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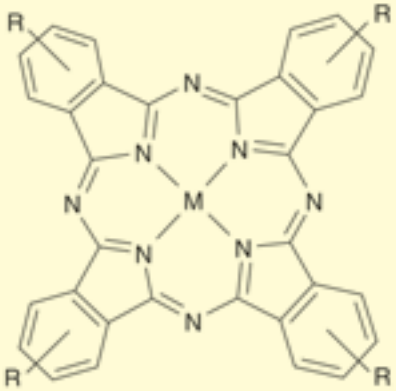
Tetrapyrroles

Photophysical Properties and Light Induced Transfer Processes

Tetrapyrroles (Fig. 1) play an important role in most biological processes of energy and electron transfer like photosynthesis or in the respiratory chain. On the other hand beside natural tetrapyrroles artificial tetrapyrroles like phtalocyanines become more and more important not only as colours but also as photoactive compounds in photovoltaic, photomedicine, molecular electronics and others. Because of their key role for light induced transfer processes in nature and their importance in constructing biomimetic systems the scientific interest of the group is focused on the investigation of electronic properties of these substances under very different conditions.



Artificial tetrapyrrole: phthalocyanin (M: 2H or metal)

Fig. 1
Structural formula of tetrapyrroles

The actual work is focused on the following problems:

1. Characterization of higher excited electronic states of tetrapyrroles

This includes the investigation of non-linear absorption (use as saturable absorbers) as well as that of anisotropy changes of molecules and molecular aggregates in different microenvironments (transient dichroism).

2. Development of 3rd generation photosensitisers for Photodynamic Therapy (PDT)

The goal of these investigations is the development of efficient modular carrier systems containing high branched multiplying units.

3. Investigation of different interaction mechanisms of tetrapyrroles in molecular assemblies after light absorption

These activities are focused on characterization of the assemblies organization, the investigation of energy and electron transfer processes and the structure-activity-relation (experiment and theory) after embedding of functionalised tetrapyrroles in inert molecular structures.

TETRAPYRROLE – Photophysikalische Eigenschaften und lichtinduzierte Transfer-Prozesse

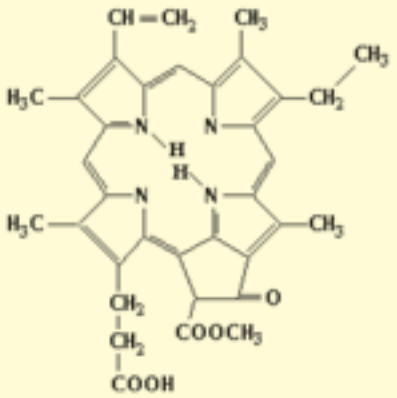
Im Mittelpunkt des Interesses der Arbeitsgruppe »Photobiophysik« stehen die besonderen elektronischen Eigenschaften von Tetrapyrrolen, die eine Schlüsselstellung in Energie- und Elektronentransferprozessen lebender Systeme einnehmen. Neben Untersuchungen zu grundlegenden klassischen und nichtlinearen optischen Eigenschaften von Einzelmolekülen werden insbesondere Fragen der Wechselwirkung zwischen Tetrapyrrolmolekülen (zweidimensionale Aggregate) in und mit ihrer mikroheterogenen Umgebung (z.B. Liposomen, LB-Schichten) untersucht. Im Mittelpunkt stehen folgende Probleme:

- Vergleichende Charakterisierung elektronisch höher angeregter Zustände von Metallo-Porphyrinen und -Phthalocyaninen;
- Entwicklung und photophysikalische Charakterisierung von Photosensibilisator-Carrier-Komplexen für die PDT;
- Charakterisierung und Optimierung biomimetischer Systeme für die lichtinduzierte Elektronenübertragung in anisotropen Medien (Photosynthese-Modellverbindungen).

