Photodynamic therapy

(PDT)

Basic principles

- What is PDT?
- History of PDT
- Photosensitizers of 1st generation
- Photosensitizers of 2nd generation

Principles of tumor treatment

Traditional

Surgery – surgical removal of tumors
documented already in 3000 b.c.

Radiotherapy – cell destruction by ionizing radiation

Chemotherapy – cytotoxic drugs

Principles of tumor treatment

New developments

Monoclonal antibodies

Antigene or antisense therapy

Gene therapy

Stem cells

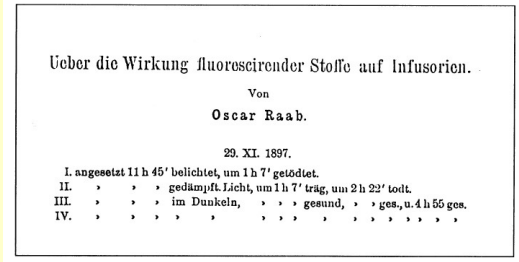
Boron neutron capture therapy

Photodynamic therapy

History of photodynamic tumor therapy

1900 – O. Raab: inactivation of acridin labeled paramecium by light - *Photosensitization*

1906 – H. von Tappeiner recognizes the crucial role of oxygen in the process – *Photodynamic effect*



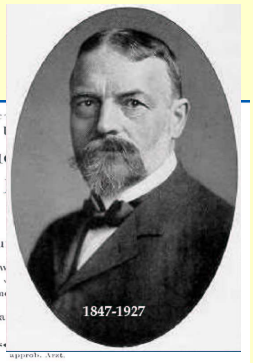
Ueber die Wirkung fluorescirender Stoffe auf Infusorien.

Von

Oscar Raab.

29. XI. 1897.

I. angesetzt 11 h 45′ belichtet, um 1 h 7′ getödtet.
II. › › › gedämpft. Licht, um 1 h 7′ träg, um 2 h 22′ todt.
III. › › › im Dunkeln, › › › gesund, › › ges., u. 4 h 55 ges.
IV. › › › › › › › › › › › › › › ›



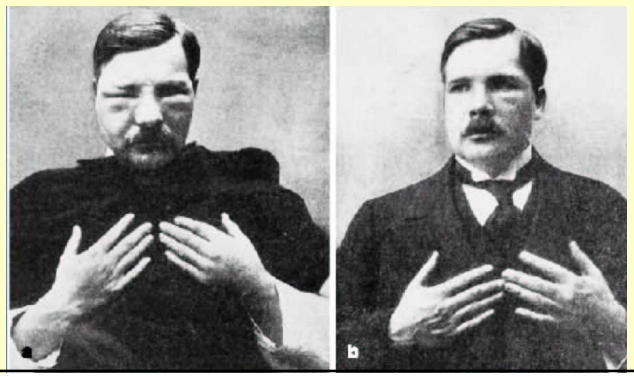
1847-1927

History of PDT

1908 – *Hausman:* Photo-toxicity of chlorophyll

1911 – *Hausman:* Photo-toxicity of porphyrins in mice

1913 – *Meyer-Betz:* injected 3 mg/kg porphyrin



History of PDT

1924 – *A. Policard:* Porphyrin enriched tissue exhibits red fluorescence upon illumination with UV radiation

1925 - *H. Fischer:* porphyrins in the structure of human pigments (Nobel-price 1930)

Nobel-price 1930

„Fischer's scientific work was mostly concerned with the investigation of the constitutive properties of the pigments in blood, bile, and also leaves, as well as with the chemistry of pyrrole. The main reason for the latter investigation was the synthesis of these natural pyrrole pigments. Of special importance was his synthesis of bilirubin"

History of PDT

1974 – T. Dougherty: first systematic clinical studies

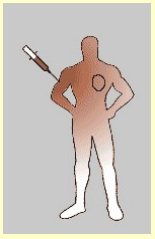
T. Dougherty: Activated dyes as antitumor agents. J. Natl. Cancer. Inst. 1974

What is PDT

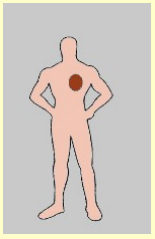
Combined application of a photosensitizer and light

in the presence of oxygen

Steps of PDT according Dougherty et al.

