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EDITORIAL

President's Letter

Dear EPA members

A wonderful year of photochemical science with many inspiring conferences (see conference reports in this Newsletter) is about to close. Good science should be publishable, but this letter is devoted to the dark side of publishing, to plagiarism and academic fraud in photochemistry. In fact, 2013 has been an impressive year if it comes to scientifically unethical behaviour. The first case concerns an embarrassing note in the published supporting information section in which the senior author encourages the student author as follows: "Emma, … for this compound, just make up an elemental analysis…".[1] A second case affects an associate editor of a reputed organic journal, who published peculiar, apparently manipulated NMR spectra with "missing chunks of signal" in his/her own journal as well as in other ones.[2] A third case is ridiculing publishing as well as science funding at its best.[3] The authors appear in fake moustaches and wigs on their portrait photos and the paper (accepted and printed in Metalugia International) contains more nonsense than a reviewer or editor can imagine, starting from the title "Evaluation of Transformative Hermeneutic Heuristics for Processing Random Data" to references from Bernoulli and Laplace with recent publication years. This is highly recommended reading.

Having recently been called as an expert in a plagiarism case, and reading further through the most recent convicted and pending cases of academic fraud or plagiarism,[4] it occurred to me that there is nothing substantial to be found in the area of photochemistry and photophysics. I offer a few alternative explanations for this here, in addition to the lack of fraud being a coincidence: 1) Maybe photochemists adhere to the highest moral and ethical academic standards. 2) The scientific results in our field are possibly too difficult to manipulate. 3) Nobody investigates the validity of data obtained by other photochemists once they have been published. 4) Competition and publication pressure in photochemistry is perhaps not sufficiently fierce such that authors are less tempted to massage data to get them published more quickly in a higher-impact journal. If any EPA member is aware of recent or past major cases of academic

fraud in photochemistry, please bring them to the attention of all readers (write a story for the EPA Newsletter). Personally, I would be really relieved if none of the reasons quoted above was actually valid.

Cases of academic fraud or severe plagiarism, once proven, usually result in retractions of the papers, something which is thought to be associated with a decrease in reputation of the authors (and the journal, whose quality control apparently failed). In the cases mentioned above, retractions have not yet happened, the investigations are on-going. Recently, a meaningful correlation between the retraction index of a journal (number of retractions per 1000 published articles) and its impact factor has been found,[5] which suggests that those journals which are conceived as best either attract more fraudulent manuscripts or, conversely, are more under scrutiny by competitors after publication. Be this as it may, the retraction index of our society journal, Photochemical and Photobiological Sciences, remains low, if not vanishing. Again, depending on the viewpoint, this may be a good or a bad sign. And to the best of my knowledge (and senior EPA members, please correct me), there has not been a single article ever which had to be retracted from EPA Newsletters. With this peaceful insight, I wish all EPA members a splendid new year full of publishable but real discoveries in research.

Prof. Werner Nau
Jacobs University, Bremen

1) http://blog.chembark.com/2013/08/06/a-disturbing-note-in-a-recent-si-file/

2) http://blog.chembark.com/2013/08/19/some-very-peculiar-nmr-spectra-in-organic-letters/

3) http://www.scribd.com/doc/167706815/EVALUATION-OF-TRANSFORMATIVE-HERMENEUTIC-HEURISTICS-FOR-PROCESSING-RANDOM-DATA