Mit diesen Untersuchungen können zum einen Beiträge zum besseren Verständnis von Struktur-Wirkungs-Beziehungen dieser Moleküle geleistet und andererseits Erkenntnisse zur Herstellung und Optimierung biomimetischer Systeme gewonnen werden.

Characterization of higher excited electronic states of tetrapyrroles – *Polarization Sensitive Jablonski Diagram*

Beside other photophysical parameters photoinduced anisotropy and its dynamics are widely studied in the laser spectroscopy of organic dyes. Anisotropy data particularly provide an insight into the nature of interactions between the dye and its microenvironment. For this reason, time-resolved experiments on photoinduced anisotropy are well-established methods in fluorescence and absorption spectroscopy [1] and are widely used in biophysical research [2]. A convenient scheme to describe the kinetics of electronic relaxation is the Jablonski diagram shown in Fig. 2a. It describes intra- and intermolecular electronic transitions by a set of differential rate equations. Contrarily, the kinetics of the photoinduced anisotropy in liquids is usually described in terms of a rotational Brownian motion of free rigid bodies, yielding in an equation of rotational diffusion [3]. Due to the complexity of the problem, the solution of this diffusion equation is critically dependent on the molecular symmetry, the number of relevant molecular electronic levels, and the initial anisotropy after excitation. Extensive work has been done on fluorescence anisotropy [2–5], for which the population dynamics of only one electronic state (S_1) needs to be taken into account, and the small signal limit applies (i.e. a $\cos^2$ distribution of initially excited molecules). Certainly, the small signal limit approximation cannot be employed in nonlinear spectroscopy, such as transient absorption experiments. Moreover, the population dynamics of more than just one electronic state are monitored in most spectroscopic techniques. Ansari and Szabo [6] developed a unified treatment of non-$\cos^2$ type initial distributions for



Natural occurring tetrapyrrole: pheophorbide a

transient absorption experiments using power expansions to solve the equation of rotational diffusion.

In 1971, Weber [7] suggested an alternative approach to rotational diffusion, which he called the Discontinuous Distribution Approach (DDA). A major advantage of DDA is that the initial conditions enter the calculation only as a set of numerical parameters, thus not affecting the algorithm as in the diffusion approach. So far, DDA has been rarely applied and exclusively to evaluate fluorescence anisotropy experiments. Yet, the idea to model the process of rotational diffusion by a series of arbitrary jumps between discrete states fits nicely into the concept of the Jablonski diagram. In [8], we developed a generalization of the Jablonski diagram to account for rotational diffusion using a DDA like method, which we called the »*Polarization Sensitive Jablonski Diagram*« (PSJD). The main advantage of this model is its easy customization to special requirements, as the Jablonski diagram itself can be easily extended to any possible electronic states and transitions in (supra-) molecular systems. For this reason, the PSJD appears to be much more suitable for applications as transient absorption spectroscopy, which are monitoring polarization dependent various states and transitions.

The model presented in [8] is restricted to the simple case of spherical rotors. However, it can be easily expanded to account for rotors of lower symmetry. A typical Jablonski diagram is shown in Fig. 2a, summarizing the essential electronic transitions after photoexcitation of the molecule. To account for rotational diffusion of a spherical rotor, the Jablonski diagram is expanded as shown in Fig. 2b. According to DDA, rotational diffusion can be described by 90-degree jumps between the principal molecular orientations in relation to the laboratory coordinates [7].

For spherical rotors, one principal molecular orientation per axis has to be considered. Each electronic state of the Jablonski diagram has thus to be divided into three sublevels which represent the projections of the orientational distribution of molecules in this state onto the laboratory axes *x*, *y*, and *z*. Rotational diffusion is only considered between sublevels of the same electronic state. The case of a simultaneous electronic and »rotational« transition is excluded because the electronic transition time of a single molecule is short compared to rotational motion.