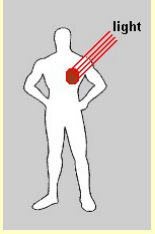


Systemic administration of photosensitizer (PS)

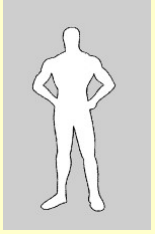


Selective accumulation of PS in tumor

Directed irradiation by light

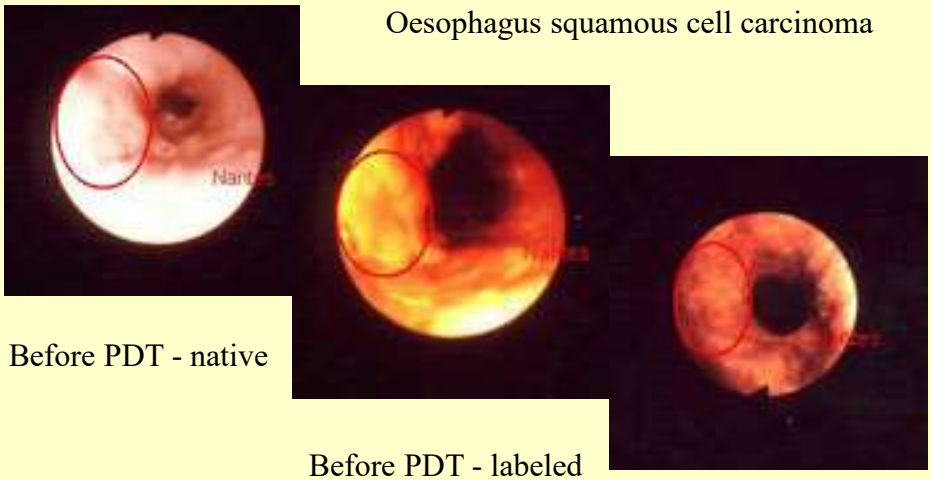


Selective tumor destruction



An example of HpD-PDT

Oesophagus squamous cell carcinoma



Before PDT - native

Before PDT - labeled

After HpD-PDT

Photochemistry of PDT

Indirect photochemical reactions

Electron transfer

$$D \xrightarrow{hf} D^*$$

$$D^* + A \longrightarrow D^+ + A^-$$

Type I reaction
Generation of reactive radicals

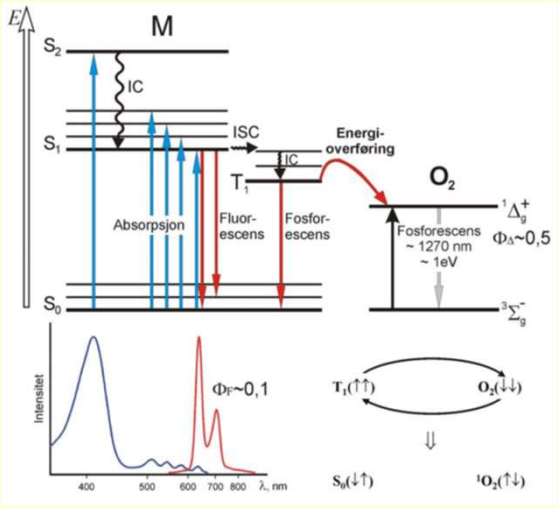
Energy transfer

$$D \xrightarrow{hf} D^*$$

$$D^* + A \longrightarrow D + A^*$$

Type II reaction
Generation of singlet oxygen

Photophysics of PDT



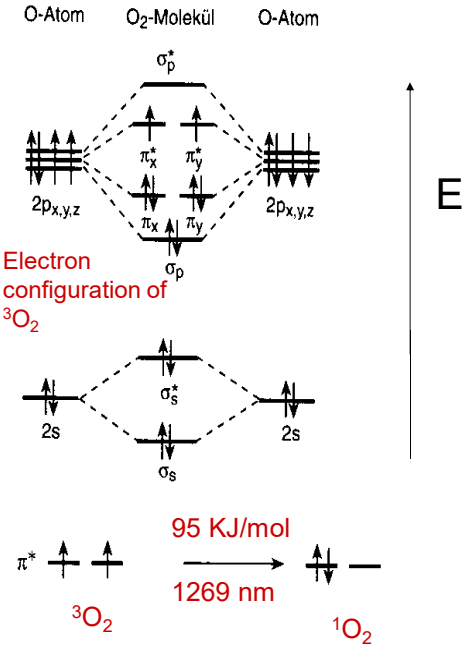
$^1O_2$ diffusion pathlength < 0.1 µm

Relevant sizes:

Human cell ~ 10 µm,
Nucleus ~ 2 µm

What is $^1O_2$?

- O_2 is paramagnetic (in triplet state) in the ground state (according to Hund rule)
- Because of spin restriction triplet oxygen $^3O_2$ participates only in non-selective radical reactions
- Singlet oxygen $^1O_2$ is **very reactive** and selective



