the triplet ground state of oxygen ($^3O_2$) and by unavoidable loses ΦΔ results less than 1. However, absorption and fluorescence spectra of GQDs revealed that the excited singlet state (S1) was 49.3 kcal mol^{-1} above the ground state and the T1 between 22.5 and 26.5 kcal mol^{-1} above the ground state. Since the excitation of $^3O_2$ to $^1O_2$ requires an energy input of 22.5 kcal mol^{-1}, both relaxations of S1 to T1 and from T1 to ground state are able to provide that input. Therefore, the group proposed that it is this additional pathway of $^1O_2$ generation that allows for $^1O_2$ yield greater than 1. The relaxation processes described, are schematically depicted in fig. 7C in comparison with the $^1O_2$ generation mechanism of conventional PS. The new mechanism was termed multistate sensitization. (MSS). In overall, this work revealed the immense potential of GQDs for PTD by verifying its outstanding $^1O_2$ generation, sufficient stability and suitable biocompatibility. Following this report, many labs have prepared various systems containing GQDs which continue to live up to their expectations and produce positive results.

A possible way to improve the performance of QQDs even more is through nitrogen doping. Doping graphene with nitrogen (N) was been suggested in the past that results in excellent photochemical activities[47]. Inspired by these results, Kuo et al.(2017)[48], in an attempt to obtain improved confinement, tested the N doping of GQDs. In vitro studies were performed against E.coli and the system was irradiated for 3 minutes with 670 nm light. To their pleasure the N-doped GQDs exhibited a 100% elimination of the bacterial. When tested with lower concentration of N-doping the killing efficiency had dropped, indicating that it is the N-C bonding that enhanced the antimicrobial effect. In addition to the improved therapeutic properties, it seemed that N-doping equipped the system with sufficient intrinsic photoluminescence at the NIR region which arised from defect states. Therefore the system offers quite promising possibilities in the simultaneous treatment and bio imaging.

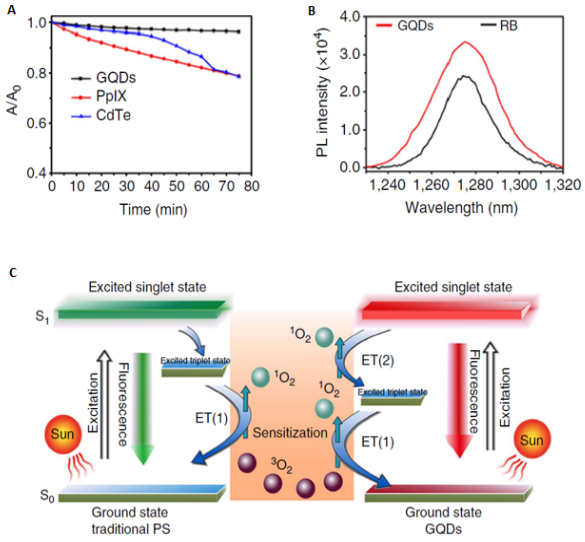

Fig. 7[(modified from 45)] **A.** Ratio of the absorbance versus time over the initial absorbance for graphene quantum dots (GQDs), photoporphyrin IX (PpIX) PS and CdTe QDs. Even though the CdTe systems exhibited obvious degradation, no obvious decline was observed for the GQSs. The plot demonstrate the superior photostability of the GQD system. **B.** Comparative results for the T8O7 photoluminescence of $^1O_2$ generated from QGDs and Rose Bengal (RB) PS. By comparing the peak intensity, ΦΔ was calculated using the known corresponding value of 0.76 for the RB system. **C.** Comparison between the $^1O_2$ generation mechanism in conventional PS (left) and in GQDs (right). In addition to the energy transfer resulting from the relaxation from the excited triplet state of the PS to its ground state (ET1), GQDs provide an extra energy input for the generation of $^1O_2$, originating from the relaxation of the excited singlet state to the excited triplet state (ET2). The additional pathway allows for $^1O_2$ yield greater than 1.

Except of their role as a direct PS, GQDs could function in combination with other PS. Towards this perspective Nwahara et al. (2017)[49], tried to combine the (widely used as PS) metal conjugated phthalocyanines (aluminum tetrasulfonated in this case) with GQDs hoping to achieve enhanced combined results. The GQDs (covalently linked with the common targeting ligand folic acid) were capped with glutathione which allows the CLALTSPc (aluminium tetrasulfonated phthalocyanines) to be non-covalently absorbed (via π-π interactions) in the surface of the system. The 1O2 yield value was calculated 0.52, improved compared to the value of 0.42 that the CLALTSPc

achieved alone. Therefore the group demonstrated that the presence of GQDs can improve the performance of clinically approved PS like CLALTSPc. As an extension to this approach, Fomo at al.(2018)[50] very recently proposed an in-situ, one-pot (the reactants undergo successive reactions in the same volume) route to synthesize GQDs-Pc supramolecular hybrids achieving ΦΔ of 0.74, improved when from the value of 0.70 of the phthalocyanine alone.

