PDT – sources, light dosimetry and combined applications PDI – photodynamic inactivation – principles and applications

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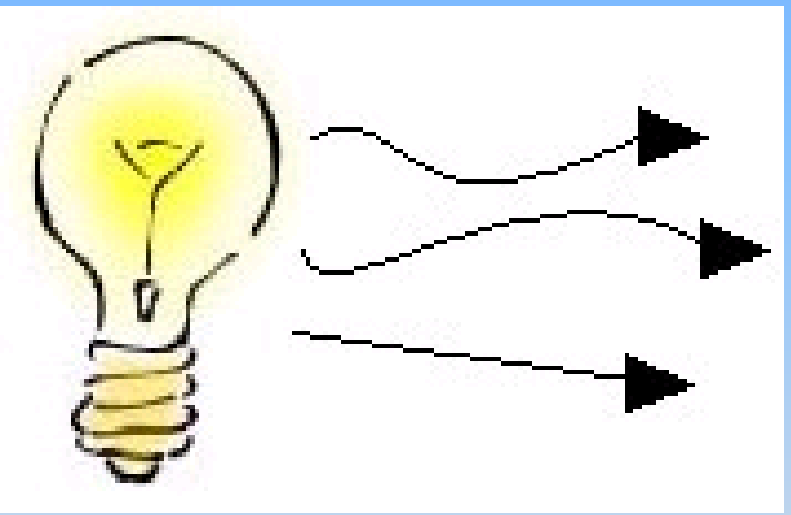
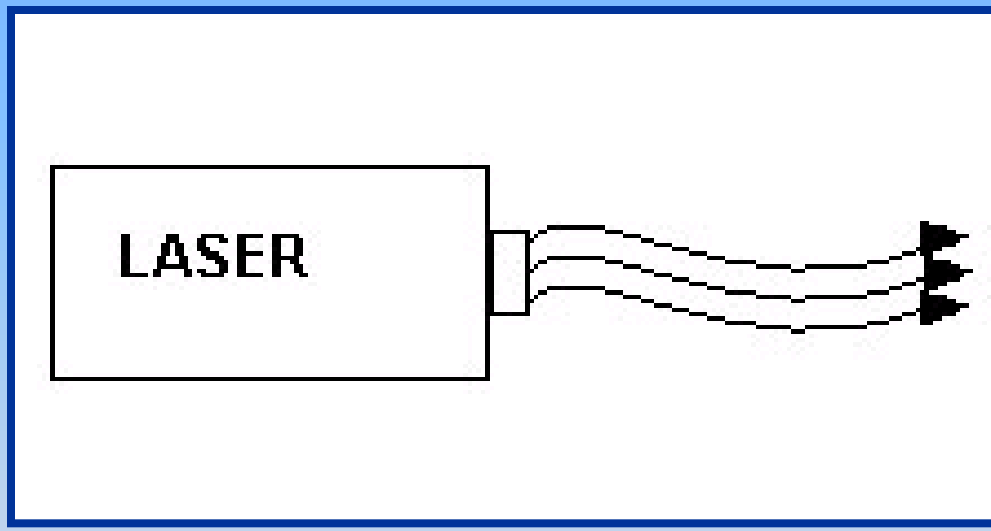
Light sources used for PDT
Incandescent vs. Laser Light

Light from bulbs are due to spontaneous emission





1. **Many wavelengths**
2. **Multidirectional**
3. **Incoherent**

1. **Monochromatic**
2. **Directional**
3. **Coherent**

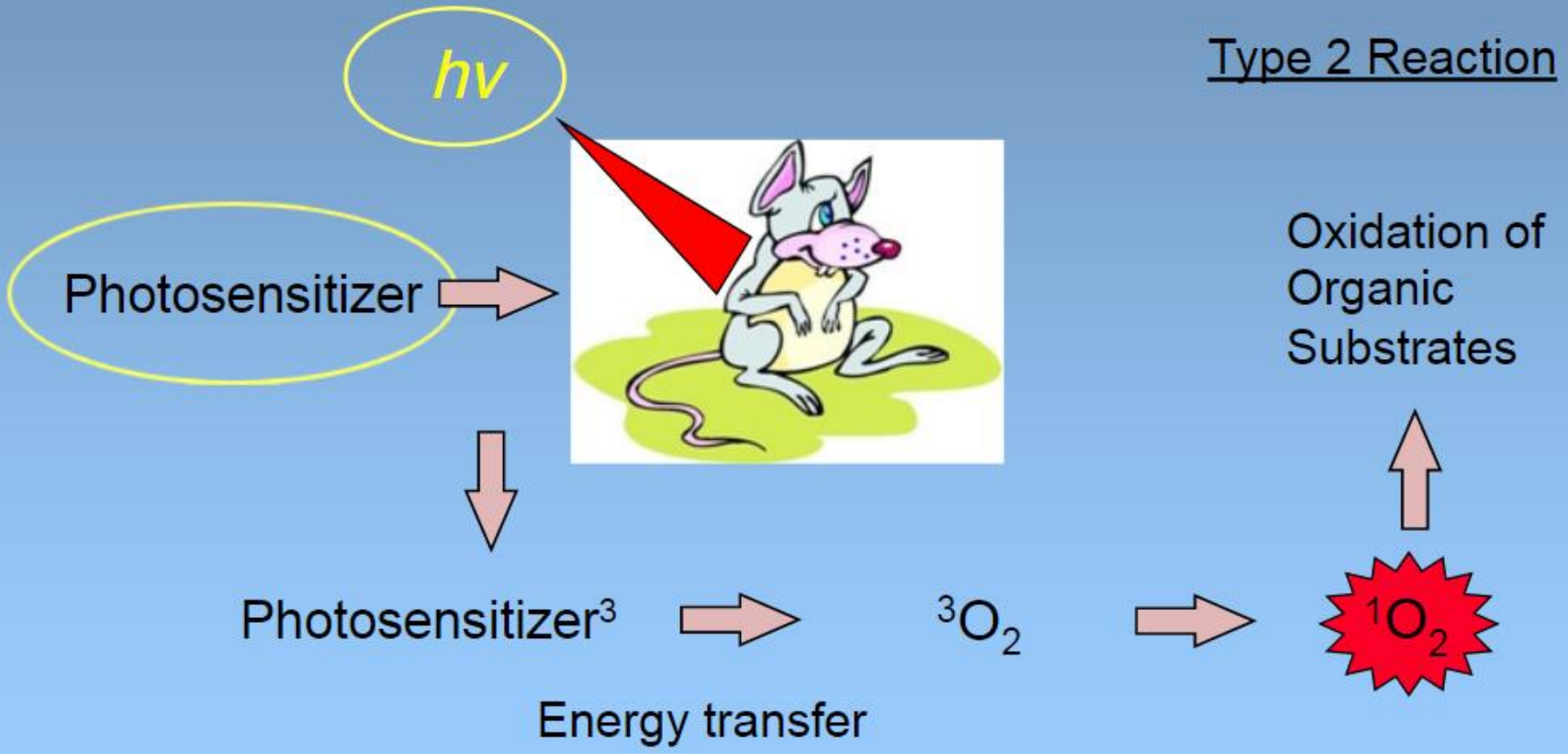
Lasers have *unique characteristics* as light sources:

- Directionality
- Monochromaticity
- Brightness
- Coherence
- Other properties:
  - Pulsed or continuous operation
  - Tunable
  - Polarised light

Why are these properties useful?

Characteristic	Advantage	Applications
High power	Multiphoton process Improved signal High scattering intensity	Nonlinear spectroscopy Improved sensitivity Raman scattering
Monochromatic	High resolution State selection	Spectroscopy Isotope separation
Collimated beam	Long path lengths	Sensitivity
Coherent	Interference between separate beams	CARS Focused in fiber optics
Pulsed	Precise timing of excitation	Pump-probe studies Relaxation processes Energy transfer Fast reactions

How does PDT work?



How does PDT work?

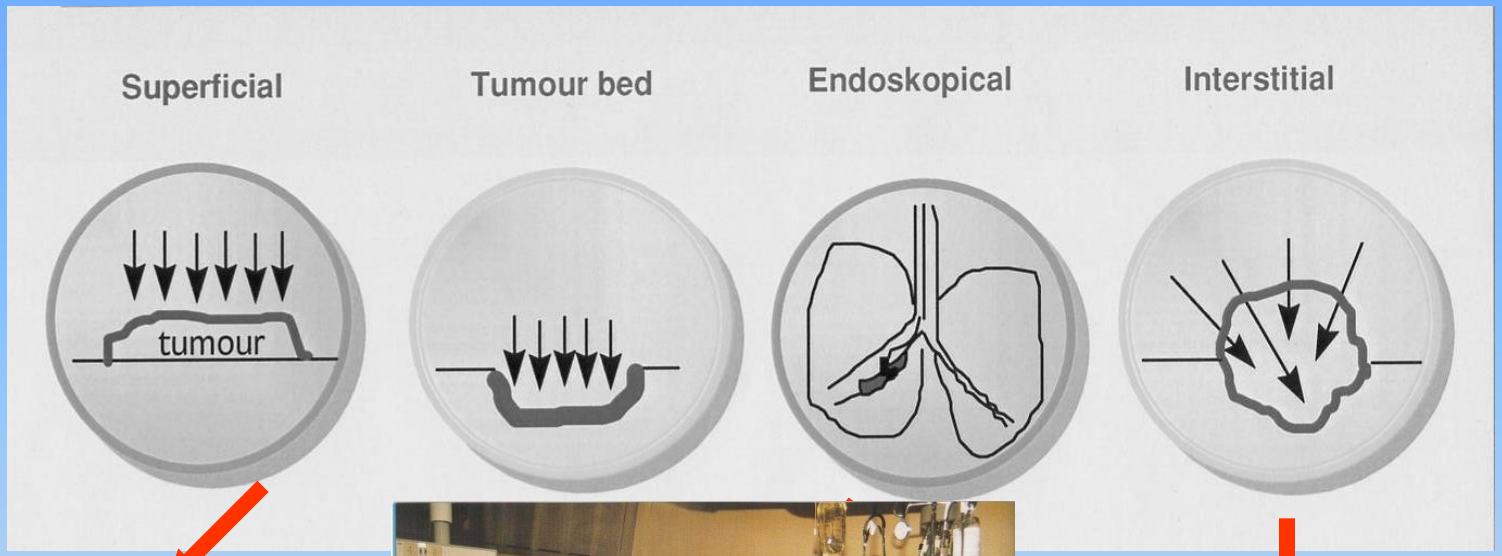
- Mechanisms of PDT action
  - Direct Cell Effects
    - Direct $^1O_2$-mediated toxicity to tumor cells
  - Indirect Effects
    - Vascular damage
      - During light treatment
      - Delayed development within several hours after light treatment
    - Stimulation of host immune responses.
- Cell death may occur by apoptosis, necrosis, and/or autophagy

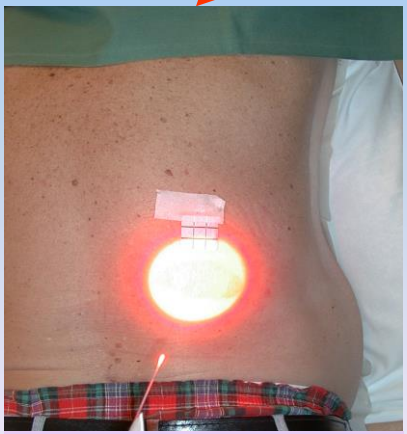
PDT variables

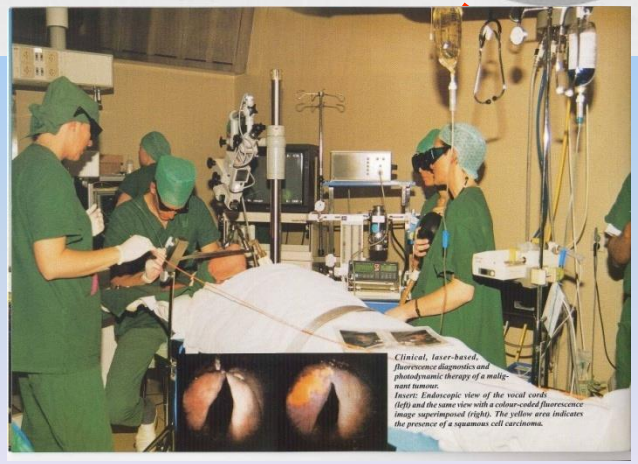
- **Photosensitizer**
  - Type of sensitizer
  - Dose of drug
  - Drug-light irradiation interval

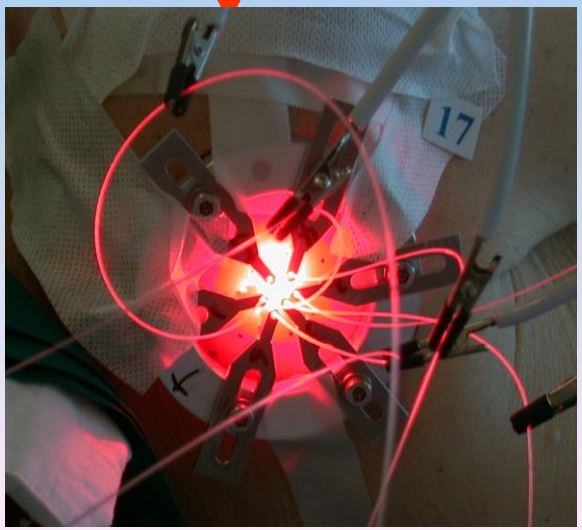
- **Light delivery**
  - Geometry of irradiation
  - Irradiation wavelength
  - Fluence
  - Fluence rate (Light dose)

Geometry - how to deliver the light to the tumour?