Quantum yield of singlet oxygen generation

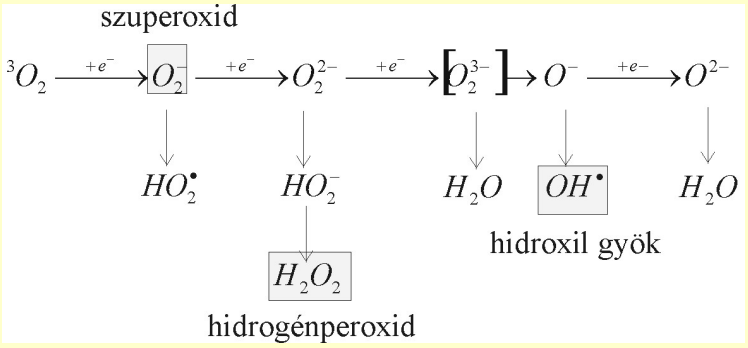
Rh	<0,03
Rb	0,8
Metilénkék	0,5
PpIX	0,6
HpD	0,3
Chlorin e6	0,8
mTHPC	0,4
AlPcS	0,2-0,4
BpD	0,7

Targets of $^1O_2$

DNA, unsaturated fatty acids, carotenoids, amino acids: methionine, tryptophan, cysteine, histidine.

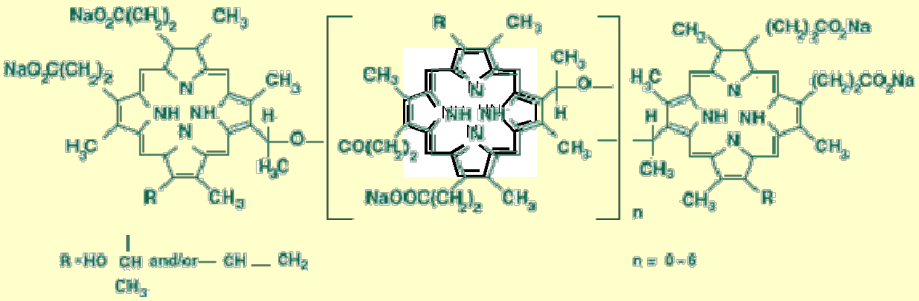
Electron transfer → ROS

Reactive oxigen species



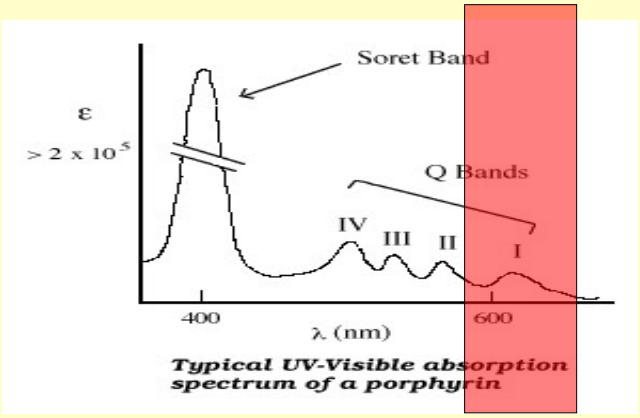
Photosensitizers of the first generation

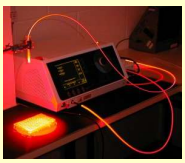
Chemical structure: Derivatives and oligomers of
Hematoporphyrin (Hp)
Hematoporphyrin derivatives (HpD)



Precise composition is unpredictable

Photofrin®.

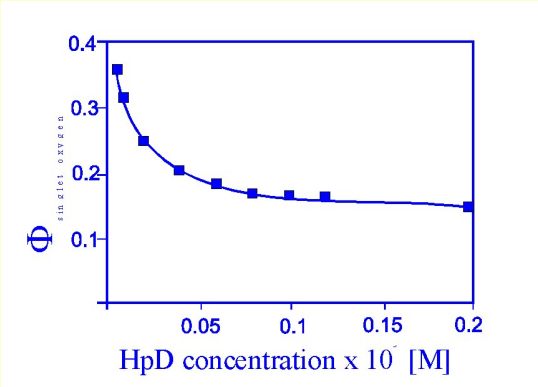




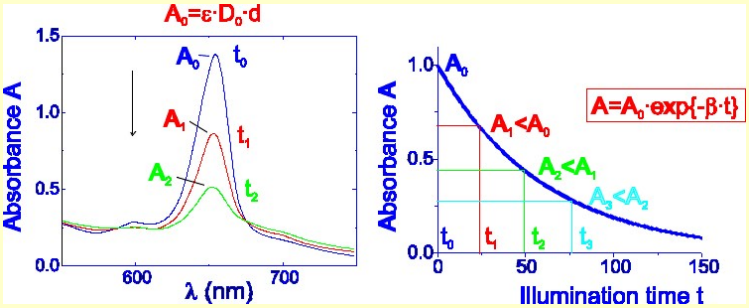
Irradiation: high power monochromatic red light

- Absorption at 405, 505, 525, 565 and 630 nm
- Emission around 635 and 700 nm
- Accumulation in tumors (?)

