

PROGRESS IN BIOMEDICAL OPTICS  
**EUROPTO**  
SERIES

*Proceedings of*

---

***Photodynamic Therapy  
of Cancer II***

**Daniel Brault  
Giulio Jori  
Johan Moan  
Benjamin Ehrenberg**  
*Chairs/Editors*

**Abraham Katzir**  
*Biomedical Optics Series Editor*

**9–10 September 1994**  
**Lille, France**

*Sponsored by*

The Commission of the European Communities, Directorate General for Science,  
Research, and Development

INSERM—Institut National de la Santé et de la Recherche Médicale

The Biomedical Optics Society

ELA—The European Laser Association

EOS—The European Optical Society

SBLM—La Société Belge de Laser Médical

SFLM—La Société Française des Lasers Médicaux

SPIE—The International Society for Optical Engineering

*Published by*

SPIE—The International Society for Optical Engineering



**Volume 2325**

SPIE (The Society of Photo-Optical Instrumentation Engineers) is a nonprofit society dedicated to the advancement of optical and optoelectronic applied science and technology.

*P198  
A  
6935*

*1995*



The papers appearing in this book comprise the proceedings of the meeting mentioned on the cover and title page. They reflect the authors' opinions and are published as presented and without change, in the interests of timely dissemination. Their inclusion in this publication does not necessarily constitute endorsement by the editors or by SPIE.

Please use the following format to cite material from this book:

Author(s), "Title of paper," in *Photodynamic Therapy of Cancer II*, Daniel Brault, Giulio Jori, Johan Moan, Benjamin Ehrenberg, Editors, Proc. SPIE 2325, page numbers (1995).

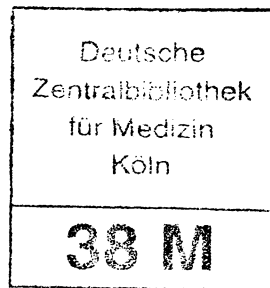
Library of Congress Catalog Card No. 94-67401  
ISBN 0-8194-1658-4

Published by  
**SPIE—The International Society for Optical Engineering**  
P.O. Box 10, Bellingham, Washington 98227-0010 USA  
Telephone 206/676-3290 (Pacific Time) • Fax 206/647-1445

Copyright ©1995, The Society of Photo-Optical Instrumentation Engineers.

Copying of material in this book for internal or personal use, or for the internal or personal use of specific clients, beyond the fair use provisions granted by the U.S. Copyright Law is authorized by SPIE subject to payment of copying fees. The Transactional Reporting Service base fee for this volume is \$6.00 per article (or portion thereof), which should be paid directly to the Copyright Clearance Center (CCC), 222 Rosewood Drive, Danvers, MA 01923. Other copying for republication, resale, advertising or promotion, or any form of systematic or multiple reproduction of any material in this book is prohibited except with permission in writing from the publisher. The CCC fee code is 0-8194-1658-4/95/\$6.00.

Printed in the United States of America.



AKZ. Nr.

~~528212~~

# Contents

ix *Conference Committee*

## SESSION 1 CHEMISTRY OF PHOTOSENSITIZERS

---

- 2 **Synthetic approaches to long-wavelength photosensitizers for photodynamic therapy and their preliminary biological activity** [2325-01]  
R. K. Pandey, Roswell Park Cancer Institute (USA); N. Jagerovic, Univ. of California/Davis (USA); T. J. Dougherty, Roswell Park Cancer Institute (USA); K. M. Smith, Univ. of California/Davis (USA)
- 13 **In vitro photobiological activity of a new series of photosensitizers: the glycoconjugated porphyrins** [2325-02]  
M. Momenteau, D. Oulmi, P. Maillard, A. Croisy, Institut Curie (France)
- 24 **Tetrabenzoporphyrins for PDT** [2325-03]  
Ma. A. Vallés, A. Ma. Gómez, Univ. de Barcelona (Spain)
- 29 **Selective synthesis and photophysical properties of tailor-made chlorins for photodynamic therapy** [2325-04]  
F.-P. Montforts, B. Gerlach, G. Haake, F. Höper, D. Kusch, A. Meier, G. Scheurich, Univ. Bremen (FRG); H.-D. Brauer, K. Schiwon, G. Schermann, Univ. Frankfurt (FRG)
- 40 **Chlorin-type photosensitizers derived from vinyl porphyrins** [2325-05]  
D. Brault, Muséum National d'Histoire Naturelle (France); B. Aveline, O. Delgado, C. Vever-Bizet, INSERM (France)
- 47 **Intra- and intermembrane distribution of chlorin e6 derivatives** [2325-06]  
V. P. Zorin, T. E. Zorina, I. S. Mikhalovsky, I. I. Khludeyev, Byelorussian State Univ.
- 58 **Photomodification of ALA-induced protoporphyrin IX in cells in vitro** [2325-62]  
G. Streckytė, Vilnius Univ. (Lithuania); K. Berg, J. Moan, Institute for Cancer Research (Norway)

## SESSION 2 PHYSICAL CHEMISTRY AND SPECTROSCOPY OF PHOTOSENSITIZERS

---

- 68 **Spectroscopic studies of photosensitization in solutions and in cells** [2325-08]  
B. Ehrenberg, L. Roitman, A. Lavi, Y. Nitzan, Z. Malik, Bar-Ilan Univ. (Israel); J. L. Sessler, Univ. of Texas/Austin (USA)
- 80 **Photophysical characterization of far-red absorbing photosensitizers in cyclodextrin solutions** [2325-09]  
B. Röder, C. Zimmermann, R. Herter, Humboldt Univ. Berlin (FRG)
- 92 **Carrier systems in PDT: on the way to novel antitumor drugs** [2325-10]  
J. G. Moser, A. Heuermann, P. Oehr, Heinrich-Heine-Univ. (FRG); H. Scheer, Ludwig-Maximilians-Univ. (FRG); A. Vervoorts, S. Andrees, Heinrich-Heine-Univ. (FRG)