Very recently (2018), Zhang et al.[51] , in a very interesting approach tried to combine the efficient $^1O_2$ generation of GQDs with UCNP (upconversion nanoparticles) to achieve deeper penetration. UCNP have the ability to absorb light of certain wavelength and emit in shorter wavelength regions. Thus, both deep penetration (irradiation with long wavelength) and sufficient $^1O_2$ generation (emission is short wavelengths) is possible. In the previous years, graphitic-C3N4 quantum dots have been tested in similar approaches using NaYF4:Yb3+/Tm3+ upconversion nanoparticles which is a famous family of UCNP doped rare earth upconversion nanoparticles and in-vitro studies had shown positive results[52]. In this case UCNP of composition NaYF4:59.5% Y^{3+}, 40% Yb^{3+}, 0.5% Tm^{3+}) were used and covalently linked with the GQDs. Another innovation of the group was to modify the system such as it will be specifically targeted to attach the mitochondria of the target cells. Mitochondria are intracellular organelles that play a crucial role in cell apoptosis, as damage of mitochondria rapidly promotes it. Mitochondria specificity was achieved by covalently linking TRITC (tetramethylrhodamine-5-isothiocyanate), which has proved to be effective for that purpose[53]. In vivo studies against 4T1 tumors in mice, revealed that upon NIR irradiation (980 nm), the growth rate of the tumors were considerably quenched. Thus, it was verified that energy was indeed transferred from the UCNP to the GQDs (because the GQDs do not absorb in that region) and sufficient $^1O_2$ was generated close to the mitochondria which resulted in the cell apoptosis. Therefore the group demonstrated an effective way to perform specific treatment of deep sited tumors, combining the effects of UCNP, GQDs and targeting moieties.

Putting things all together GQDs have been proved to be one of the most promising photosensitizers or energy donors tested up to date. Combining the biocompatibility arising from their "carbon nature", with their suitable energy level spacing that give rise to a new $^1O_2$ generation mechanism, GQDs' potential far exceed the possibilities of every other system containing a QD. Utilizing GQDs in different way (N-doping, combination with upconversion nanoparticle) has also been proved successful, which creates hope for even further improvement in the future.

3.2.4 Graphene QDS in Two photon excitation

Following the remarkable results that GQDs provided under one photon excitation, it was a matter of time until they would also be tested in TPE. Towards that direction, Kuo et al (2016)[54] first reported a successful study using GQDs under TPE. For their studies GQDs were synthesized via the Hummer method (generation of graphene oxide by adding potassium permanganate to a solution of graphite, sodium nitrate and sulfuric acid). Initially the antibacterial properties of GQDs were tested in vitro, under illumination of 800 nm, using E. coli and MRSA bacteria. The results were positive and almost all bacteria of both types were killed after irradiation for 15 seconds. In addition, the cross section of TPE of the GQDs was determined as high as 47903 GM, demonstrating their potential to be used us contrast probes during operation at dip sited areas. To investigate that possibility, the group obtained TPL (Two Photon Photoluminescence) images of GQD-treated bacteria in different depths, embedded in a collagen matrix to limit the 3D biological environment. The obtained images can be seen on fig.8. for the cases of both bacteria.

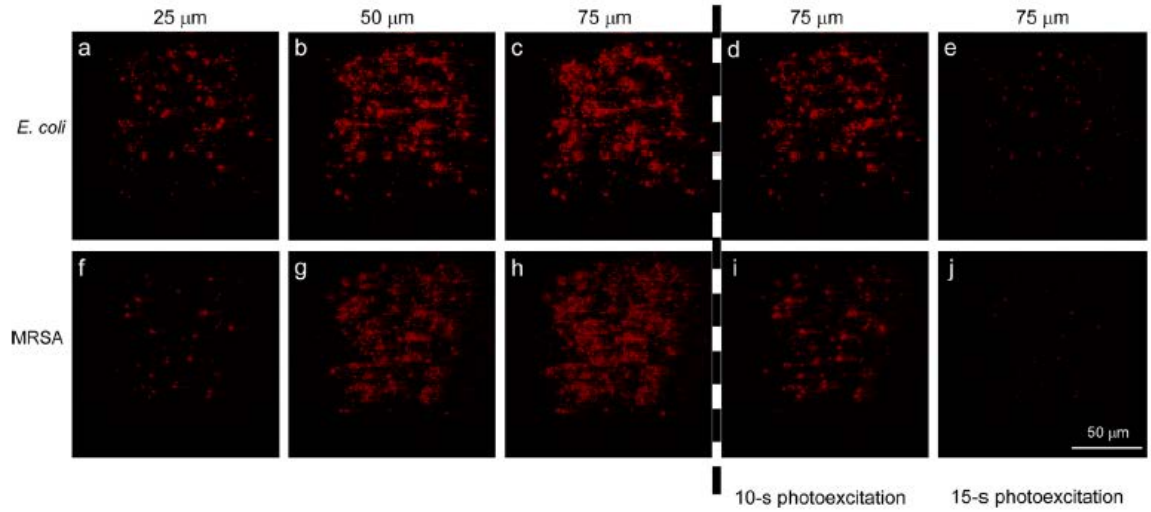

Fig. 8[reproduced from 54] Photoluminescence images of E. coli and MSRA bacteria as a function of depth. The bacteria's behavior at 75 μm was monitored against time by obtaining two images at 10 and 15 seconds of irradiation. The decrease of photoluminescence in this interval of time reveals the fatal effect that the GQDs had on the bacteria.