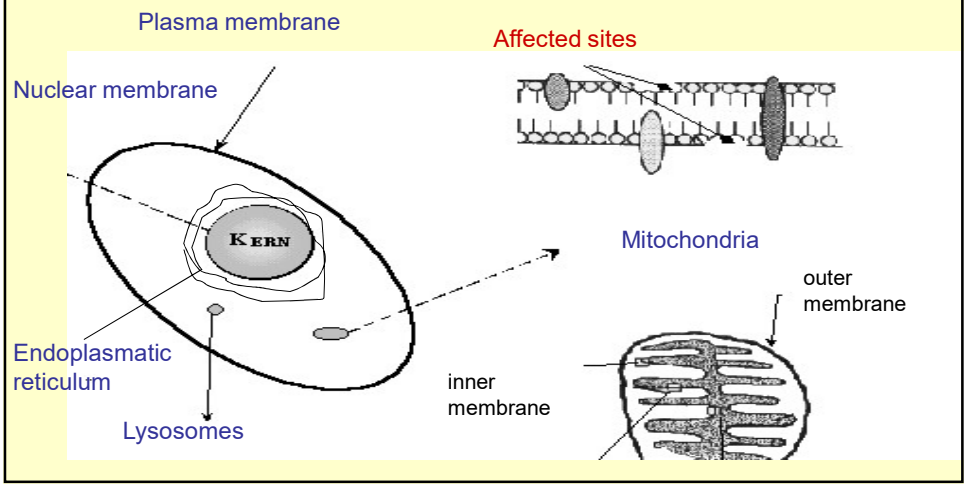
Aggregation – reduced efficiency,
increased photodegradation
unpredictable chemical dose



Photobleaching



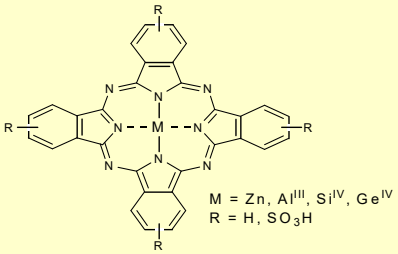
Intracellular localization: HpD accumulates preferentially in membranes



Pros and cons of the 1st generation of photosensitizers

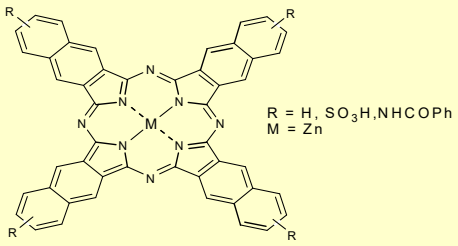
- Photophysics: high ϕ_{isc} and ϕ ($^1O_2$), but relatively short wavelength absorption with a low absorption coefficient
- *in vivo* activity: low dark activity, high photodynamic activity, but relatively low selectivity of absorption in tumors; aggregation

Photosensitizers of the second generation



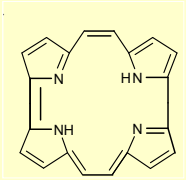
Phthalocyanin (Pc)

maximal λ_{ex} = 740 nm



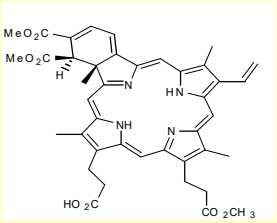
Naphthalocyanin (Nc)