- 100 **Photodynamic therapy of human skin tumors using topical application of 5-aminolevulinic acid, dimethylsulfoxide (DMSO), and edetic acid disodium salt (EDTA)** [2325-72]  
A. Orenstein, G. Kostenich, H. Tsur, Sheba Medical Ctr. (Israel); L. Roitman, B. Ehrenberg, Z. Malik, Bar-Ilan Univ. (Israel)
- 106 **Comparative in vitro and in vivo measurements of hydrophilic and hydrophobic porphyrins** [2325-12]  
H. Schneckenburger, Univ. Ulm and Fachhochschule Aalen (FRG); R. Sailer, M. H. Gschwend, K. Kunzi-Rapp, A. C. Rück, W. S. L. Strauß, Univ. Ulm (FRG)
- 116 **Time-resolved detection of singlet oxygen luminescence in red cell ghosts generated by photosensitizers via excitation in the far red** [2325-13]  
S. Oelckers, T. Hanke, Humboldt Univ. Berlin (FRG); J. G. Moser, Heinrich-Heine-Univ. (FRG); B. Röder, Humboldt Univ. Berlin (FRG)
- 121 **High-average-power tunable laser system and its long-term performance for photodynamic therapy** [2325-14]  
M. V. Ortiz, T. D. Coleman, R. M. Pon, Laserscope Surgical Systems (USA)
- 133 **Two-step excitation in photosensitized tumor therapy** [2325-15]  
R. Rotomskis, V. Mickūnaitis, D. Juodzevičius, A. S. Piskarskas, Vilnius Univ. (Lithuania)
- 144 **Working out the early diagnostics and controls for the cancer treatment method with the use of photosensitizer of modeling action** [2325-16]  
V. B. Loschenov, General Physics Institute (Russia); R. W. Steiner, Univ. Ulm (FRG)

---

**SESSION 3 ROUNDTABLE: NEW PERSPECTIVES IN PHOTODYNAMIC THERAPY**

---

- 150 **New fluorinated photosensitizers based on tetrakis(hydroxyphenyl)porphyrins** [2325-17]  
R. Bonnett, S. P. Songca, Queen Mary and Westfield College (UK)
- 155 **Immune modulation using transdermal photodynamic therapy** [2325-18]  
J. G. Levy, R. K. Chowdhary, Quadra Logic Technologies Inc. (Canada); L. Ratkay, D. Waterfield, Univ. of British Columbia (Canada); M. Obochi, S. Leong, D. Hunt, A. H. Chan, Quadra Logic Technologies Inc. (Canada)
- 166 **Photodynamic decontamination of blood for transfusion** [2325-19]  
E. Ben-Hur, H. Margolis-Nunno, P. Gottlieb, S. Lustigman, B. Horowitz, New York Blood Ctr. (USA)
- 174 **Lethal photosensitization of *Helicobacter* species** [2325-20]  
C. E. Millson, Univ. College London Medical School (UK); M. Wilson, Eastman Dental Institute for Oral Health Care Sciences (UK); A. J. MacRobert, Univ. College London Medical School (UK); W. Thurrell, Whittington Hospital (UK); P. Mlkvy, C. Davies, S. G. Bown, Univ. College London Medical School (UK)

---

**SESSION 4 PHOTODYNAMIC THERAPY**

---

- 182 **Primary targets in photochemical inactivation of cells in culture** [2325-21]  
K. Berg, S. G. Jones, Institute for Cancer Research (Norway); K. Prydz, Univ. of Oslo (Norway); J. Moan, Institute for Cancer Research (Norway)

- 189 **Kinetics of cellular uptake and retention of the benzoporphyrin derivative (BPD): relevance to photodynamic therapy** [2325-22]  
A. M. Richter, Quadra Logic Technologies Inc. (Canada); H. Meadows, A. K. Jain, A. J. Cnaan, Univ. of British Columbia (Canada); J. G. Levy, Quadra Logic Technologies Inc. (Canada)
- 198 **Comparison of the cell photodynamic sensitivity for the aluminum phthalocyanine of different intracellular localizations** [2325-23]  
E. B. Chernyaeva, M. Yu. Poroshina, Moscow State Univ. (Russia); J. Greve, A. M. van Leeuwen, B. G. de Grooth, Univ. of Twente (Netherlands); A. P. Savitsky, A.N. Bach Institute of Biochemistry (Russia)
- 212 **Discrepancy between photodynamic injuries and pheophorbide A accumulation in digestive tissues** [2325-25]  
S. Evrard, M. Koenig, C. Damgé, J. Marescaux, M. Aprahamian, Hôpitaux Universitaires (France)
- 220 **Pharmacokinetics of the far red absorbing octa- $\alpha$ -butyloxy-zinc phthalocyanine in Lewis lung carcinoma bearing mice** [2325-26]  
C. Dressler, Freie Univ. Berlin and Laser-Medizin-Zentrum Berlin GmbH (FRG); M. S. Ismail, Freie Univ. Berlin (FRG); C. Nowak, Max-Delbrück-Centrum (FRG); R. Herter, Technische Fachhochschule Berlin and Humboldt Univ. Berlin (FRG); R. G. Senz, Laser-Medizin-Zentrum Berlin GmbH and Technische Fachhochschule Berlin (FRG); R. Hagemann, Laser-Medizin-Zentrum Berlin GmbH (FRG); B. Röder, Humboldt Univ. Berlin (FRG); H.-P. Berlien, Freie Univ. Berlin and Laser-Medizin-Zentrum Berlin GmbH (FRG)
- 228 **Active immunotherapy of Walker-256 carcinosarcoma by tumor-infiltrating lymphocytes associated with photodynamic therapy** [2325-27]  
V. F. Dima, Cantacuzino Institute (Romania); V. Vasiliu, Institute of Atomic Physics (Romania); D. Laky, Victor Babes Institute (Romania); P. Ionescu, Military Central Hospital (Romania); S. V. Dima, Cantacuzino Institute (Romania)
- 240 **Clinical photodynamic therapy of malignant neoplasms** [2325-32]  
E. E. Stranadko, O. K. Skobelkin, G. Litwin, T. Astrakhankina, State Research Ctr. for Laser Medicine (Russia)