The decrease of fluorescence observed in images d,e,I,j of figure 8 after irradiation for 10-15 seconds, reveals that the bacteria were oxidized and deteriorated by the generation of ROS which caused morphological damage (including the desorption of the GQDs of their surface which caused the decrease in fluorescence). By repeating the measurements with one-photon excitation with a 594 nm laser, the bacteria were not able to be observed. Therefore their studies proved the potential of GQDs to simultaneously act both as ROS generation during PTD and as two-photon contrast agent for observing bacteria at greater depths.

Very recently, the behavior of GQDs in the presence of another PS was investigated by Sun et al.(2018)[55] under TPE. The group had already reported nitrogen-doped GQDs (N-GQDS) with TPAC of 4.8 10^4 GM. In the past[56], so following the same synthetizing methods they synthesized N-GQDs and additionally they covalently linked them to Rose Bengal (RB) (via a 4,7,10-trioxa-1,13-tridecanediamine). RB was selected due to its absorption maximum at 560 nm, conveniently coinciding with the emission maximum of the N-GQDs at 525 nm and increasing the FRET efficiency. The cytotoxicity of the system was tested in vitro using MCF-7 cells and the change in their morphology, upon two-photon irradiation of 800 nm confirmed the cytotoxic effect of the N-GQD-RB complex after TPE. Finally they tested the precision of imaging in deep lengths by injected the complex into a mouse via the tail vein. By comparing the TPL of blood vessels, with the corresponding images obtained with one-photon irradiation, they were able to confirm the higher signal to noise ration and precision in the case of TPE. Therefore the group demonstrated that GQDs can be used not only as direct photosensitizers under TPE but also in conjugation with typical PS providing sufficient cytotoxic effects, deeper penetration and higher precision.

As the TPE of QDs is a quite new field, no safe conclusion can be made. However, judging from these successful studies and from their amazing performance under one-photon excitation there is no reason why GQDs could not be successfully implemented in PTD under TPE.

4. Multifunctionality

Despite the various treatments that have been developed over the year to deal with cancer (PTD, photothermal therapy, radiotherapy, chemotherapy) none of them has actually managed to deal with it alone. Thus, many efforts have been conducted towards combining different methods to efficiently tackle this long resisting disease. However, function of different methods simultaneously at the same spot is very complicated to operate. Therefore the fabrication

of a “one in all” multifunctional system, that will be able to combine harmonically different techniques and efficiently deal with cancer once and for all, has been the dream of the scientific community for many years.

A combination of therapies that could greatly be benefited by such a multifunctional system is simultaneous PTD with Photothermal therapy (PTT). PTT uses a photothermal agent (PT) to achieve selective local heating of the diseased region upon irradiation. This local heating caused denaturation (modification of their natural function) of proteins and the cell membrane which possibly leads to the complete destruction the tissues. Since both PTD and PTT utilize light radiation to trigger the therapy it should be possible to combine the two therapies and achieve improved results compared to its method separately. However, when one tries to combine PTD and PTT, he faces the problem of mismatch in the absorption spectra of PS and PT (most PTs absorb in the region of 700-2000 nm). Using two lasers to separately excite the PS and the PT is complicated to operate and also it is hard to focus two laser beams on the same site.[57] Therefore, the fabrication of a sole common agent that behaves simultaneously like PS and PT upon a single illumination seems like the only solution to the problem.

QDs, these tiny magic nanoparticles that seem promising for a large range of applications, of course could not stay out of the this “multifunctional game”. Very recently (2017) Ge et al.[58] first showed that CDs, despite their inefficiency in PTD without the presence of an additional PS, they can achieve remarkable results when combined with PTT. The group first synthesized CDs capable of acting both as a PS (verified by detecting generated $^1O_2$, even though with a small ΦΔ of 0.27) and as PT (verified by monitoring the temperature changes of a solution) under illumination by a 625 nm laser. In order to understand the mechanism that makes thermal generation possible, the group measured the singlet and triplet energy levels of the CDs. According to their fluorescent spectra, the excited singlet state of the CDs is 54.8 kcal mol^{-1} above the ground state. At the same time the triplet state is between 34.4 and 42.6 kcal mol^{-1} above the ground state and between 12.2 and 20.4 kcal mol^{-1} under the excited state. As singlet oxygen requires a formation energy of 22.5 kcal mol^{-1}, generation of $^1O_2$ is only able through the relaxation of the triplet state to the ground state. Since ISC is not the most probable relaxation pathway (due to different spin configuration) most of the energy released after the CD excitation is of the form of radiation or quite possibly heat. Thus simultaneous heat and $^1O_2$ generation was possible (even though with small ΦΔ) under single illumination. The system was finally tested in vivo using mice bearing HeLa tumor. Despite the low $^1O_2$ generation, the synergetic function with the thermal effects caused the complete tumor ablation in a two – week period, as verified with fluorescent studies after illumination with 625 nm laser.