maximal λ_{ex} = 820 nm



Porphycen

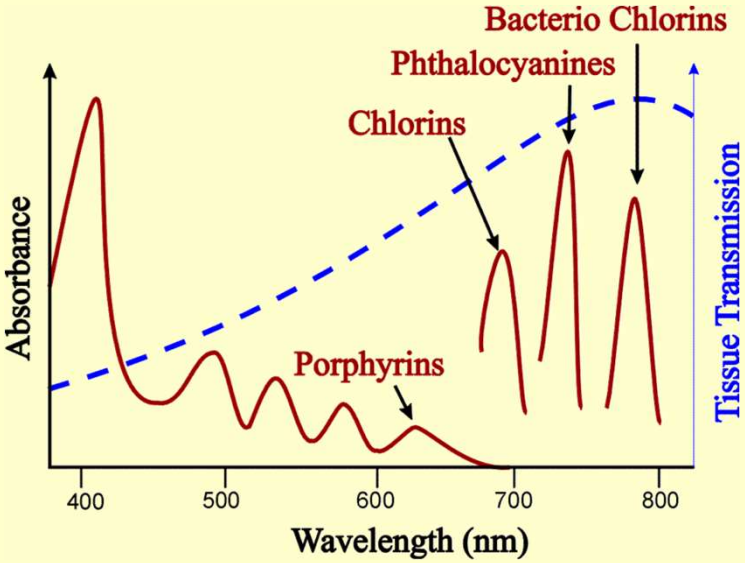
maximal λ_{ex} = 710 nm



Chlorin e_6

maximal λ_{ex} = 750 nm

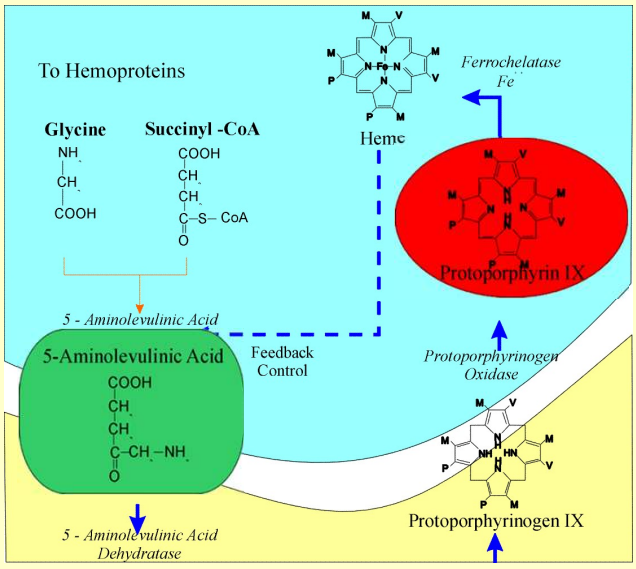
Absorption spectra



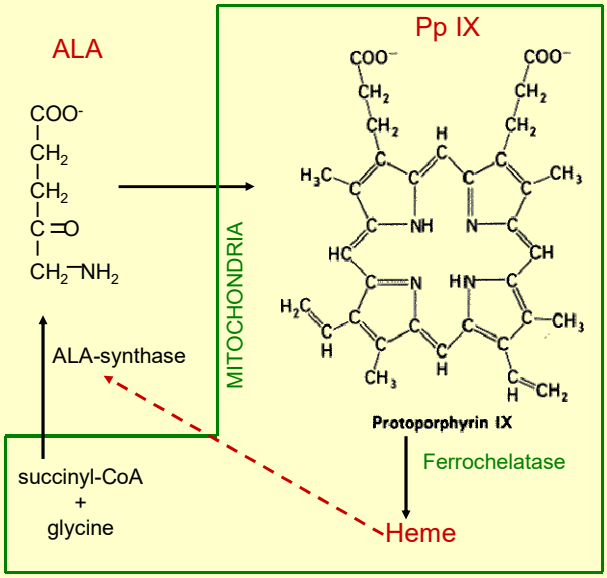
Pros of the 2nd generation of photosensitizers

- Chemical stability
- Long wavelength (600-900 nm) absorption with large extinction coefficient
- Good accumulation in tumor
- Possibility of targeting
- Smaller side effects (short retention in skin)

ALA- induced PDT



Endogenous PS: Cells produce their own PS



- Photosesitizer protoporphyrin IX (Pp IX) is an intermediate of heme synthesis
- The physiological concentration of Pp IX is low because of a controlled expression of its precursor 5-aminolevulinic acid (ALA)
- The expression of ALA is feedback controlled via heme concentration

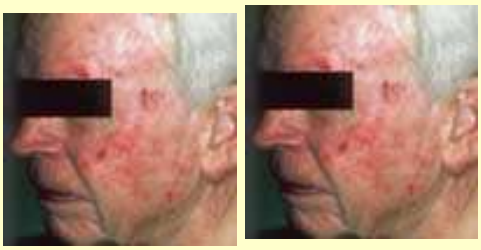
Pros and cons of ALA-PDT

- Local application
- No photosensitization in skin
- Repeated treatments
- in situ fluorescence control

- Good tumor selectivity
- PpIX rapid degradation
- Excitation at 630 nm
- Uptake of ALA is opposed by skin

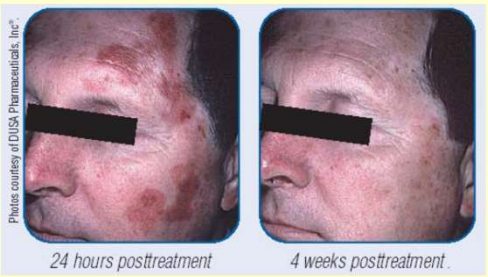
2001
Metvix® (ALA-derivative) topically

www.photocure.com



2000
Levulan® topically

www.dusapharma.com



Compound	Name	Marketing	λ max	Phase of development
Porfimer sodium	*Photofrin®*	QLT pharmaceuticals, Sanofi	630 nm	***FDA approval,*** France (april 1996) USA (december 1995)
5-aminolevulinic acid (ALA)	*Levulan®*	DUSA pharmaceuticals, Schering AG	630 nm	***FDA approval***: (USA dec 99) multiple actinic keratoses ***Clinical trials:*** I/II: psoriasis, basal cell carcinoma,
Benzoporphyrin	*Visudyne®*	Sanofi,	690 nm	***FDA approval***: (USA nov 99, France octobre 00) mascular degeneration (ARMD)
	Verteporfin®	Cibavision,		***Clinical trials:*** I/II: anti HIV agent, non melanoma skin cancer, II: II: cutaneous oncology III: immunosuppressant
Hydroxyphenyl chlorine (m-THPC)	*Foscan®*	Scotia	652 nm	***Clinical trials:*** II: chest, GI, pancreas cancers III: head, neck, laryngeal cancers, adjunct to surgery and radiotherapy in late stage cancers