---

**SESSION 5 POSTER SESSION**

- 248 **Quantum yields of photodegradation of protoporphyrin IX in solution: luminescent, spectrophotometric, and biological testing of photobleaching** [2325-34]  
L. Bezdetsnaya, Ctr. Alexis Vautrin (France) and Russian State Medical Univ.; N. Zeghari, M. Barberi-Heyob, J.-L. Merlin, Ctr. Alexis Vautrin (France); A. Potapenko, Russian State Medical Univ.; F. H. Guillemin, Ctr. Alexis Vautrin (France)
- 259 **Tetrasulphonated aluminum phthalocyanine as a pH-sensitive probe** [2325-36]  
E. B. Chernyaeva, M. Yu. Poroshina, L. V. Zhorina, A. V. Agronskaya, A. Yu. Chikishev, V. A. Schinov, S. Yu. Ardgantsev, Moscow State Univ. (Russia); M. G. Galpern, Moscow Institute of Organic Intermediates and Dyes (Russia)
- 270 **Fluorescence detection system using a krypton laser: application to cancer diagnosis in photodynamic therapy** [2325-37]  
M.-L. Diller, Y. Granjon, Ctr. de Recherche en Automatique de Nancy (France); F. H. Guillemin, Ctr. Alexis Vautrin (France); E. Yvroud, Ctr. de Recherche en Automatique de Nancy (France)

- 277 **Comparative analysis of different phthalocyanine photosensitizers for experimental PDT of cancer** [2325-39]  
M. G. Galpern, I. Novozhilova, G. Sokolova, G. N. Vorozhtsov, Moscow Institute of Organic Intermediates and Dyes (Russia); N. N. Zharkova, D. N. Kozlov, General Physics Institute (Russia); V. V. Sokolov, G. M. Sukhin, P.A. Herten Moscow Research Oncology Institute (Russia)
- 281 **Dynamics of phthalocyanine Al accumulation in stomach cancer photodynamic therapy** [2325-40]  
S. S. Kharnas, Moscow Medical Academy (Russia); V. B. Loschenov, A. A. Stratonnikov, T. A. Kramarenko, General Physics Institute (Russia); O. V. Artemjeva, Moscow Medical Academy (Russia); V. P. Bakonin, M. I. Kuzin, V. Ya. Zavodnov, General Physics Institute (Russia); R. W. Steiner, Univ. Ulm (FRG)
- 290 **Photodynamic therapy of colorectal cancer using a new light source: from in vitro studies to a patient treatment** [2325-41]  
H. Kashtan, R. Haddad, Y. Skornick, Tel-Aviv Medical Ctr. and Sackler School of Medicine (Israel)
- 297 **New chlorin and bacteriochlorine-type photosensitizers for photodynamic therapy** [2325-47]  
A. N. Kozyrev, A. V. Efimov, O. A. Efremova, P. Yu. Perepyolkin, A. F. Mironov, M.V. Lomonosov Moscow State Academy of Fine Chemical Technology (Russia)
- 306 **Combination of photodynamic therapy and x-irradiation: a study on 5-aminolevulinic acid (ALA) radiomodifying properties** [2325-49]  
Z. Luksiene, Lithuanian Oncological Ctr.; K. Berg, J. Moan, Norwegian Radium Hospital
- 312 **Synthesis and properties of ether-bonded porphyrin-chlorin dimers** [2325-50]  
A. F. Mironov, E. G. Levinson, M.V. Lomonosov Moscow State Academy of Fine Chemical Technology (Russia)
- 321 **Fluorescence detection of MSH-receptors in melanoma as a target of hormone-directed photosensitization in PDT** [2325-51]  
S. Roehrs, J. G. Moser, Heinrich-Heine-Univ. (FRG); Y. Salomon, Weizmann Institute of Science (Israel)
- 326 **Photodynamic therapy of human bladder carcinoma cells in vitro with liposomes as a carrier for protoporphyrindisodiumsalt** [2325-52]  
K. Schmidt, U. K. Wenderoth, E. Reich, R. E. Hautmann, Univ. Ulm (FRG)
- 339 **Spectroscopic studies on Si-phthalocyanines and Si-naphthalocyanines** [2325-53]  
C. von Schönemark, A. Volkmer, Humboldt Univ. Berlin (FRG); S. Müller, D. Wöhrle, Univ. Bremen (FRG); B. Röder, Humboldt Univ. Berlin (FRG)
- 349 **Interesting method for the synthesis of monofunctionalized phthalocyanines via subphthalocyanines for photodynamic therapy of cancer** [2325-54]  
R. G. Senz, Technische Fachhochschule Berlin and Laser-Medizin-Zentrum Berlin GmbH (FRG); R. Herter, Technische Fachhochschule Berlin (FRG)
- 351 **Multiple laser irradiation of ORL organ tumors in photodynamic therapy** [2325-55]  
V. V. Shental, N. E. Edynak, N. A. Abdulin, Y. P. Kuvshinov, B. K. Poddubnyi, T. D. Tabolinskaya, Oncological Scientific Ctr. (Russia); V. B. Loschenov, P. V. Poleshkin, E. A. Luk'yanets, General Physics Institute (Russia)