4) http://retractionwatch.com

5) http://iai.asm.org/content/79/10/3855.full

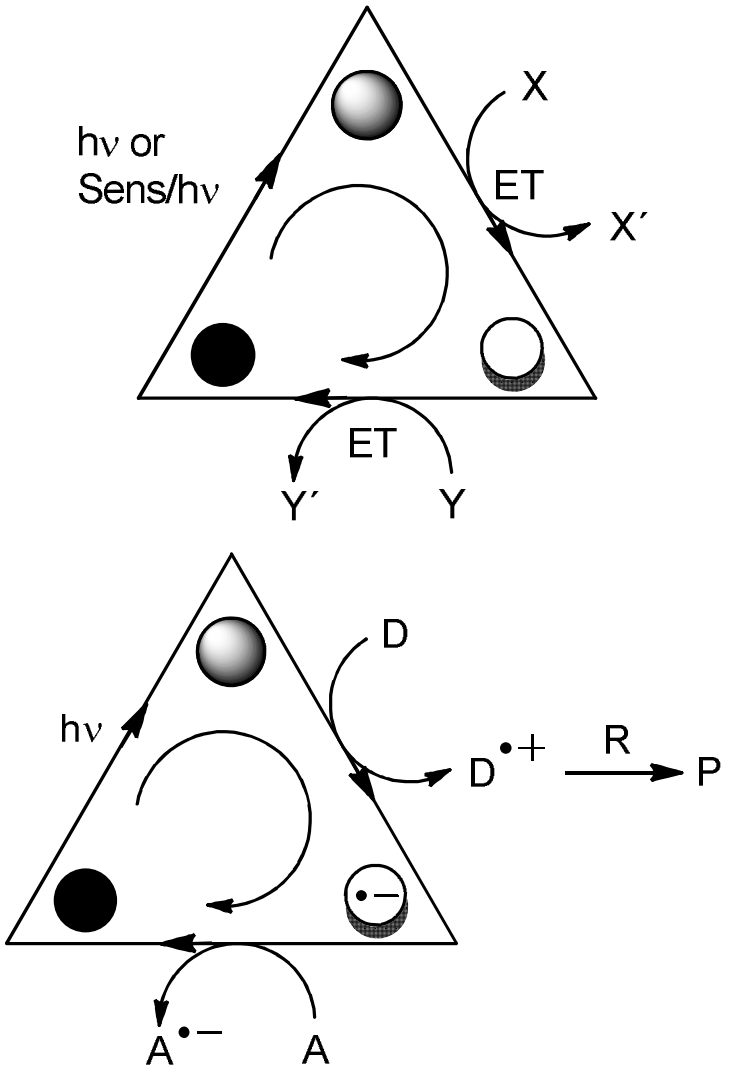
PUBLICATIONS

Synthetic Organic photochemistry – Grown up and applicable for life sciences

Axel G. Griesbeck,* Maria Bräutigam, Sebastian Hanft, Margarethe Kleczka, Sabrina Molitor, Melissa Reckenthäler, and Sarah Sillner
University of Cologne, Department of Chemistry, Griesbeck Photochemistry Group, Greinstr. 4, 50939 Cologne/Köln, Germany; griesbeck@uni-koeln.de

Organic photochemistry is a bridging discipline that profits by the constantly increasing knowledge obtained in neighbouring areas such as photophysics, photobiology and -medicine, spectroscopy, theory and catalysis research. In recent years, especially the rapid development of catalytic techniques (bio-, organo-, transition metal catalysis) has opened new fields of applications for organic synthesis. When searching for new applications, the (photo) synthetic community can take advantage from numerous data compilations of photophysical properties of dyes, electrochemical properties of reagents and kinetic data on charge separation and charge shift reactions. The impact of apparently new concepts is the greater the more well established concepts are integrated, i.e. photoredox-catalysis coupled with concepts of transition-metal catalysis or organocatalysis. The use of trivial organic dyes as catalysts enables the use of visible light, e.g. from LED light sources, for the in-situ generation of highly redox-active species – eventually you can get everything what is needed for photoredox catalysis in the hardware store (in german: Baumarkt) – and enantioselective synthesis can be realized with the help of established chiral organocatalysts. Photoactive catalysts can either transfer the excitation energy directly to a substrate molecule or indirectly via sequential electron transfer (ET forward and back) steps. The latter process can be depicted by a catalytic triangle that combines two ET steps.[1] The version on the right shows the donor-acceptor sequence of a single ET sensitizer in a photoredox catalytic reaction sequence. Understanding the basic principles of energy and electron transfer catalysis and combining this knowledge with the principles of general and special catalysis can lead to new reaction types as well as

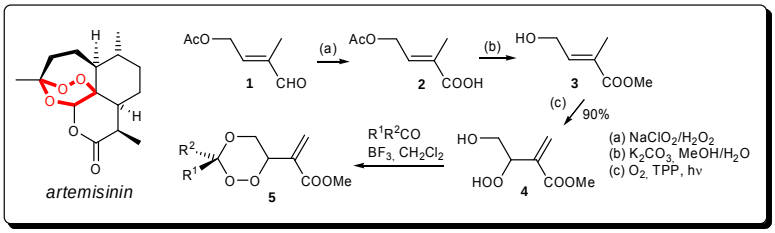
to applications of these reactions in problems related to life sciences. We herein outline briefly six ongoing research approaches in the context of our *photochemistry for organic synthesis* project.



1. Photoredox Catalysis for Organic Syntheses. M. Reckenthäler and A. G. Griesbeck, *Adv. Syn. Cat.* **2013**, *355*, 2727-2744.