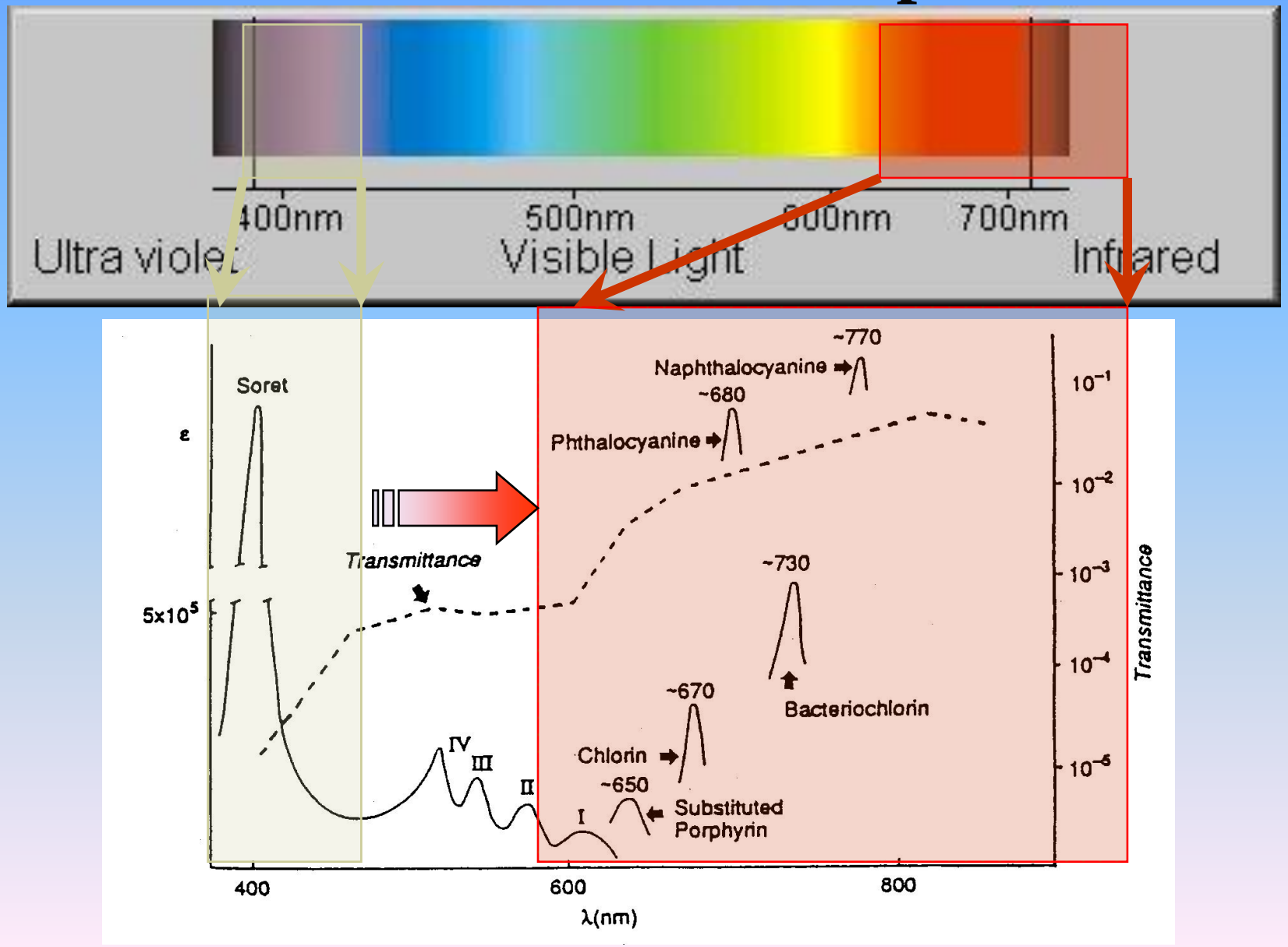




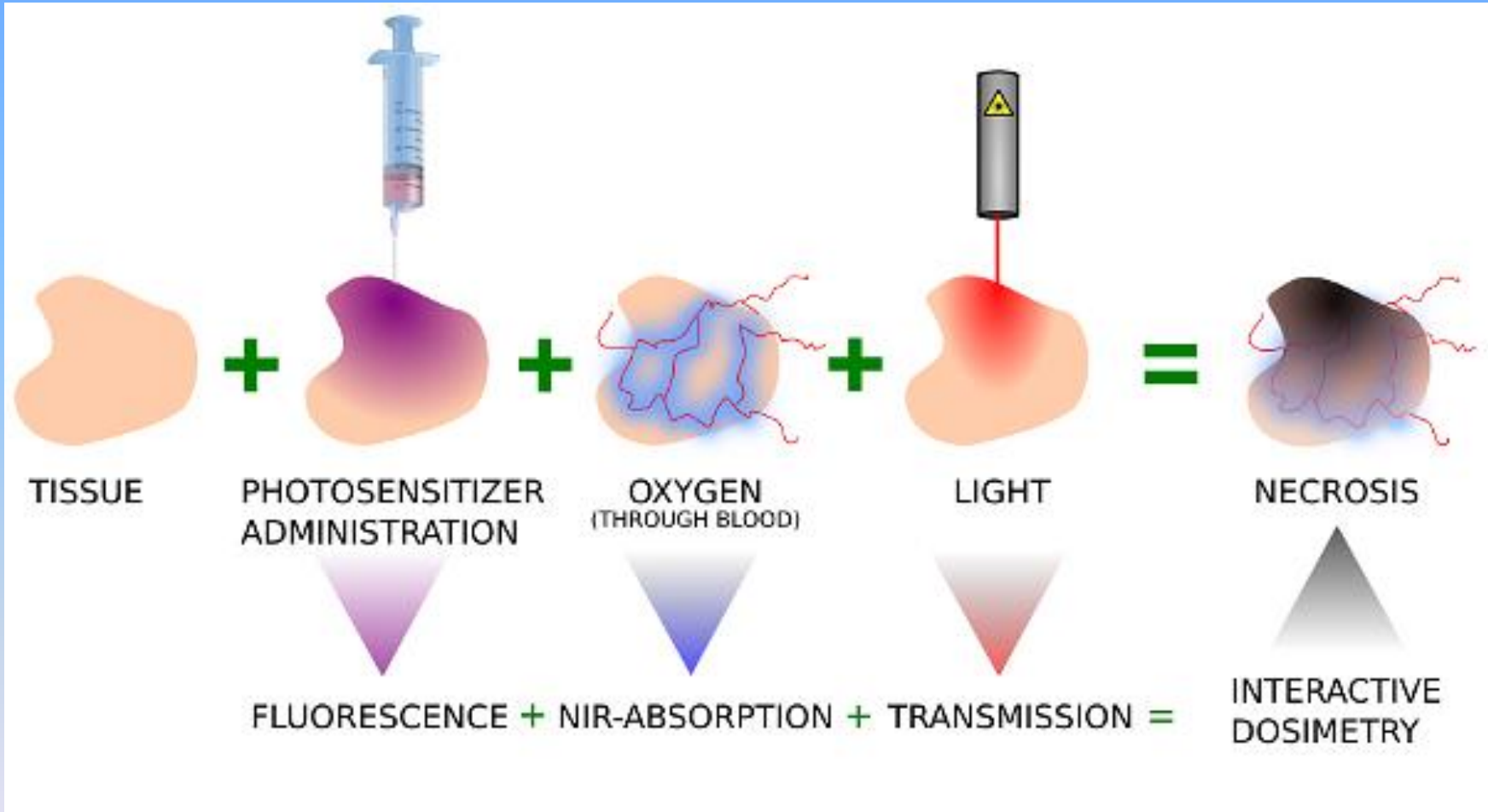




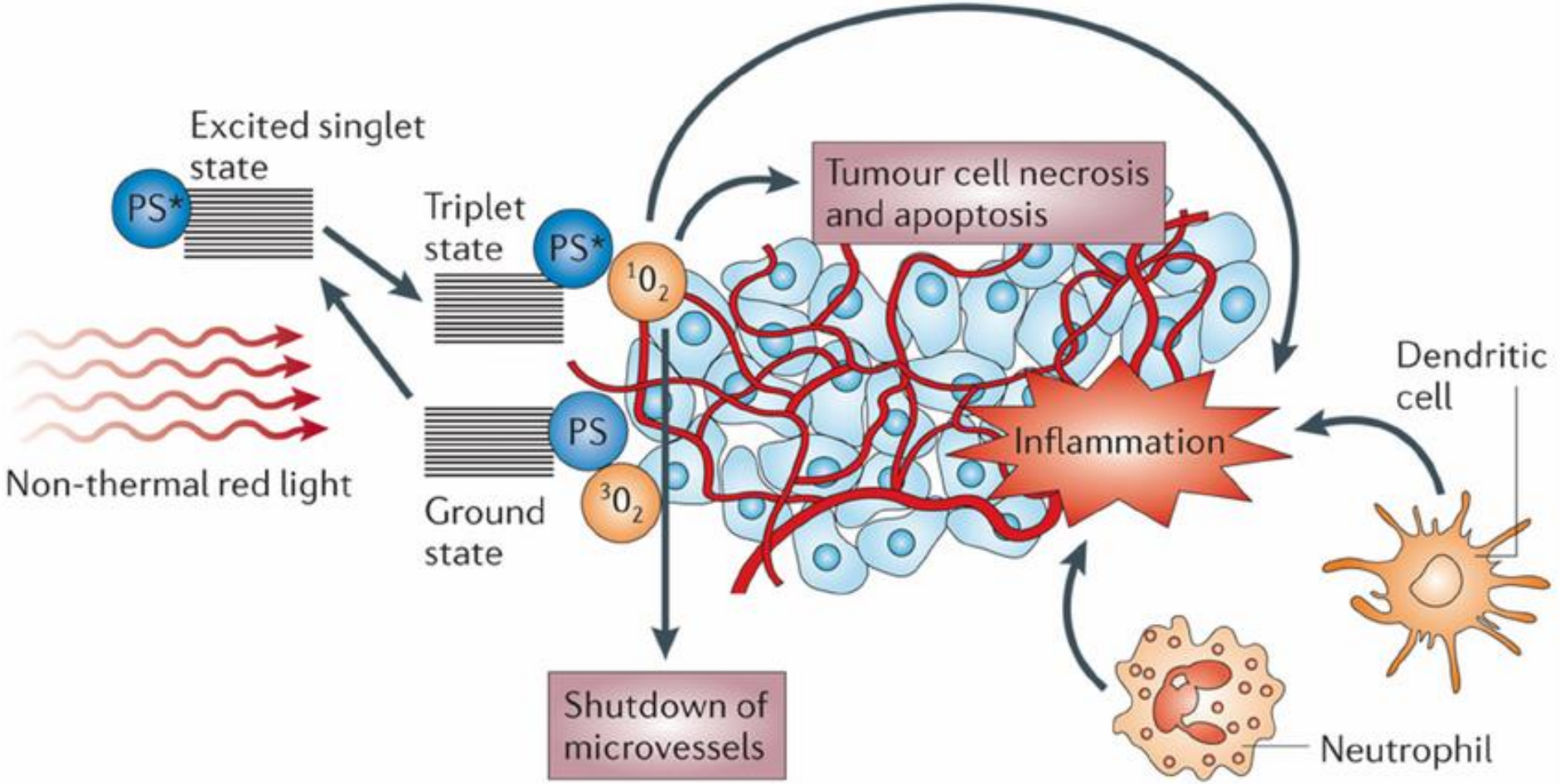
Irradiation wavelengths – therapeutic window of the tissues+absorption of PS



PDT – light dosimetry



http://www.atomic.physics.lu.se/biophotonics/research/photodynamic-therapy/realtime-pdt-dosimetry/



The mechanism of action on tumours in PDT. The photosensitizer (PS) absorbs light and an electron moves to the first short-lived excited singlet state. This is followed by intersystem crossing, in which the excited electron changes its spin and produces a longer-lived triplet state. The PS triplet transfers energy to ground-state triplet oxygen, which produces reactive singlet oxygen ($^1O_2$). $^1O_2$ can directly kill tumour cells by the induction of necrosis and/or apoptosis, can cause destruction of tumour vasculature and produces an acute inflammatory response that attracts leukocytes such as dendritic cells and neutrophils.

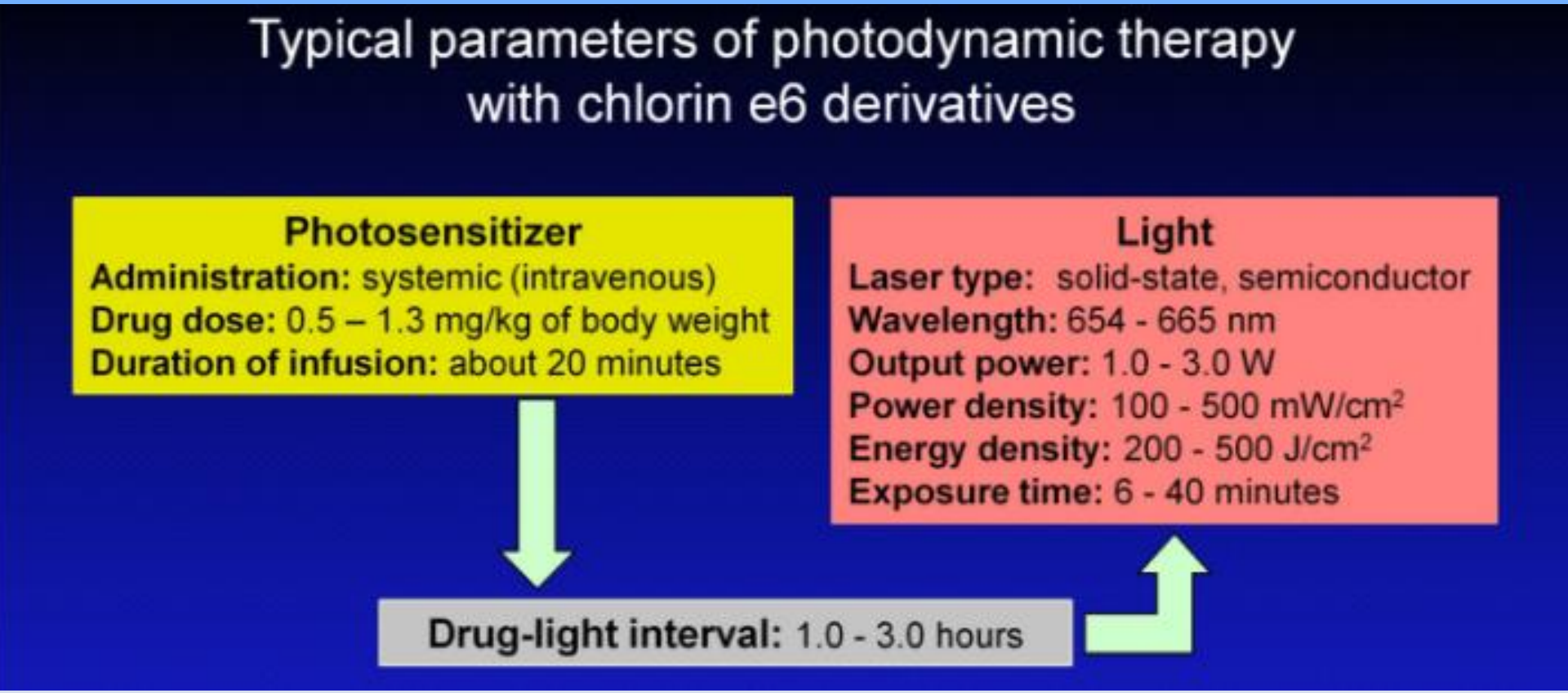
PDT dose

- Light dose rate and dose
  - Fluence rate – irradiance: W/cm^2
  - Fluence – radiant exposure: J/cm^2
- Photosensitizer dose
  - Systematic: mg/kg, or mg/m^2
- PDT dose = {Fluence}x(PS dose}

PDT dosimetry

- Treatment parameters
  - Fluence rate and fluence
- In real-time regime
  - Light dosimetry system
- Post treatment
  - Biomarkers for necrosis and apoptosis

Light regimes and dose diversity



http://www.magicray.ru/PDT_Photodynamic_therapy/Oncology_lectures/Historical_aspects_of_PDT_development.html

High or low?

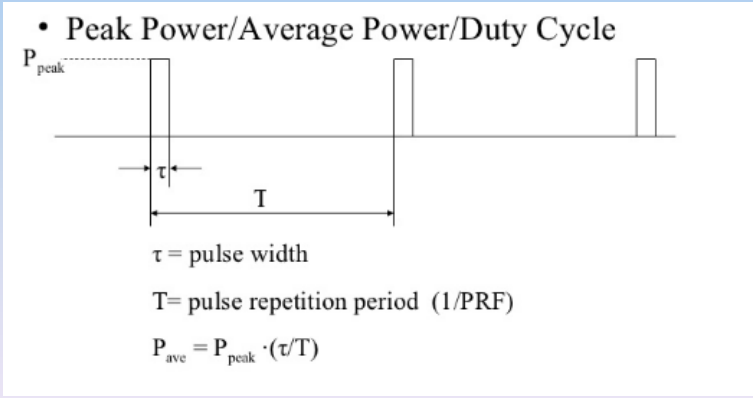
Rationale:

- Lowering PDT fluence rate reduces the rate of photochemical oxygen consumption.
  - Better maintenance of tumor oxygenation during illumination.
  - Improves long-term tumor responses
    - Enhanced direct cell kill
    - Enhanced vascular shutdown in the treatment field

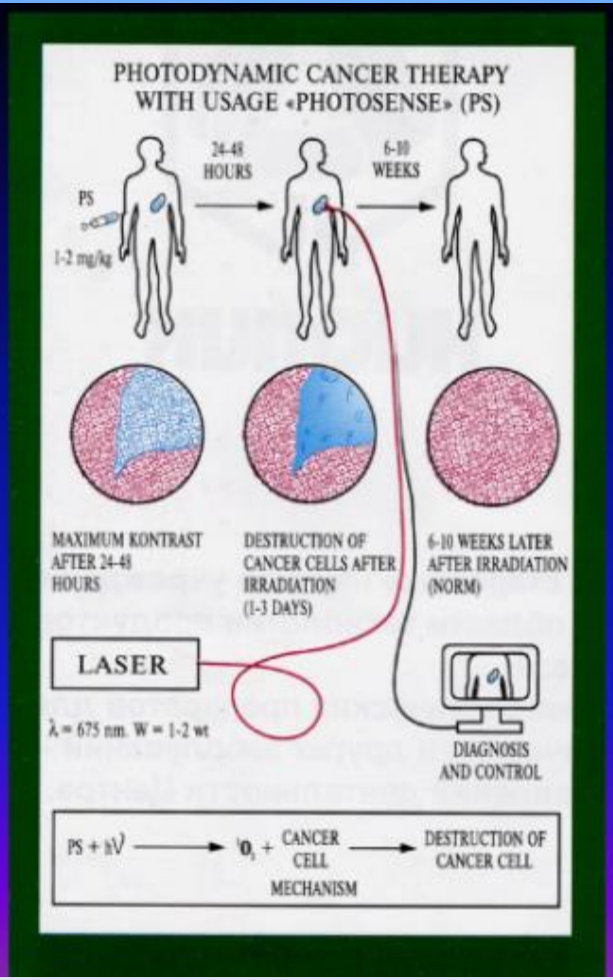
Light dose

- CW irradiation
  - Light power – P [W]
  - Beam cross-section – S [cm^2] (depends from beam shape) (circular shape $S=pi*r^2$)
  - Power density (light fluence) – F = P/S [W/cm^2]
  - Irradiation time t [s]
  - **Light dose D=F*t = (P/S)*t [J/cm^2]**

- Pulsed irradiation
  - Pulse width τ [s]
  - Pulse repetition period – T [1/PRF]
  - Average power $P_{aver} = P_{peak}*(\tau /T)$
  - **Light dose $D = (P_{aver}/S)*t$ [J/cm^2]**



PS in use in Russia



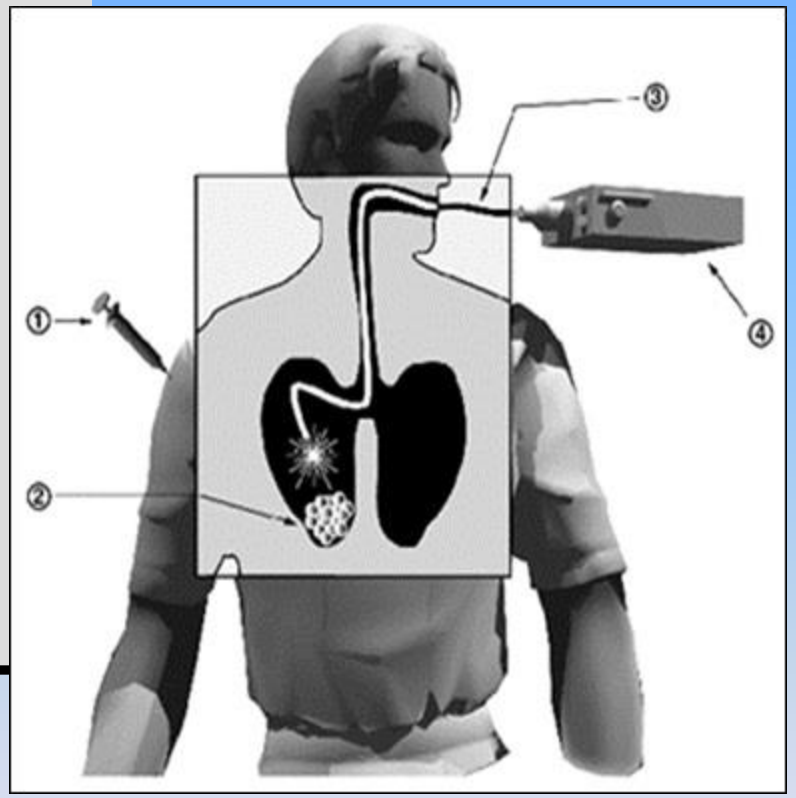
Photosense - 675 nm

Alasense - 630 nm

Photoditazine - 0.5% water solution for i.v. administration

Radachlorin - 0.35% water solution for i.v. administration

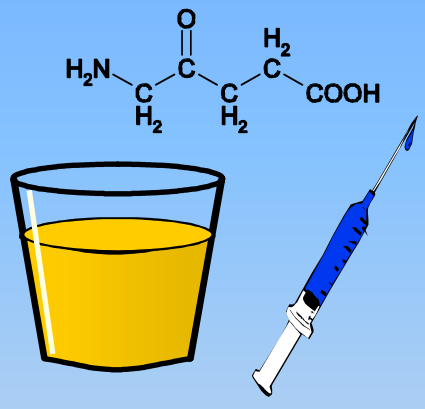
Photodynamic therapy of tumors - basic steps for PS



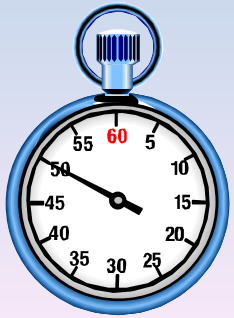
1. In the body is introduced light-sensitive chemical compound (photosensitizer)
2. The drug selectively accumulates in the tumor tissue where it is irradiated with a laser of appropriate wavelength
3. The photosensitizer transmits the laser energy and creates a toxic form of oxygen and free radicals
4. Tumor cells are destroyed with a minimal damage to surrounding healthy cells