Table 1. Fundamental clinical characteristics of the photosensitizers presently in clinical or preclinical trials

Photosensitizer	Wavelength (nm)	Extinction coefficient (M^{-1} cm^{-1})	Mode of delivery	Delivery vehicle	Typical dose (mg kg^{-1})	Light dose (J cm^{-2})	Time post-injection	Duration of skin photosensitivity
Haematoporphyrin-derivative	630	3.0×10^3	IV or topical	5% Dextrose	2.0–5.0	100–200	24–48 h	2–3 months
Methylene blue	668	9.5×10^4	*Ex vivo*	Water-soluble	1 μM	50,000 lux	n/a	n/a
5-Aminolaevulinic acid (protoporphyrin IX)	635	$<5.0\times10^3$	Topical, oral or IV	Water-soluble	<60 (orally) <30 (IV)	100–200	–	1–2 days
Verteporfin	690	3.5×10^4	IV	Liposomal	0.1–2.0	100–200	30–150 min	3–5 days
Tin etiopurpurin	660	2.8×10^4	IV	Lipid emulsion	1.0–2.0	100–200	24 h	Up to 1 month
Temoporfin	652	3.0×10^4	IV	PEG/ ethanol/ water	0.1–0.3	8–12	24–48 h	Up to 6 weeks
Texaphyrins	732	4.2×10^4	IV	Water-soluble	0.6–7.2	150	3–5 h	Minimal
Phthalocyanines	670–680	2.5×10^5	IV	Liposomal or water-soluble	0.5–2.0	100	24–72 h	8–10 days
Naphthalocyanines	750–780	$>10^5$	IV	Liposomal	–	–	–	–
N-aspartyl chlorin e6	664	4.0×10^4	IV	Water-soluble	0.5–3.5	25–100	4 h	3–7 days
Rhodamines	511	2.0×10^4	*Ex vivo*	Water-soluble	25 μM	1–10	n/a	n/a
Porphycenes	630	5.2×10^4	Topical	Liposomal	1.0–3.0	–	n/a	–
Hypericin	590	4.4×10^4	Topical	Liposomal	–	–	–	–

Abbreviations: IV, intravenous; PEG, polyethylene glycol.

Furthe applications of photodynamic reactions

Antimicrobial PDT

Treatment of arterial diseases

Treatment of maculadegeneration

Treatment of psoriasis

Photorejuvenation

Antimikrobial PDT

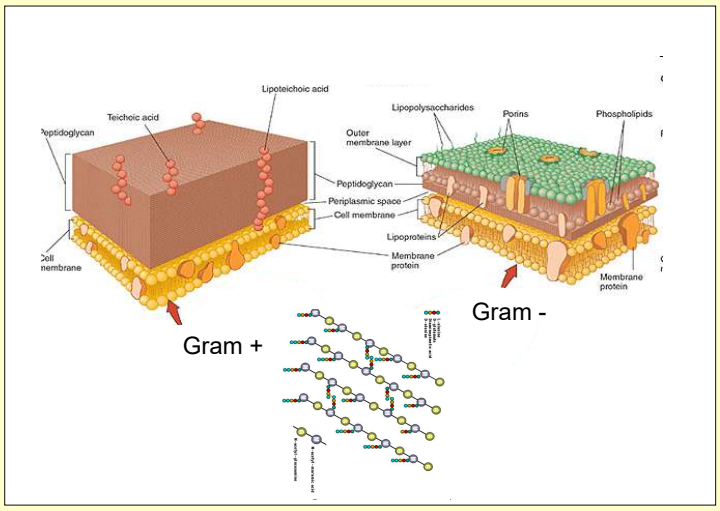
Photoinactivation of

Bakteria

Yeasts

Viruses

Different feasibility in Gram+ and Gram- bacteria



Photodynamic therapy

(PDT)

PS targeting

Ames of targeted PDT

Increased selective accumulation in tumor cells/bacteria

Increased amount of incorporated PS

Modulation of intracellular distribution

Save/increase phototoxicity

Oligopeptide conjugates

1. methods targeting amino groups

   reaction with carboxilic or isothiocyanate groups of PS

2. methods targeting thiol groups

   thioether linkage with maleimide derivatives

3. bioorthogonal ligation strategies

   introduction of proper functional groups to the conjugation partners

Oligopeptide conjugates

Oligopeptide conjugates for PDT

Nuclear localization signaling entities (NLS)

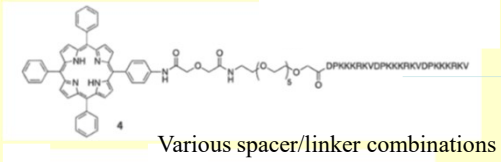
Cell penetrating peptide (CPP)