During the last year (2017) except of the aforementioned CDs, there has been a number of publications, using GQDs[59] [60], upconversion carbon nitride QDs[61], MoO_{3-x} QDs[62], $CU_2(OH)PO_4$ QDs[63] or Black Phosphorus QDs[64] that all successfully combined the advantages of PTD and PTT, judging from positive in vitro and in vivo results. Despite that the field of utilizing QDs as multifunctional agents for synergetic PTD and PTT is quite new, these initial promising results allow us to hope or the realization of efficient multifunctional systems that will combine the advantages of the two methods and improve their performance when functioning individually.

5. The big picture

As aforementioned, the aim of the present report is to describe the way the QDs can be utilized to increase the potential of PTD, to critically assess various approaches that have been conducted towards that goal and to highlight promising paths for the future.

In overall the implementation of QDs in PTD aims on dealing with certain problems that the currently clinically used PS are unable to deal with. More specifically, they can prevent the PS from aggregating by acting as carriers, improve the efficacy of the technique owing to their superior optical properties (high absorption and tunability) and deal with the problem of low penetration in

the body (TPE or conjugation with upconversion nanoparticles.

Going through various efforts reported the last 15 years, even though absolute conclusions are difficult to be made, the distinction between promising and unpromising paths is quite clear. Starting with semiconductor QDs, even though they introduced us to the possibility of applying QDs in PTD, the systems are the furthest from being clinically used in the future. Despite various modifications on their surface that limit their toxicity at a certain degree, the desired biocompatibility could not yet been achieved. In addition, even if the biocompatibility problem is to be solved, their $^1O_2$ generation is not sufficient to achieve effective treatment. On the other hand, CDs, owing to the hydrophilic functional groups on their surface they possess the desired biocompatibility. Even though their poor intrinsic optical properties do not allow them to act as photosensitizers alone, they have been effectively used as energy donors in the presence of other PS, generating sufficient amount of $^1O_2$. Among QDs that are based on carbon, GQDs clearly stand out as they combine the characteristic solubility of the carbon family, suitable intrinsic optical properties and impressive stability that give birth to effective, biocompatible and stable systems. Furthermore GQDs are compatible with various approaches that further enhance their performance (N-doping, conjugation with upconversion nanoparticles). All these advantage make graphene quantum dots superior to any other QD bearing candidate for PTD agent and future efforts could focus on enhancing its performance even more by optimizing its synthesis routes or investigating its interaction with different additional photosensitizers. Regarding multifunctionality, CDs are very promising as they hold the potential to function as double agents for PTD and PTT. Despite their low production of ROS when functioning alone, they seem to be able to simultaneously produce heat under a single illumination which allows them to act as photosensitizer and photothermal agent at the same time. As a final conclusion, the presented studies undoubtedly establish the immense possibilities of Nanoscience in medical applications like Photodynamic Therapy. However, at the same time they demonstrate that that the path towards clinical applications if filled limitations which will require numerous studies in the future in order to allow this promising field to unfold its true potential.

6. Acknowledgments

At this point I would like to warmly thank my supervisor Dr. A. Salvati for the time she invested on my project, her flexibility towards the topic of my choice, her accurate and without a doubt helpful remarks and our interesting conversations. Furthermore I would like to express my gratitude to Prof.dr.ir. P. Onck for coordinating our course, to prof. dr. T. Banerjee for her lecture in scientific integrity, to prof. dr. R.C. Chiechi for his useful lectures in scientific writing and research and to the PhD student S. Soni for his lecture in Latex.

7. Table with abbreviations

Table 2. Abbreviations.

FULL NAME	ABBREVIATION
Photodynamic Therapy	PTD
Quantum Dot	QD
Photosensitizer	PS
Reactive Oxygen Species	ROS
Intersystem Crossing	ISC
Singlet oxygen yield	ΦΔ
Yield of Triplet Formation	ΦT
Singlet Molecular Oxygen	$^1O_2$
Triplet Molecular Oxygen	$^3O_2$
Two Photon Excitation	TPE
Two Photon Photoluminescence	TPL
Two Photon Absorption Cross section	TPAC
Aluminum sulfonated phthalocyanine	ALPcS
Chlorin e6	Ce6
Upconversion Nanoparticles	UCNP
Photothermal Therapy	PTT
Photothermal agent	PT
Intersystem Crossing	ITC

8. References

[1] Photodynamic therapy for the treatment of nonmelanomatous cutaneous malignancies. Allison RR, Mang TS, Wilson BD. Semin Cutan Med Surg ;17(2):153-63. (1998)

[2] Redox – Active Therapeutics, Ines Batinic Haberle, Julio S. Reboucas, Ivan Spasojevic, Oxidative Stress in Applied Basic Reasearch and Clinical Practice, (2016)