Cyclic peroxides by singlet oxygen reactions: chemotherapeutic agents

A relatively small group of organic peroxides existing in nature exhibit biological properties. Some of these compounds are used as drugs in ancient folk medicine since centuries, for example the well-known *artemisinin* as an antimalarial medicine. Motivated by this fact numerous derivatives of cyclic peroxides, namely 1,2-dioxanes, 1,2-dioxetanes, 1,2,4-trioxanes, 1,2,5-trioxolanes, and 1,2,4,5-tetroxanes were synthesized in the last decades and their pharmacological potentials were tested. Beside multi-antiparasitic activities, also antibiotic, antiviral as well as tumor-therapeutic properties were detected. We are currently studying polar 1,2,4-trioxanes as potential inhibitors of several forms of glutathione-transferases, enzymes that are up-regulated in tumor cells and thus reduce the potency of chemotherapy drugs by conjugation to glutathione. The 1,2,4-trioxanes **5** are available by a photooxygenation route, the singlet oxygen ene reaction with appropriate allylic alcohols **3** and a subsequent peroxyacetalization step. Photochemistry is a tool here that enables the incorporation of molecular oxygen in organic molecules in a controlled process, a triplet-triplet energy transfer from excited organic dyes (such as porphyrins) to triplet oxygen. The polar trioxanes **5** show promising pharmacological properties and are investigated as enzyme inhibitors for tumor therapeutic applications as well as for their antiparasitic activities.[2]



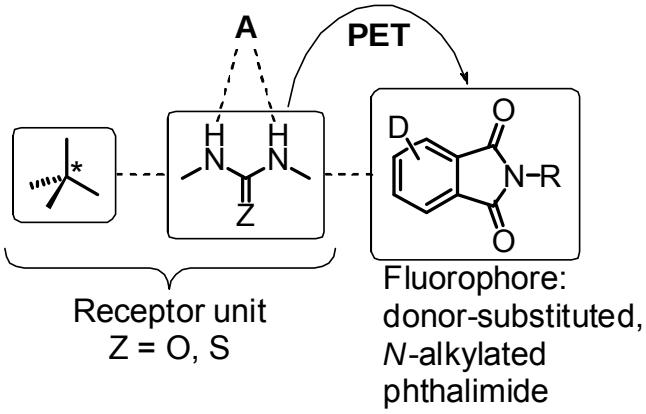
2. Functionalized polar 1,2,4-trioxanes as building blocks by singlet oxygenation of 4-hydroxy tiglic acid using the deuterium isotope trick.

A. G. Griesbeck, V. Schlundt and J. M. Neudörfl, RSC Adv. 2013, 3, 7265-7270.

Maria Bräutigam

Fluorescence sensors for chiral analyte detection

Enantioselective fluorescence-based analysis of chiral analytes is a hot topic in molecular sensor research. The basic concept underlying most approaches to fluorescence sensors is the connection of a fluorophore to a receptor unit that is capable of influencing the photophysical properties of the fluorophore upon binding to analytes. Consequently, analytes can be detected by means of fluorescence spectroscopy.
In order to achieve detectable interactions with anionic species, we concentrated on phthalimide fluorophores attached to anion sensitive (thio-)urea receptors.[3] The phthalimide moiety becomes an easily accessible fluorophore through alkylation of the nitrogen and attachment of a donor moiety to the six-membered ring. Both are essential features to enhance the phthalimide emission and to get a detectable fluorescence. The urea group fulfills both, the need for an electron donating moiety to enhance phthalimide fluorescence and the ability to act as a hydrogen bond donor. The formation of hydrogen bonds to analytes leads to an increase of electron density at the urea which causes subsequent electron transfer to the excited phthalimide (photoinduced electron transfer, PET) that quenches the fluorescence of the latter.



For enantioselective recognition of chiral analytes we further extended the receptor with groups containing stereogenic centers from the pool of natural compounds such as amino acids, 1,2-diamines and 1,2/1,3-amino alcohols. These were chosen either to produce steric hindrance and/or to enable secondary binding interactions with analytes.

3. Colorimetric Detection of Achiral Anions and Chiral Carboxylates by a Chiral Thiourea-Phthalimide Dyade. A. G. Griesbeck, S. Hanft and Y. Díaz-Miara, *Photochem. Photobiol. Sci.* **2010**, *9*, 1385-1390.

