

The basics of ALA-PDT



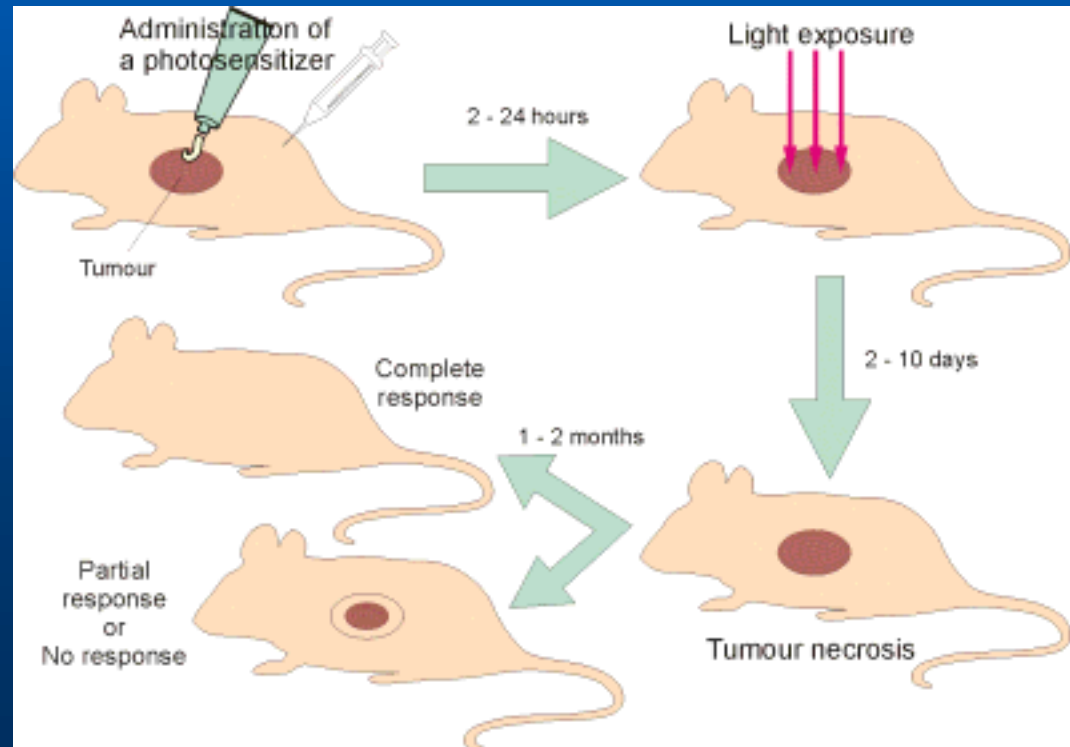
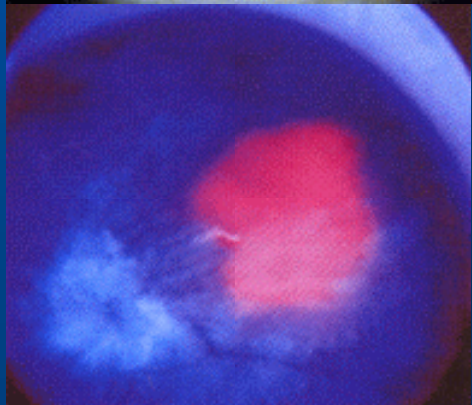
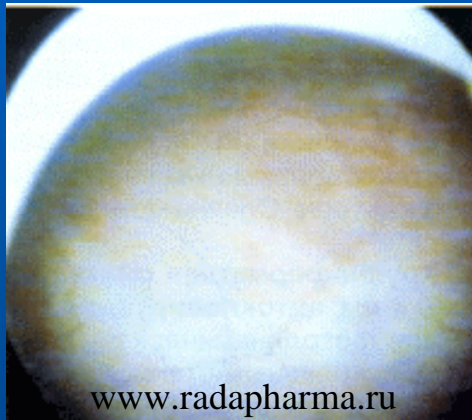
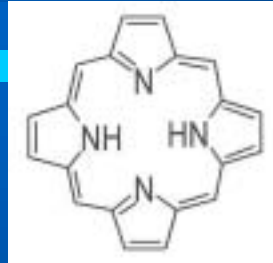
Petras Juzėnas

2003.06.17



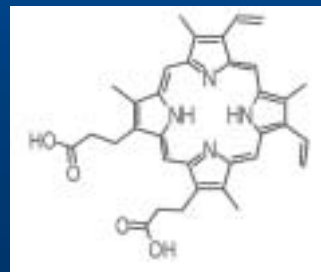
Department of Biophysics
PDT and Photobiophysics Group
<http://folk.uio.no/petrasj/photobiology>

Photodynamic Therapy (PDT) Fluorescence Diagnosis (FD)

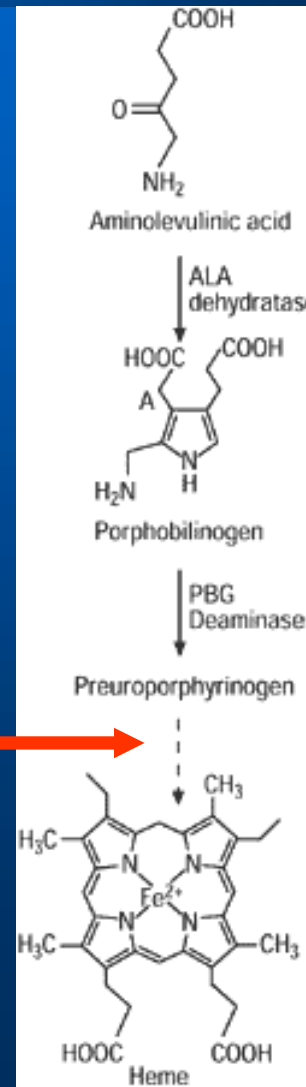


ALA-PDT

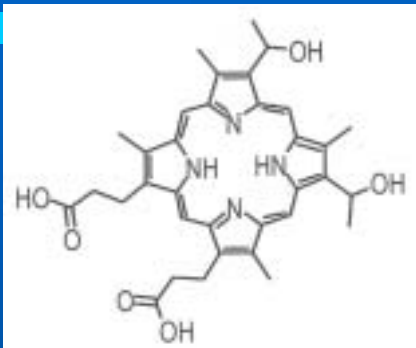
ALA 131,0 g/mol
ALA-HCl 167,6 g/mol



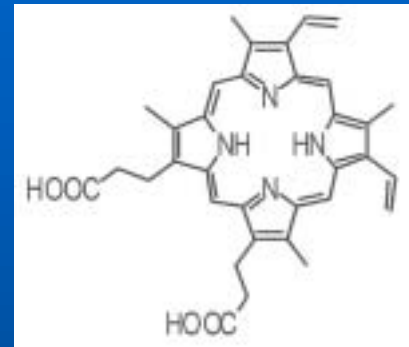
Protoporphyrin IX (PpIX)



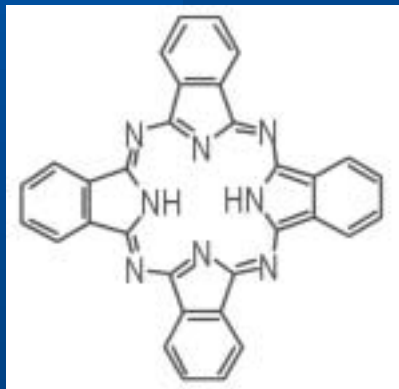
Porphyrins



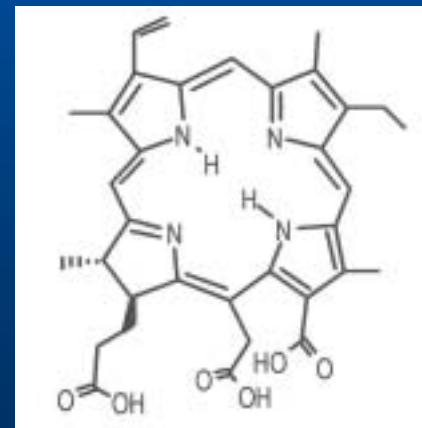
Hematoporphyrin (Hp)



Protoporphyrin IX (PpIX)



Phthalocyanines (Pc)



Chlorins

A Brief History

- 1897-1904 - O.Raab and H. von Tappeiner: photosensitization
- 1895-1903 m. - N.R.Finsen – phototherapy (Nobel prize in 1903)
- 1931 - H.Kautsky and G.Herzberg: active oxygen
- 1960-1967 - R.Lipson and E.Baldes: HpD
- 1964 - C.S.Foote et al.: singlet oxygen
- 1983-1993 – T.J.Dougherty et al.: Photofrin®
- 1980's - J.Kennedy and R.Pottier: ALA for PpIX induction
- 1987 - Z.Malik and H.Lugaci; Q.Peng. J.Moan, et al.
- 1990 - J.Kennedy and R.Pottier: clinical application of ALA
- 1997 - J.-M.Gaullier, J.Moan et al.: derivatives of ALA
- 2000 - DUSA Pharmaceuticals Inc.: Levulan®
- 2001 - PhotoCure ASA: Metvix®

Photosensitization

1905

5% eosin solution topically

50 year old patient with *Ulcus rodens*.

G.Kick *et al.* (1996) *Hautarzt* 47, 644–649.



1912

0.2 g hematoporphyrin i.v.

A “self-experiment” of Dr. F.Meyer-Betz

G.Kick *et al.* (1996) *Hautarzt* 47, 644–649.