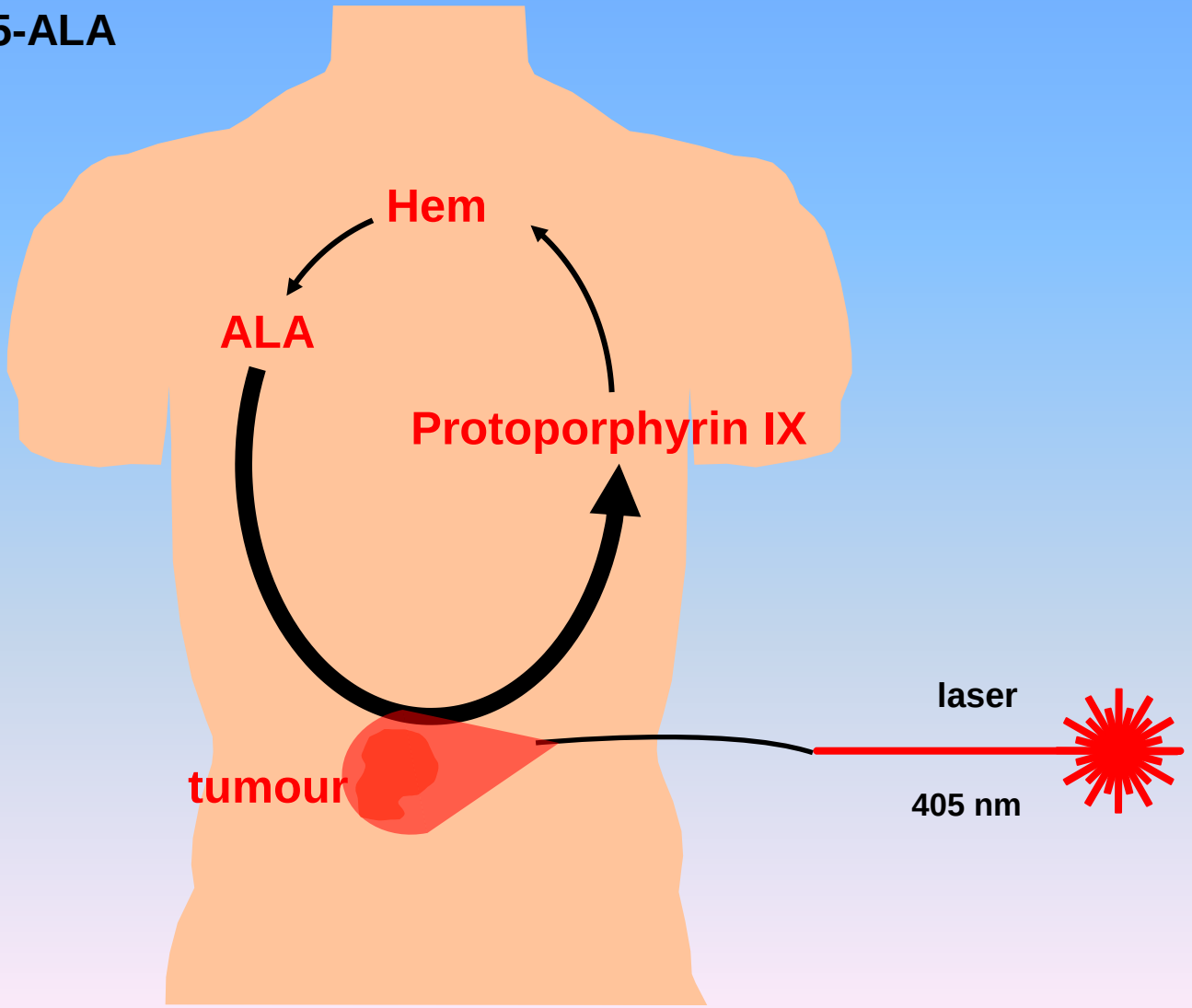
Photodynamic therapy of tumors – using 5-ALA/PpIX

Administration of 5-ALA



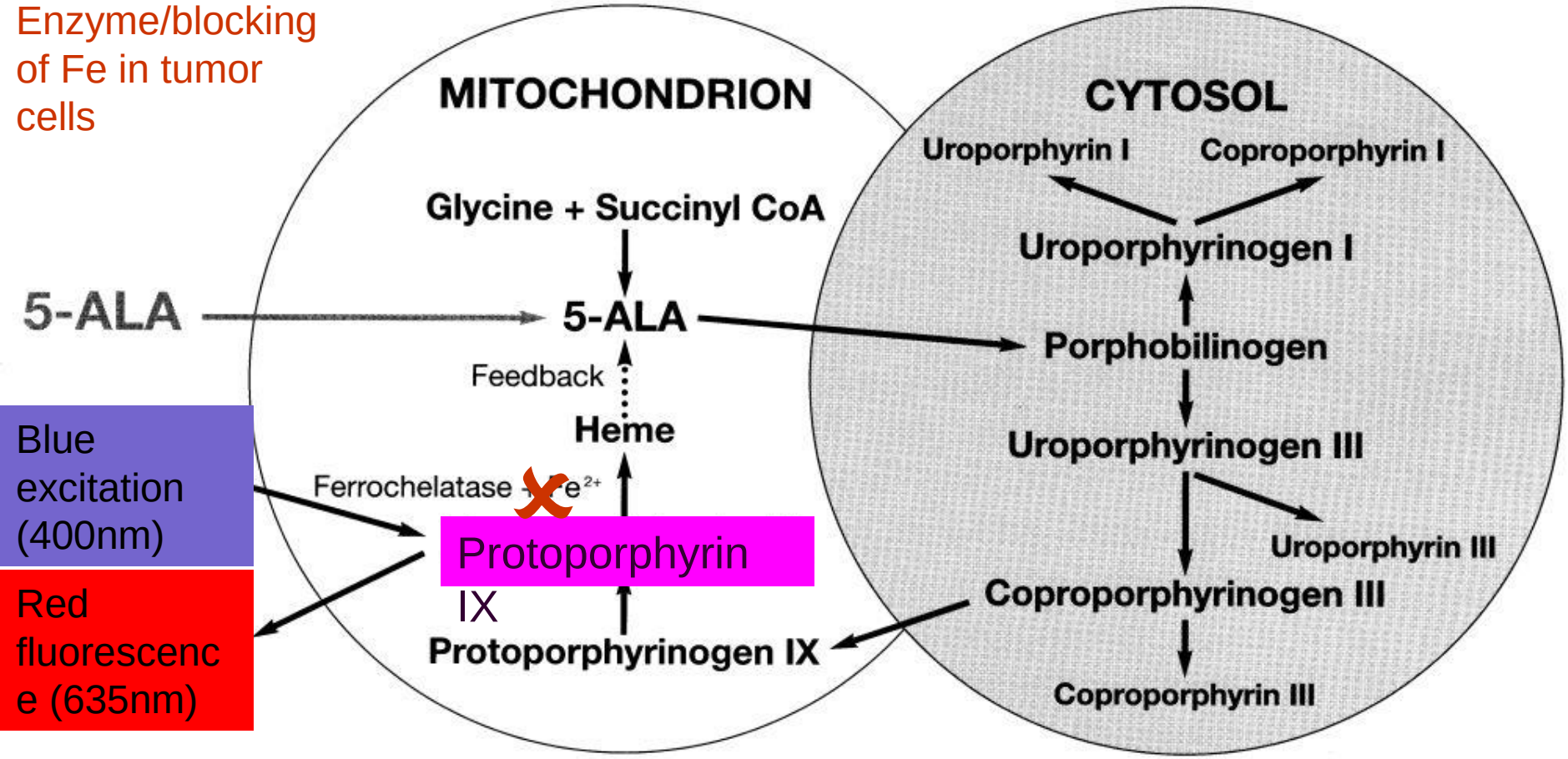
Synthesis Protoporphyrin IX





Metabolism of δ-ALA in the cells

Enzyme/blocking of Fe in tumor cells



Blue excitation (400nm)

Red fluorescence (635nm)

PDT-combined applications

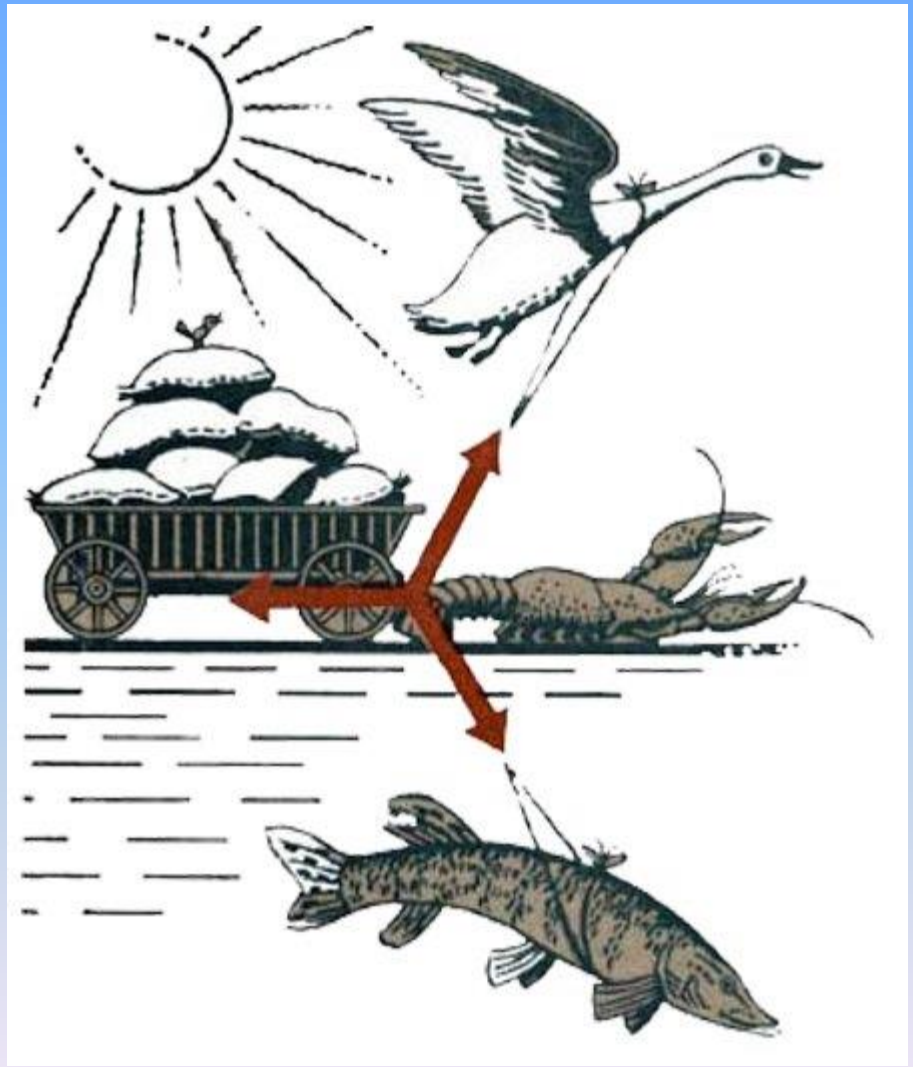


Photo-immunology

- Photo-immunology is introduced as a biomedical discipline studying the effect of non-ionizing electromagnetic radiation and, above all, ultraviolet radiation on the human immune system.
- Psoriasis - PUVA therapy
- Light irradiation of blood
- Photo-immune PDT

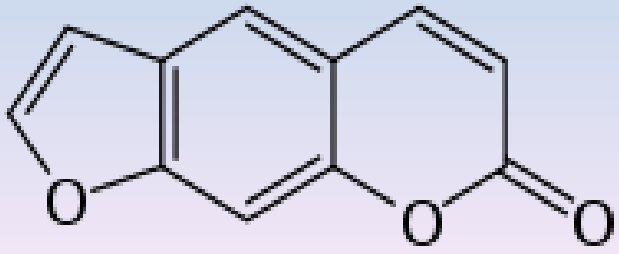
365 nm

405 nm

630-670 nm

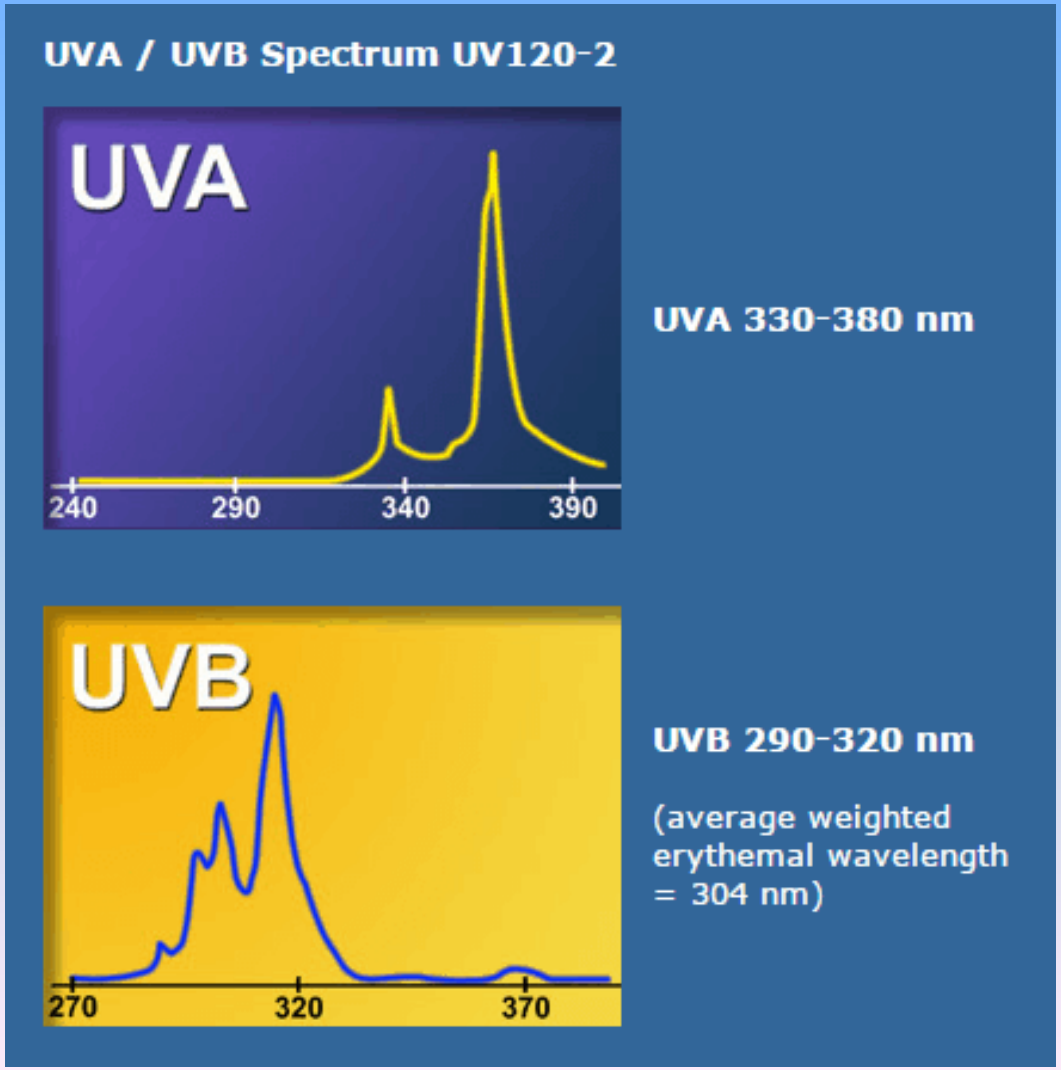
PUVA therapy

- Psoralen UltraViolet A (PUVA) - therapy
- Psoralen - for psoriasis, vitiligo dermatitis, eczema, T-cell lymphoma and others. skin diseases
- Without exposure to UV-A, psoralen and its derivatives do not interact with the skin
- Time between intake of the substance and irradiation - 1-2 hours



Light sources for PUVA and PUVB therapy

- PUVB (280-320 nm) - most commonly used in the treatment of vitiligo
- PUVA (320-400 nm) - for other types of skin problems
- Narrow and broad-band irradiation



PUVA-therapy - short-term and long-term effects

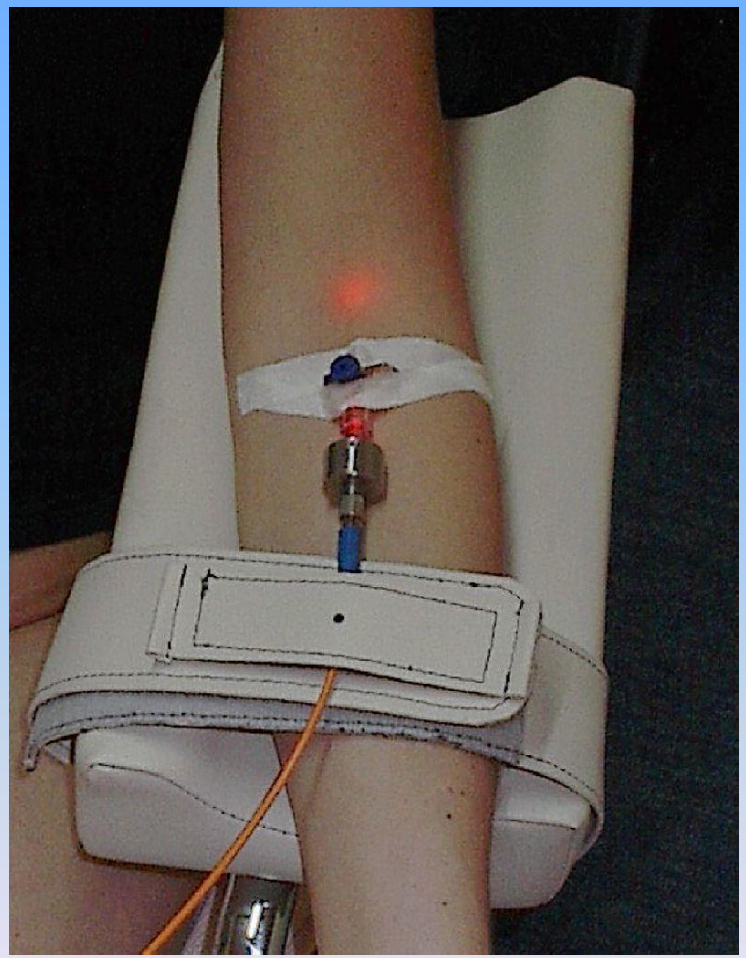
- Erythema
- Tann
- Nausea
- Itching

- Skin cancer
- Cataract
- Wrinkles (photo-aging)
- Freckles and pigment spots

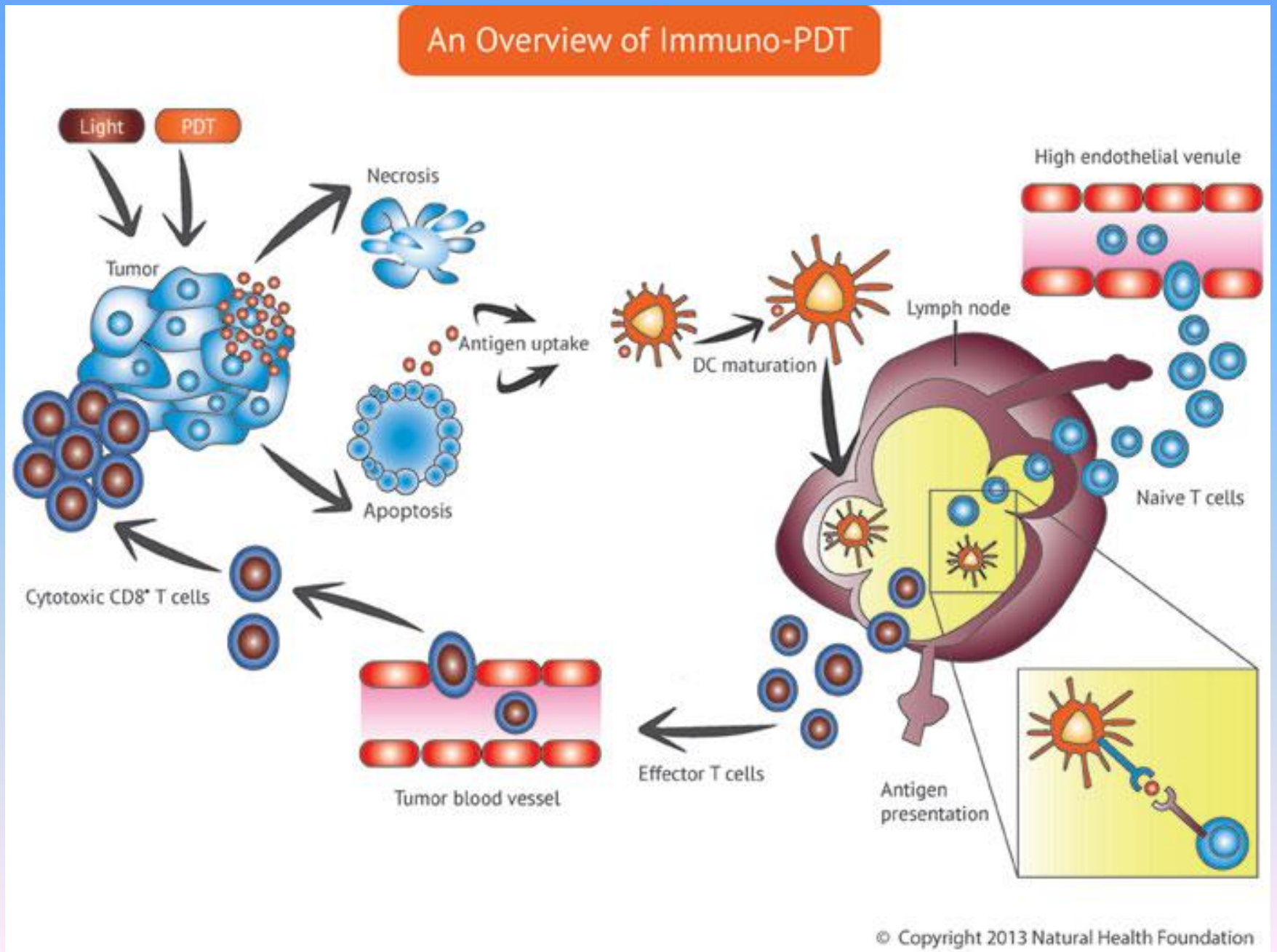
Light irradiation of blood

Light radiation therapy is a procedure in which the blood is exposed to low intensity red light (most commonly laser radiation). It is applied clinically in Germany, Russia and China, and clinical trials are conducted in the United Kingdom and the United States on the feasibility of this type of therapy. It can be applied:

1) Transcutaneous (through the skin) for patients with problematic blood vessels
2) Can be administered via a catheter into a vein
3) by irradiating of the surface vessels of mucous membranes - inside the nose, throat, etc.