- 355 **Influence of photodynamic therapy on the delay of metastasis development in Lewis lung carcinoma** [2325-56]  
V. Mantareva, Institute of Organic Chemistry (Bulgaria); K. Kassabov, National Ctr. of Oncology (Bulgaria); M. Shopova, Institute of Organic Chemistry (Bulgaria); S. Müller, D. Wöhrle, Univ. Bremen (FRG)
- 364 **First clinical results with a new drug for PDT** [2325-57]  
V. V. Sokolov, V. I. Chissov, E. V. Filonenko, R. I. Yakubovskaya, D. G. Sukhin, P.A. Herten Moscow Research Oncology Institute (Russia); M. G. Galpern, G. N. Vorozhtsov, Moscow Research Institute of Organic Intermediates and Dyes (Russia); A. V. Gulin, M. B. Zhitkova, Research Institute Poljus (Russia); N. N. Zharkova, D. N. Kozlov, V. V. Smirnov, General Physics Institute (Russia)
- 367 **Photodynamic therapy of cancer with the photosensitizer PHOTOGEM** [2325-58]  
V. V. Sokolov, V. I. Chissov, E. V. Filonenko, G. M. Sukhin, R. I. Yakubovskaya, T. A. Belous, P.A. Herten Moscow Research Oncology Institute (Russia); N. N. Zharkova, D. N. Kozlov, V. V. Smirnov, General Physics Institute (Russia)
- 375 **Clinical fluorescence diagnostics in the course of photodynamic therapy of cancer with the photosensitizer PHOTOGEM** [2325-59]  
V. V. Sokolov, V. I. Chissov, E. V. Filonenko, P.A. Herten Moscow Research Oncology Institute (Russia); N. N. Zharkova, D. N. Kozlov, V. V. Smirnov, General Physics Institute (Russia)
- 381 **Experimental studies of the combination of PDT and tumor chemotherapy or <sup>60</sup>Co irradiation** [2325-60]  
J. Didžiapetrienė, G. Prasmickienė, D. Šukelienė, Lithuanian Oncology Ctr.; R. Rotomskis, G. Streckytė, V. Atkočius, L. Stačiokienė, V. Smilgevičius, Vilnius Univ. (Lithuania)
- 389 **Investigation of phtalocyanine Al photosensitizer and blood interaction** [2325-63]  
N. L. Torshina, V. B. Loschenov, A. A. Stratonnikov, A. Yu. Duplik, A. M. Posypanova, General Physics Institute (Russia)
- 391 **Quantitative data on blood flow during tumor PDT obtained by laser Doppler spectroscopy in the hen's egg test system** [2325-65]  
A. Vervoorts, A. Rood, Heinrich-Heine-Univ. (FRG); M. Klotz, Klotz Analytical Registration Techniques (FRG); J. G. Moser, Heinrich-Heine-Univ. (FRG); M. Rosenbruch, Bayer AG (FRG)
- 400 **Fluorescence observations of patients in the course of photodynamic therapy of cancer with the photosensitizer PHOTOSENS** [2325-67]  
N. N. Zharkova, D. N. Kozlov, V. V. Smirnov, General Physics Institute (Russia); V. V. Sokolov, V. I. Chissov, E. V. Filonenko, D. G. Sukhin, P.A. Herten Moscow Research Oncology Institute (Russia); M. G. Galpern, G. N. Vorozhtsov, Moscow Research Institute of Organic Intermediates and Dyes (Russia)
- 404 **Likely mechanism of selective photosensitizer accumulation in malignant tumors: the mathematical model** [2325-07]  
N. V. Stepanova, L. V. Zhorina, E. B. Chernyaeva, Moscow State Univ. (Russia)
- 416 **Pheophorbides as photosensitizers for the photodynamic therapy of tumors** [2325-11]  
C. Tanielian, C. Wolff, Ecole Européenne des Hautes Etudes des Industries (France); M. Kobayashi, Univ. of Tokyo (Japan)
- 425 **Photodynamic therapy of human tubulo-villous adenomas** [2325-73]  
T. Warloe, Q. Peng, H. Heyerdahl, H. Wæhre, J. Moan, H. Steen, K.-E. Giercksky, Norwegian Radium Hospital
- 437 *Author Index*





# Conference Committee

## *Conference Chairs*

**Daniel Brault**, Muséum National d'Histoire Naturelle (France)

**Giulio Jori**, University of Padova (Italy)

**Johan Moan**, Norwegian Radium Hospital (Norway)

**Benjamin Ehrenberg**, Bar-Ilan University (Israel)

## *Session Chairs*

- 1 Chemistry of Photosensitizers  
**Daniel Brault**, Muséum National d'Histoire Naturelle (France)
- 2 Physical Chemistry and Spectroscopy of Photosensitizers  
**Benjamin Ehrenberg**, Bar-Ilan University (Israel)
- 3 Roundtable: New Perspectives in Photodynamic Therapy  
**Giulio Jori**, University of Padova (Italy)
- 4 Photodynamic Therapy  
**Giulio Jori**, University of Padova (Italy)  
**Johan Moan**, Norwegian Radium Hospital (Norway)

## Carrier systems in PDT: On the way to novel anti-tumor drugs

Jörg G.Moser<sup>1</sup>, Anja Heuermann<sup>1</sup>, Peter Oehr<sup>1</sup>, Hugo Scheer<sup>2</sup>, Anja Vervoorts<sup>1</sup>,  
and Sonja Andrees<sup>1</sup>

<sup>1</sup>Institute of Laser Medicine, Heinrich-Heine-University, Universitaetsstr.1  
D-40225 Duesseldorf (Germany)

<sup>2</sup>Institute of Botany, Ludwig-Maximilians-University, Menzinger Str. 67  
D-80638 Munich (Germany)

### Abstract

A novel mode to apply photosensitizing drugs specifically to tumor tissue using the principles of polyphasic tumor therapies is lined out. Key compounds are tumor specific functionalized antibodies with reduced immunogenicity. These bind to drug inclusion complexes in a multiplicative manner. Drug inclusion complexes are designed on the basis of tethered functionalized  $\beta$ -cyclodextrin dimers with maximum affinity to porphyrinoid photosensitizers forming monomeric clathrates. To enhance porphyrin-cyclodextrin interaction peripheral groups of the porphyrin have to be chemically modified. The development of the method is not yet completed. First results are demonstrated.

### Introduction

In a tumor-bearing organism there are two general routes by which photosensitizers find their target tissue: water soluble sensitizers (e.g. the polysulphonated m-tetraphenyl porphines or phthalocyanines) enrich in tumor tissue according to pH-equilibria of their differently ionized forms (1). Apolar photosensitizers use the lipoprotein pathway to be taken up as complexes via the lipoprotein receptors of the tumor cells (2). Both these pathways allow for targeting of healthy organs and lead to the wellknown side effects of PDT as well as of general chemotherapy.

To avoid these side effects we started with the simple consideration to construct a porphyrinoid -containing *inert drug which cannot be taken up by any living cell*. Such a drug should be functionalized in a manner that it can be bound exclusively to tumor cells. The stability of such a drug should be sufficiently high to prevent interaction with the known distribution pathways.

### Water soluble complexes: cyclodextrins

Cyclodextrins are wellknown to form soluble complexes with several water-insoluble drugs (3). For example,  $\alpha$ -cyclodextrin is admitted as an aid to solubilize prostaglandin E 1 for injections,  $\gamma$ -cyclodextrin has been used successfully to solubilize tin etio-purpurin for PDT purposes (4). In general, aggregates of apolar photosensitizers dissolve in cyclodextrin solutions: into monomeric host-guest complexes (fig. 1). Highly symmetrical apolar phthalocyanins and porphycenes achieve an orientation towards the substructures of the cell membrane, and this results in a greatly enhanced uptake velocity by the cell (fig. 2). Despite the considerable stability of the 1:1 host-guest complexes (fig. 3) an interaction with the lipoprotein pathway is pre-programmed (4).

Fig.1 Disaggregation equilibria of pheophorbide a at rising CD concentrations.