2001

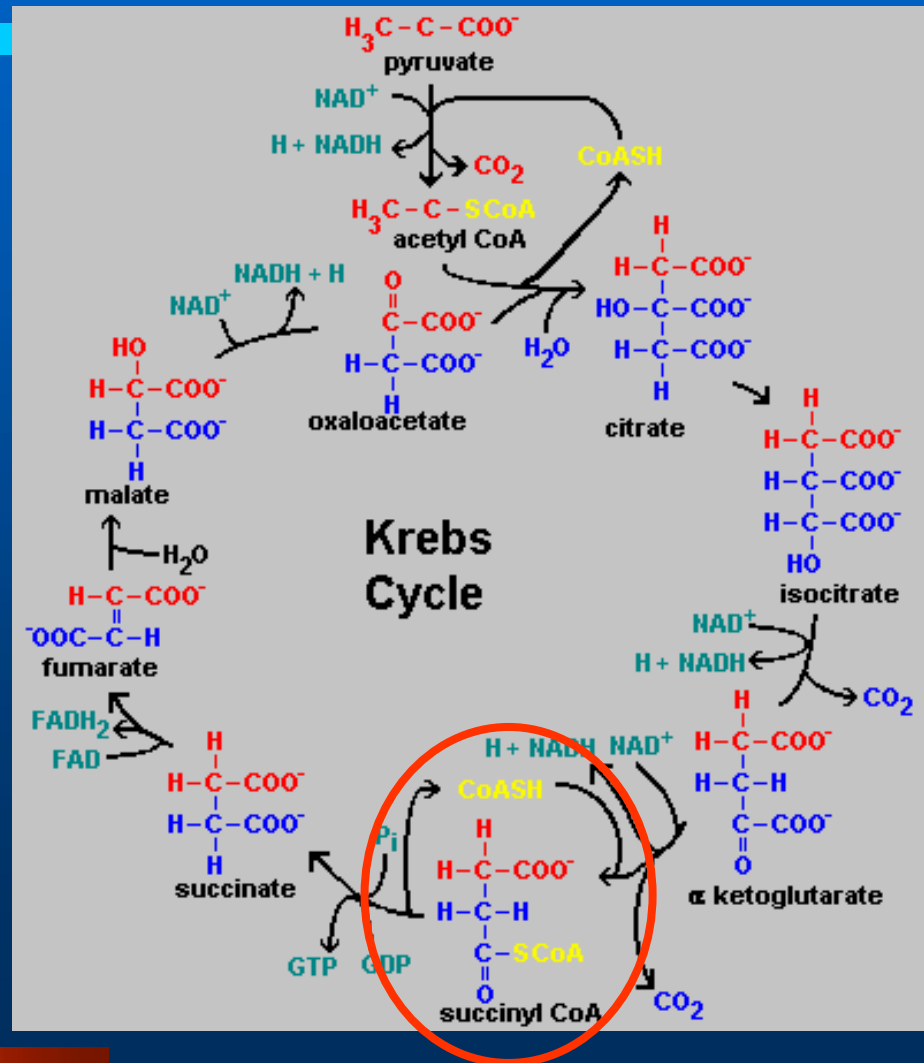
Metvix® (ALA-derivative) topically

www.photocure.com

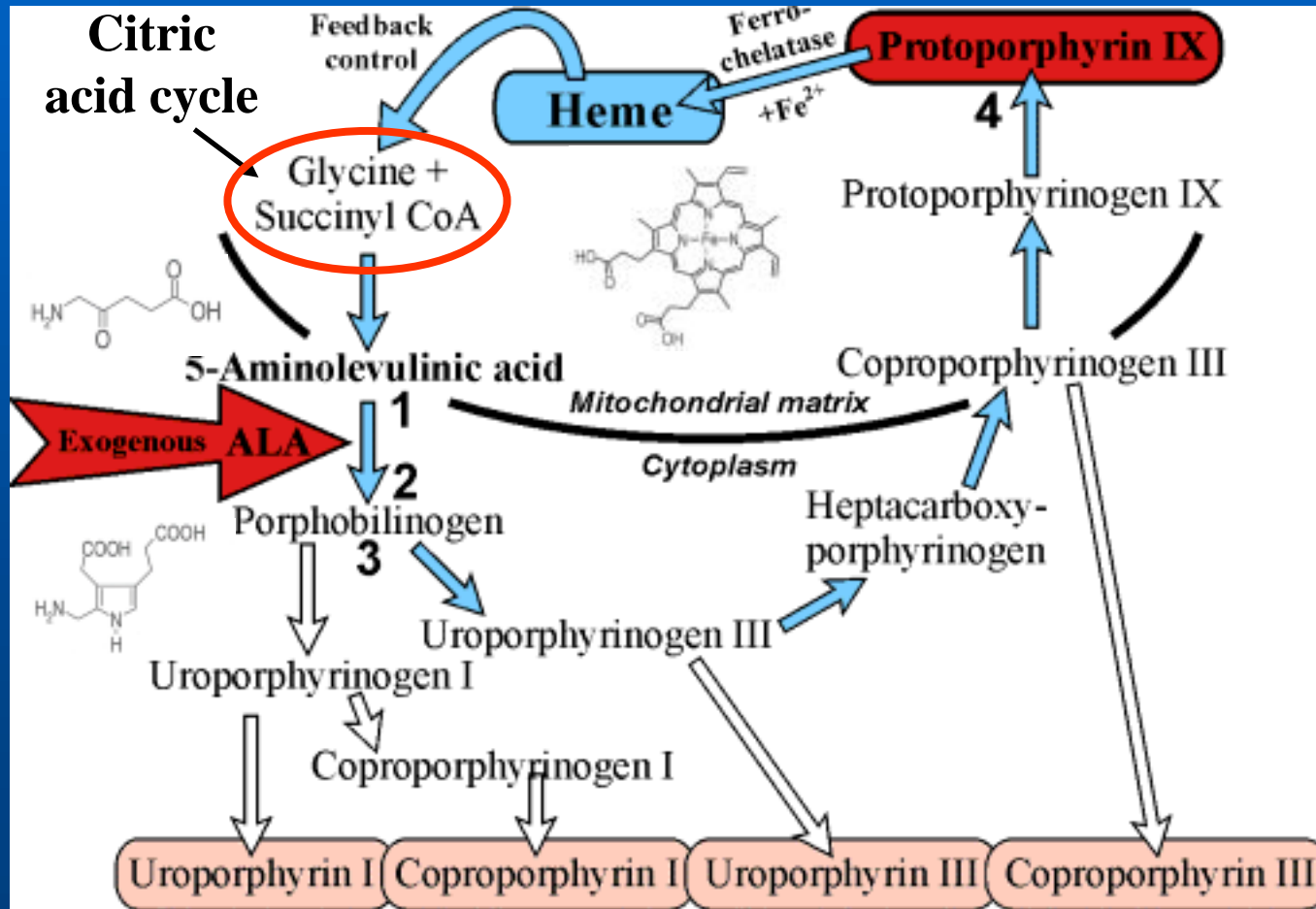
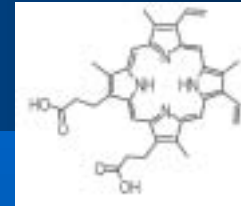


Citric Acid (Krebs) Cycle

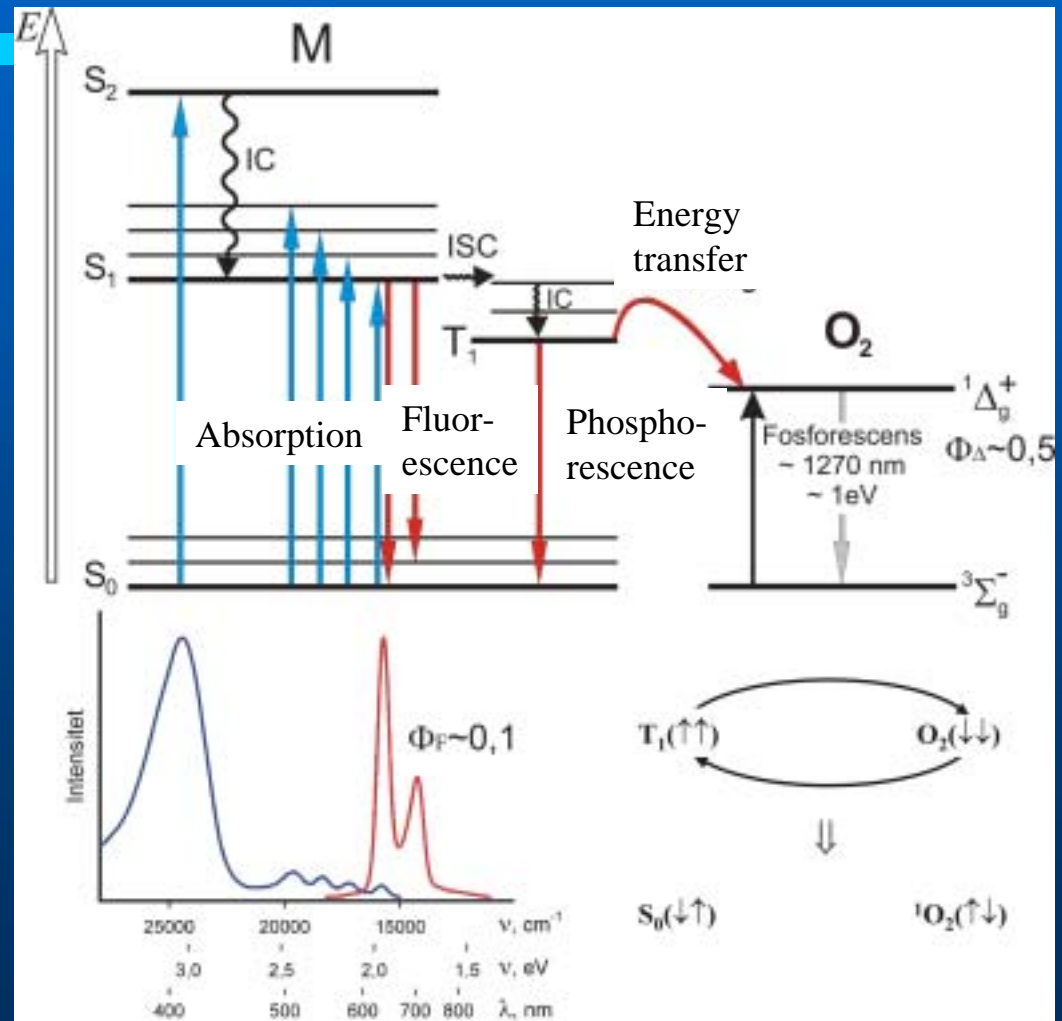
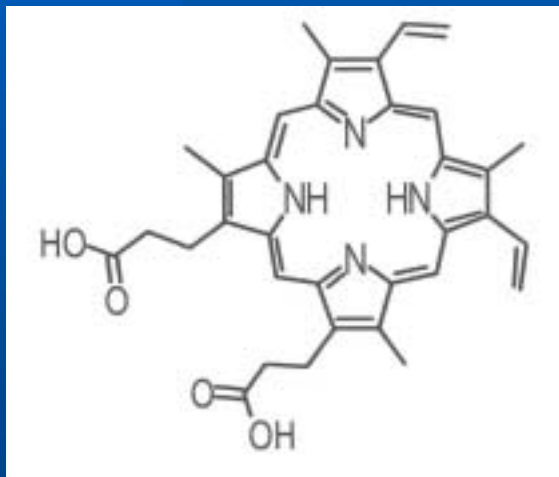
**Precursors of
 δ -aminolevulinic acid
or δ -aminolevulinate
(ALA)**



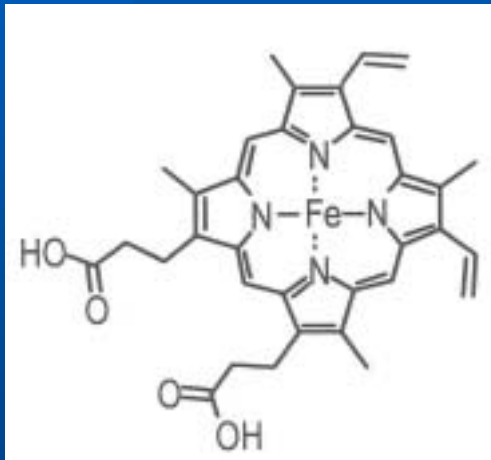
Heme synthesis



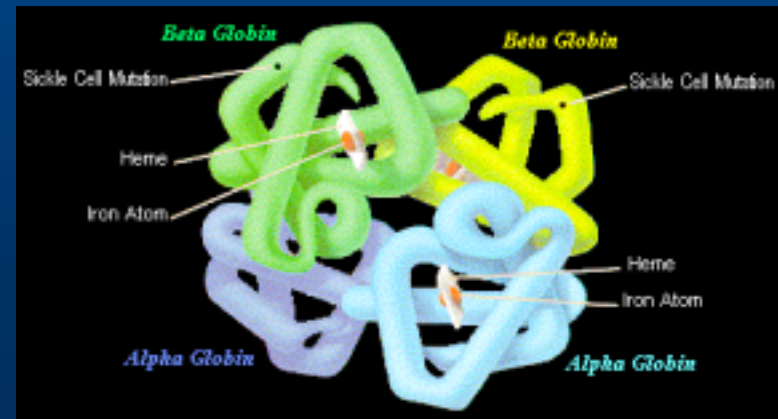
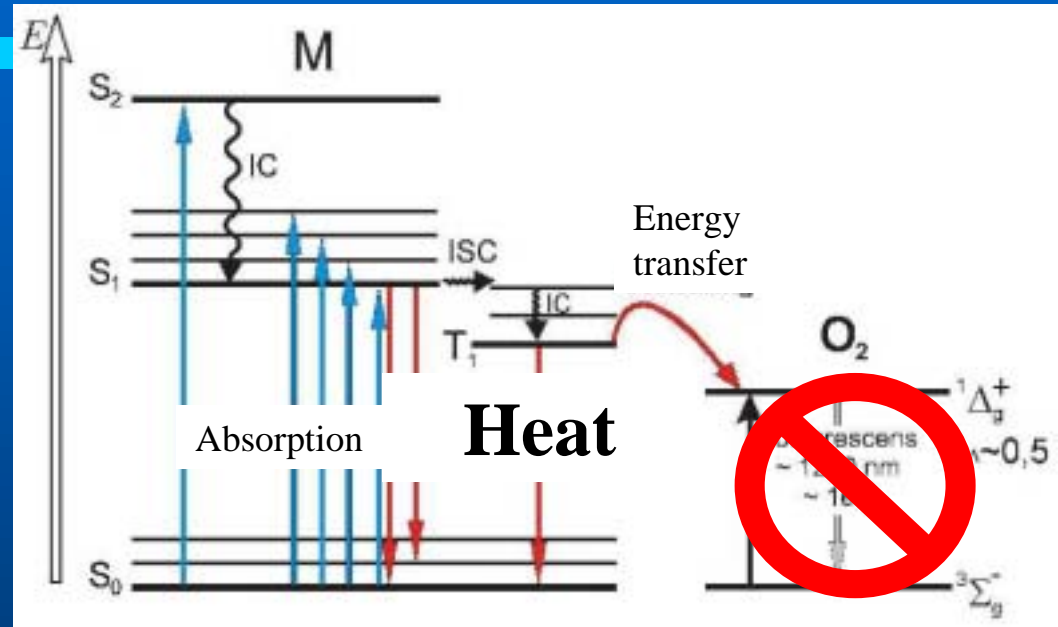
Photosensitization with PpIX