In case of spherical rotors, the jump rates between the sublevels of a single electronic state are equal. However, they might differ for different electronic states, as reported in the literature [1]. Thus, rotational diffusion is modeled by a constant »flow« of molecules between the three sublevels, represented by three identical rate constants K^i_{rot} for the *i*th electronic state. If the populations of the three sublevels are different, i.e. in case of an anisotropic orientational distribution in the *i*th state, the net flow leads to an equalization of populations. In case of an isotropic distribution, the net flow vanishes. Thus, given an initial distribution of molecules, the state of the system at subsequent times can be derived easily from solving (analytically or numerically) the system of rate differential equations arising from the PJSD.

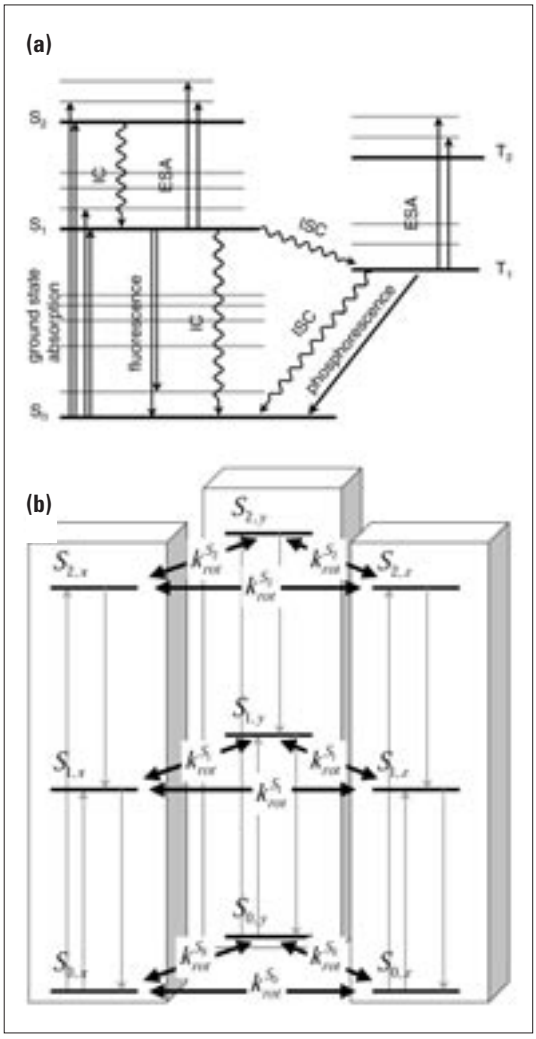


Fig. 2
(a) Jablonski diagram of organic dyes: IC – internal conversion, ESA – excited state absorption, ISC – intersystem crossing. (b) Polarization Sensitive Jablonski Diagram for a spherical rotor: the three sublevels of each electronic states are connected by »rotational« transitions (to avoid confusion, only the singlet system from Fig. 2 is shown). [After 8]

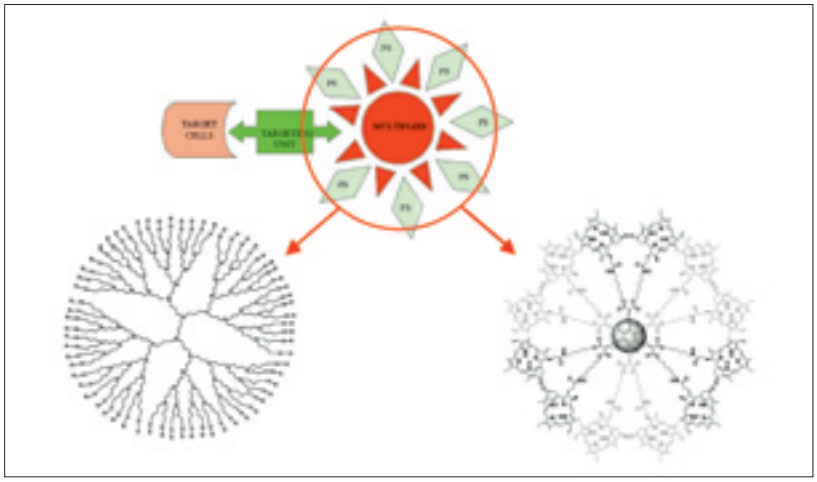


Fig. 3
Principle schematic draw of a Modular Carrier System. Starburst dendrimers and fullerene-dendrons were found to be very suitable for use as multiplying units in such systems. (Source: see [8]; Reproduced by permission of the PCCP Owner Societies)