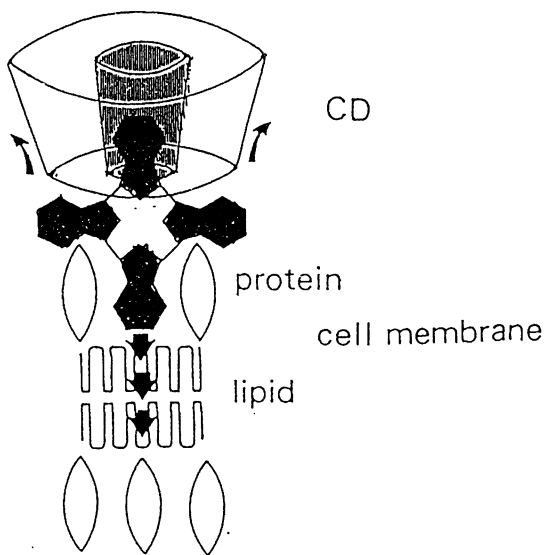
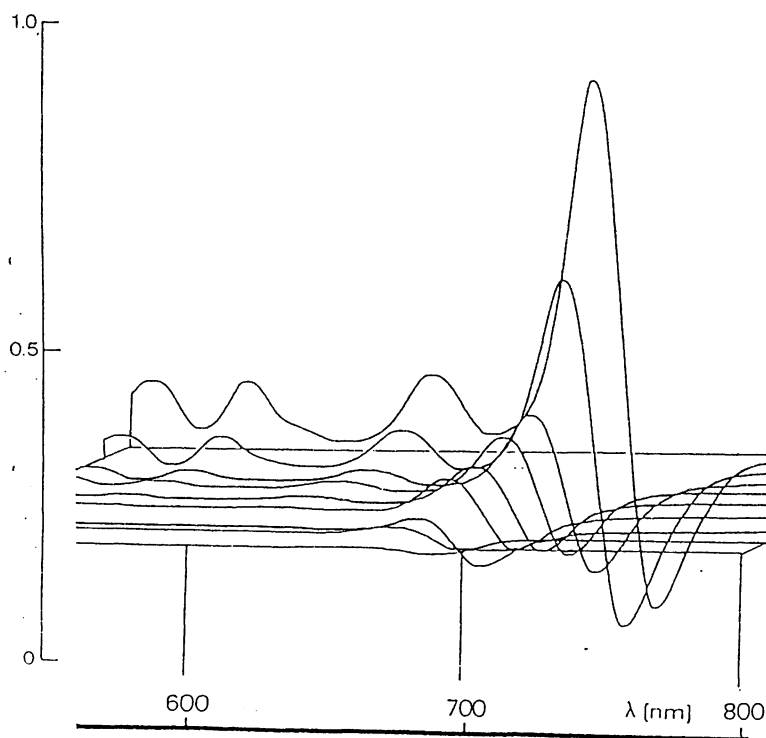


fig.2 Acquiring polarity by binding to CD of an apolar sensitizer, phthalocyanine.

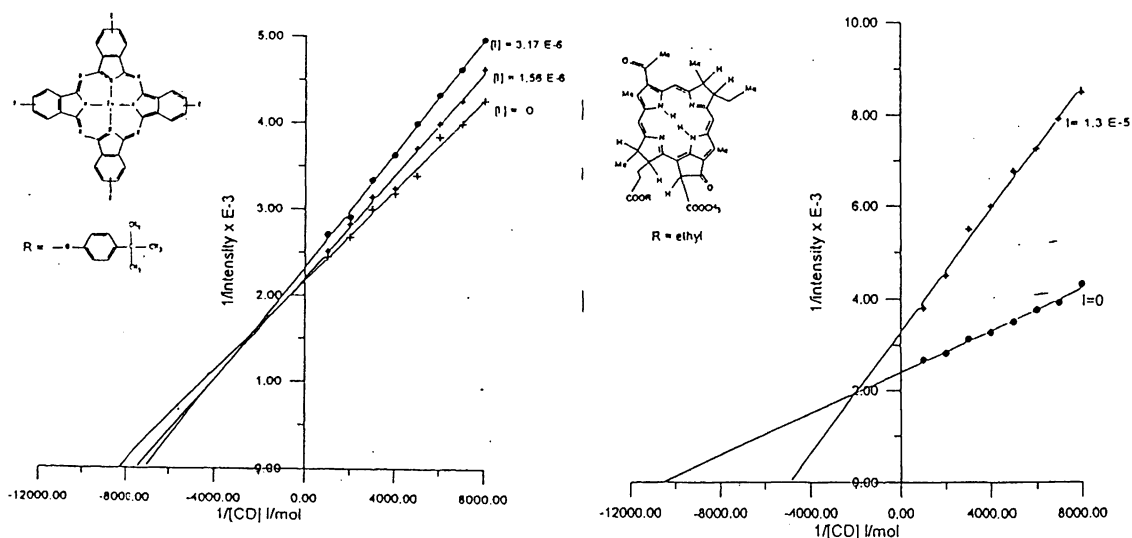


fig. 3 Association equilibria of tetra-t-butyl-phenoxyphthalocyanine and BPEE as revealed by TNS-exclusion fluorescence technique.  $K_D$  (TTBP) =  $14.8 \times 10^{-6}M$   
 $K_D$  (BPEE) =  $8.3 \times 10^{-6}M$

## Stabilization of the complex: cyclodextrin oligomers

As a signpost towards stable complexes, BRESLOW and CHUNG (5) investigated the complexation of a very simple symmetrical diester, propane diol p-tert.butyl benzoic acid diester, with a dimeric "clamshell"  $\beta$ -cyclodextrin. The stability of the complex exceeded the expected value by two orders of magnitude and resulted in a  $K_D = 10^{-10}M$  comparable with the complex stability between antibodies and their antigens. In the mean time several other groups constructed cyclodextrin dimers mainly to study and to mimic enzyme-like activities. Our first model of a more flexible cyclodextrin dimer to complex porphyrinoid compounds (6) has been reconstructed to a box-of-bricks principle (fig. 4): starting from tosylated  $\beta$ -cyclodextrin (at positions 2 or 6) we substitute by nucleophilic attack with alkyl diamines of short chain length and connect the resulting N-alkyl amines with homobifunctional reagents to form  $\beta$ -cyclodextrin dimers with variable extension including the possibility to introduce preformed break-points to finally break the complex at the target site.