Photothermal sensitization



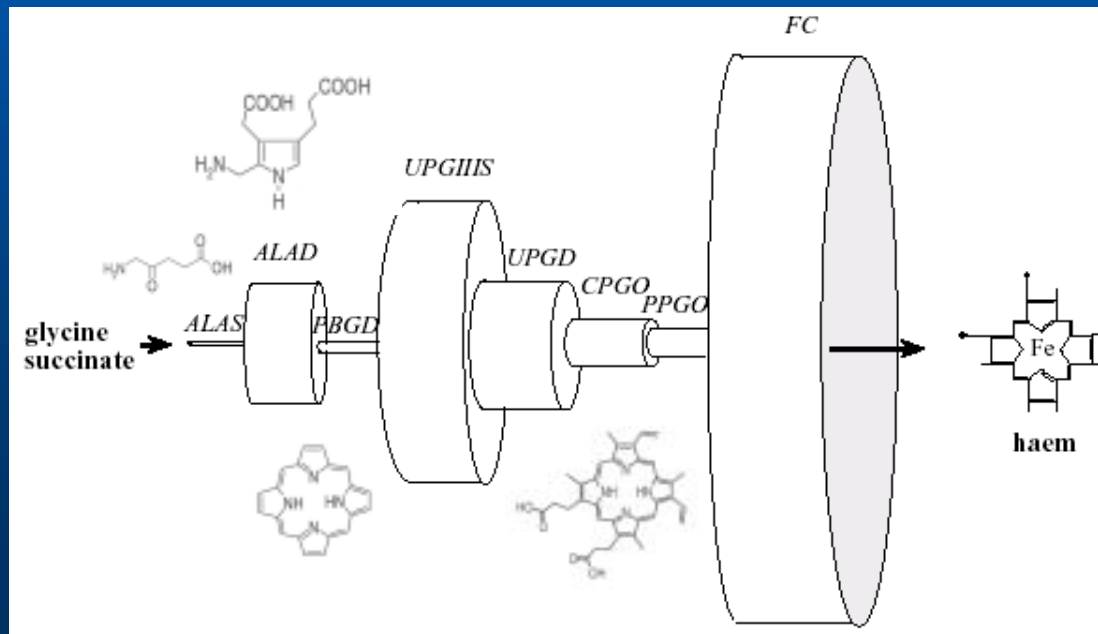
Heme



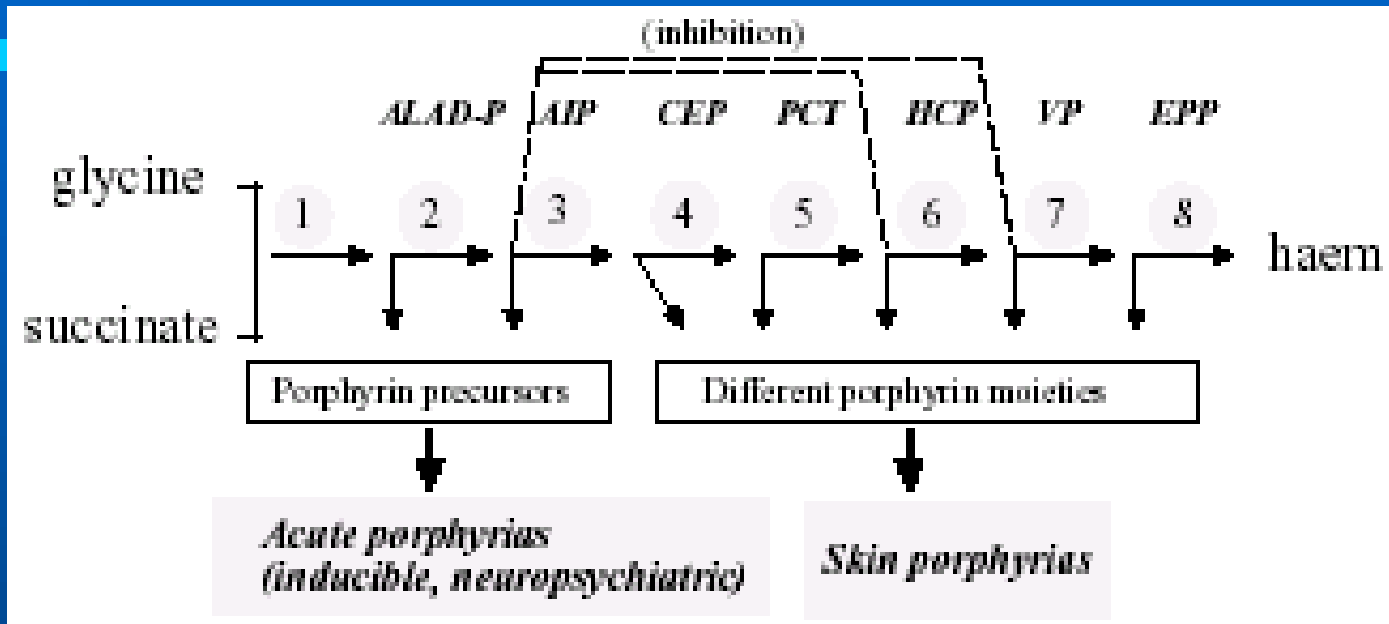
Enzymes of the heme synthesis

ALAS: 5-aminolevulinate synthase
ALAD: 5-aminolevulinate dehydratase
PBGD: porphobilinogen deaminase
UPGIIS: uroporphyrinogen III synthase

UPGD: uroporphyrinogen decarboxylase
CPGO: coproporphyrinogen oxidase
PPGO: protoporphyrinogen oxidase
FC: ferrochelatase



Porphyrias



ALAD-P – ALA-Dehydratase deficiency porphyria

AIP – acute intermittent porphyria

CP – coproporphyria

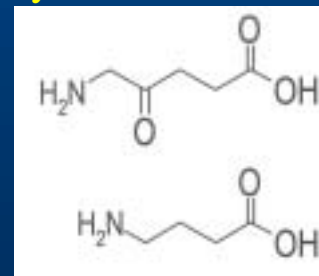
CEP – congenital erythropoietic porphyria

PCT – porphyria cutanea tarda

HCP – hereditary coproporphyria

VP – variegate porphyria

EPP – erythropoietic protoporphyria



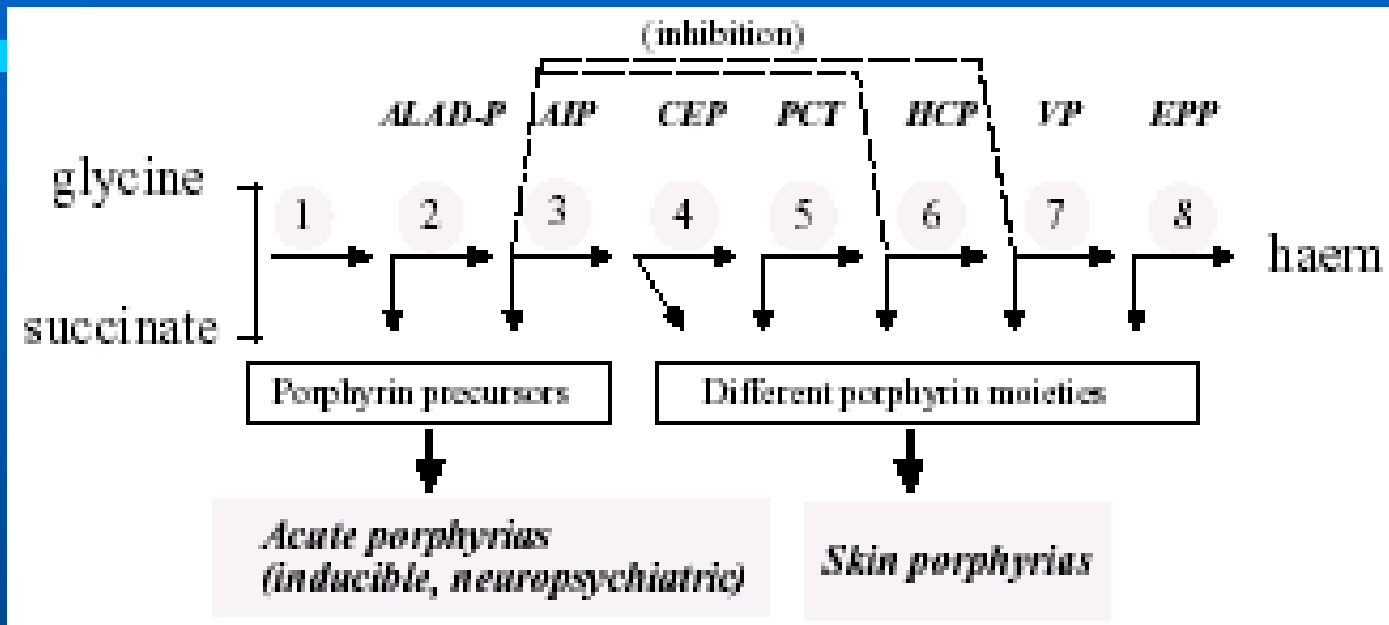
5-aminolevulinic acid
(ALA)

γ-aminobutyric acid
(GABA)

M.R.Moore et al. (1987) Disorders of Porphyrin Metabolism, Plenum Publishing Corp.

S.Thunell (2000) Scand. J. Clin. Lab. Invest 60, 509-540

Porphyrias



ALAD-P – ALA-Dehydratase deficiency porphyria

AIP – acute intermittent porphyria

CP – coproporphyria

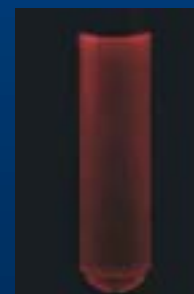
CEP – congenital erythropoietic porphyria

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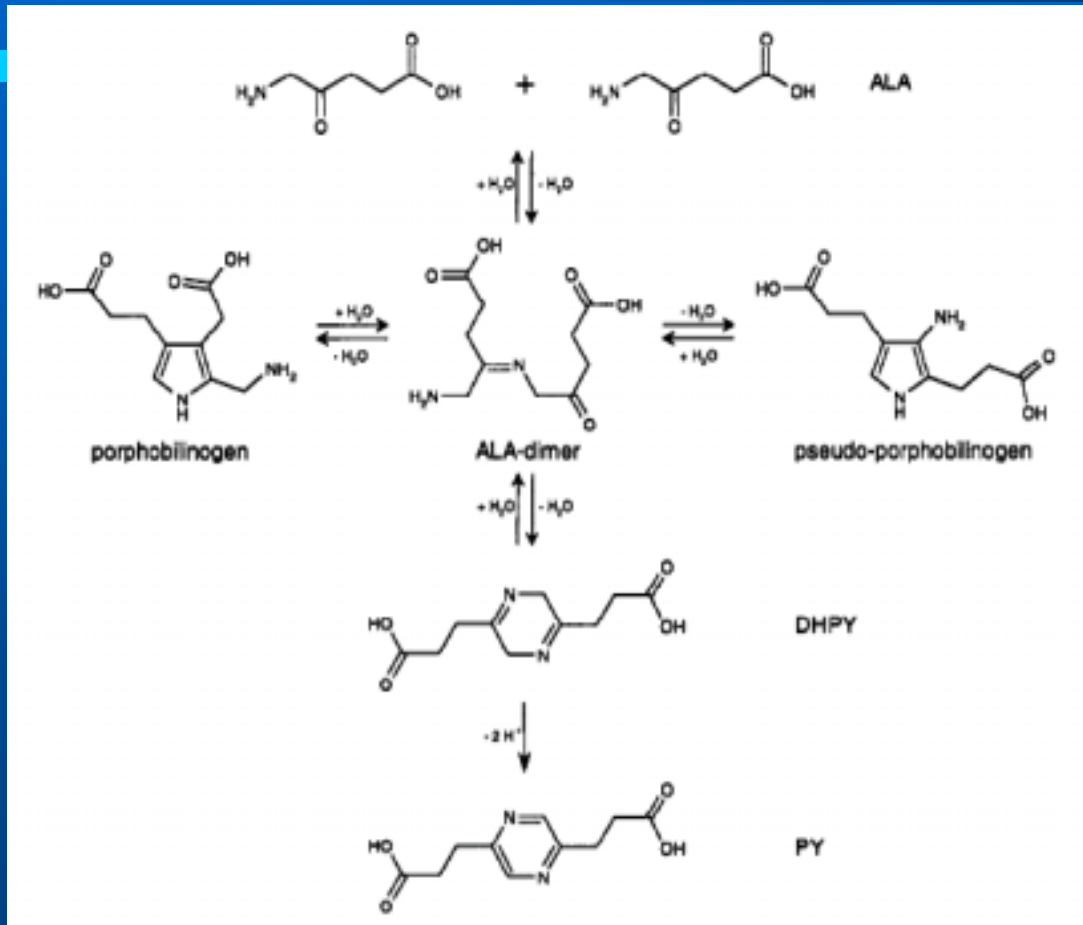
HCP – hereditary coproporphyria

VP – variegate porphyria

EPP – erythropoietic protoporphyria



Stability of ALA



2,5-(dicarboxyethyl)dihydropyrazine

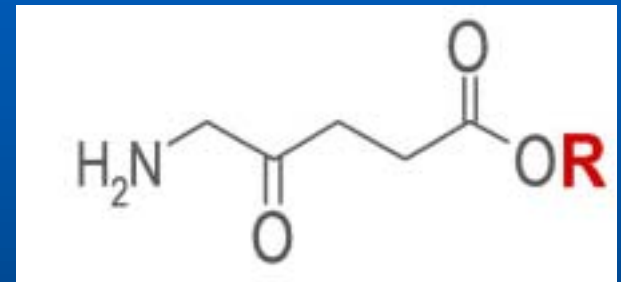
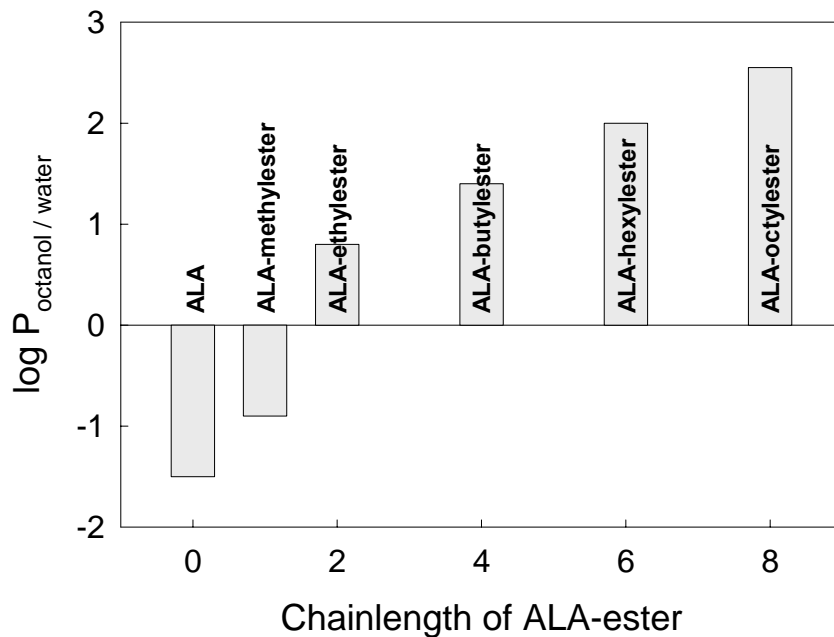
2,5-(dicarboxyethyl)pyrazine

The products lead to yellow colour

A. Bunke *et al.* (2000) *J. Pharmac. Sci.* 89, 1335-1341.

A. W. de Blois *et al.* (2002) *Lasers Med. Sci.* 17, 208-215.

Derivatives of ALA



n=0 - ALA

n=1 - ALA-Me

n=6 - ALA-Hex

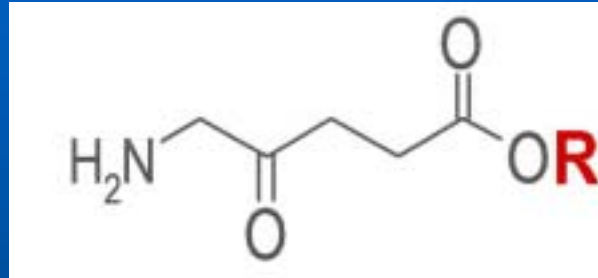
n=8 - ALA-Oct

J.Kloek et al. (1996) Photochem. Photobiol. 64, 994-1000.