Immune PDT


An Overview of Immuno-PDT
Light
PDT
Necrosis
Tumor
Antigen uptake
Apoptosis
DC maturation
High endothelial venule
Lymph node
Naive T cells
Cytotoxic CD8+ T cells
Tumor blood vessel
Effector T cells
Antigen presentation
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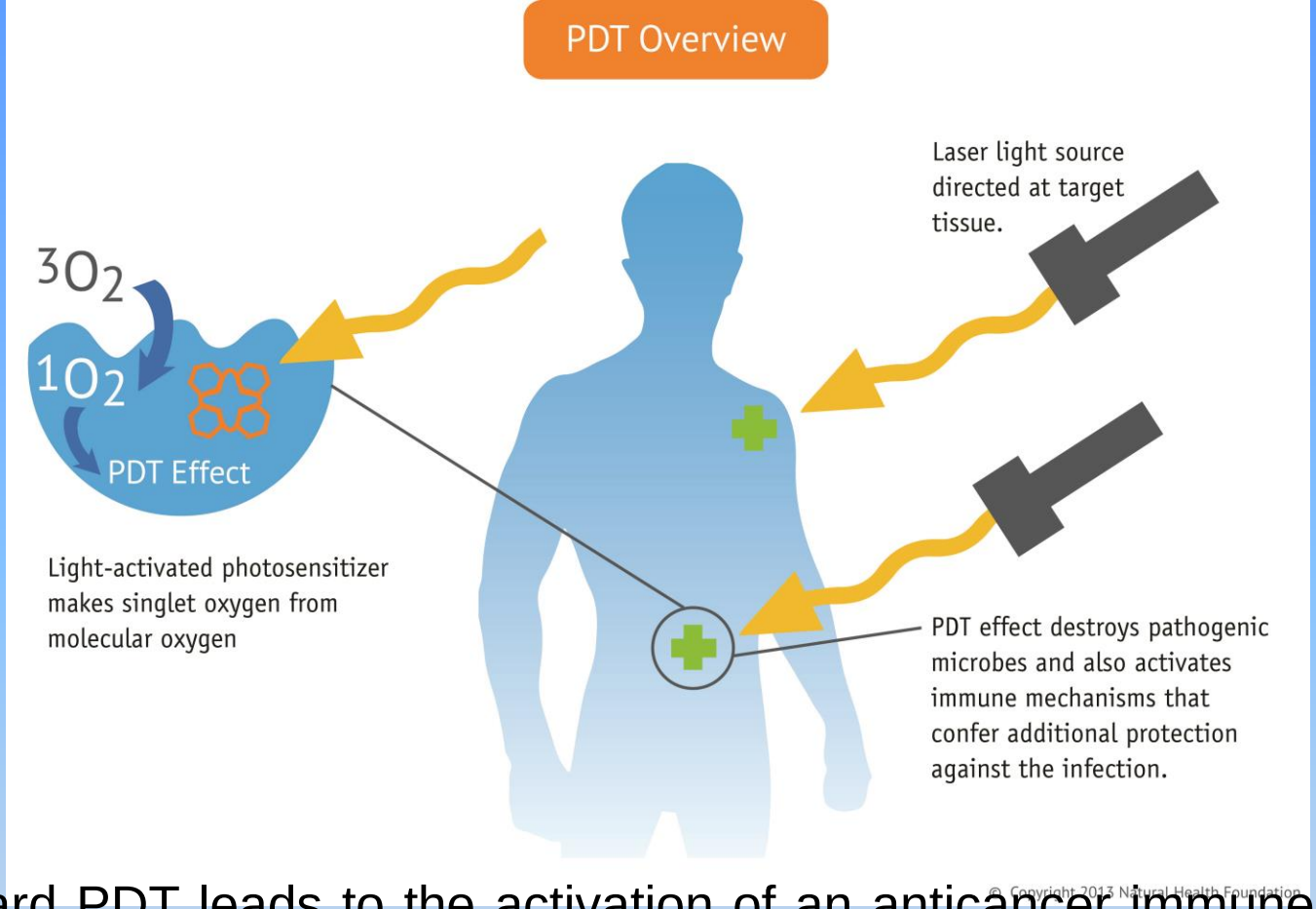
Immune PDT

- In Immuno-PDT immune defense is specifically activated to induce an effective response to cancer cells. PDT induces the release of antigens from the tumor during the necrosis it induces. Dendritic cells are able to capture these tumor antigens and then migrate to the lymph nodes, where they interact with T-cells, which triggers an aggressive immune response against cancer.

Immune PDT - types

- The four main types of immune photodynamic therapy include:

- Low dose of PDT with immune adjuvants
- Antibodies-based photoimmunotherapy
- PDT vaccines
- Systemic light treatment



- Standard PDT leads to the activation of an anticancer immune response to the body. However, this reaction is usually too weak to have a significant effect on metastasis. A low dose of PDT may be combined with immunomodulatory agents known as immune adjuvants to help the immune system better recognize cancer cells and react more actively.
- It is important to understand that immune adjuvants are by themselves largely ineffective against cancer. Without the addition of PDT, the cancer cells can not be recognized and attacked accordingly.

PDT with antibodies

- By binding the photosensitizer to the antibody more selectively, the drug is delivered to the tumor tissue. Each antibody is specific for the particular type of protein found on the tumor cell membrane so that the antibody binds highly specifically to the tumor. After irradiation with light - a photodynamic effect is induced.
- In ideal case, the photosensitizer-antibody complex should reach the most vulnerable parts of cancer cells, such as lysosomes that contain a digestive enzyme. Destruction of lysosomes causes cell degradation, as a result of enzyme release. These results are more selective and result in a more focused therapeutic effect, and allow lower doses of photosensitizer and lower light irradiation doses to be used.

Systematic PDT

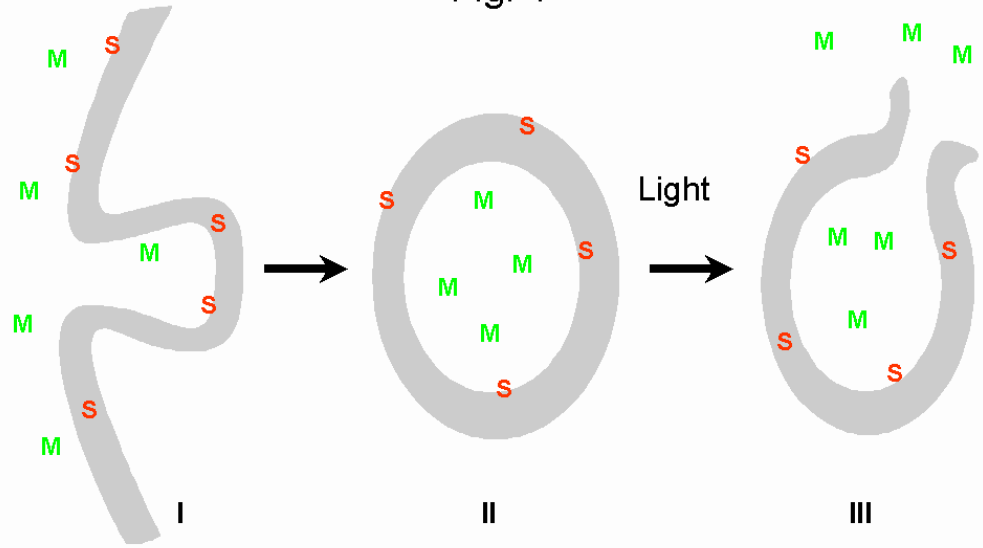
- Systemic irradiation is a new approach to cancer therapy originally developed in Russia. The underlying principle is the activation of the photosensitizer, which circulates inside the circulatory system with whole body exposure without irradiating directly to produce a cytotoxic effect on tumor cells.
- In this way, the photosensitizer passes from the essentially oxidized state within the body volume. The oxidized photosensitizer accumulates in diseased areas - cancer or infection. During the PDT system, light is applied to the patient by laser or LED matrices, luminescent tubes, or even sunlight. The aim is to illuminate as much as possible the body,
- Clinical observations to nowadays indicate that S-PDT is effective against most of the cancers. The great advantage of this approach is that primary tumor and metastatic tumors are treated with equal efficacy.

Photochemical internalization

Photochemical internalization is used to transport macromolecules to cells, as well as drug resistance is observed

The photosensitizer is located in the membrane of the liposome or vesicle transporting the drug substance. After the vesicle endocytosis inside the cell and under light irradiation, the membrane is destroyed and macromolecules are released.

Fig. 1

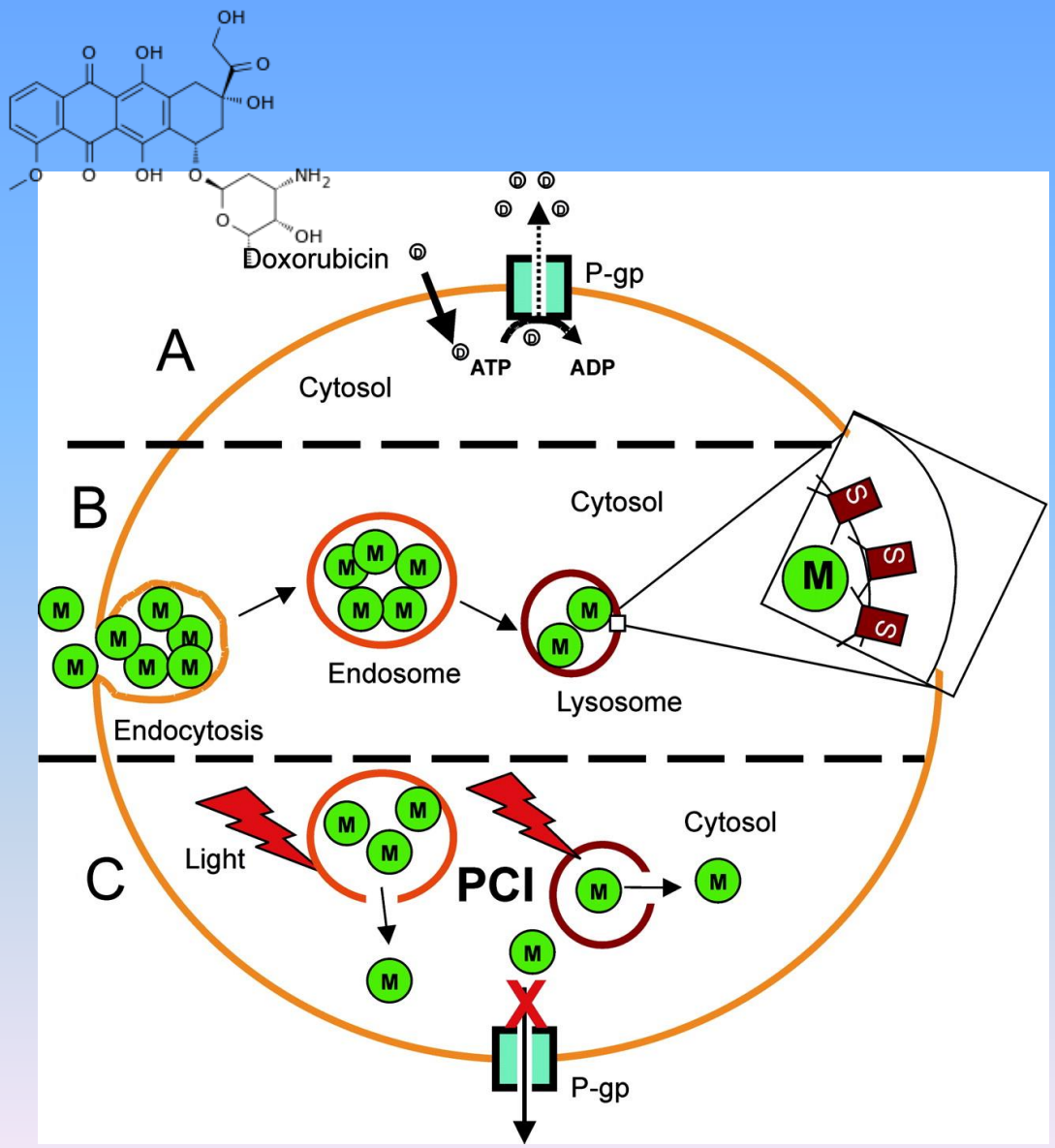


I) Endocytosis of the photosensitizer (S) and the macromolecule (M) of interest.

II) Localization of the photosensitizer and macromolecules in the same endocytic vesicles.

III) Rupture of membranes of endosomes/lysosomes upon light exposure and subsequent cytosolic release of the macromolecules.

Schematic diagram of the PCI in the cell

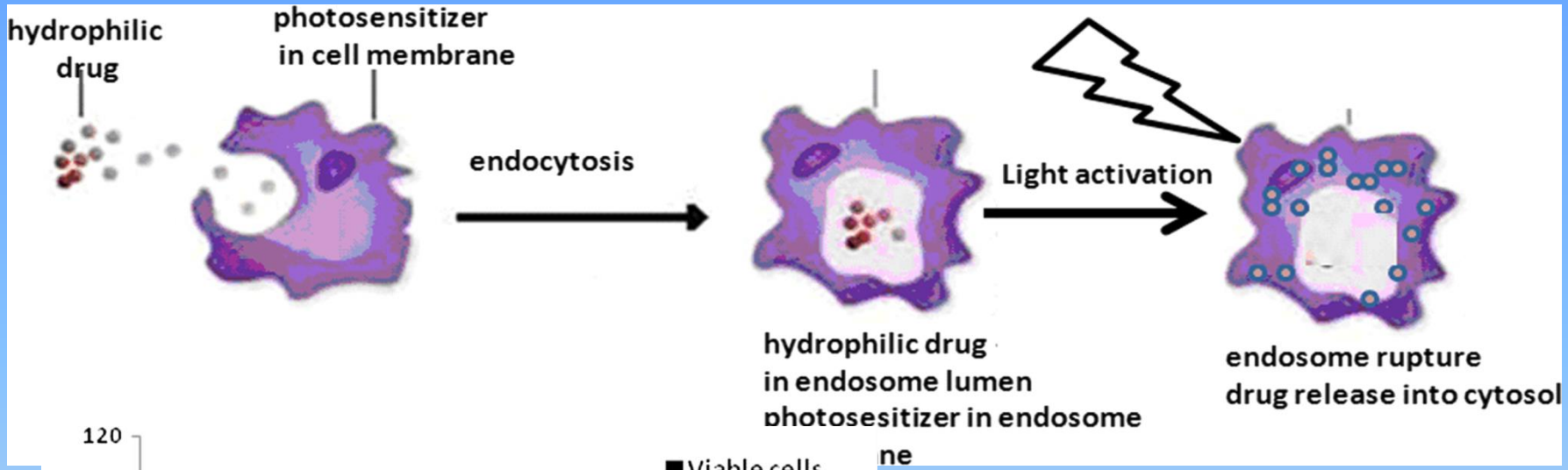


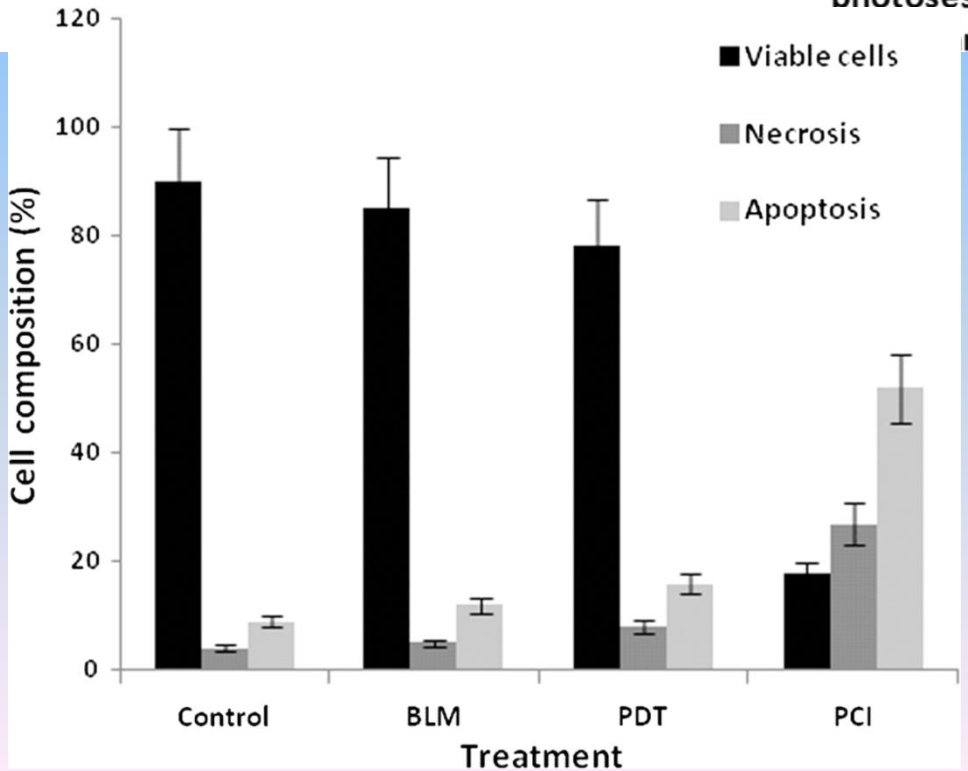
PCI principle for insertion of macromolecular drugs into tumor cells: A-P-gp pumps that export doxorubicin (D) from the tumor cell, which dramatically reduces the effectiveness of chemotherapy and represents a major barrier to treatment.