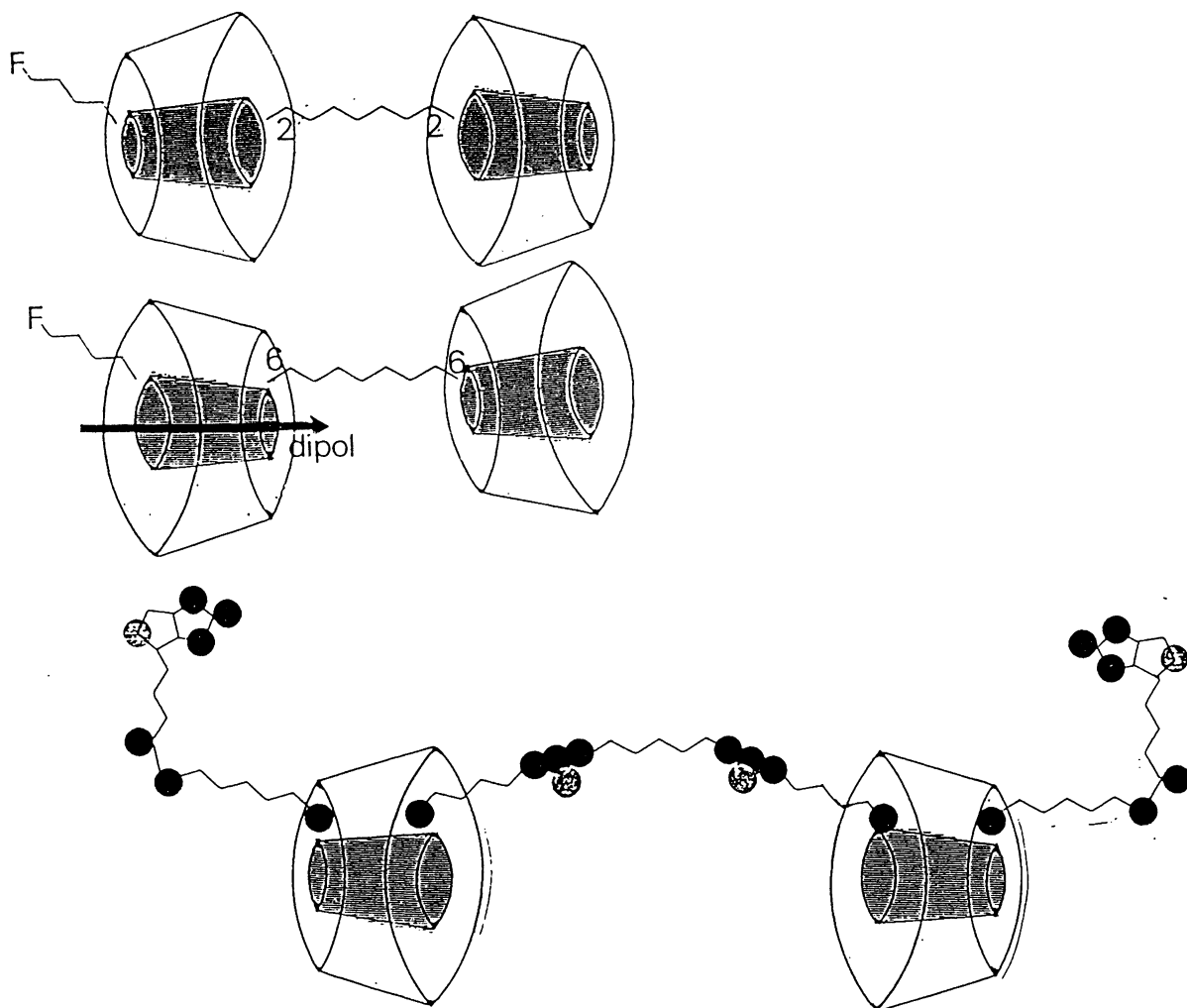


fig. 4 Orientation of CD's against guests and bridge forming modalities to achieve optimum binding

## High affinity complexes: anchor structures

Porphyrins normally contain very short side chains which allow only poor interactions with the extended lipophilic cavity of  $\beta$ -cyclodextrin. More voluminous side chains, like the p-tert.butyl phenyl group, may allow for much higher stability of the host-guest complex, and by the same time may enhance the dipole momentum of the included drug (7). At the moment we try to substitute pheophorbide a in C-3<sup>1</sup> with a p-tert.butyl phenyl ether according to the general method given by PANDEY et al (8) in order to better understand anchor functions (fig.5).

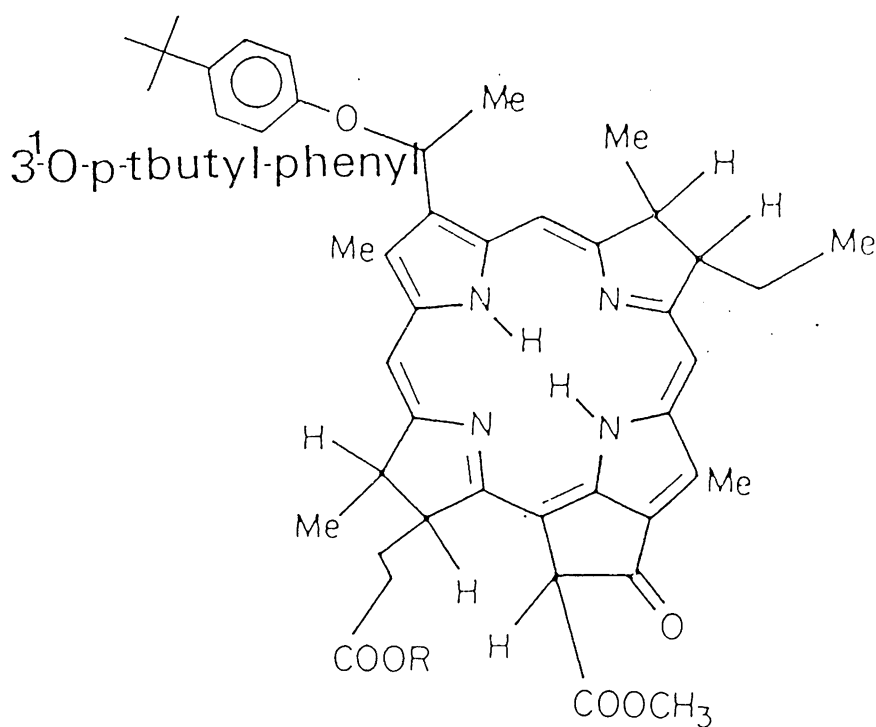


fig. 5 Substitution of apolar side chains may enhance complex stability

## Functionalization of the complex: biotin-avidin complexes

The biotin-avidin system is wellknown to improve considerably the tumor localization by radioscintigraphic methods (9). Thus, biotinylation of the drug-cyclodextrin complexes as well as of monoclonal tumor-localizing antibodies is investigated in our group. An anti-CEA antibody, BW 431/26, has been biotinylated successfully with up to 7 biotin molecules without losing its affinity to the antigen localized on the surface of LoVo adenocarcinoma cells. Biotinylation of  $\beta$ -cyclodextrins in position 6 over a  $C_6$ -spacer was easily achieved using the methods yet described.