J.-M.Gaullier et al. (1997) Cancer Res. 57, 1481-1486.

P.Uehlinger et al. (2000) J. Photochem. Photobiol. B: Biol. 54, 72-80.

Derivatives of ALA

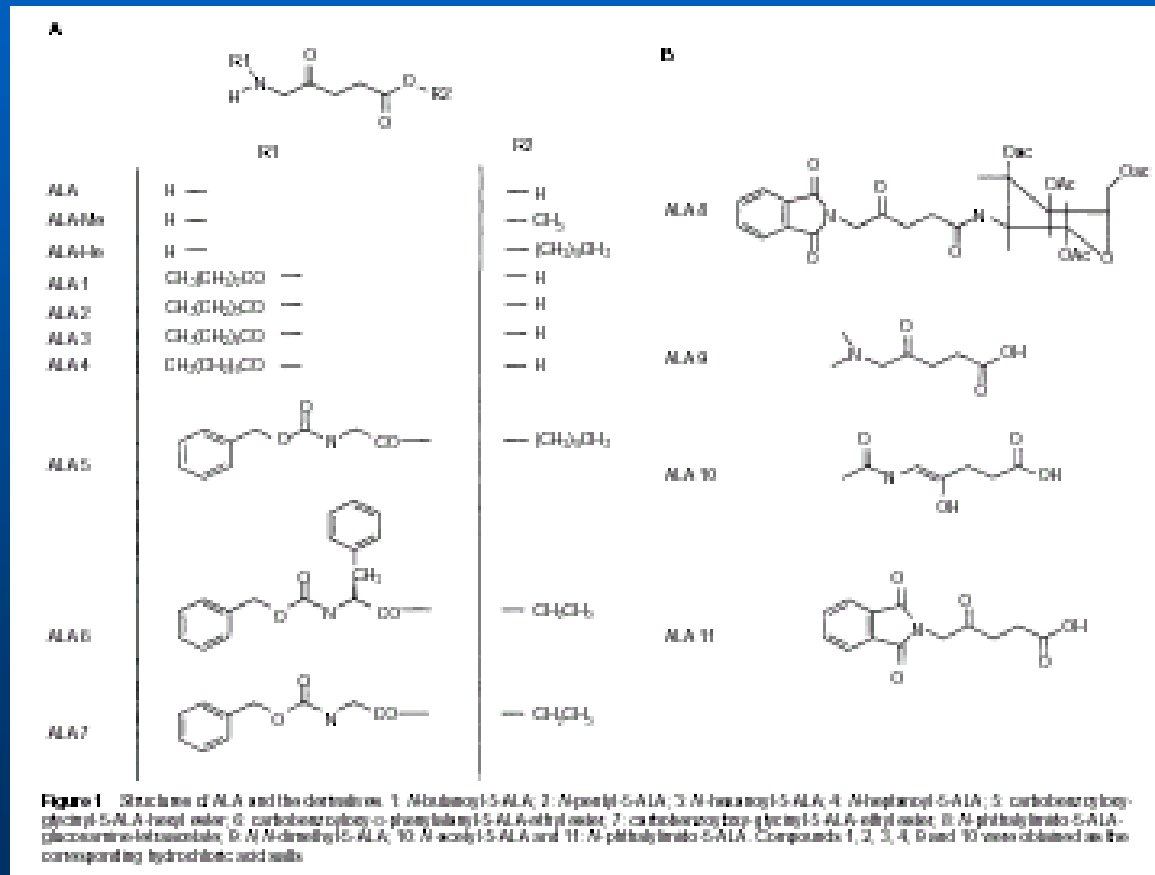


ALA – 5-aminolevulinic acid
 δ -aminolevulinic acid
 δ -aminolevulinate

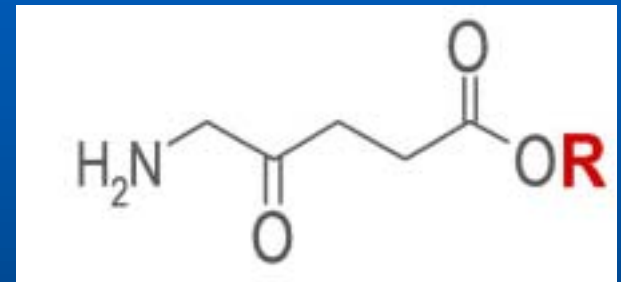
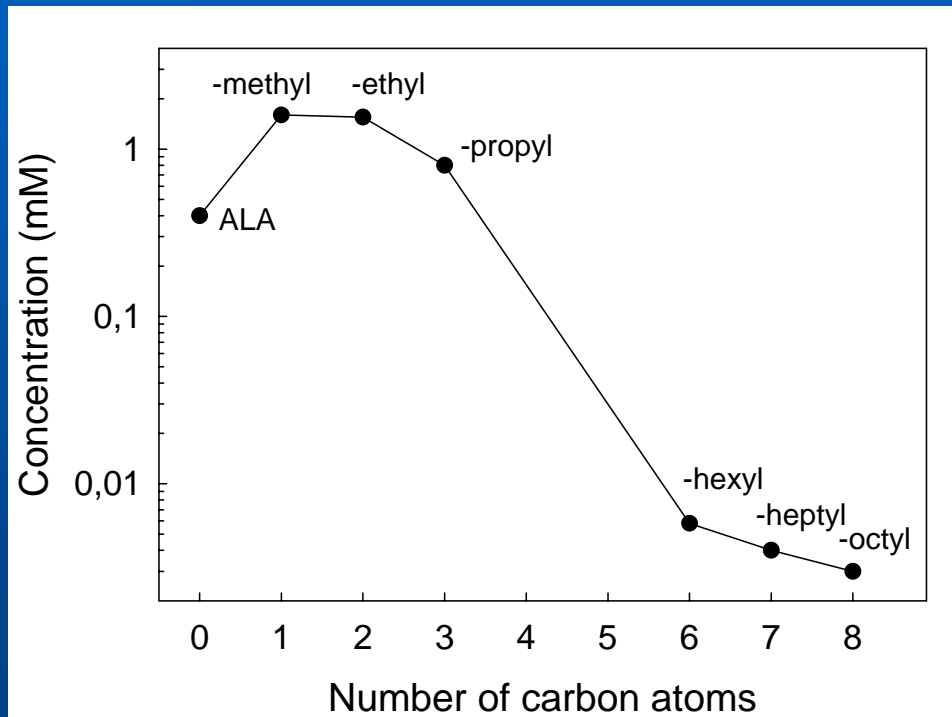
ALA-Me – aminolevulinic acid methylester
methyl aminolevulinate (m-ALA; MAL)

ALA-Hex – aminolevulinic acid methylester
hexyl aminolevulinate (h-ALA; HAL)

Derivatives of ALA



Derivatives of ALA *in vitro*



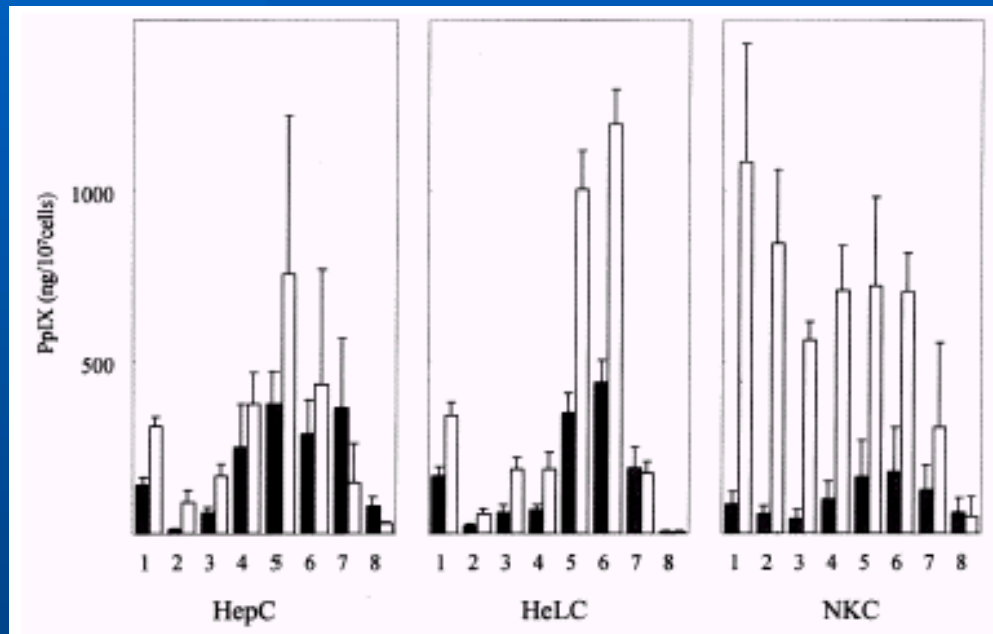
$n=0$ - ALA

$n=1$ - ALA-Me

$n=6$ - ALA-Hex

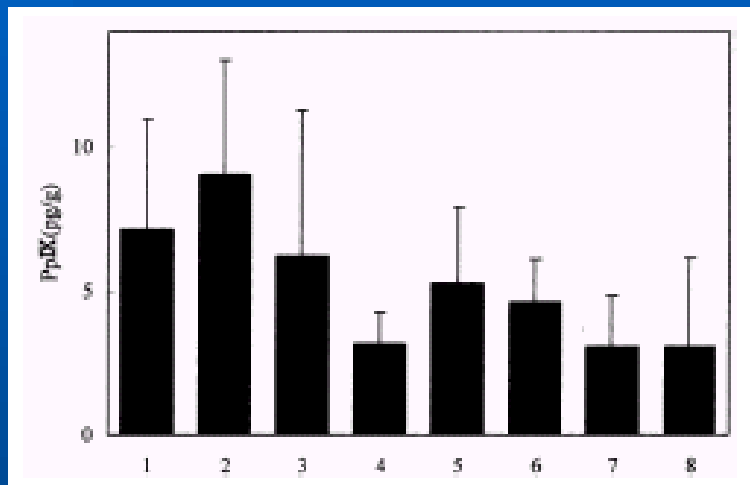
$n=8$ - ALA-Oct

Derivatives of ALA *in vitro*



| | |
|--------------------|---|
| 1 ALA | $\text{NH}_2\text{CH}_2\text{CO}(\text{CH}_2)_2\text{COOH}\cdot\text{HCl}$ |
| 2 ALA methyl ester | $\text{NH}_2\text{CH}_2\text{CO}(\text{CH}_2)_2\text{COOCH}_3\cdot\text{HCl}$ |
| 3 ALA ethyl ester | $\text{NH}_2\text{CH}_2\text{CO}(\text{CH}_2)_2\text{COOC}_2\text{H}_5\cdot\text{HCl}$ |
| 4 ALA propyl ester | $\text{NH}_2\text{CH}_2\text{CO}(\text{CH}_2)_2\text{COOC}_3\text{H}_7\cdot\text{HCl}$ |
| 5 ALA butyl ester | $\text{NH}_2\text{CH}_2\text{CO}(\text{CH}_2)_2\text{COOC}_4\text{H}_9\cdot\text{HCl}$ |
| 6 ALA pentyl ester | $\text{NH}_2\text{CH}_2\text{CO}(\text{CH}_2)_2\text{COOC}_5\text{H}_{11}\cdot\text{HCl}$ |
| 7 ALA hexyl ester | $\text{NH}_2\text{CH}_2\text{CO}(\text{CH}_2)_2\text{COOC}_6\text{H}_{13}\cdot\text{HCl}$ |
| 8 ALA acetamide | $\text{CH}_3\text{CONHCH}_2\text{CO}(\text{CH}_2)_2\text{COOH}$ |