Dual targeting strategy - CPP+NLS

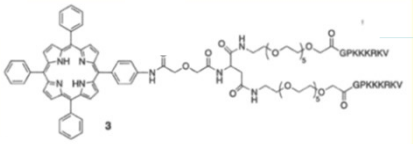
Bombesin (BBN) – interaction with gastrin-released peptide receptors

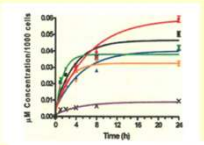
Oligopeptide conjugates for PDT

Nuclear localization signaling entities (NLS)

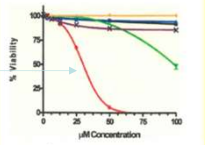


Various spacer/linker combinations

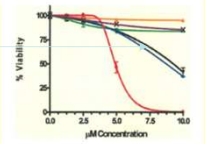




Uptake



Dark toxicity



Phototoxicity

Sibrian-Vazquez et al., Influence of the number and distribution of NLS peptides on the photosensitizing activity of multimeric porphyrin–NLS, Org. Biomol. Chem., 2010,8, 1160–1172

Oligopeptide conjugates for PDT

Nuclear localization signaling entities (NLS)

Despite the NLS peptide PS is localized in endoplasmatic reticulum

Intracellular integrity of conjugate was not investigated (!!!)

Sibrian-Vazquez et al., Influence of the number and distribution of NLS peptides on the photosensitizing activity of multimeric porphyrin–NLS, Org. Biomol. Chem., 2010,8, 1160–1172

Oligopeptide conjugates for PDT

Cell penetrating peptide (CPP)

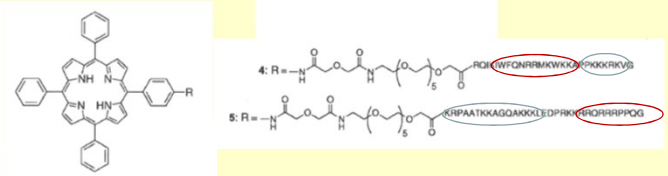
Comparison of NLS and CPP:

CPP-conjugates accumulate more

NLS-conjugates have higher phototoxicity

Oligopeptide conjugates for PDT

Dual targeting strategy - CPP+NLS



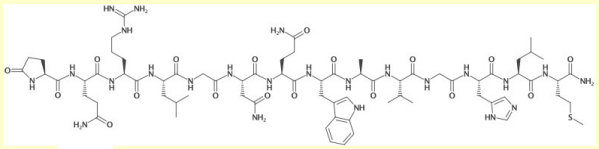
The least hydrophobic conjugates accumulated the most

Despite the NLS peptide PS was not localized in the nucleus

Sehgal et al., Photoinduced Cytotoxicity and Biodistribution of Prostate Cancer Cell-Targeted Porphyrins, J. Med. Chem. 2008, 51, 6014–602

Oligopeptide conjugates for PDT

Bombesin

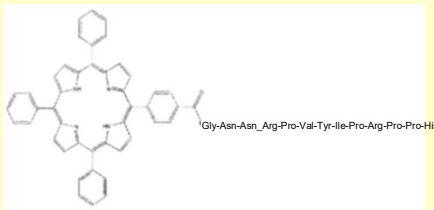


Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH2

Dubuc et al., Targeting gastrin-releasing peptide receptors of prostate cancer cells for photodynamic therapy with a phthalocyanine–bombesin conjugate. Bioorg Med Chem Lett 18 (2008) 2424–2427

Oligopeptide conjugates for antimicrobial photochemotherapy

Cationic antimicrobual peptides (CAMPs)

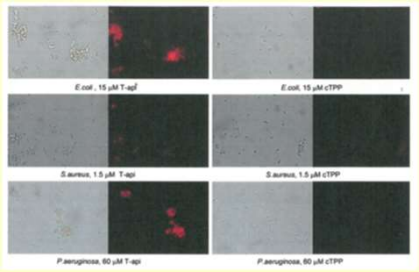


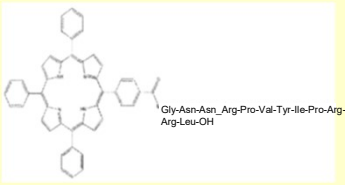
Apidaecin Ib

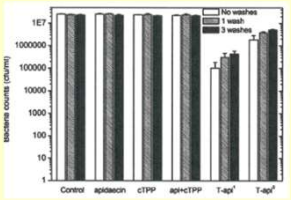
Pro-rich antimicrobial peptide

non-specific binding of the peptides to an outer membrane component

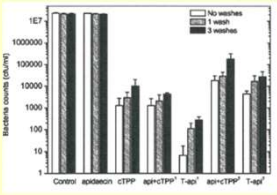
Dosselli et al. Porphyrin-Apidaecin Conjugate as a New Broad Spectrum Antibacterial Agent, ACS Med. Chem. Lett. 2010, 1, 35