The Polarization Sensitive Jablonski Diagram provides a highly flexible tool to model experiments generating an anisotropy in the orientational distribution of photo-excited molecules. It facilitates the calculation of the time-dependent population densities and orientational distributions of molecules in any desired electronic levels including the ground state, and works for arbitrary initial distributions of excited molecules. Thus, it is easy to include other initial distributions than the $\cos^2$ distribution of the small signal limit, a fact that makes this approach surpass others. In addition, it is expected to obtain a more precise determination of transition dipole moments from excited states by applying the new model to polarization-dependent pump-probe experiments. To accommodate for such experiments, the total anisotropy of the signal can be derived from the superposition of the anisotropies of the single transitions that are probed. In addition, the influence of the probe pulse on the population density and the anisotropy of the considered electronic level can be calculated with this model.

Photodynamic Therapy

Photodynamic Therapy (PDT) is an effective treatment of different types of cancer, virus infections, skin diseases and others using photosensitisers (PS) which are non toxic in the dark but become phototoxic following activation by low energy light. The PS is applied topically or systemically and should be selectively accumulated in the target tissue. The molecular mechanism of the therapy is based on the *Type II* pathway of photosensitization – the photodynamic effect: following light absorption energy is transferred from the photosensitiser to molecular oxygen generating singlet molecular oxygen ($^1O_2$) which causes directly or indirectly the photodamage of the target tissue.

The limiting factors of the efficiency for this treatment are the photophysical properties of the PS, the oxygen concentration during the treatment and the localization of the PS molecules in the tissue. Due to their special electronic properties – high intersystem crossing and singlet oxygen quantum yields – the most important chemical class of potent photosensitisers are tetrapyrroles. After twenty years of clinical PDT a variety of very efficient tetrapyrrole based PS exist with excellent photophysical properties. The unsolved problem today is the »right« location of PS in the cell or at the cell surface. To solve this problem different strategies are employed. One possibility is the use of drugs specifically designed for treatment of one disease. Another possibility to get high concentrated and selective biologically defined accumulation of PS is the use of modular carrier systems (Fig. 3). The intention of this concept is the attachment of PS molecules to one addressee and one multiplying unit – the so-called 3rd generation PS [9].