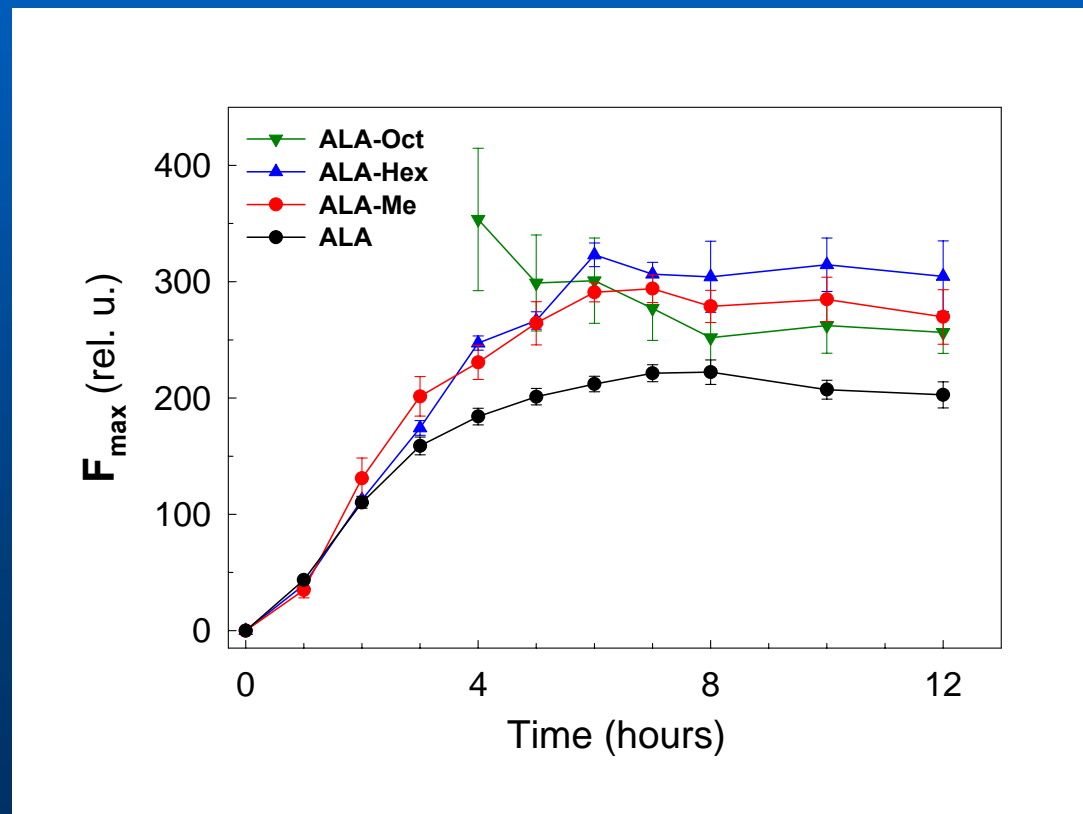
Derivatives of ALA *in vivo*



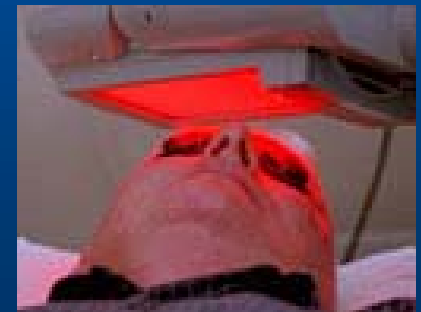
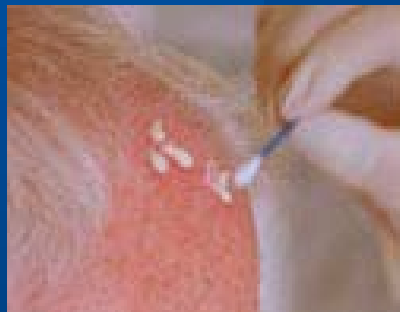
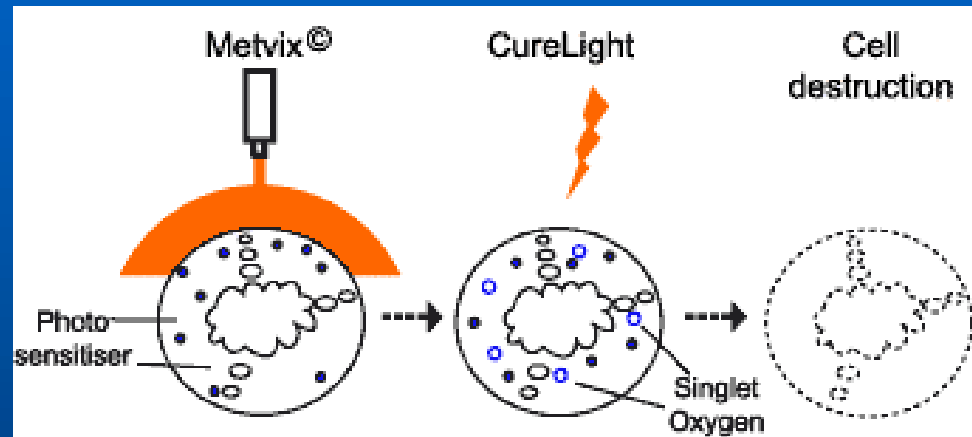
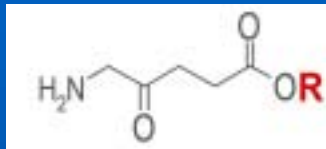
| | |
|--------------------|---|
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| 2 ALA methyl ester | $\text{NH}_2\text{CH}_2\text{CO}(\text{CH}_2)_2\text{COOCH}_3\cdot\text{HCl}$ |
| 3 ALA ethyl ester | $\text{NH}_2\text{CH}_2\text{CO}(\text{CH}_2)_2\text{COOC}_2\text{H}_5\cdot\text{HCl}$ |
| 4 ALA propyl ester | $\text{NH}_2\text{CH}_2\text{CO}(\text{CH}_2)_2\text{COOC}_3\text{H}_7\cdot\text{HCl}$ |
| 5 ALA butyl ester | $\text{NH}_2\text{CH}_2\text{CO}(\text{CH}_2)_2\text{COOC}_4\text{H}_9\cdot\text{HCl}$ |
| 6 ALA pentyl ester | $\text{NH}_2\text{CH}_2\text{CO}(\text{CH}_2)_2\text{COOC}_5\text{H}_{11}\cdot\text{HCl}$ |
| 7 ALA hexyl ester | $\text{NH}_2\text{CH}_2\text{CO}(\text{CH}_2)_2\text{COOC}_6\text{H}_{13}\cdot\text{HCl}$ |
| 8 ALA acetamide | $\text{CH}_3\text{CONHCH}_2\text{CO}(\text{CH}_2)_2\text{COOH}$ |

Under *in vitro* conditions (SCC tumour), PpIX expression was efficiently induced by long chain ALA esters, while better *in vivo* PpIX induction was obtained with short chain ALA esters. In this study, ALA methyl ester was found to be the best among the ALA derivatives in inducing PpIX expression *in vivo*, and would be more effective in treatment of skin cancers than ALA.

Derivatives of ALA *in vivo*



Application of ALA and derivatives



Photosensitizers for PDT

Table 1 | **Photosensitizers for malignant diseases**

| Sensitizer | Trade name | Potential indications | Activation wavelength |
|---|-------------|---|-----------------------|
| HPD (partially purified), porfimer sodium | Photofrin | Cervical*, endobronchial*, oesophageal*, bladder* and gastric cancers*, and brain tumours | 630 nm |
| BPD-MA | Verteporfin | Basal-cell carcinoma | 689 nm |
| m-THPC | Foscan | Head and neck tumours*, prostate and pancreatic tumours | 652 nm |
| 5-ALA | Levulan | Basal-cell carcinoma, head and neck, and gynaecological tumours Diagnosis of brain, head and neck, and bladder tumours | 635 nm 375- 400 nm |
| 5-ALA-methylester | Metvix | Basal-cell carcinoma* | 635 nm |
| 5-ALA benzylester | Benzvix | Gastrointestinal cancer | 635 nm |
| 5-ALA hexylester | Hexvix | Diagnosis of bladder tumours | 375- 400 nm |
| SnET2 | Purlytin | Cutaneous metastatic breast cancer, basal-cell carcinoma, Kaposi's sarcoma, prostate cancer | 664 nm |
| Boronated protoporphyrin | BOPP | Brain tumours | 630 nm |
| HPPH | Photochlor | Basal-cell carcinoma | 665 nm |
| Lutetium texaphyrin | Lutex | Cervical, prostate and brain tumours | 732 nm |
| Phthalocyanine-4 | Pc 4 | Cutaneous/subcutaneous lesions from diverse solid tumour origins | 670 nm |
| Taporfin sodium | Talaporfin | Solid tumours from diverse origins | 664 nm |

*Indications that are registered in one or more countries (all other indications are in development). 5-ALA, 5-aminolevulinic acid; BPD-MA, benzoporphyrin derivative-monoacid ring A; HPD, haematoporphyrin derivative; HPPH, 2-(1-hexyloxyethyl)-2-devinyl pyropheophorbide-alpha; mTHPC, meta-tetrahydroxyphenylchlorin; SnET2, tin ethyl etiopurpurin.

D.E.J.G.J. Dolmans *et al.* (2003) *Nature Reviews: Cancer* 3, 380-387.

Photosensitizers for PDT

| | | | |
|-------------------|---------|---|-----------------------|
| 5-ALA | Lewulan | Basal-cell carcinoma, head and neck, and gynaecological tumours Diagnosis of brain, head and neck, and bladder tumours | 635 nm 375- 400 nm |
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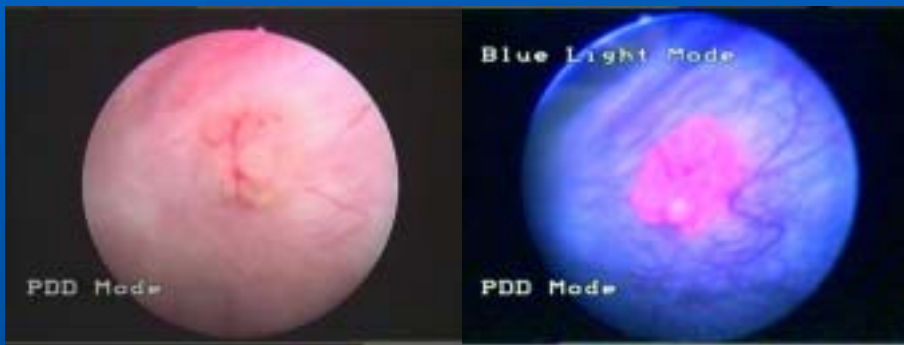
Approved drugs for PDT

Table 2 | **Type of cancer and approved drugs (2003)**

| Disease | Drug | Country |
|--------------------------|-----------------|---|
| <i>Pre-cancer</i> | | |
| Actinic keratosis | Levulan, Metvix | EU |
| Barrett's oesophagus | Photofrin | EU, USA |
| Cervical dysplasia | Photofrin | Japan |
| <i>Cancer</i> | | |
| Basal-cell carcinoma | Metvix | EU |
| Cervical cancer | Photofrin | Japan |
| Endobroncheal cancer | Photofrin | Canada, Denmark, Finland, France, Germany, Ireland, Japan, The Netherlands, UK, USA |
| Oesophageal cancer | Photofrin | Canada, Denmark, Finland, France, Ireland, Japan, The Netherlands, UK, USA |
| Gastric cancer | Photofrin | Japan |
| Head and neck cancer | Foscan | EU |
| Papillary bladder cancer | Photofrin | Canada |

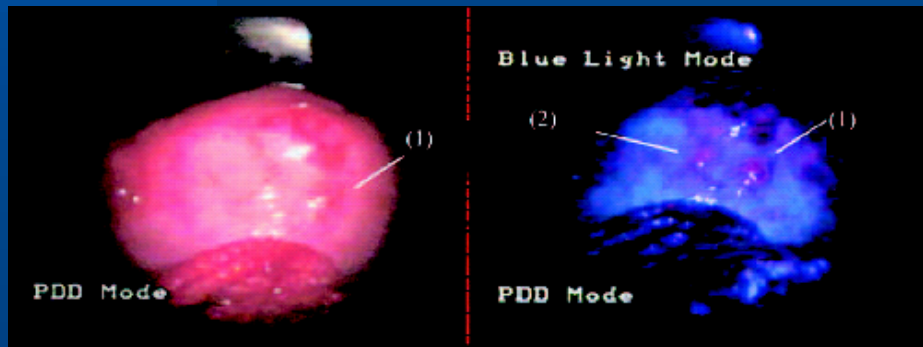
Photodynamic therapy has also been undertaken in other countries with haematoporphyrin derivative and porphyrin mixtures (China and India), and phthalocyanines (Russia and India). EU, European Union; UK, United Kingdom; USA, The United States.

Fluorescence diagnosis (FD)



ALA-Hex

H. van den Bergh et al. (1999) *Photodynamics News* 2(1), 4-8.



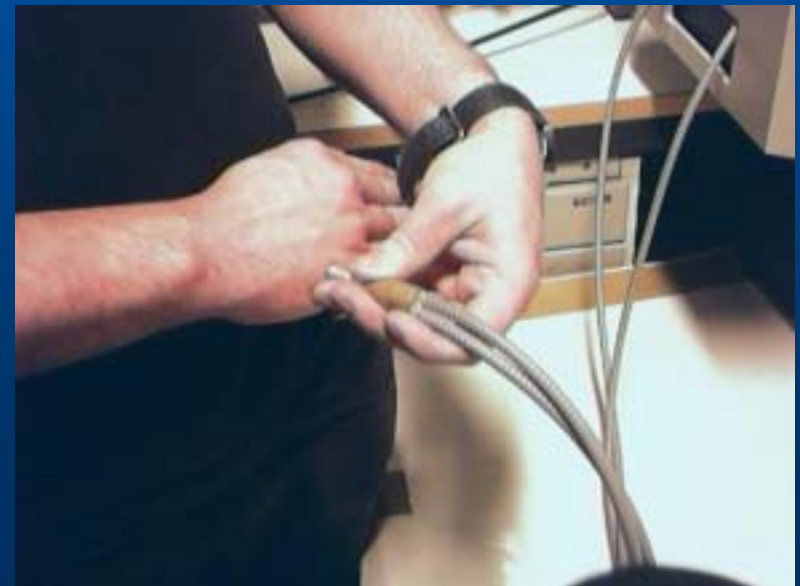
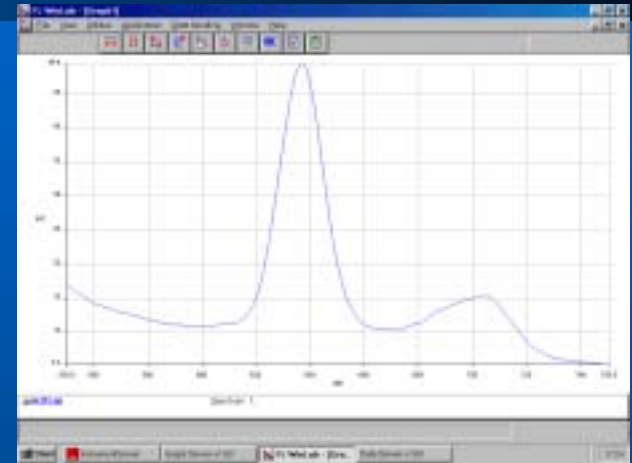
ALA

A.L.Major et al. (2002) *Lasers Med. Sci.* 17, 2-5.