[3] Basic principles of photodynamic therapy, IAN J. MACDONALD and THOMAS J. DOUGHERTY, Photodynamic Therapy Center, Roswell Park Cancer Institute, Buffalo, NY 14263, USA, (2000)

[4] How does photodynamictherapy work?, Henderson BW, Dougherty TJ, *Photochem. Photobiol.*; **55**: 147–157, (1992)

[5] Laser and Non-laser Light Sources for Photodynamic Therapy, L. Brancaleon and H. Moseley, Lasers Med Sci, 17:173–186 (2002)

[6] New Photosensitizers for Photodynamic Therapy, Heidi Abrahamse, Miachael R. Hamblin, Biochem J. February 15;473 (4): 347-364. Doi 10.10 42/BJ20150942, (2016)

[7] Photosensitizers in clinical PDT, Ron R Allison, Gordon H Downie, Rosa Cuenca, Xin-Hua Hu, Carter JH Childs, Claudio H Sibata, Photodiagnosis and Photodynamic Therapy 1. 27-42 (2004)

[8] Can nanotechnology potentiate photodynamic therapy?, Ying-Ying Huang, Sulbha K. Sharma, Tianhong Dai, Hoon Chung, Anastasia Yaroslavsky, Maria Garcia-Diaz, Julie Chang, Long Y. Chiang, Michael R. Hamblin, Nanotechnoly Rev. March ; 1(2): 111–146. (2012)

[9] Semiconductor quantum dots for biological applications, Santos BS, Farias PMA, Fontes A., Elsevier,p.773-98. (2008).

[10] Semiconductor Quantum Dots for Photodynamic Therapy, Anna C.S. Samia, Xiaobo Chen, Clemens Burda, 15736 J. AM. CHEM. SOC. 125, 15736-15737, (2003)

[11] Measurement of 1268 nm Emission for Comparison of Singlet Oxygen ($^1\Delta_g$) Production Efficiency of Various Dyes, Kumi ARAKANE, AkemiRYU, Kenji TAKARADA, Takuji MASUNAGA, Kouichi SHINMOTO, Reijirou KOBAYASHI, Shinrou MASHIKO, Tetsuo NAGANO, Masaaki HIROBE, Chem. Pharm. Bull44 (1), 1, (1996)

[12] Quantum dot-mediated photoproduction of reactive oxygen species for cancer cel annihilation. Chen J-Y at al., Langmuir.;23(4):1974-80 (2007)

[13] New Light on Quantum Cytotoxicity, James M. Tsay and Xavier Michalet, Chemistry & Biology, Vol. 12, November, (2005)

[14] Singlet Oxygen Production by Peptide-Coated Quantum Dot-Photosensitizer Conjugates, James M. Tsay, Michael Trzoss, Lixin Shi, Xiangxu Kong, Matthias Selke, Michael E. Jung, and Shimon Weiss, J. AM. CHEM. SOC., 129, 6865-6871 9 6865 (2007)

[15] Generation of Singlet Oxygen via the Composites of Water-Soluble Thiol-Capped CdTe Quantum Dots Sulfonated Aluminum Phthalocyanines, Jiong Ma, Ji-Yao Chen, Mopelola Idowu and Tebello Nyokong, J. Phys. Chem. B, Vol. 112, No. 15, (2008)

[16] Rakovich A, et al. CdTe quantum dot/dye hybrid system as photosensitizer for photodynamic therapy. Nanoscale Res Lett.;5(4):753–60. (2010)

[17] Quantum dot–folic acid conjugates as potential photosensitizers in photodynamic therapy of cancer, Vincent Morosini, Thierry Bastogne, C´eline Frochot, Rapha¨el Schneider, Aur´elie Franc¸oisFranc¸ois Guillemin and Muriel Barberi-Heyob, 842 | Photochem. Photobiol. Sci., 10, 842–851.(2011)

[18] Quantum dot-aluminium phthalocyanine conjugates perfotm photodynamic reactions to kill cancer cells via fluorescence resonance energy transfer, Lei Li, Jin-Feng Zhao, Nayoun Won, Ho Jin, Sungiee Kim, Ju-Yao Chen, Nanoscale Research Letters, 7:386 (2012)

[19] Complexes of functionalized Quantum Dots and chlorin e_6 in Photodynamic Therapy, R. Rotomsksis, J. Valanciunaite, A.Skripka, S. Steponkiene, G. Spogis, S. Bagdonas, G. Streckyte, Lithuaninan Journal of Physics, Vol 53, No. 1, pp. 57-68 (2013)

[20] Comparative Study on the Efficiency of the Photodynamic Inactivation of Candida albicans Using CdTe Quantum Dots, Zn(II) Porphyrin and Their Conjugates as Photosensitizers, Osnir S. Viana 1, Martha S. Ribeiro , Andréa C. D. Rodas , Júlio S. Rebouças 3, Adriana Fontes and Beate S. Santos 1, Molecules, 2015