B - if a macromolecular drug (M) containing this cytostatics is injected through liposomes, the membrane of which has a photosensitizer (S) through endocytosis, the drug enters without detection on the cell membrane and is pumped. Inside, these macromolecules fall into endocytic vesicles (another protective mechanism leading to resistance). M is released from the endosomes or lysosomes after light irradiation and reaches the cytosol of the cell where it can subsequently reach the desired intracellular target. M is too large to be thrown out of the cell via P-GP (or another ABC transporter).

Pål K. Selbo, Anette Weyergang, Anette Bonsted, Stephen G. Bown, Kristian Berg, Photochemical Internalization of Therapeutic Macromolecular Agents: A Novel Strategy to Kill Multidrug-Resistant Cancer Cells, JPET,vol. 319(2) 604-612 (2006)

Example - bleomycin for glioma lesion





PDI – principles and applications

PDI - In the beginning…

The potential of PDT against microorganisms was first established more than a century ago by O. Raab (1898) with observation of lethal effect of acridine orange and visible light on *Paramecium caudatum*.

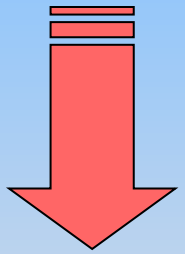
However, the potential of PDT against the microorganisms was not exploited for long years because:

a) some pathogens, like gram-negative bacteria and protozoa were poorly responsive to photosensitization with known at that time photosensitizing agents (xanthene or acridine dyes) and porphyrins;

b) discovery of antibiotics forced the believe that the microbial-induced infection would have one general solution.

Problems

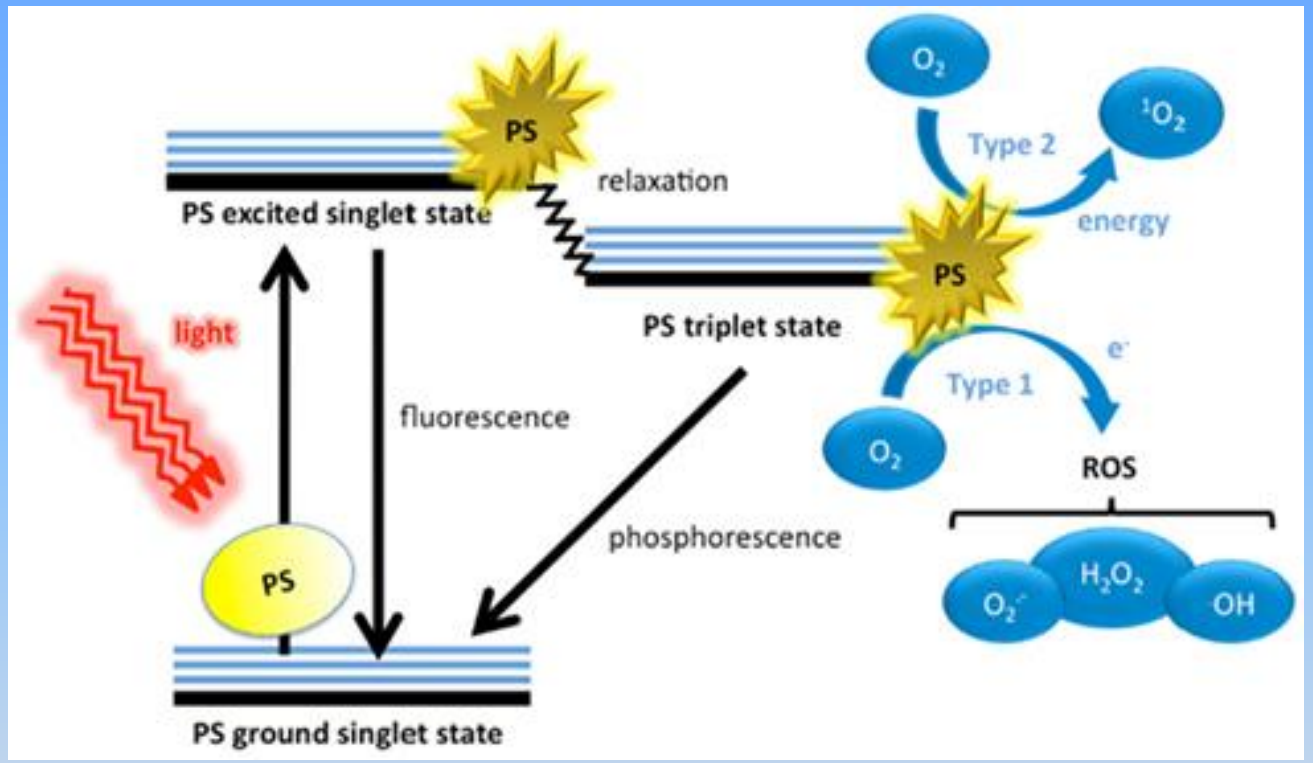
- Increased number of antibiotic-resistive pathogenic bacteria
- Multi-drug resistive bacterial strains
- Clinical infections, inflammations, resistive wounds
- Ecological multibacterial contaminations



Need for new treatment modalities

Solution? ⟶ **PDI**

Photodynamic inactivation - principles



Jablonski diagram*

Radicals:

O_2^-

H_2O_2

HOCl

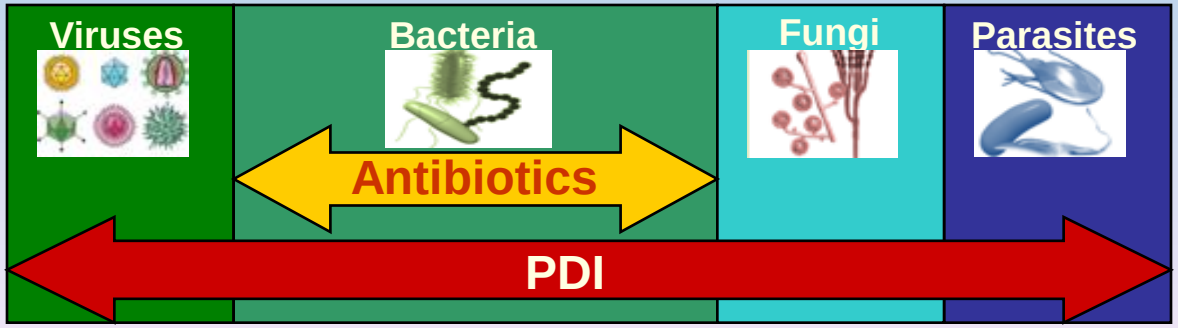
NO

$ONOO^-$

NO_2

HO

$^1O_2$



T. Dai et al., Front. Microbiol., 2012

Main features of antimicrobial PDI

- There is no specificity toward the selected microorganism, since the photosensitization can act to bacteria, fungi, viruses and etc.
- There is no dependency on the antibiotic resistance of microorganisms to the photodynamic action;
- The local treatment of the pathogenic micro-organisms proceeds with very limited damage of the host biological cells;
- The selective delivery of the photosensitizer to the infected area occurs by the suitable drug formulation and the further local irradiation with cost effective light sources, already developed for classic PDT applications

PDI Applications

1) Ecology - Waste water bacterial disinfection

2) Medicine - microorganisms treatment

3) Zoonotic disease cause treatment

Properties of an ideal disinfectant

- Broad action spectrum - active against all types of microorganisms
- Fast acting - produces rapid inactivation of pollutants
- Effective in the presence of organic matter, suspended solids and other matrices or sample constituents
- Non-hazard – toxic, flammable or explosive material
- Compatible with various materials/surfaces
- Stable or persistent for the intended exposure period
- Provides a residual
- Easy to generate/synthesize
- Convenient application
- Low price

Standard disinfectants in water and wastewater treatment

- **Free Chlorine** - strong **oxidant**; oxidizes various protein sulfhydryl groups; alters membrane permeability; oxidize/denature nucleic acid components, etc.
- **Monochloramine** - weak **oxidant**; denatures sulfhydryl groups of proteins
- **Ozone** - strong **oxidant**; ditto free chlorine
- **Chlorine Dioxide** - strong **oxidant**; ditto free chlorine

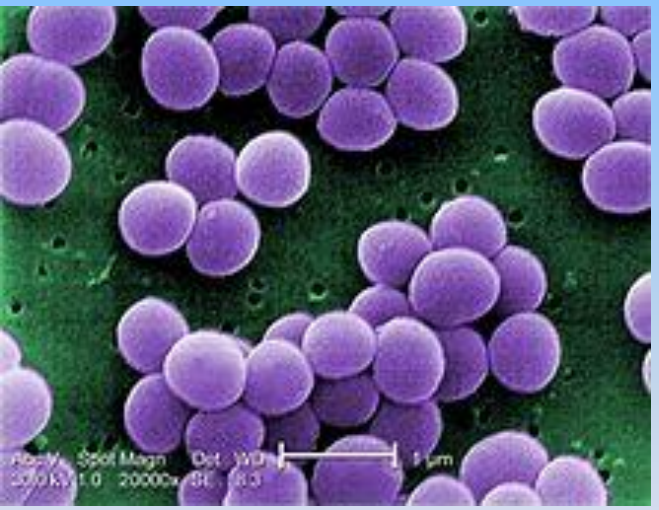
- **Electrochemically generated mixed oxidants** from NaCl - strong **oxidants**; probably ditto free chlorine
- **UV Light** - nucleic acid damage: thymidine dimer formation, strand breaks, **oxidation**,etc.

Wastewater and photodynamic inactivation

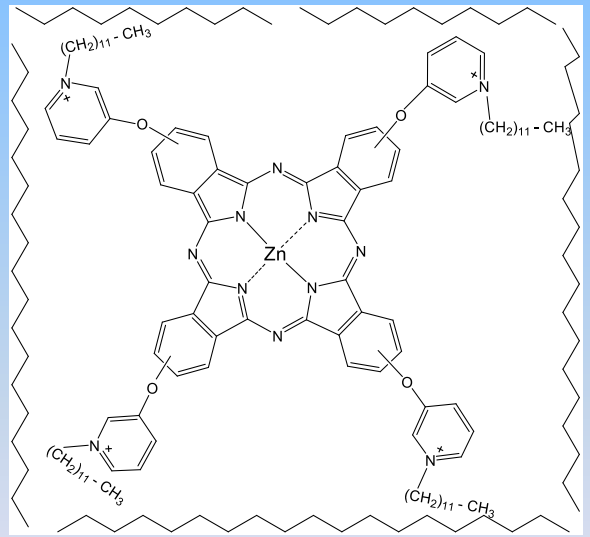
- The light exposure on a daily basis has been well accepted as a competitive method for decontamination of wastewater.
- The great numbers of studies on the photocatalytic action of TiO_2 show the high efficacy in industrial water cleaning including the usefulness against the pathogenic microorganisms.
- The photodisinfection method offers great potential to reduce the transmission of pathogens in the environment.
- Although the titanium dioxide shows high activity against pathogens, its general usage in waste water cleaning is limited due to the insufficient excitation natural light (about 3% of the solar spectrum). The specific dopants as metals and broad-band active compositions have been explored for enhancement of photoactivity.

PDI examples

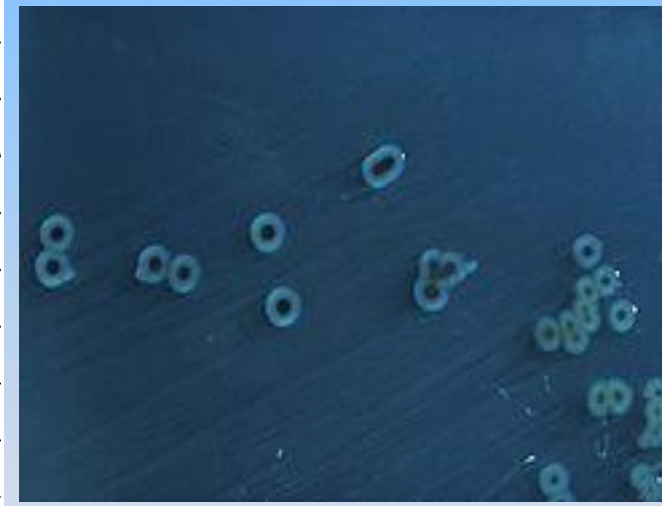
PDI on the basis of hydrophobic tetra-dodecylpyridyloxy Zn(II) phthalocyanine immobilized on TiO_2. The photodynamic efficacy of new solid composite was studied towards two pathogenic bacterial strains associated with wastewater, one Gram-positive *methicillin-resistant Staphylococcus aureus* (*MRSA*) and one Gram-negative *Salmonella enteritidis*.



*Staphylococcus aureus**



Structure of dodecylpyridyloxy Zn(II) phthalocyanine adsorbed on TiO_2 particle

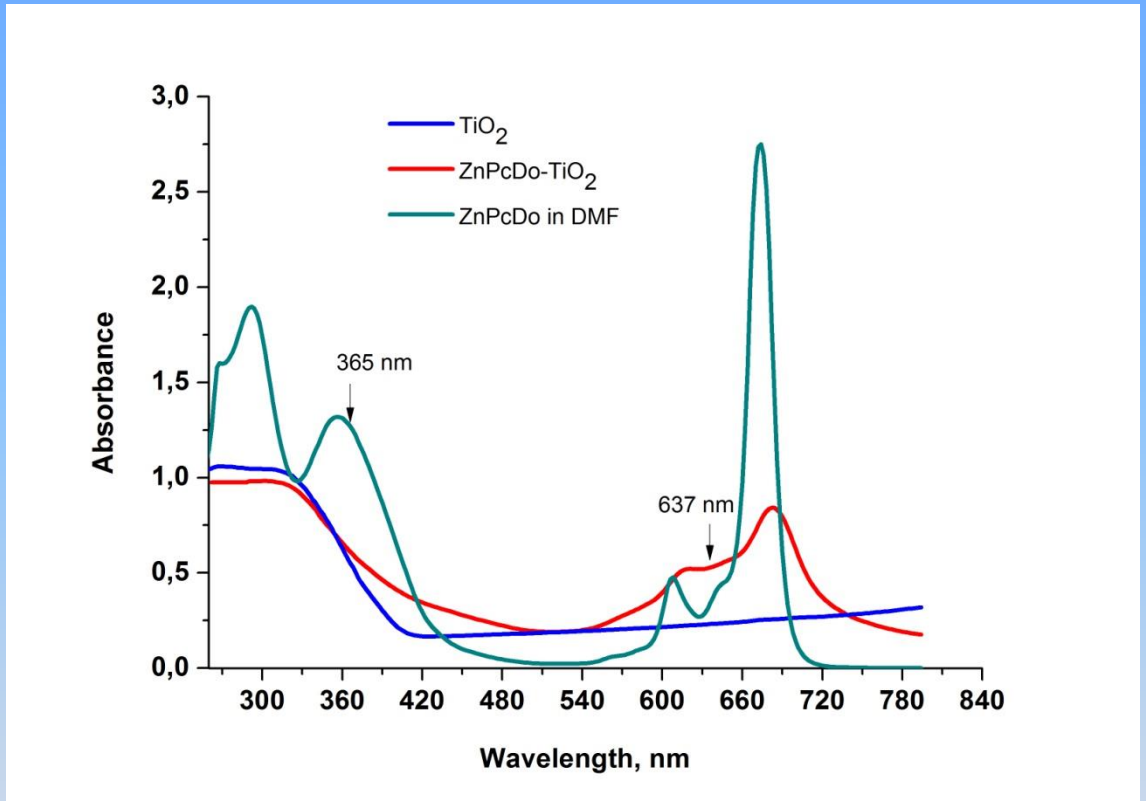


*Salmonella enteritidis**

* *http://en.wikipedia.org*

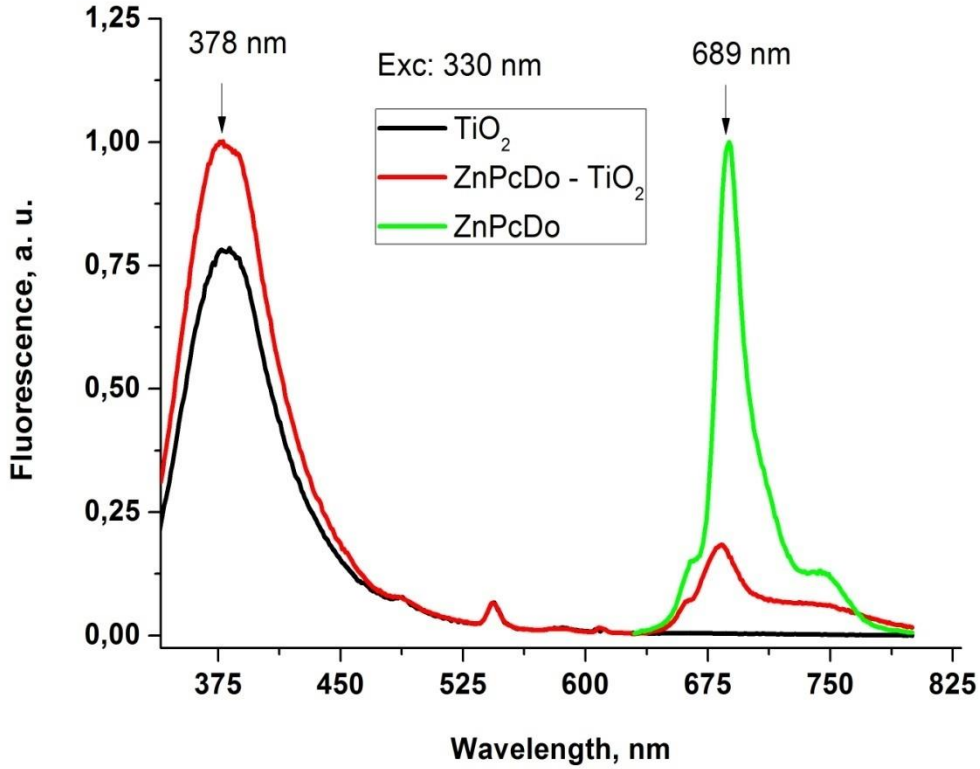
Absorption spectrum characterization

The total spectrum ZnPcDo-TiO_2 consist of two sub-spectra The visible absorption band consists of two maxima at 619 nm and 683 nm. Both maxima are batho-chromicaly shifted about 9-11 nm as compared to the ZnPcDo max. The spectra of the powder anatase TiO_2 covers the whole UV region. The resulted broad spectrum of the solid ZnPcDo adsorbed on TiO2 seems to be very useful for application for wastewater disinfection by solar light irradiation.