## Immunological problems: immunogenicity of foreign proteins

The introduction of foreign proteins, avidin from hen's egg white and mouse monoclonal antibodies, into the system raises immunological problems. BOSSLET et al.(10) circumvent the problem by constructing an ingenious fusion protein to be applied with the Antibody Directed Enzyme Prodrug Therapy (ADEPT) splitting an adriamycin glucuronide at the target site by a humanized antibody- $\beta$ -glucuronidase complex. This is beyond our today facilities.

So we study the reduction of immunogenicity by coupling our proteins with unifunctional m-polyethylene glycols (11) which in a modified form may be used as additional centers to accumulate the drug complexes.

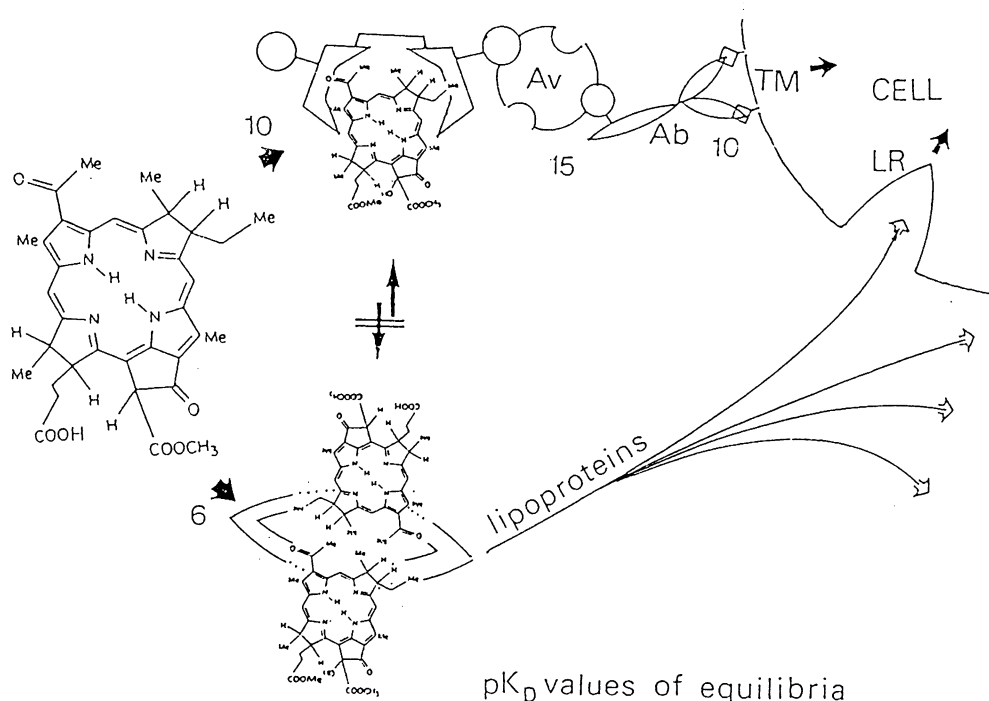


fig. 6 High stability of inclusion complexes prevents sensitizer transport on the lipoprotein pathway

## Outline of the targeting system: polyphasic PDT

The intended targeting approach is closely related to the ADEPT concept just in phase II-studies in Great Britain and in Germany. In a first step, biotinylated antibodies with tumor-localizing properties are injected and then cleared for some days as controlled by scintigraphy. In a second step avidin or just avidin-sensitizer chlathrate complexes can be injected at very low concentrations due to the high affinity to biotin. We expect concentrations as low as  $10^{-12}\text{M}$  to be able to saturate the antibody-bound biotins excluding any toxicity of  $\beta$ -cyclodextrin. The multiplicative effect due to multiple binding sites of avidin and the polyvalency of the drug complexes should allow for an accumulation of  $10^6$  to  $10^8$  molecules of sensitizer per tumor cell assuming  $5 \times 10^4$  antigenic binding sites. This is fairly in the range of phototoxic concentrations and should allow for irreversible damage by laser irradiation (fig. 6).