[21] Rational engineering of semiconductor QDs enabling remarkable $^1O_2$ production for tumor-targeted photodynamic therapy, Yizhong Shen, Yidan Sun, Runqi Yan, Erquan Chen, Huan Wang, Deju Ye, Jing-Juan Xu, Hong-Yuan Chen, Biomaterials 148, 31e40 (2017)

[22] Generation of singlet oxygen by water-stable CDSe(S) and ZnSe(S) quantum dots, Sonia Bailon-Ruiz, Oscar J. Perales-Perez, Applied Materials Today 9 (2017) 161-166

[23] Photodynamic Therapy of Human Hepatoma Using Semiconductor Quantum Dots as Sole Photosensitizer, Jiaojiao Sun, Yuming Guo, Lin Zhu, Lin Yang, Weike Shi, Kui Wang, Hua Zhang, Part. Part. Charact., 34, 1600413 (2017)

[24] Two-photon excitation nanoparticles for photodynamic therapy, Yizhog Shen, Adam J.

Shuhendler, Deju Ye, Jing-Jan Xu and Hong-Yuan Chen, Chem. Soc. Rev.. 45, 6725-6741, (2016)

[25] Semiconducton Quantum Dots as Two-Photon Sensitizers, Smita Dayal and Clemens Burda, J. AM. CHEM. SOC., 130, 2890-2891 (2008)

[26] Activation of porphyrin photosensitizers by semiconductor quantum dots via two-photon excitation, Y. N. Wen, W. S. Song, L. M. An, Y. Q. Liu, Y. H. Wang and Y. Q. Yang, Appl. Phys. Lett., 95, 143702. (2009)

[27] Biocompatible CdSe quantum dot-based photosensitizer under two photon excitation for photodynamic therapy, Zu-De Qi, Dong-Wei Li, Peng Jiang, Feng-Lei Jiang, Yue-Sheng Li, Yi Liu, Wai-Kwok Wong and Kok-Wai Cheah, J. Mater. Chem., 21, 2455–2458 | 2455 (2011)

[28] Water soluble quantum dots as hydrophilic carriers and two-photon excited energy donors in photodynamic therapy, Colin Fowley, Nikolitsa Nomikou, Anthony P. McHale, Paul A. McCarron, Bridgeen McCaughan and John F. Callan. 6456 | J. Mater. Chem., 22, 6456–6462 (2012)

[29] Femto-second laser beam with a low power density achieved a two-photon photodynamic cancer therapy with quantum dots, K. L. Chou, N. Won, J. Kwag, S. Kim and J. Y. Chen, J. Mater. Chem. B, 1, 4584–4592. (2013)

[30] Water soluble quantum dots as hydrophilic carriers and two-photon excited energy donors in photodynamic therapy, Colin Fowley, Nikolitsa Nomikou, Anthony P. McHale, Paul A. McCarron, Bridgeen McCaughan and John F. Callan, J. Mater. Chem., 22, 6456 (2012)

[31] Photo induced energy transfer in a CdTe quantum dot–copper phthalocyanine system via two-photon excitation, FangLi, ZhicongHe, MuyeLi, JunpeiZhang, JunboHan, PeixiangLu, Materials Letters132,263–266 (2014)

[32] Micelle-Encapsulated Quantum Dot-Porphyrin Assemblies as in Vivo Two-Photon Oxygen Sensors, C. M. Lemon, E. Karnas, X. Han, O. T. Bruns, T. J. Kempa, D. Fukumura, M. G. Bawendi, R. K. Jain, D. G. Duda and D. G. Nocera, J. Am. Chem. Soc., 137, 9832–9842. (2015)

[33] Review on Carbon Dots and their Applications, Mhetaer Tuerhong, XU Yang, YIN Xue-Bo, Chin J Anal Chem, 45(1), 139-150. (2017)

[34] Luminescent Carbon Nanodots: Emergent Nanolights, Sheila N. Baker and Gary A. Baker, Angew. Chem. Int. Ed. 49, 6726 – 6744 (2010)

[35] Light-Triggered Theranostics Based on Photosensitizer - Conjugated Carbon Dots for Simultaneous Enhanced - Fluorescence Imaging and Photodynamic Therapy, Peng Huang , Jing Lin , Xiansong Wang , Zhe Wang , Chunlei Zhang , Meng He, Kan Wang, Feng Chen, Zhiming Li, Guangxia Shen, Daxiang Cui, and Xiaoyuan Chen, Adv. Mater. 24, 5104–5110 (2012)

[36] Highly Biocompatible Carbon Nanodots for Simultaneous Bioimaging and Targeted Photodynamic Therapy In Vitro and In Vivo,Yuri Choi, Seongchan Kim, Myung-Ho Choi, Soo-Ryoon Ryoo, Jongnam Park, Dal-Hee Min and Byeong-Su Kim, Adv. Funct. Mater., 24, 5781–5789 (2014)

[37] Quantum-sized carbon dots for bright and colorful photoluminescence, Y. P. Sun , B. Zhou , Y. Lin , W. Wang ,K. A. S. Fernando , P. Pathak , M. J. Meziani , B. A. Harruff , X. Wang , H. F. Wang , P. J. G. Luo , H. Yang , M. E. Kose , B. L. Chen , L. M. Veca , S. Y. Xie , J. Am. Chem. Soc., 128 , 7756. (2006)