Absorption spectra of the solids ZnPcDo adsorbed TiO_2 and TiO_2, and for solution of ZnPcDo in DMSO

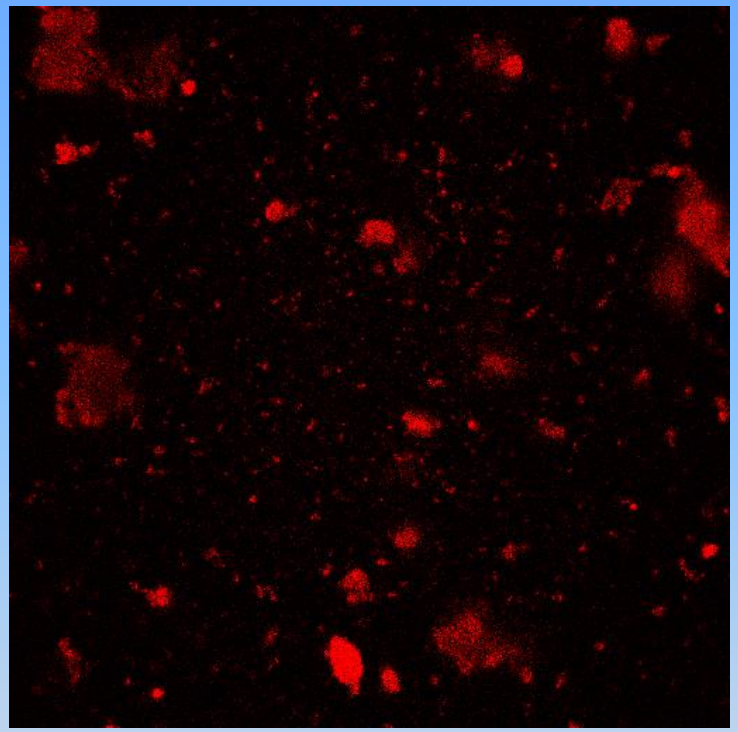
Fluorescence spectrum characterization



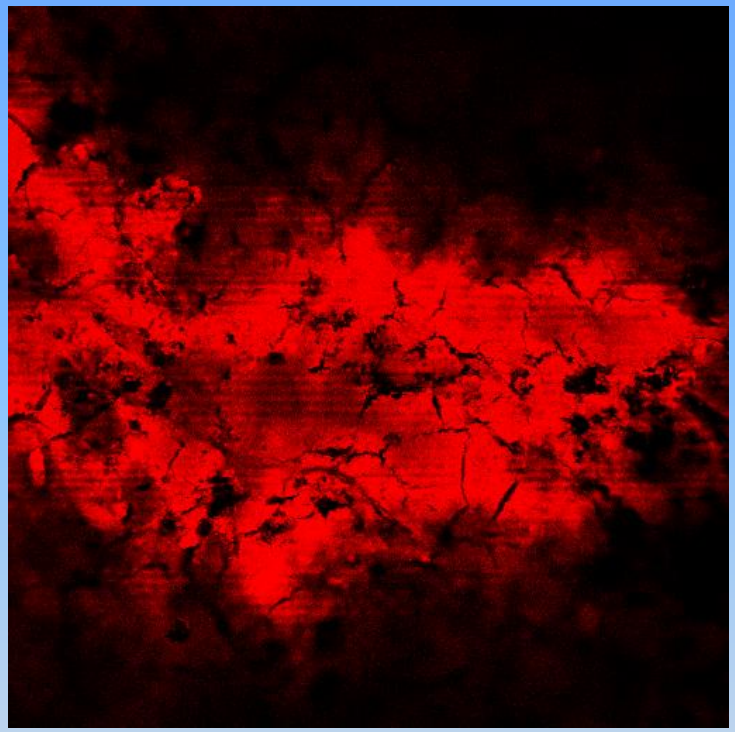
Fluorescence spectra of the solids ZnPcDo – TiO_2 and TiO_2 in suspensions and ZnPcDo in DMSO

Fluorescence spectrum of ZnPcDo in DMSO showed the typical fluorescence band, which is slightly shifted to the far red region (679 nm) vs. absorption (674 nm). The conjugate of phthalocyanine and TiO_2 has fluorescence spectra of both components with lower intensity of the maximum in far red region. The intensive peak appears in the UV spectra around 375 nm. The photoinactivation action can be combined with photodiagnosis due to intensive UVA emission maximum.

Confocal laser scanning microscopy



(a)

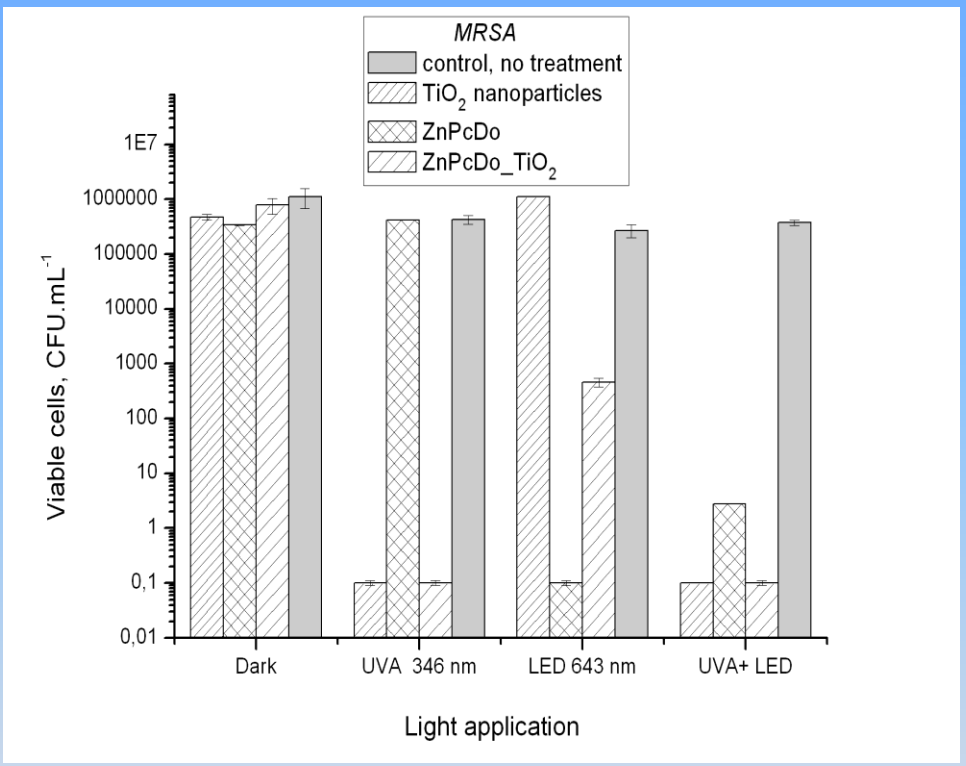


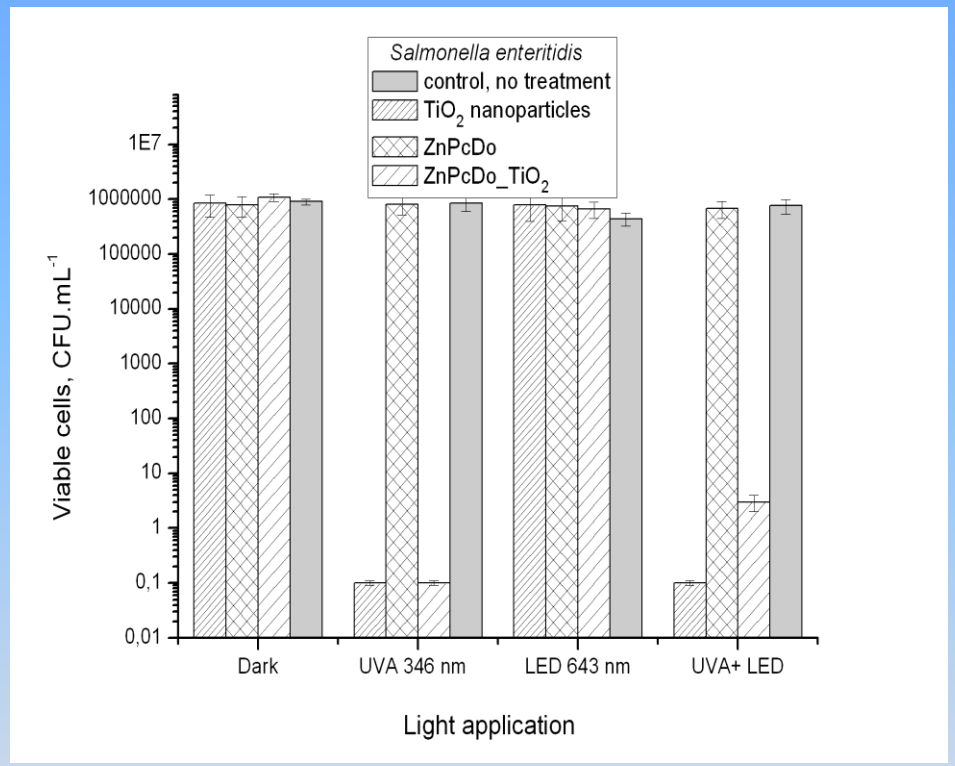
(b)

Confocal fluorescence images of ZnPcDo – TiO2 as water suspension (a) and as a dry layer on a glass (b) at exc: 635 nm (em: 650-740 nm) for visualization of the red fluorescence due to ZnPcDo. Magnifications: x 40

The red fluorescence suggested that the immobilization of the phthalocyanine is for the monomeric molecules, that is good for the photo-inactivation potential of the solid hybrid material.

Photodynamic inactivation effectiveness

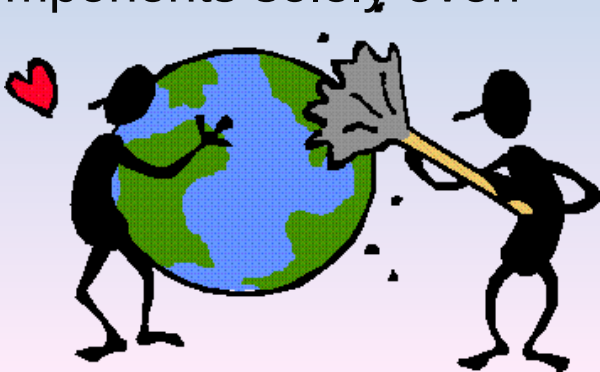




Photoinactivation with ZnPcDo adsorbed on titanium dioxide at irradiation with UVA 346 nm, LED 643 nm and UVA 346 nm + LED 643 nm of *MRSA* (a) and *S. enteritidis* (b). The constituents are added for comparison.

Discussion – PDI of waste water bacteria

- The conjugate of both photoactive compounds ZnPcDo-TiO_2 showed **2 logs inactivation** after irradiation with two light sources (UVA and red LED);
- **Gram-positive** MRSA incubated with ZnPcDo irradiated only with red light was completely inactivated;
- **Gram-negative** strain *S. enteritidis* was highly effective inactivated **only for the conjugate ZnPcDo-TiO_2** after combined UVA and red light;
- Lack of any PDI effectiveness was observed for ZnPcDo-TiO_2, ZnPcDo and TiO_2 at 643 nm irradiation only for Gram-negative strain. The simultaneous irradiation with both light sources was effective - added result of **photocatalytic action of titanium dioxide** is important factor;
- The high inactivation efficiency of the composite prepared on the basis of dodecylpyridyloxy-substituted ZnPc adsorbed on the surface of TiO_2 anatase at irradiation is mainly due to TiO_2;
- The effect is not cumulative from the effect of two components solely even by excitation with two specific light sources.



Antibiotic–resistant micro-organisms treatment

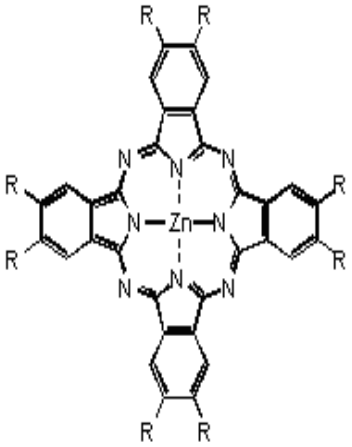
Microorganisms treated

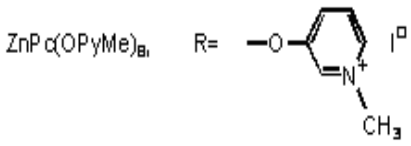
- Gram-positive Staphylococcus aureus – **methycilin sensitive**
- Gram-positive Staphylococcus aureus – **methycilin-resistant**
- Gram-negative Pseudomonas aeruginosa
- Candida albicans - fungi
- Multi-drug resistant *Aeromanas hydrophilla*

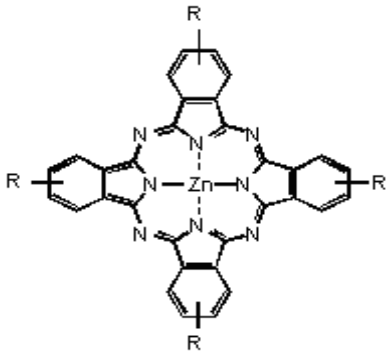
Photosensitizers applied

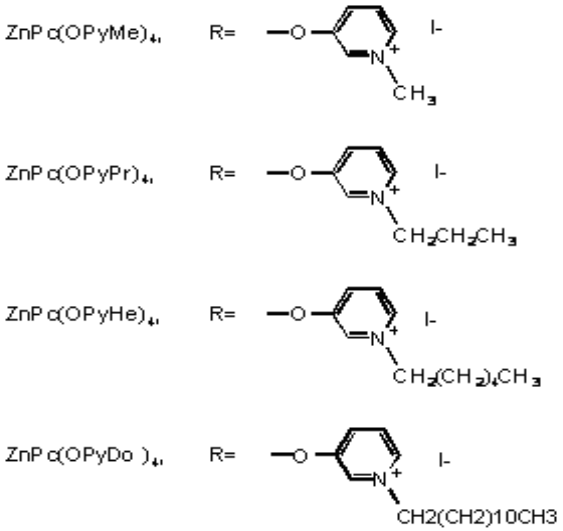
Electron-spectroscopy data for studied phthalocyanines in DMSO

Phthalo-cyanines	Q-band abs. max, nm (ε in 10^5 $M^{-1}cm^{-1}$)	Fluorescence, nm (quantum yield)
ZnPcMe	671 (1.5)	688 (0.31)
ZnPcS	673 (1.9)	690 (0.34)
ZnPcHe	675 (0.53)	688 (0.3)
AlClPc	678 (2.93)	692 (0.4)
ZnPc	672 (2.38)	679 (0.2)



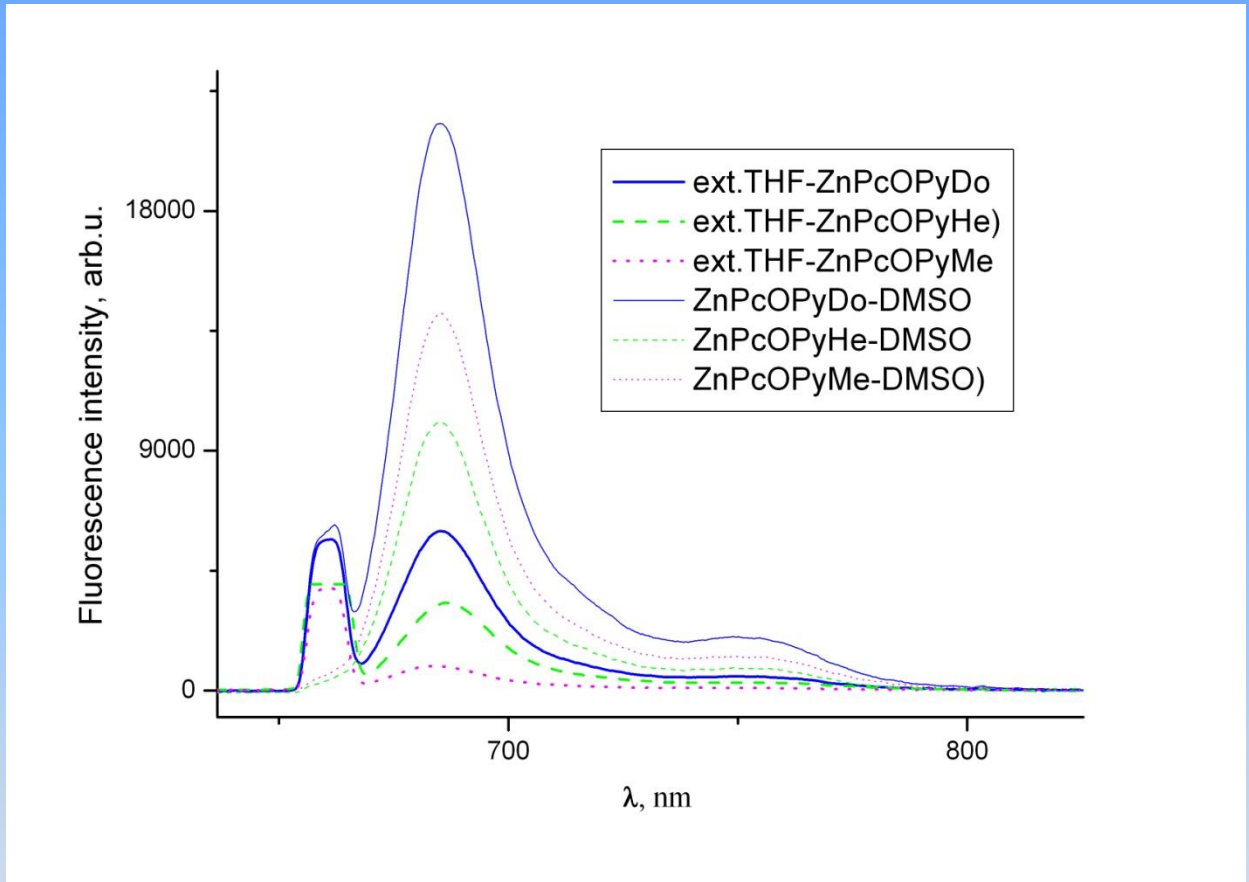






Molecular structure of tetra- and octa- cationic substituted zinc(II) phthalocyanines