Fluorescence spectroscopy



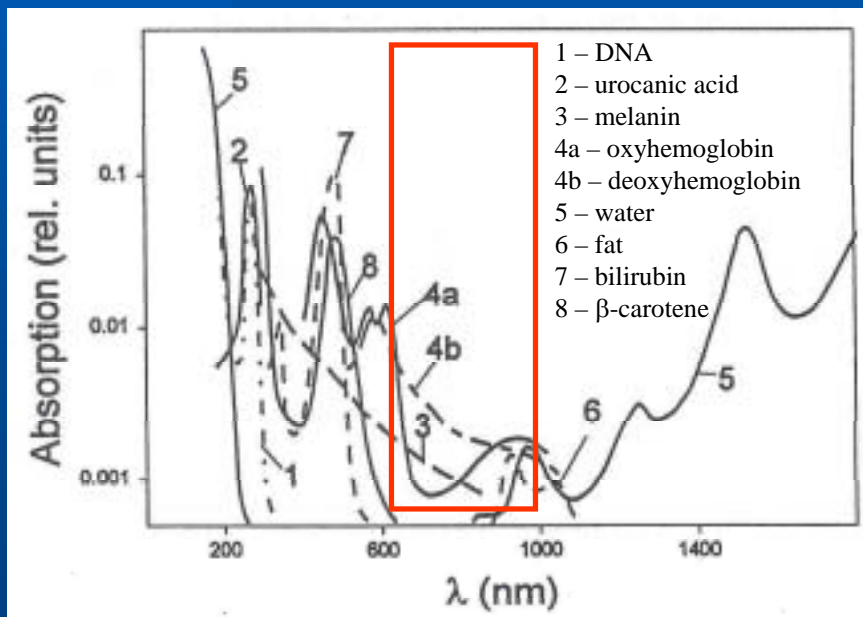
Perkin Elmer LS50B



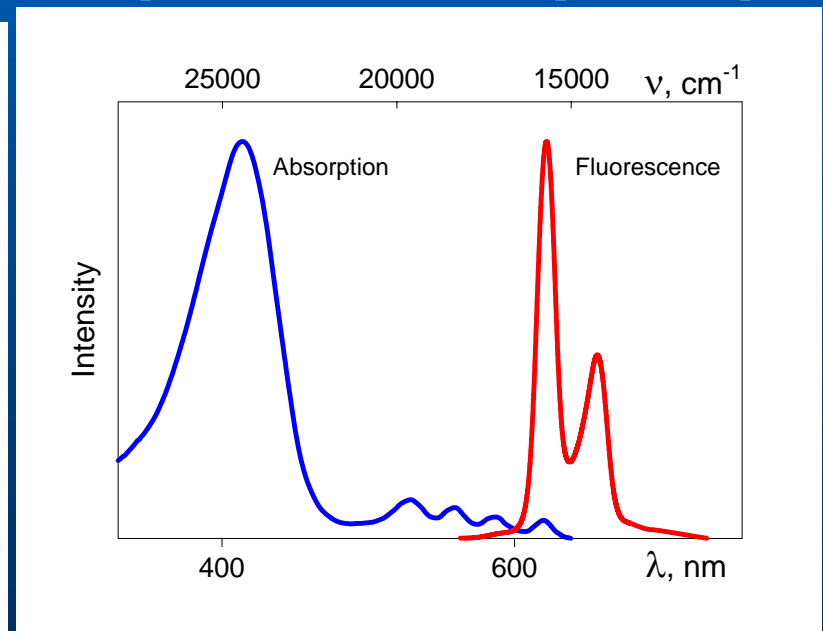
Absorption and emission of PpIX

Optimal therapeutic window

Endogenous chromophores



Absorption and fluorescence spectra of PpIX

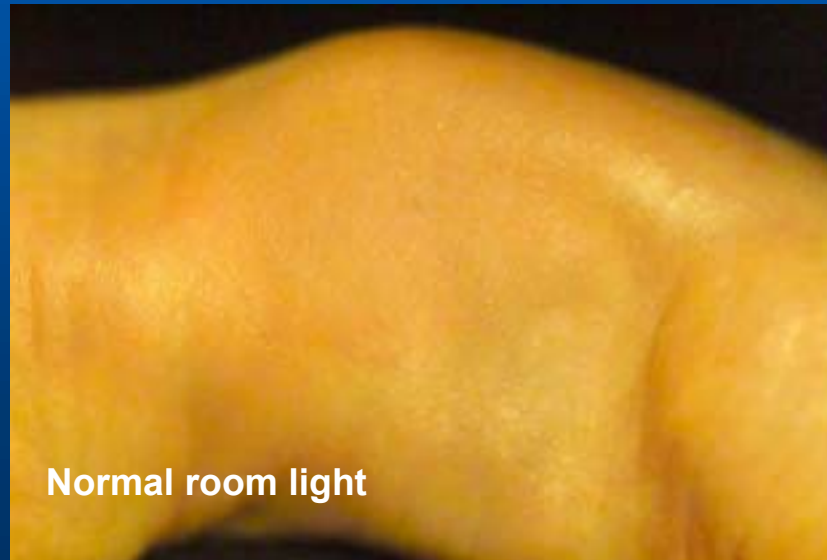


J. Moan (2001) Visible light and UV radiation. Radiation at home, outdoors and in the workplace, Scandinavian Science Publisher.

A. Juzeniene et al. (2002) Photochem. Photobiol. 76, 329-334.

Fluorescence of PpIX

Normal mouse skin treated
with ALA-Me (1 mol/g) cream for 4 h



Pictures were taken with a Kodak DCS 720x digital camera.

Fluorescence of PpIX

Normal mouse skin treated
with ALA-Me (1 mol/g) cream for 4 h

Fluorescence in
the area where
the cream was
applied



Pictures were taken with a Kodak DCS 720x digital camera.

Fluorescence of PpIX

Normal mouse skin treated
with ALA-Me (1 mol/g) cream for 4 h

Fluorescence in
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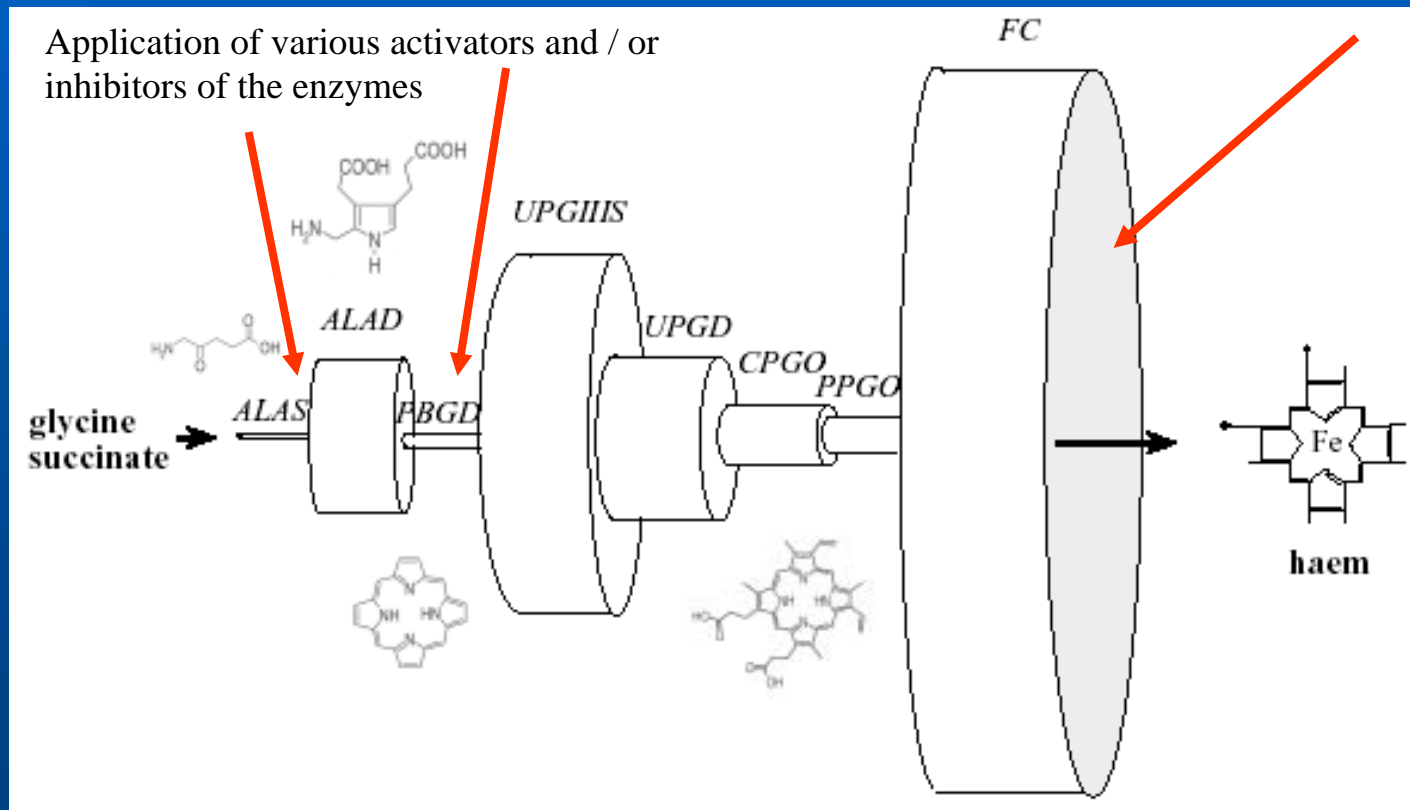
Fluorescence of PpIX



Photographed in a dark room under
350-410 nm light (Philips TLD 18W08) exposure

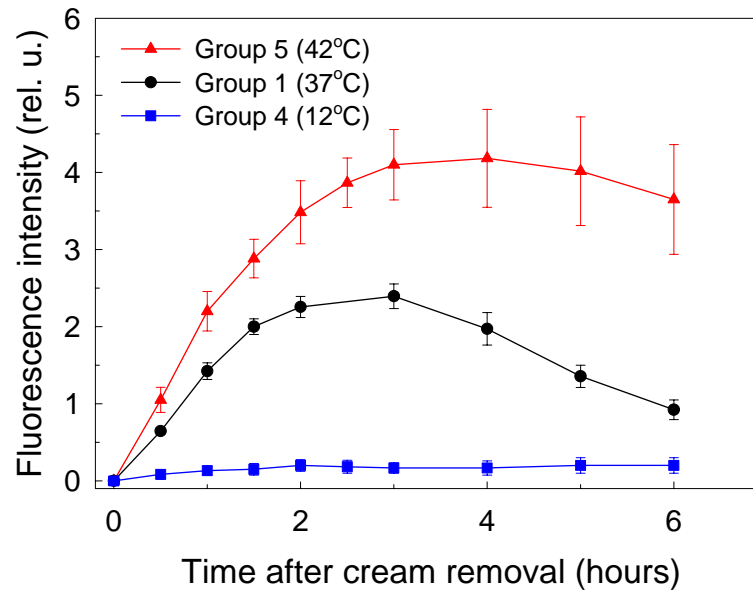
Methods to enhance PpIX production

Iron chelators, FC inhibitors: Deferoxamine, CP94, etc.