[38] Increment of the FRET efficiency between carbondots and photosensitizers for enhanced photodynamic therapy, Kun Yang, Fan Li, Wangyuan Che, Xiao Hu, Changjun Liu and Feng Tian, RSC Adv., 6, 101447–101451 | 101447 (2016)

[39] Mg/N double doping strategy to fabricate extremely high luminescent carbon dots for bioimaging, F. Li, C. Liu, J. Yang, Z. Wang, W. Liu and F. Tian, RSC Adv., 4, 3201–3205.(2014)

[40] Porphyrin-Based Carbon Dots for Photodynamic Therapy of Hepatoma, Yang Li, Xiaohua Zheng, Xiaoyu Zhang, Shi Liu, Qing Pei, Min Zheng, and Zhigang Xie, Adv. Healthcare Mater., 6, 1600924 (2017)

[41] Extending the tissue penetration capability of conventional photosensitisers: a carbon quantum dot–protoporphyrin IX conjugate for use in two-photon excited photodynamic therapy, Colin Fowley, Nikolitsa Nomikou, Anthony P. McHale, Bridgeen McCaughan and John F. Callan, Cite this: Chem. Commun.,49, 8934 (2013)

[42] Carbon nanodots featuring efficient FRET for two-photon photodynamic cancer therapy with a low fs laser power density, J. Wang, Z. Zhang, S. Zha, Y. Zhu, P. Wu, B. Ehrenberg and J. Y. Chen, Biomaterials, 35, 9372–9381.(2014)

[43] Ruthenium complex-modified carbon nanodots for lysosome-targeted one- and two-photon imaging and photodynamic therapy, Dong-Yang Zhang, Yue Zheng, Hang Zhang, Liang He, Cai-Ping Tan, Jing-Hua Sun, Wei Zhang, Xingyun Peng, Qiuqiang Zhan, Liang-Nian Ji and Zong-Wan Mao, Cite this: Nanoscale, 9, 18966 (2017)

[44] Markovic, Z. M. et al. Graphene quantum dots as autophagy-inducing photodynamic agents. Biomaterials 33, 7084–7092 (2012).

[45] A graphene quantum dot photodynamic therapy agent with high singlet oxygen generation, Jiechao Ge1, Minhuan Lan, Bingjiang Zhou, Weimin Liu, Liang Guo, Hui Wang, Qingyan Jia, Guangle Niu, Xing Huang, Hangyue Zhou, Xiangmin Meng, Pengfei Wang, Chun-Sing Lee,

Wenjun Zhang & Xiaodong Han, NATURE COMMUNICATIONS|5:4596|DOI: 10.1038/ncomms5596, (2014)

[46] Gollnick, K. & Held, S. H. Hydroxyanthraquinones as sensitizers of singlet oxygen reactions: quantum yields of triplet formation and singlet oxygen generation in acetonitrile. J. Photochem. Photobiol. A Chem. 69, 155–165 (1992).

[47] N-Doping of Graphene Through Electrothermal Reactions with Ammonia, Xinran Wang,1 Xiaolin Li, Li Zhang, Youngki Yoon, Peter K. Weber, Hailiang Wang, Jing Guo, Hongjie Dai, SCIENCE VOL 324, (2009)

[48] Graphene quantum dots with nitrogen-doped content dependence for highly efficient dual-modality photodynamic antimicrobial therapy and bioimaging, Wen Shuo Kuo, Hua-Han Chen, Shih-Yao Chen, Chia-Yuan Chang, Pei-Chi Chen, Yung-I Hou, Yu-Ting Shao, Hui-Fang Kao, Chih-Li Lilian Hsu, Yi-Chun Chen, Shean-Jen Chen, Shang-Rung Wu, Jiu-Yao WangBiomaterials 120, 185e194 (2017)

[49] Improving singlet oxygen generating abilities of phthalocyanines: aluminum tetrasulfonated phthalocyanine in the presence of graphene quantum dots and folic acid, Nnamdi Nwahara, Jonathan Britton & Tebello Nyokong, Journal of Coordination Chemistry, VOL . 70, NO . 9, 1601–1616 (2017)

[50] One-pot synthesis of graphene quantum dots–phthalocyanines supramolecular hybrid and the investigation of their photophysical propertis, Gertrude Fomo, Ojodomo J. Achadu, and Tebello Nyokong, Mater Sci 53:538–548(2018)

[51] Mitochondrial specific photodynamic therapy by rare-earth nanoparticles mediated near-infrared graphene quantum dots,m Dandan Zhang, Liewei Wen, Ru Huang, Huanhuan Wang, Xianglong Hu, Da Xing, Biomaterials 153 (2018)

[52] Near-Infrared Light-Mediated Photodynamic Therapy Nanoplatform by the Electrostatic Assembly of Upconversion Nanoparticles with Graphitic Carbon Nitride Quantum Dots, Ming-Hsien Chan, Chieh-Wei Chen, I-Jung Lee, Yung-Chieh Chan, Datao Tu, Michael Hsiao, Chung-Hsuan Chen, Xueyuan Chen, and Ru-Shi Liu, Inorg. Chem. 55, 10267−10277 (2016)