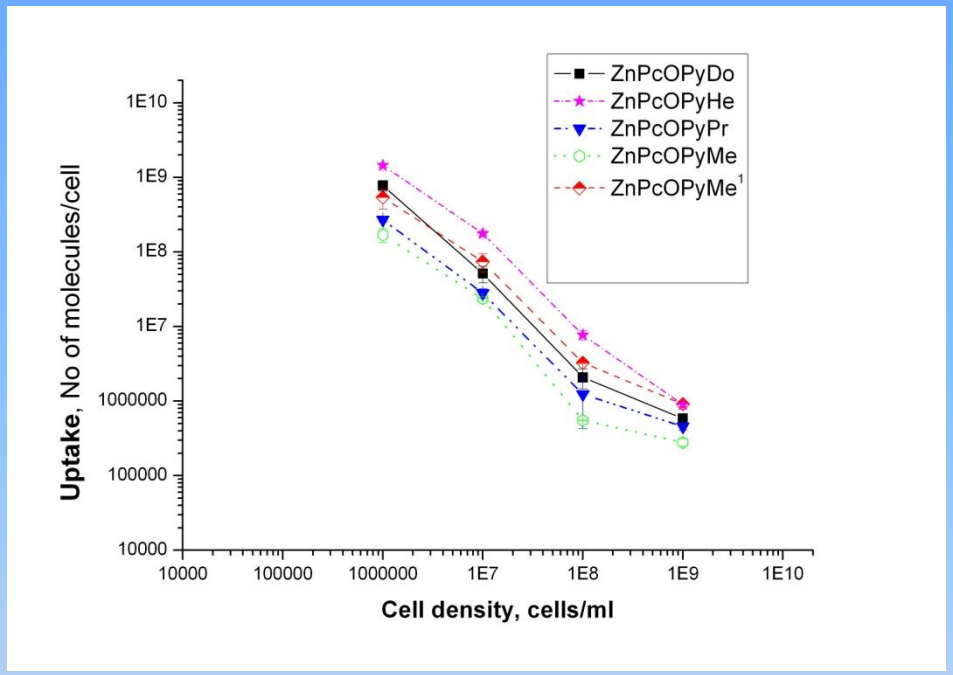
Fluorescence measurements

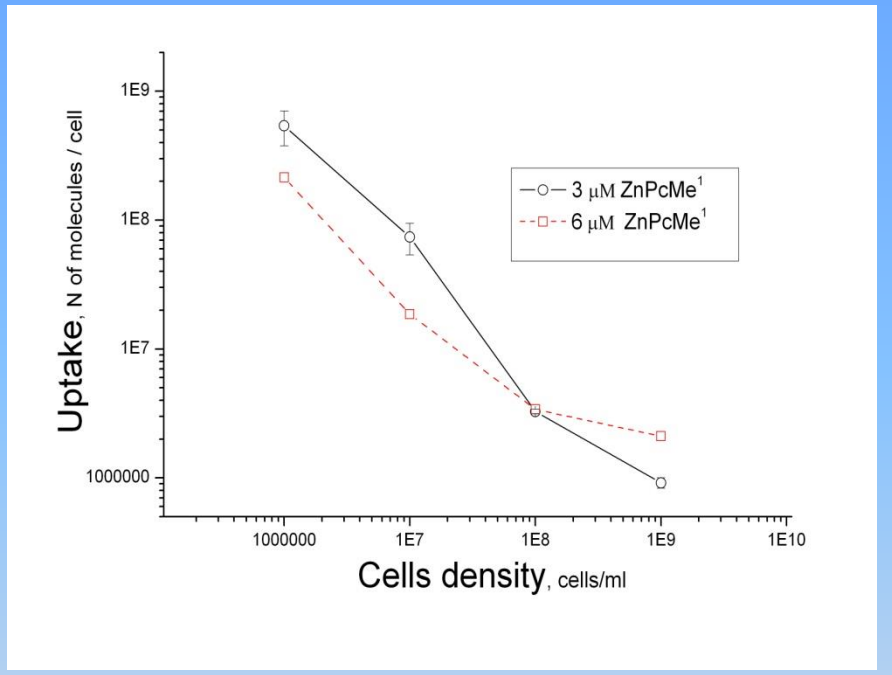


Fluorescence spectra obtained in DMSO and after bacterial chemical extraction with THF

Phthalocyanine uptake: The amount of photosensitizer bound to the microbial cells for the bacteria densities between 10^6 and 10^9 cells/mL is determined by using the fluorescence spectroscopy setup. The fluorescence signal, is obtained from extraction mixture from the ZnPc incubated cells.

Drug uptake





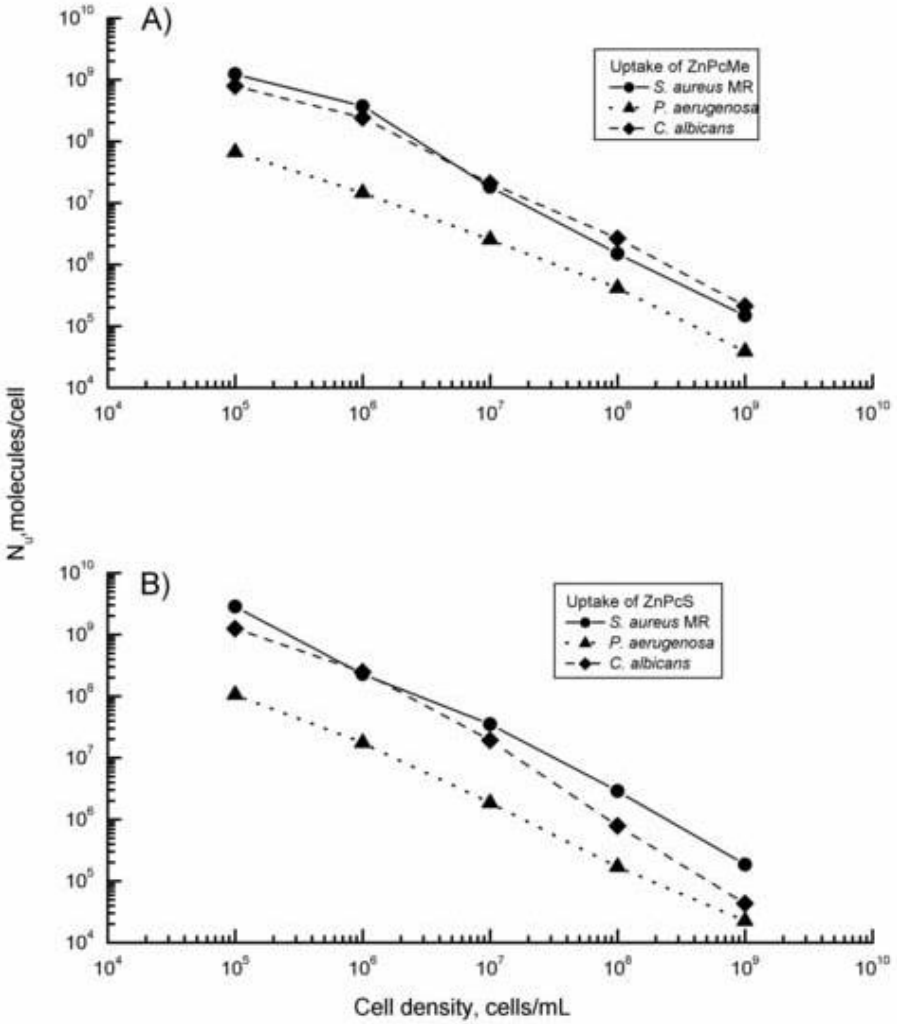
Drug-uptake dependence on cell density (left graph) and at two drug concentrations of octa-substituted ZnPcMe (right graph)

ThePcs uptake is calculated quantitatively at different densities of *bacterial* cells and at different drug concentrations.

- cell bounded compounds decrease with increasing of the cell density

- uptake increases from hydrophilic to hydrophobic complexes and it is higher for water soluble octa- substituted ZnPcMe

Pcs-cell bounding



The number of phthalocyanine molecules bound to the microorganism's cells with different cell densities and incubated for 10 min with 1.5 lM ZnPcs:
(a) ZnPcMe and (b) ZnPcS.

-The uptake by methicillin-resistant S. aureus was two orders higher compared to gram-negative P. aeruginosa and one order higher compared to the fungi C. albicans

- There was not observed charge dependency on the uptake of the studied compounds.

Photodynamic inactivation-S. aureus

Bacterial suspension with cell densities of 10^6 till 10^9 cells/mL

↓

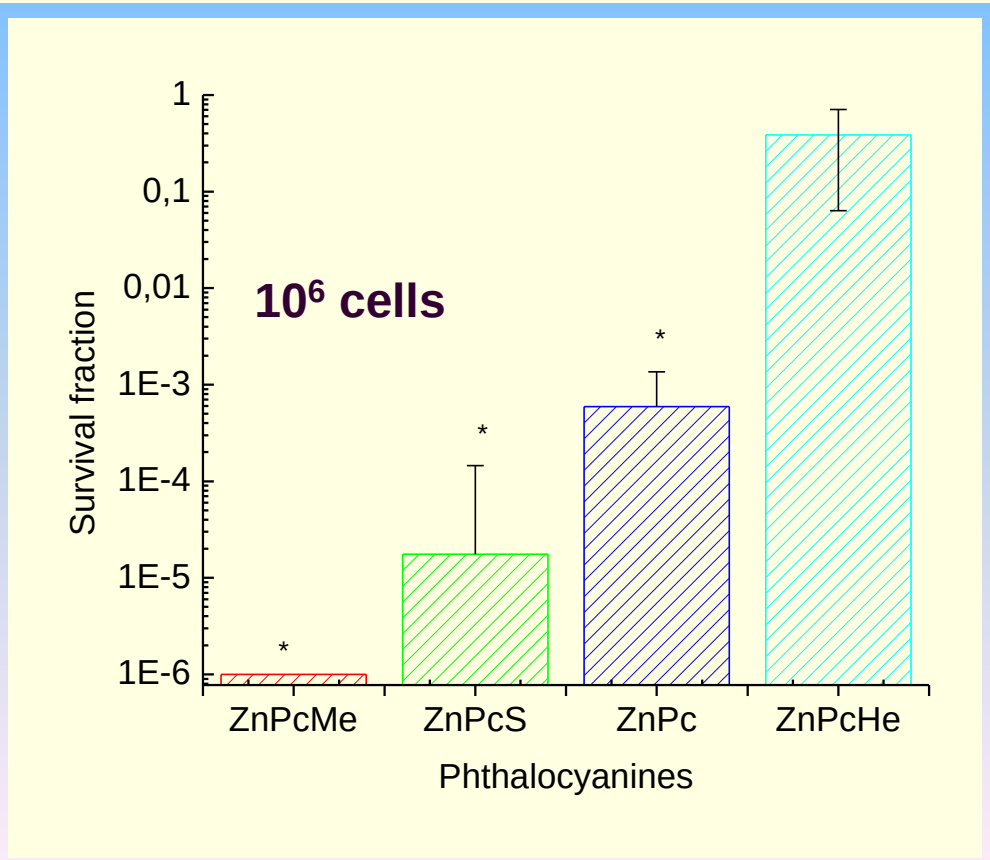
Incubation with 10^{-6} mol phthalocyanines for 10 min

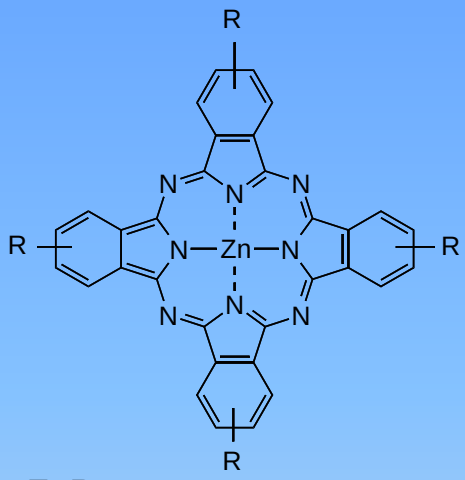
↓

Irradiation for 2 till 10 min with light 675 nm

↓

Determination of survival fractions on agar plates





ZnPc, R= —H

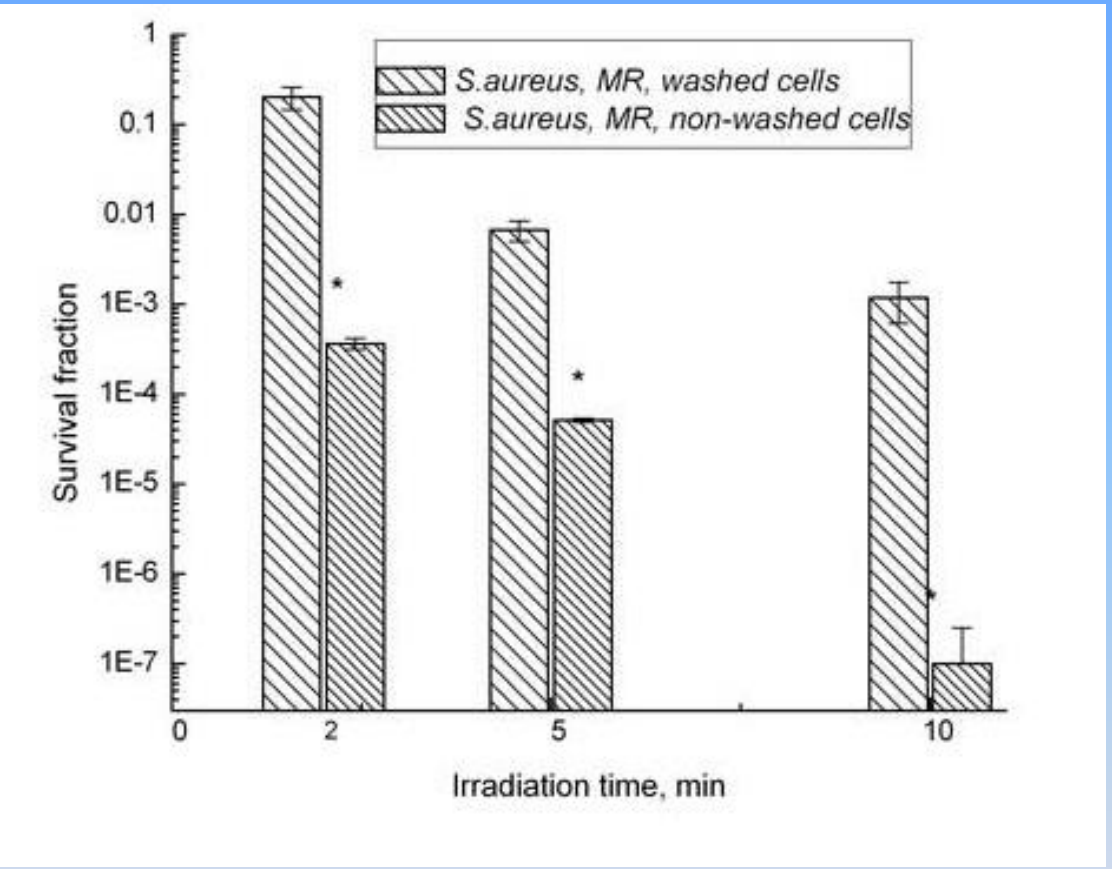
ZnPcMe, R= —O— N^+ I^- CH_3

ZnPcHe, R= —O— N^+ I^- $CH_2(CH_2)_4CH_3$

ZnPcS, R= —O— SO_3H

Photoinactivation of bacteria *Staphylococcus aureus*, after irradiation with 100 mW/cm² and 60 J/cm²

Bound-unbound Pcs

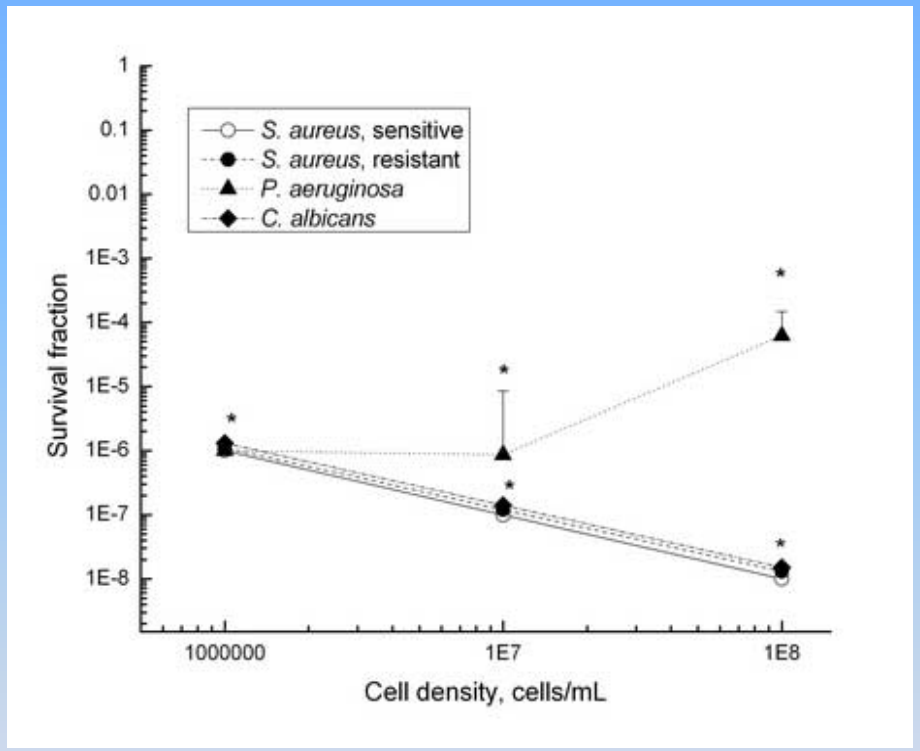


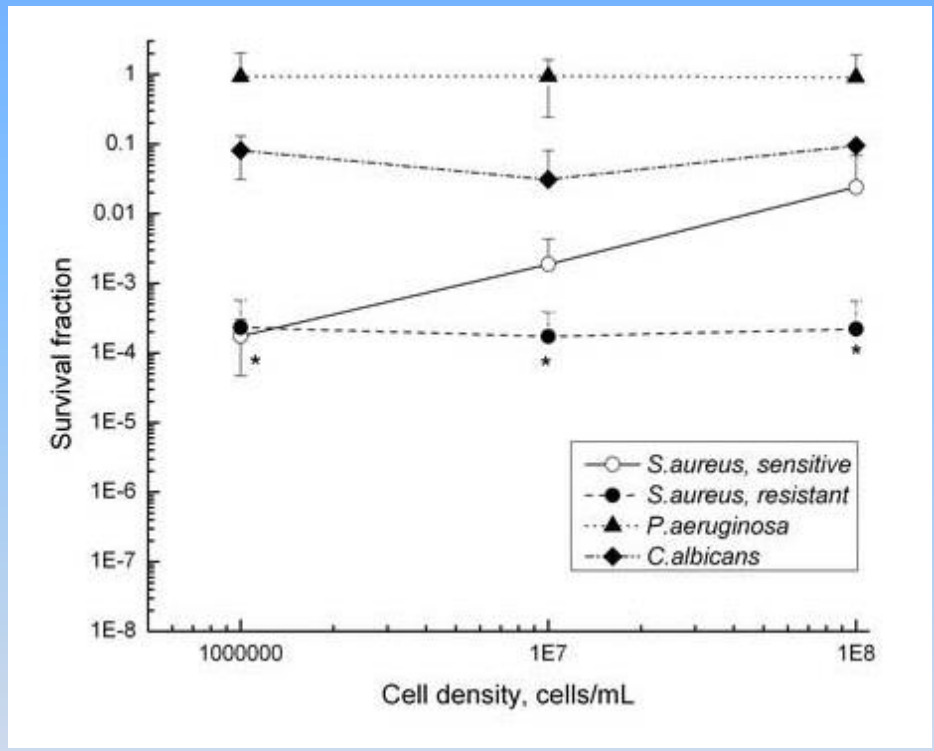
The photoinactivation with ZnPcMe was evaluated for different irradiation times after bacterial suspensions were washed out to remove the unbound photosensitizer. Result suggests that the molecules non-bound to the cells also result on the bacterial photoinactivation after irradiation.