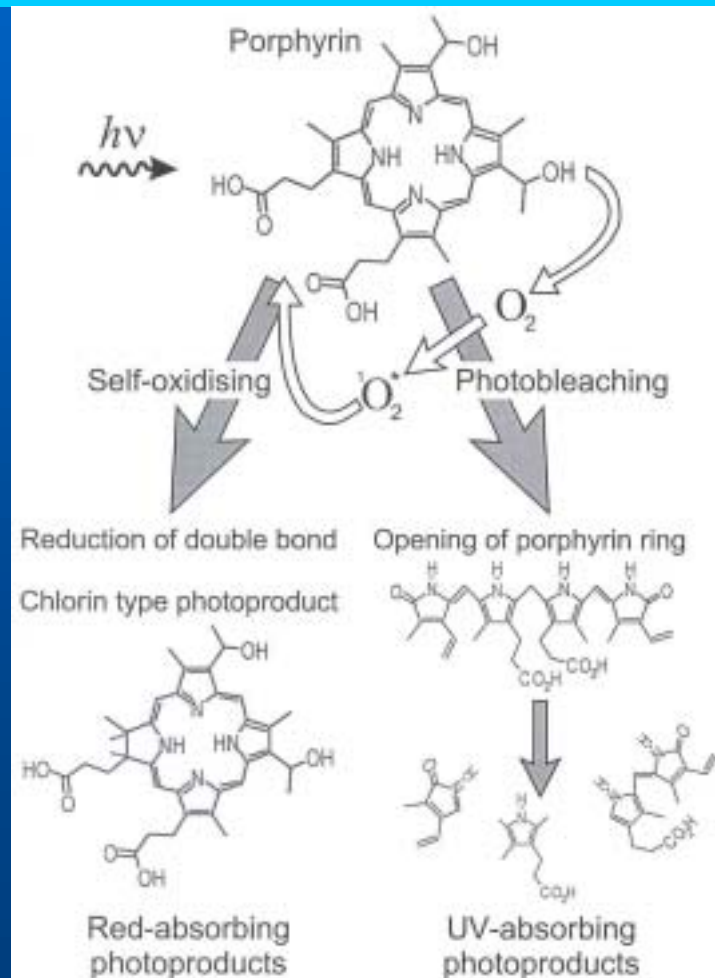


Methods to enhance PpIX production

Temperature



Phototransformations of PpIX



Inhoffen et al. 1969

Cox et al. 1982

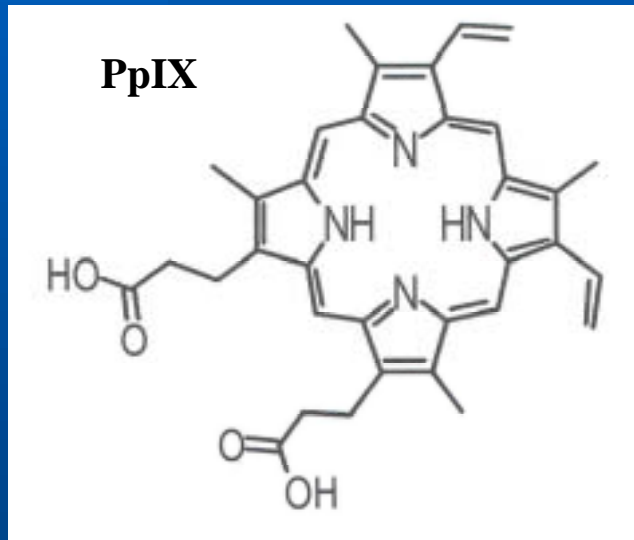
König et al. 1993

Schneckenburger et al. 1993

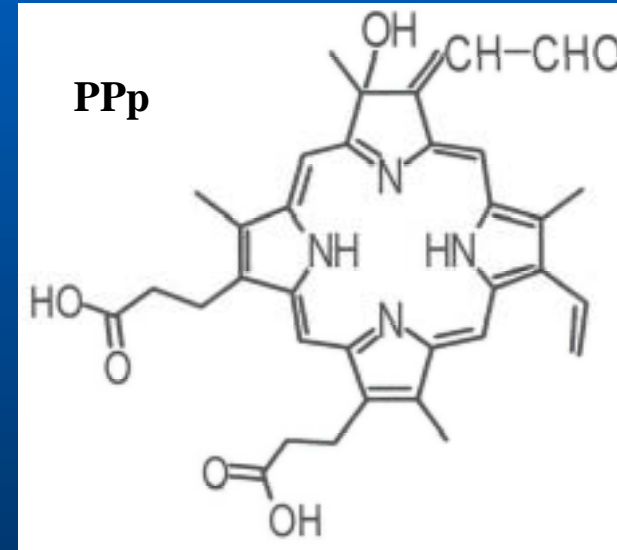
Rotomskis et al. 1997

Phototransformations of PpIX

Protoporphyrin IX

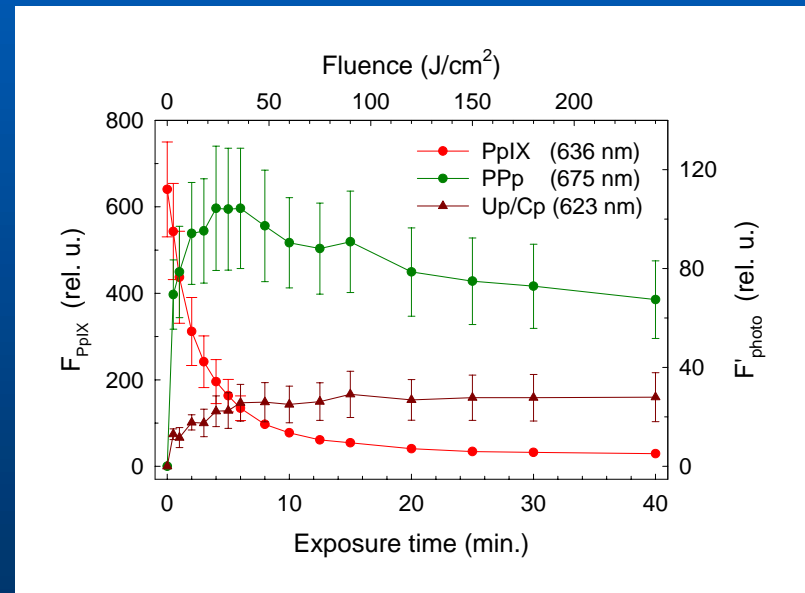
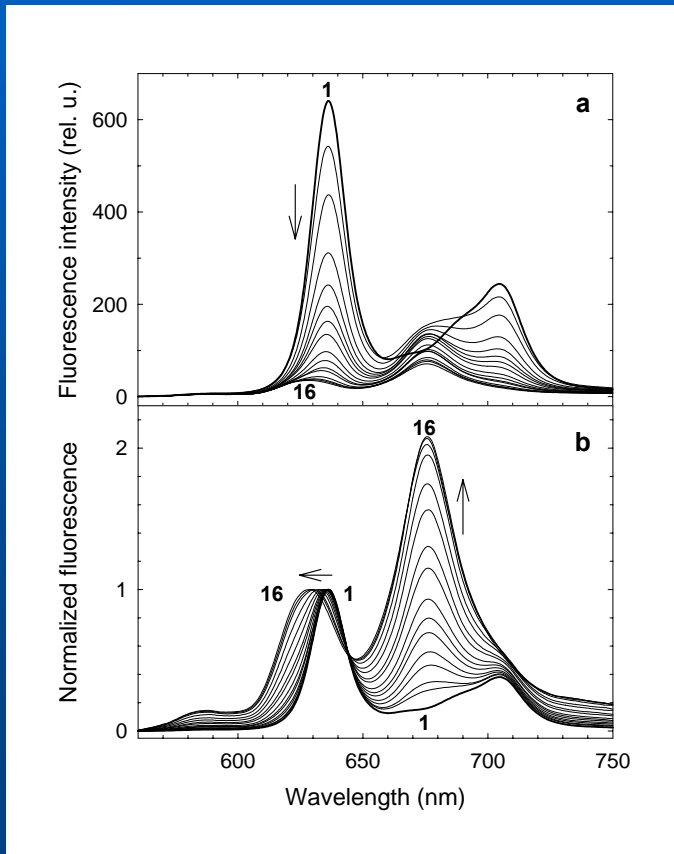


Photoporphyrin

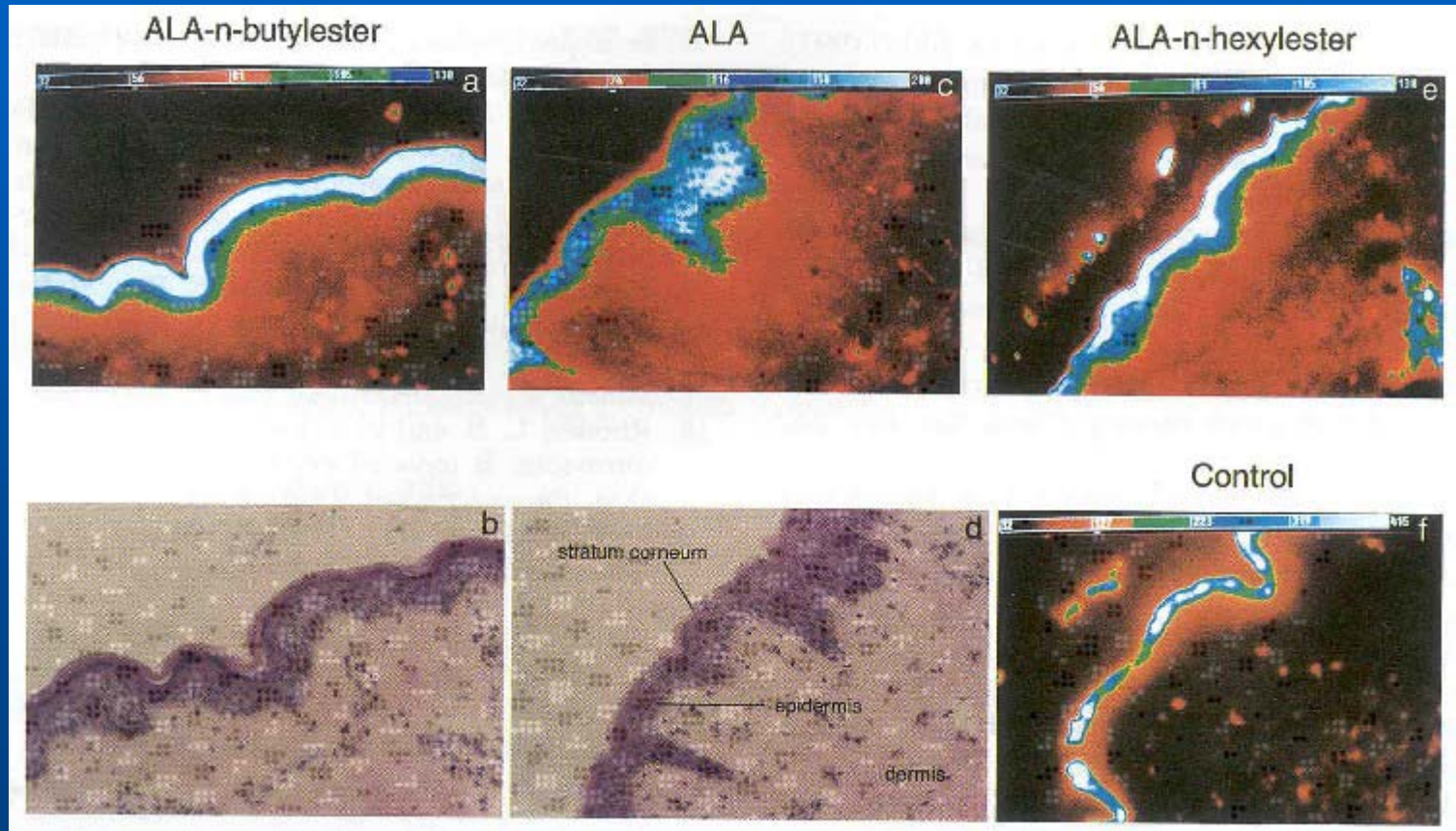


Inhoffen et al. 1969; Cox et al. 1982;
König et al. 1993; Schneckenburger et al. 1993.

Phototransformations of PpIX



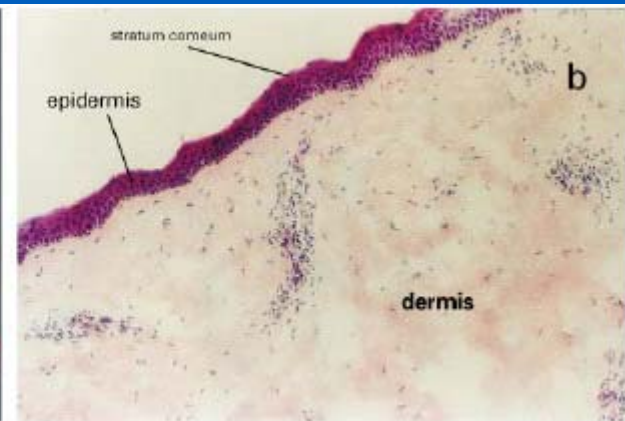
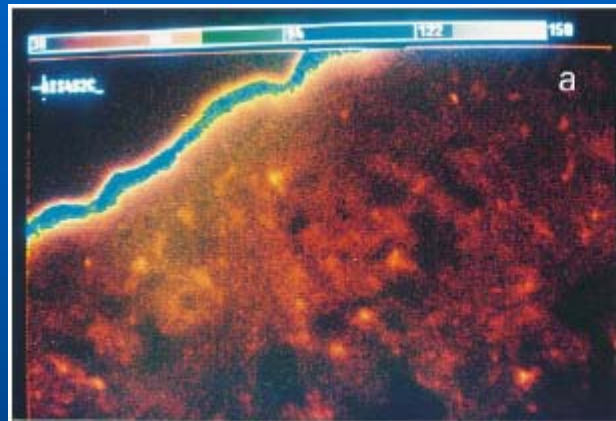
Penetration of ALA and its esters



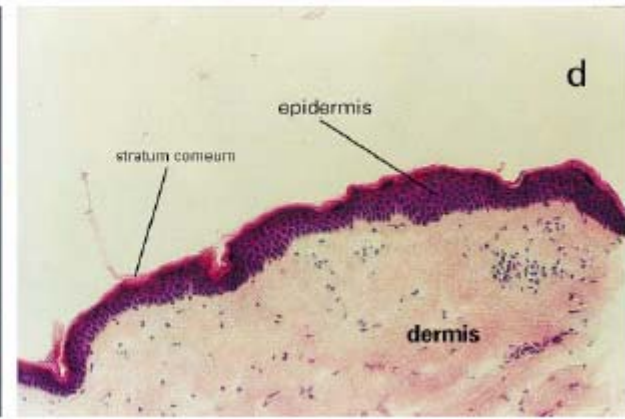
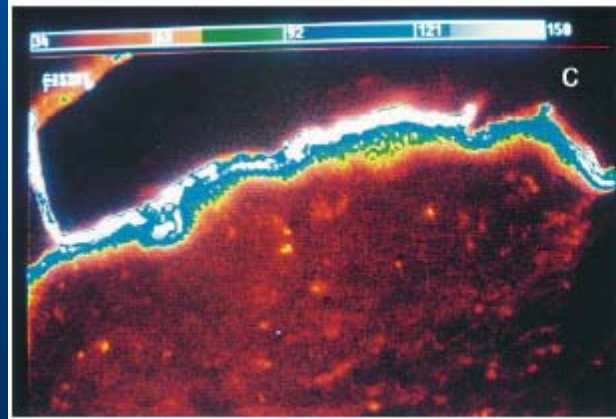
S.Gerscher et al. (2000) *Photochem. Photobiol.* 72, 569-574. (image size 500 x 880 μm)