[53] Localization of mitochondria in living cells with rhodamine, L.V. Johnson, M.L. Walsh, L.B. Chen, 123, P Natl. Acad. Sci. U. S. A 77 (1980)

[54] Two-Photon Photoexcited Photodynamic Therapy and Contrast , Agent with Antimicrobial Graphene Quantum Dots, Wen-Shuo Kuo, Chia-Yuan Chang, Hua-Han Chen, Chih-Li Lilian Hsu, Jiu-Yao Wang, Hui-Fang Kao, Lawrence Chao-Shan Chou, Yi-Chun Chen, Shean-Jen Chen, Wen-Tsan Chang, Shih-Wen Tseng, Ping-Ching Wu, and Ying-Chih Pu, ACS Appl. Mater. Interfaces, 8, 30467−30474 (2016)

[55] Nitrogen-doped graphene quantum dots coupled with photosensitizers for one-/two-photon activated photodynamic therapy based on a FRET mechanism, Jiaheng Sun, Qi Xin, Yang Yang, Hameed Shah, Hongqian Cao, Yanfei Qi, Jian Ru Gong and Junbai Li, Cite this: Chem. Commun., 54, 715 (2018)

[56] Strong two-photon-induced fluorescence from photostable, biocompatible nitrogen-doped graphene quantum dots for cellular and deep-tissue imaging, Q. Liu, B. D. Guo, Z. Y. Rao, B. H. Zhang and J. R. Gong, Nano Lett., 13, 2436–2441 (2013)

[57] MoO_{3-x} quantum dots for photoacoustic imaging guided photothermal/photodynamic cancer treatment, Dandan Ding,‡ Wei Guo, Chongshen Guo, Jianzhe Sun, Nannan Zheng, Fei Wang, Mei Yan and Shaoqin Liu, Nanoscale, 9, 2020 (2017)

[58] Carbon Dots with Intrinsic Theranostic Properties for Bioimaging, Red-Light-Triggered Photodynamic/ Photothermal Simultaneous Therapy In Vitro and In Vivo, Jiechao Ge, Qingyan Jia, Weimin Liu, Minhuan Lan, Bingjiang Zhou , Liang Guo , Hangyue Zhou, Hongyan Zhang, Ying Wang, Ying Gu, Xiangmin Meng, and Pengfei Wang, Adv. Healthcare Mater. 5, 665–675 (2016)

[59] Multifunctional graphene quantum dots for combined photothermal and photodynamic therapy coupled with cancer cell tracking applications, Mukeshchand Thakur, Mukesh Kumar Kumawat and Rohit Srivastava, RSC Adv., 7 5251–5261 | 5251 (2017)

[60]Aptamer-Conjugated Graphene Quantum Dots/Porphyrin Derivative Theranostic Agent for Intracellular Cancer-Related MicroRNA Detection and Fluorescence-Guided Photothermal/Photodynamic Synergetic Therapy, Yu Cao,† Haifeng Dong, Zhou Yang, Xiangmin Zhong, Yi Chen, Wenhao Dai, and Xueji Zhang, ACS Appl. Mater. Interfaces, 9, 159−166 (2017)

[61]MultifunctionalUCNPs@MnSiO3@g-C3N4 nanoplatform:improved ROS generation and reduced glutathione levels for highly efficient photodynamic Therapy, Lili Feng, Fei He, Yunlu Dai, Shili Gai, Chongna Zhong,a Chunxia Lib and Piaoping Yang, Cite this: Biomater. Sci., 5, 2456 (2017)

[62] MoO3−x quantum dots for photoacoustic imaging Guided photothermal/photodynamic cancer treatment, Dandan Ding, Wei Guo,Chongshen Guo,Jianzhe Sun, Nannan Zheng, Cite this: Nanoscale, 9, 2020, Fei Wang,a,c Mei Yana and Shaoqin Liu (2017)

[63] Multifunctional Theranostic Agent of Cu2(OH)PO4 Quantum Dots for Photoacoustic Image-Guided Photothermal/Photodynamic Combination Cancer Therapy, Wei Guo, Zhenyu Qiu, Chongshen Guo, Dandan

Ding, Tianchan Li, Fei Wang, Jianzhe Sun, Nannan Zheng and Shaoqin Liu, ACS Appl. Mater. Interfaces, 9, 9348−9358 (2017)

[64]Multifunctional Nanoplatform Based on Black Phosphorus Quantum Dots for Bioimaging and Photodynamic/Photothermal Synergistic Cancer Therapy, Yi Li, Zhiming Liu, Yuqing Hou, Guangcun Yang, Xixi Fei, Henan Zhao, Yanxian Guo, Chengkang Su, Zhen Wang, Huiqing Zhong, Zhengfei Zhuang, and Zhouyi Guo, ACS Appl. Mater. Interfaces, 9, 25098−25106 (2017)