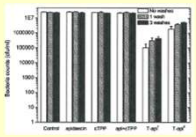




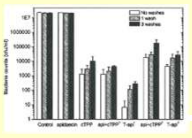
P.aeruginosa



S.aureus



P.aeruginosa



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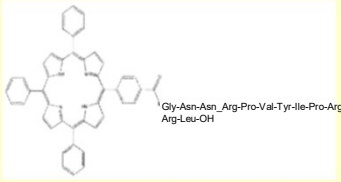


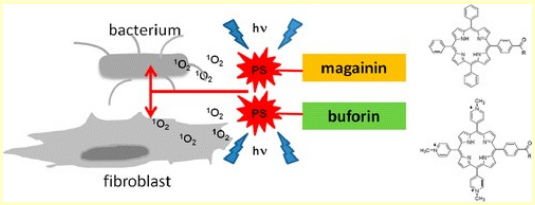
The quantum yields of $^1O_2$ production decrease by 20−30-fold or even 100-fold

The lifetime of $^1O_2$ for conjugates in deuterated PBS is shorter than that for free porphyrin

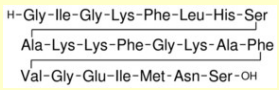
Dosselli et alSynthesis, characterization, and photoinduced antibacterial activity of porphyrin-type photosensitizers conjugated to the antimicrobial peptide apidaecin 1b, J. Med. Chem. 56, (2013) 1052-1063.

Oligopeptide conjugates for antimicrobial photochemotherapy

Cationic antimicrobual peptides (CAMPs)



Dosselli et al. Synthesis, Spectroscopic, and Photophysical Characterization and Photosensitizing Activity toward Prokaryotic and Eukaryotic Cells of Porphyrin-Magainin and -Buforin Conjugates, J. Med. Chem., 2014, 57 1403–1415



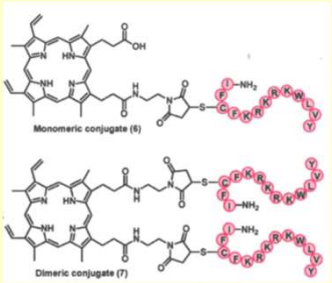
Pore-forming ability
due to α-helical structure

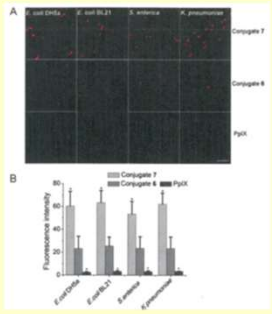
H-Thr-Arg-Ser-Ser-Arg-Ala-Gly-Leu-Gln-Phe-Pro-Val-Gly-Arg-Val-His-Arg-Leu-Leu-Arg-Lys-OH

Non-membranolytic,
DNA and RNA binding

Oligopeptide conjugates for antimicrobial photochemotherapy

Cationic antimicrobual peptides (CAMPS)

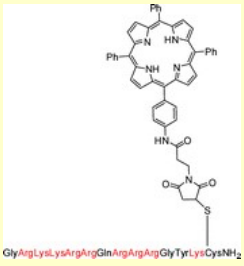


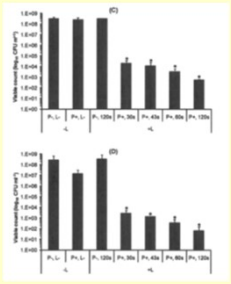


Liu et al. Lipopolysaccharide Neutralizing Peptide−Porphyrin Conjugates for Effective Photoinactivation and Intracellular Imaging of GramNegative Bacteria Strains, Bioconjugate Chem. 2012, 23, 1639−1640.

Oligopeptide conjugates for antimicrobial photochemotherapy

Cell penetrating peptides (CPPs)



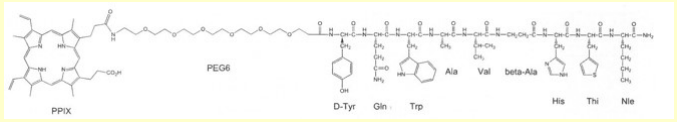


P.aeruginosa

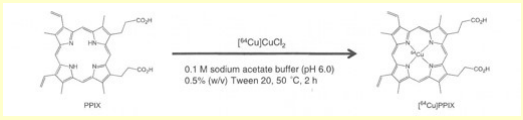
E.coli

Bourré, et al., Effective photoinactivation of Gram-positive and Gram-negative bacterial strains using an HIV-1 Tat peptide-porphyrin conjugate, Photochem Photobioll Sci 2010, 9(12),. 1613-1620

Positron Emission Tomography (PET)



The structure of protoporphyrin IX conjugated with a bombesin analog (PPIX-PEG6-BBN analog)



A schematic of the synthesis of [^{64}Cu]PPIX