Penetration of ALA and its esters

ALA



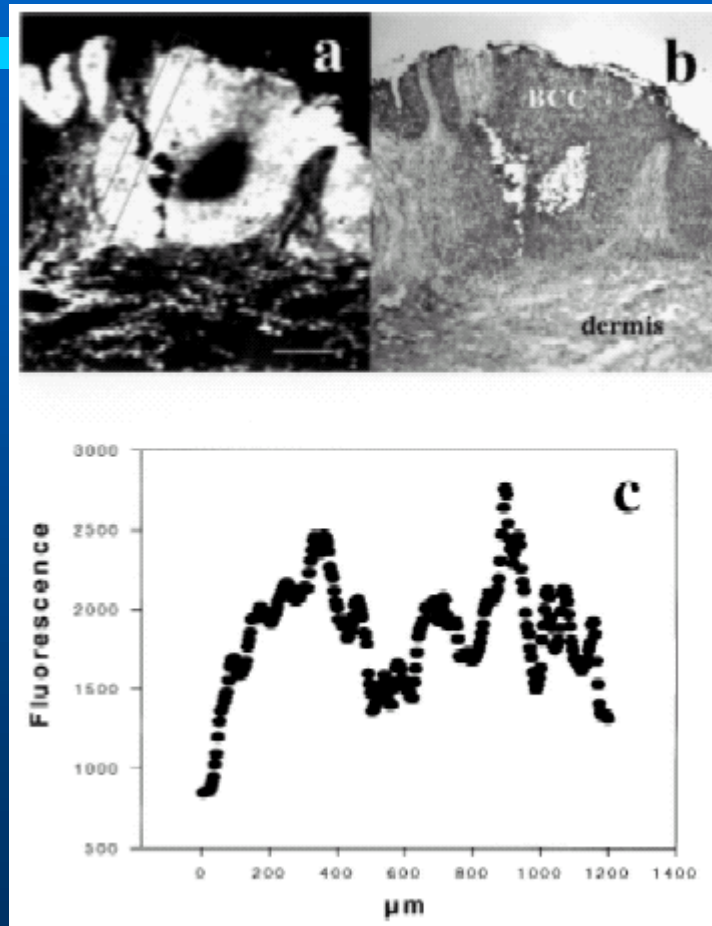
ALA-
pentylester



S.Gerscher et al. (2001) Br. J. Dermatol. 144, 983-990. (image size 500 x 880 μm)

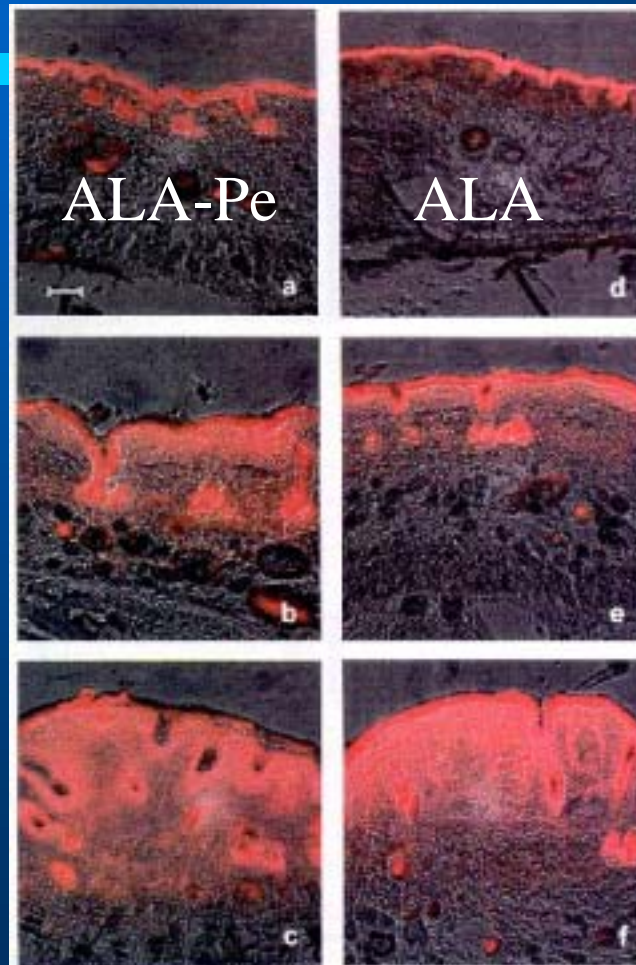
Penetration of ALA and its esters

ALA-Me



Q.Peng et al. (2001) Photochem. Photobiol. 62, 140-145. (bar represents 300 μm)

Penetration of ALA and its esters



Normal skin

UVB-altered skin

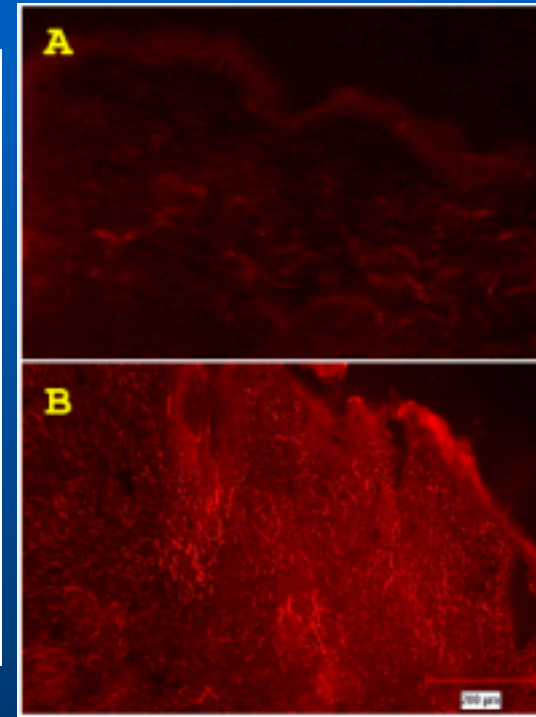
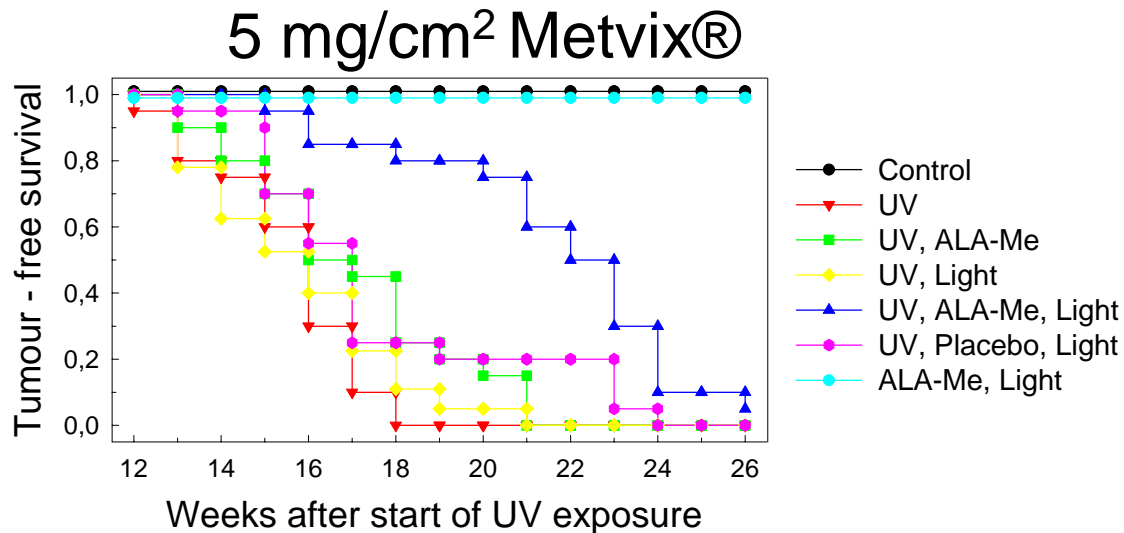
UVB-induced cancer

J. van den Akker et al. (2000) *Photochem. Photobiol.* 72, 399-406. (bar represents 100 μm)

Anticarcinogenic action of ALA

I.M.Stender, N.Bech-Thomsen, T.Poulsen, H.C.Wulf (1997)
Photodynamic therapy with topical δ -aminolevulinic acid
delays UV photocarcinogenesis in hairless mice.
Photochem. Photobiol. 66(4), 493-496 .

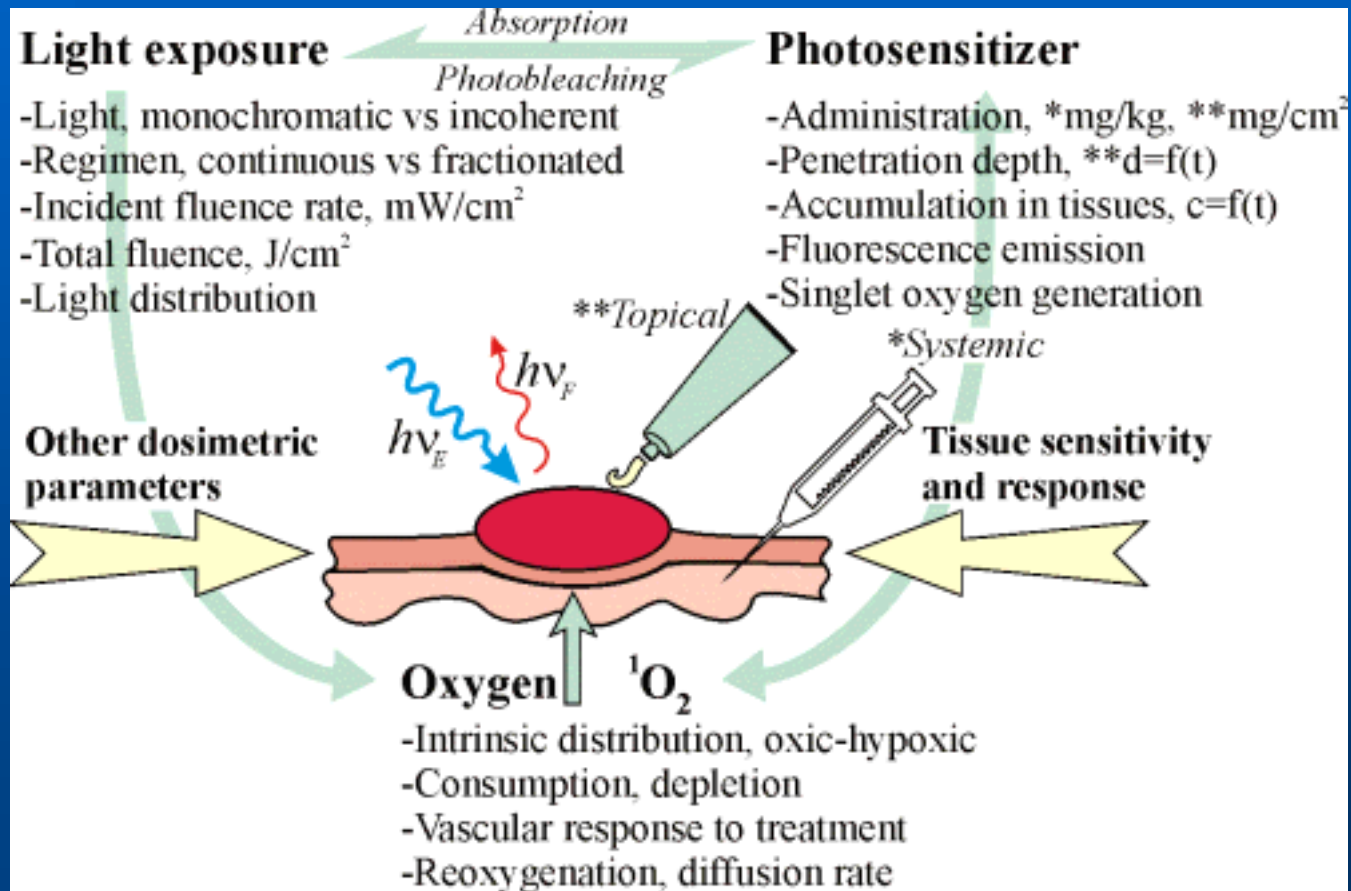
Anticarcinogenic action of ALA-Me



- Some groups of mice were weekly treated on a dorsal skin with 8% ALA-Me cream (5mg/cm², Metvix®, PhotoCure ASA) and 2 hrs later exposed to a white light source at a fluence rate 20 mW/cm² (fluence per mouse 1.2 J/cm²).

S.Sharfaei et al. (2002) Arch. Dermatol. Res. 294, 237-242.

General “conclusion”



Adapted from: B.C.Wilson et al. (1997) Lasers Med. Sci. 12, 182-199.