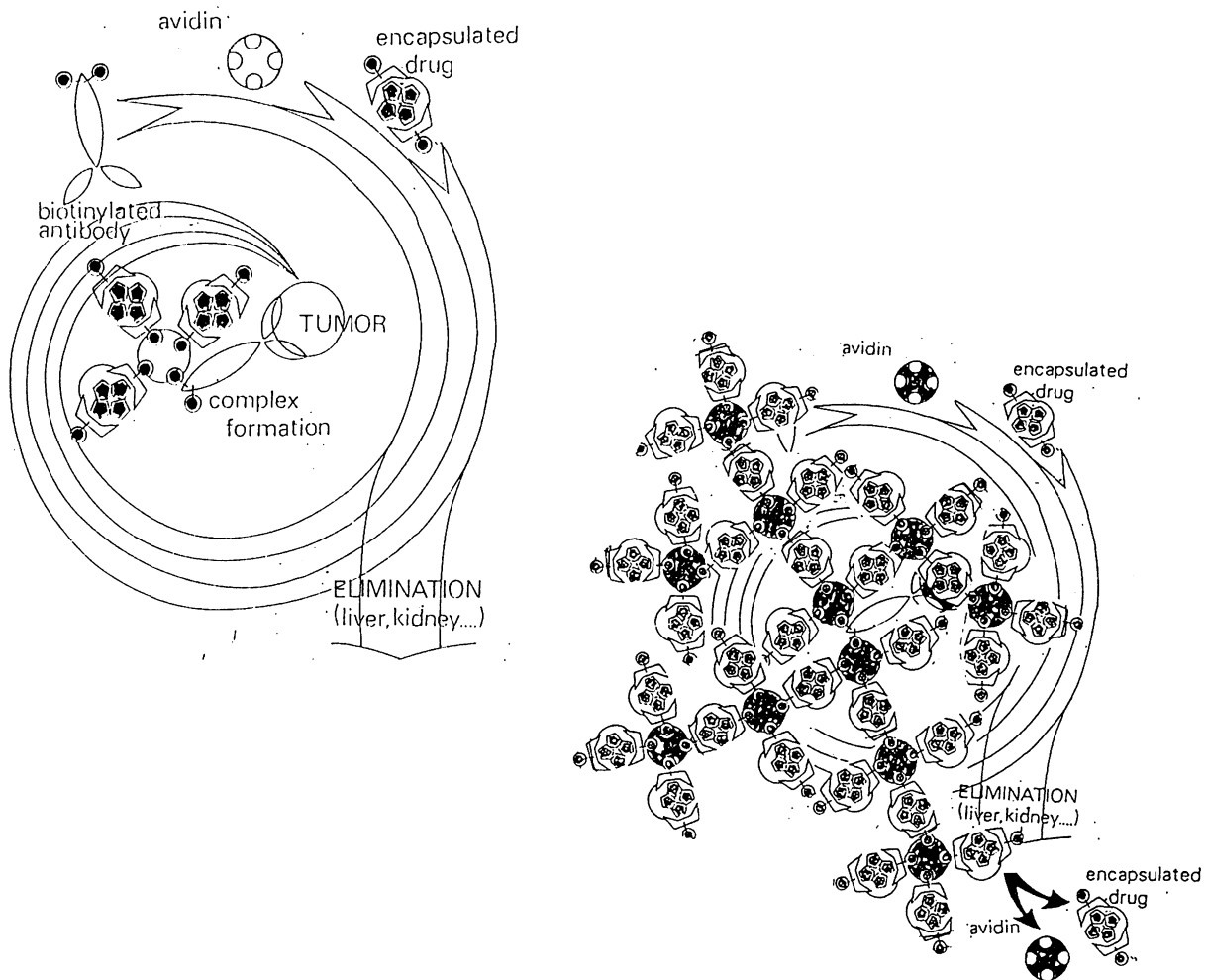


fig. 7 Sequential administration of antibodies and drug complexes leads to accumulation of sensitizer due to polyvalency of the single compounds

## A glance over the plate's border: tumor chemotherapy

We should take in mind that complexation of drugs by cyclodextrins is originally invented to solubilize water-insoluble drugs in general. Porphyrinoid binding is only one of the possible applications. Looking over the manifold of today anti-cancer agents there are several potent but harmful drugs which could be detoxified by inclusion into cyclodextrin oligomers and, by chance, transferred to its target without considerable side effects. Moreover, there are several potent anti-cancer drugs which cannot be given to a patient because of their high general toxicity. Some of them are porphyrin derivatives. So, in future such a system may be helpful to revive and to modify general chemotherapy to a curative modality without its inherent side effects.

### Literature cited

- (1) Pottier R, Kennedy JC (1990): The possible role of ionic species in selective biodistribution of photochemotherapeutic agents toward neoplastic tissue. *J. Photochem. Photobiol.* **8**, 1-16
- (2) Hamblin MR, Newmann EL (1994): On the mechanism of the tumor-localizing effect in photodynamic therapy. *J. Photochem. Photobiol.* **23**, 3-8
- Jori G, Reddi E (1993): The role of lipoproteins in the delivery of tumour-targeting photosensitizers. *Int.J.Biochem.* **25**, 1369-1375
- (3) Pitha J (1989): Cyclodextrins: solutions to insolubility. *Neurotransmissions* **5**, (1) 1-4
- (4) Kessel D, Morgan A, Garbo GM (1991): Sites and efficiency of photodamage by tin etiopurpurin in vitro using different delivery systems. *Photochem. Photobiol.* **54**, 193-196
- (5) Breslow R., Chung S (1990): Strong binding of ditopic substrates by a doubly linked occlusive C<sub>1</sub>"clamshell" as distinguished from an aversive C<sub>2</sub>"loveseat" cyclodextrin. *J.Am.Chem.Soc.* **112**, 9659-9660
- (6) Moser JG, Haller C., Herchenbach B, Vervoorts A (1991): How to prevent loss of photosensitizer into non-cancer tissues? *Abstr. 4th Congr.ESP, Amsterdam.* A 12
- Moser JG, Vervoorts A, Billen A (1992): Molecular encapsulation of pheophorbides as chlathrates in dimeric cyclodextrins. *Photodynamic Therapy and Biomedical Lasers* (Spinelli ed.) 760-763
- (7) Schnurpfeil G.(1994) unpublished results
- (8) Pandey RK, Shian FY, Sumlin AB, Dougherty TJ, Smith KM (1992): Structure/activity relationship among photosensitizers related to pheophorbides and bacteriopheophorbides. *Bio.Medicinal Chem.Lett.* **2**, 491-496
- (9) Oehr P., Liu Q, Schultes B, Altreuther M, Pollok J, Biersack HJ (1990): Effect of streptavidin on the localization of tumors by 99m-Tc-NHS-biotin. *Conf.Proc. Bad Gastein Vol.* 19



- (10) Bosslet K, Czech J, Lorenz P, Sedlacek HH, Schuermann M, Seemann G.(1992):  
Molecular and functional characterization of a fusion protein suited for tumour specific  
prodrug activation.  
Br.J.Cancer **65**, 234-238
- Bagshawe KD (1987): Antibody directed enzymes revive anticancer prodrugs concept.  
Br.J.Cancer **56**, 531
- (11) Abuchowski A, Van Es T, Palczuk NC, Davis FF (1977): Alteration of immunological  
properties of bovine serum albumin by covalent attachment of polyethylene glycol.  
J.Biol. Chem. **252**, 3578-3581

#### Acknowledgements

This work is granted by a fellowship of the Ministry for Research and Technology (BMFT) of the Federal Republic of Germany. The fruitful cooperation with all groups of the cooperation "Photodynamische Tumortherapie", in particular the groups of B. Roeder (Berlin), D. Wöhrle (Bremen), A Rück (Ulm), and E. Vogel (Köln) is thankfully acknowledged.