V. Mantareva et al. / Bioorg. Med. Chem. 15 (2007) 4829–4835

Photosensitizer-cell binding dependency on the photoinactivation of S. aureus, methicillin-resistant (10^7 cell/mL) after 10 min incubation with 1.5 lM ZnPcMe and irradiation with a fluence rate of 50 mW cm^2 for different times, respectively, the light doses (*P < 0.05, compared with untreated bacteria).

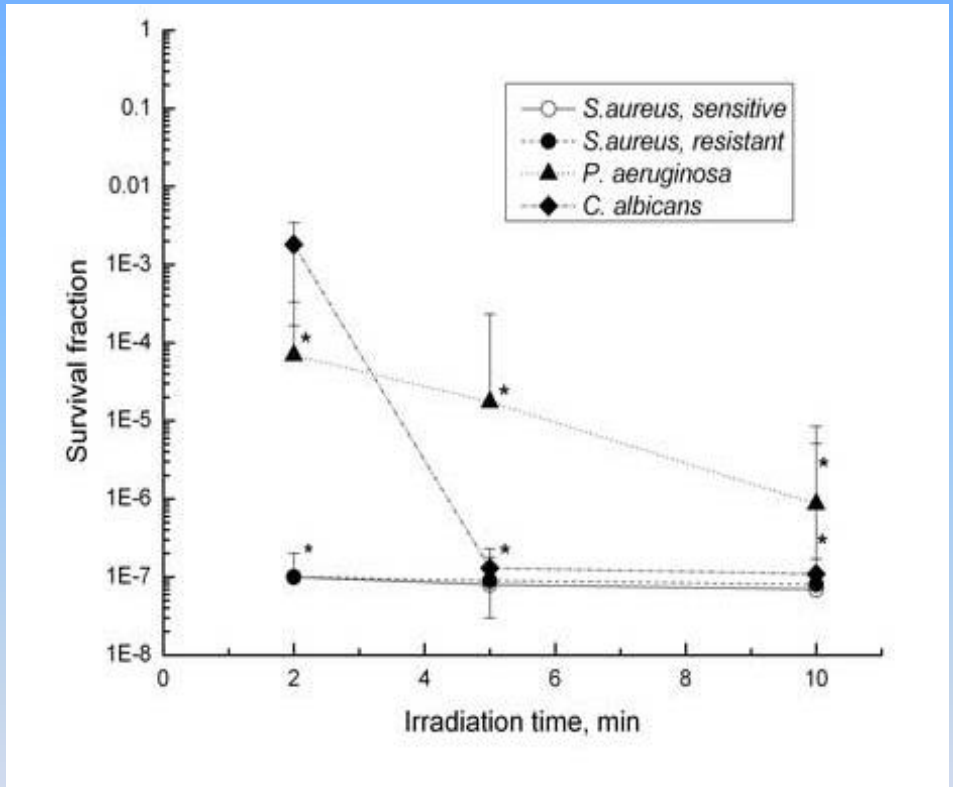
Survival of bacterial strains-cell density

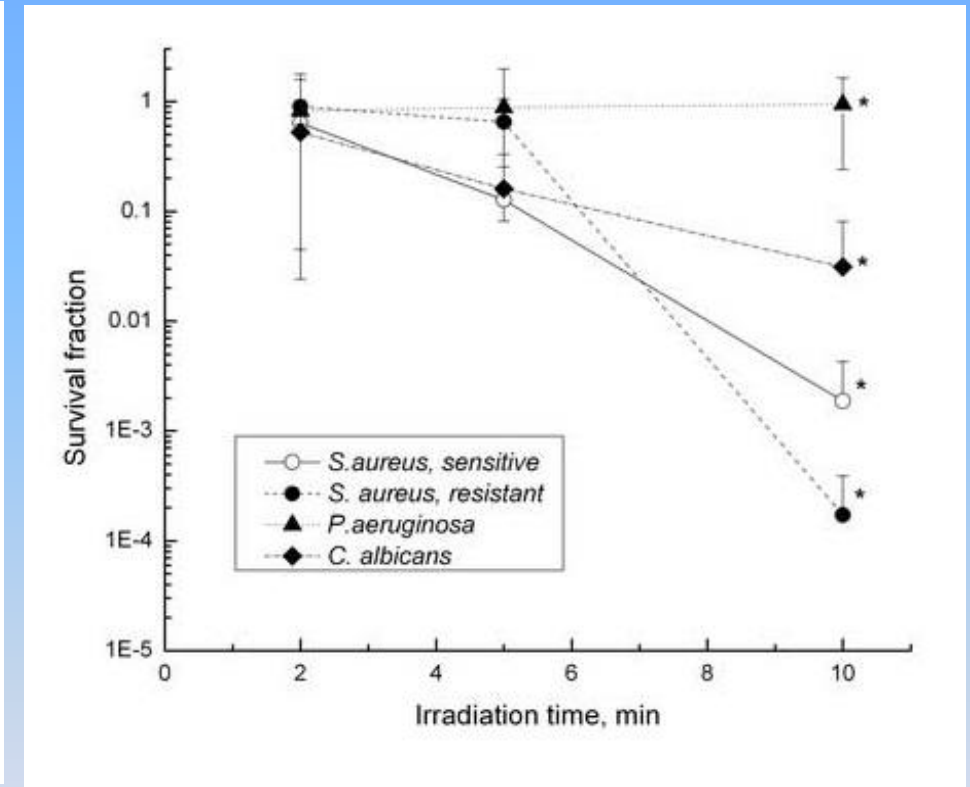




The photoinactivation of microorganisms with different cell densities irradiated with a fluence rate of 100 mW /cm^2 for 10 min after 10-min incubation with 6 lM ZnPcs: (a) ZnPcMe and (b) ZnPcS (*P < 0.05, compared with untreated bacteria).

Survival of bacterial strains – irradiation time

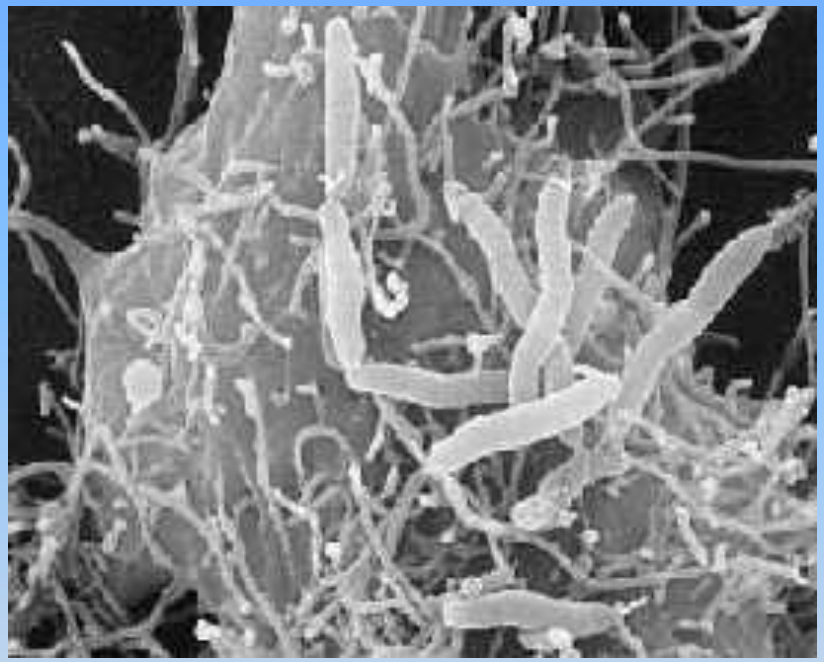




Effect of the irradiation time at fluence rate of 100 mW /cm^{-2} on the survival of the microorganisms (10^7 cell/mL) after 10 min incubation with 6 μM ZnPcs: A) ZnPcMe and B) ZnPcS

Aeromonas hydrophila

- Gram-negative, anaerobic, non-spore-forming, rod-shaped bacteria;
- Can cause diseases in fish, reptiles, amphibians, birds, mammals and humans;
- Aeromonas hydrophila is the cause of zoonotic diseases (i.e. diseases that can be spread from animals to humans and vice versa);
- Common clinical presentations are acute gastrointestinal illness, soft tissue infections, sepsis and traumatic and aquatic wound infections;
- Causes a disease in fish known as hemorrhagic septicemia, or ulcer disease, and is one of the most common bacteria present in aquatic environments throughout the world.



Scanning electron micrograph of *Aeromonas hydrophila* attached to a human intestinal epithelial cell line

Photodynamic inactivation-A.hydrophila

Bacterial suspension with cell densities of 10^6 cells/mL

↓

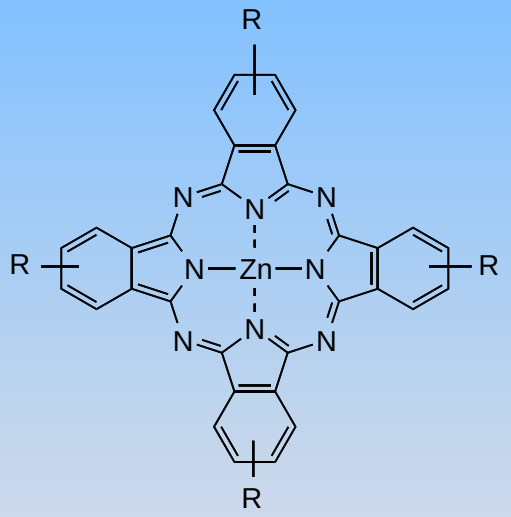
Incubation with ~10^{-6} mol phthalocyanines for 10 min

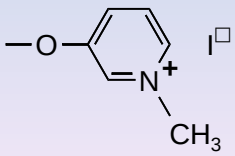
↓

Irradiation for 2 till 10 min with light 635 nm (100 mW/cm^2, 30 J/cm^2

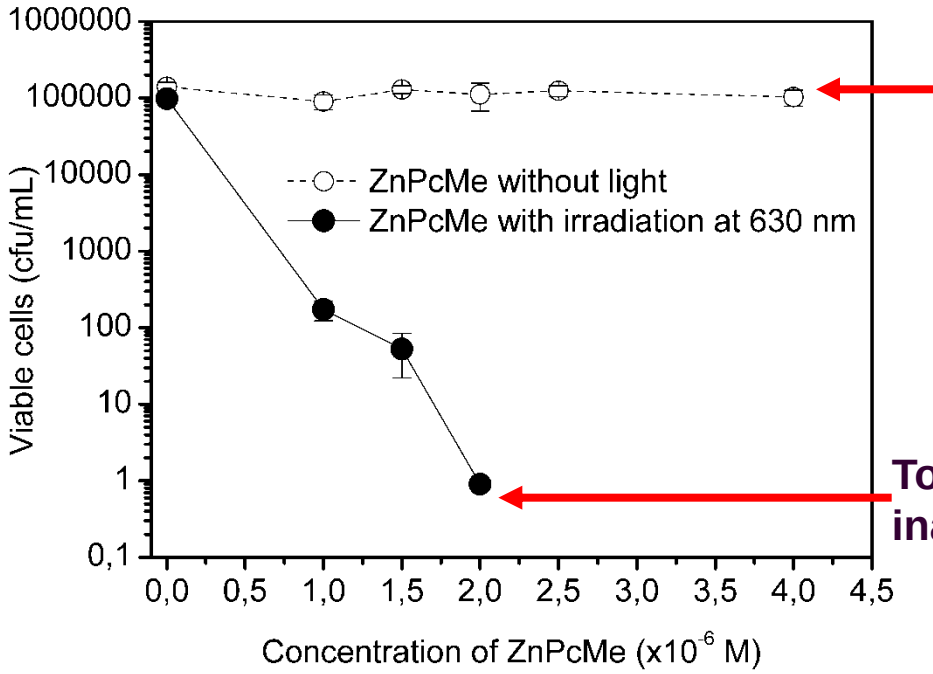
↓

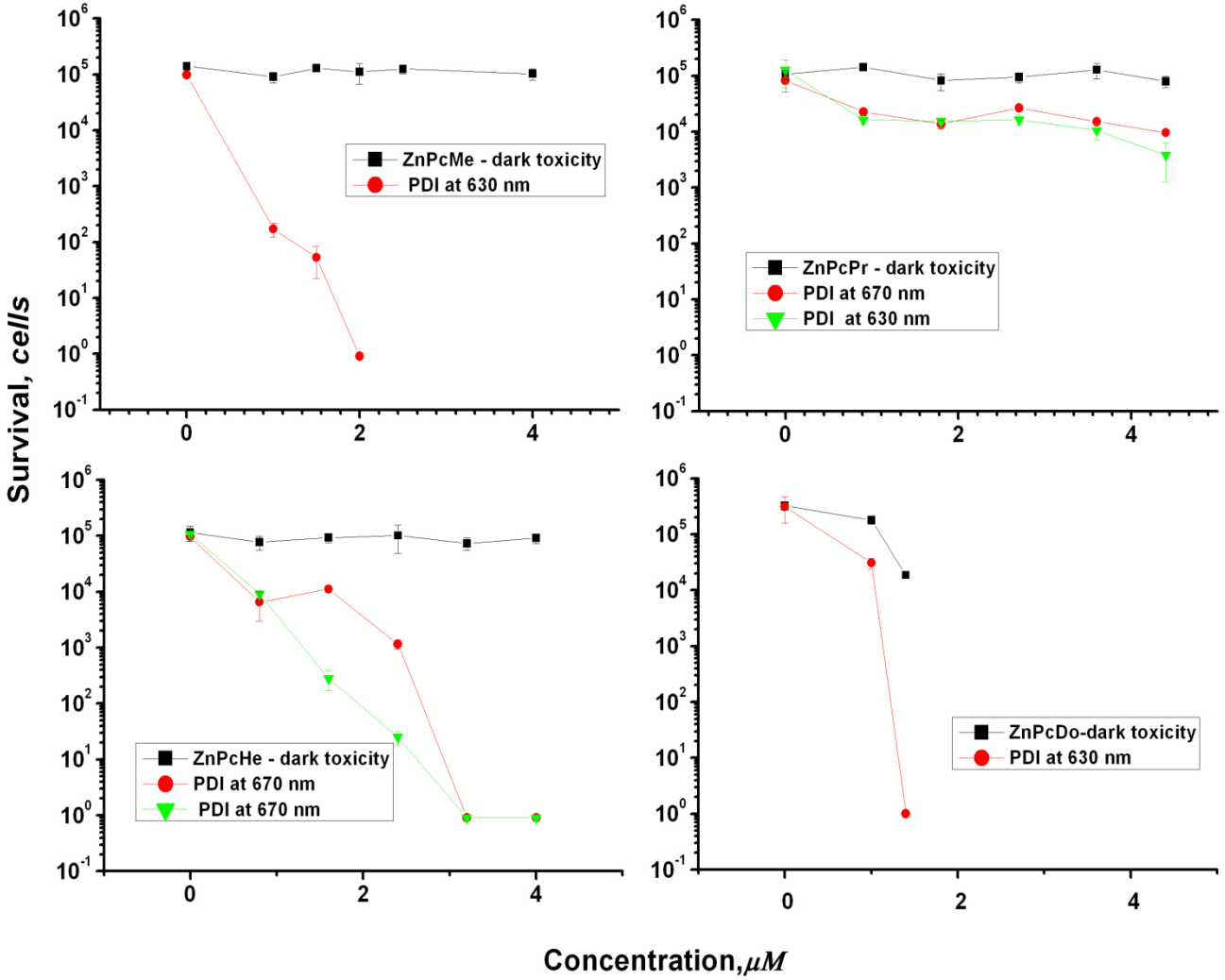
Determination of survival fractions on agar plates



ZnPcMe, R=  $I^{\square}$

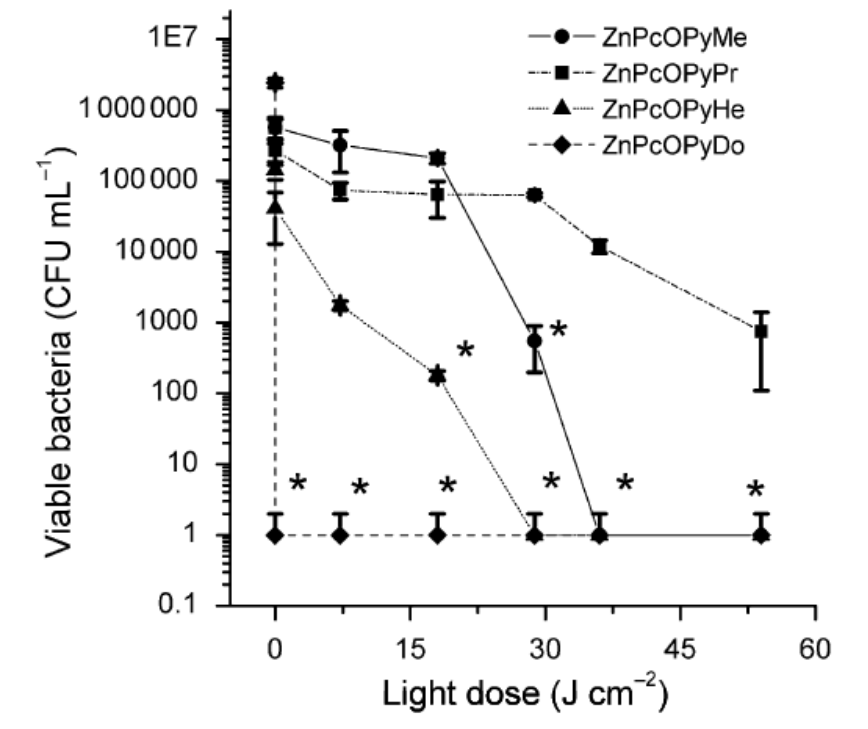
Kussovski et al, EMS Microbiol Lett 294 (2009) 133–140





Photoinactivation of Aeromonas hydrophila (10^6 cells mL^{-1}) with irradiation of 60mW/cm^2 at light dose 50 J /cm^2 for different concentrations of the respective ZnPcs: (a) ZnPcOPyMe; (b) ZnPcOPyPr; (c) ZnPcOPyHe; and (d) ZnPcOPyDo.

General overview



Photoinactivation of A. hydrophila (10^6 cell mL^{-1}) with 3.0 mM ZnPcs and irradiation with a fluence rate of 100mW/cm^2 at different light doses

- Cationic photosensitizers are more PDI active than corresponding anionic or nonionic compounds;

- Following the phthalocyanine solubility from hydrophilic to hydrophobic complexes, the accumulation capacity increases;

-Nature of chemical compounds and their hydrophilic/lipophilic nature strongly affects the dye binding to the cells and the cytotoxic efficiency;

- Uptake of phthalocyanines by the Aeromonads showed an inverse dependence on the cell density;

-The most promising photodynamic properties of (methyl)-pyridyloxy-substituted phthalocyanine suggests further study for its potential use in antimicrobial therapy of animals and humans.

Thank you very much for your attention!