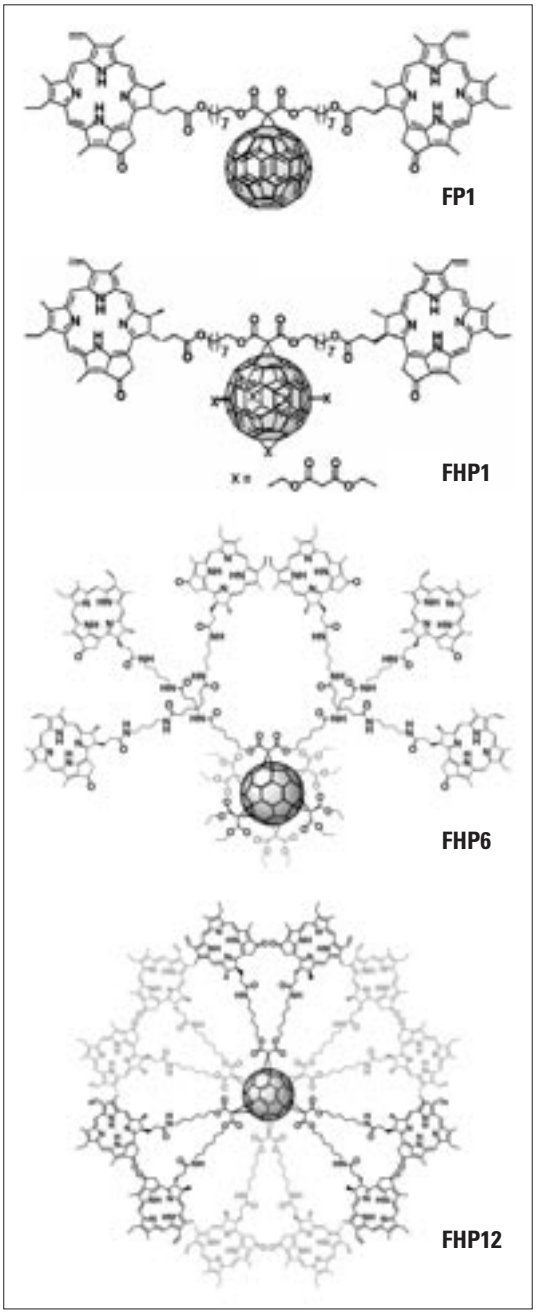
The sense of these systems is the spatial separation of PS from the localizing part of the carrier system and the attachment of a large number of PS molecules to one carrier unit. In this context we investigated dendrimers of different generation. 3rd generation starburst dendrimers were covalently linked on an average of twelve pheophorbide molecules [10] and their photophysical and photobiological properties were studied. First in vitro results suggest that dendrimers could become very efficient multiplying units [11]. An additional advantage of this system consists in the light-induced abstraction of PS molecules from the dendrimer. That is, a large number of PS can be transferred directly to target tissue by one carrier unit which has no immune activity. These results are patented [12].

Beside dendrimers also fullerenes are very promising molecules for using as multiplying units in modular drug delivery systems for PDT. It was shown that C_{60} is a versatile building block for the construction of globular dendrimers [13]. Furthermore, an antibody or an antibody fragment can be linked to the fullerene molecule [14]. These structural modifications increase the solubility of such C_{60} containing super molecules. Taking advantage of these facts fullerene can be used as functional core of an efficient multiplier in modular drug delivery systems for PDT. For this we studied the photophysical properties of novel monofullerene-bis(pyropheophorbide a) complexes (Fig. 4) [15, 16].

First we showed that if the conjugation of the π-electron system of the fullerene is broken up, the fullerene core looses its electron accepting ability [26]. It was revealed that in the pyropheophorbide a (pyroPheo)-C_{60} molecular system (FP1) strong quenching of the first excited singlet state of the pyroPheo and, as result, dramatically decrease of photosensitized singlet oxygen generation occur by efficient photoinduced electron transfer to the fullerene molecule. In contrast the fullerene hexaadduct-bis(pyroPheo) system (FHP1), which possesses five diethyl malonate addends in the remaining octahedral positions (Fig. 4),

shows a high singlet oxygen quantum yield due to the reduced fullerene chromophore that does not act as an electron acceptor anymore. Further investigation of extended fullerene-dendron-dye-complexes showed that increasing the number of pyroPheo molecules (Fig. 4: FHP6 and FHP12) causes decreasing singlet oxygen quantum yields. Nevertheless, in vitro experiments on Jurkat cell suspensions have shown increasing phototoxicity with increasing dye number per fullerene unit.

Fig. 4
Structural formula of novel monofullerene-dendron-pyropheophorbide a complexes.



So it can be concluded that dendrimers as well as fullerene-dendron-supramolecules are versatile building blocks for modular carrier systems. The optimisation of these building blocks as well as the construction of whole carrier systems are actual problems under investigation. On the other hand we are proofing the use of these high branched and high loaded with dye molecules complexes as light harvesting systems for artificial photosynthetic units.

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