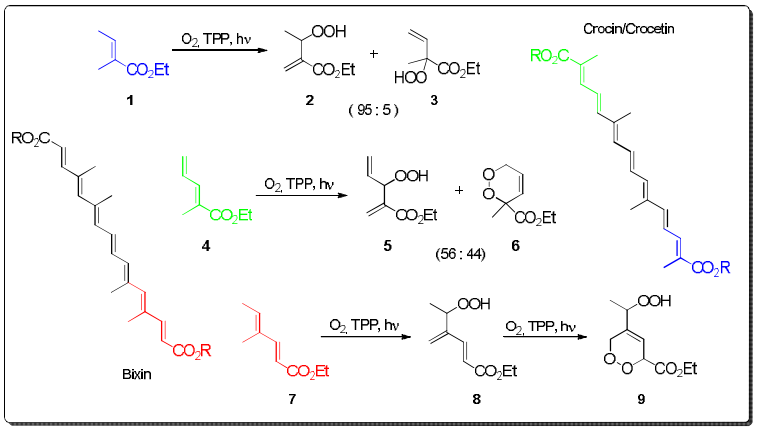
Sebastian Hanft

JLJLJL Tandem reactions with singlet oxygen for polyoxyfunctionalization

Natural antioxidants are often based on polyene structures such as the carotenoids and apocarotenoids. These compounds do react with singlet oxygen (and other reactive oxygen species = ROS) with near diffusional rate constants. This process is described either as physical quenching, without the formation of new products, or, chemical quenching, i.e. with the formation of peroxides such as hydroperoxides, endoperoxides or dioxetanes. The latter process obviously leads to degradation of the antioxidants and to the formation of biologically undesired peroxides.

In order to identify the product structures of the singlet oxygen reaction of carotenoids, we started to investigate the photooxygenation of 1,3-diene esters. The analyzed 1,3-diene esters **4** and **7** are formal building blocks of the crocetin or bixin families, respectively, and were reacted with singlet oxygen in non-polar solvents and meso-tetraphenylporphyrin (TPP) as sensitizer. In contrast to ethyl tiglate **1**, which shows a high *gem*-regioselectivity in reaction with singlet oxygen, the Schenck-ene product **5** and in almost equal parts the [4+2]-cycloaddition product **6** were obtained by the photo-oxygenation of **4**. The 1,3-diene ester **7**, without a methyl group in α-position but in γ and δ-position, undergoes a first regioselective reaction with singlet oxygen to the hydroperoxide **8**. Hydroperoxide **8** is able to react with a second equivalent of singlet oxygen in a [4+2]-cycloaddition to hydroperoxy-endoperoxide **9** in a tandem / sequential ene/4+2 singlet oxygen process.[4] Currently, we are searching for

similar synthetic or naturally occurring systems, which show comparable tandem reactivity.



4. A new directing mode for singlet oxygen ene reactions: the vinylogous gem effect enables a 1O2 domino ene/[4+2] process. A. G. Griesbeck and A. de Kiff, *Org. Lett.* **2013**, *15*, 2073-2075.

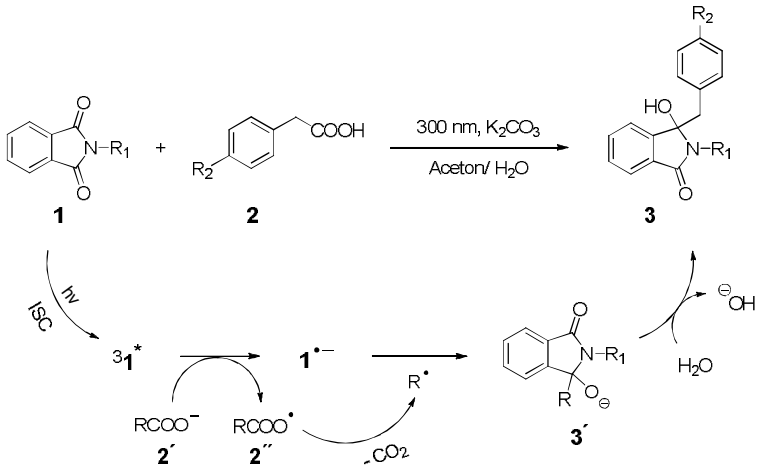
Margarethe Kleczka

Photocatalytic benzylic radical generation and reactions

Photoredox catalysis is a re-discovered "new" concept that can be applied for numerous bond forming steps. Among these, carbon-carbon bond formation is one of the most important synthetic key steps in organic synthesis. When performed in a photocatalytic fashion, metal-organic reagents or (transition)metal-catalyzed steps can be circumvented by the use of cheaper dyes as sensitizing catalysts and light as reagent.

For specific synthetic purposes, the combination of the sensitizing group and the primary radical (ions) formed by PET is desirable. We have demonstrated this version for the alkylation of phthalimides using sensitized excitation of N-alkylated phthalimides in the presence of alkylcarboxylates.[5] The high regioselectivity of the photocatalytic process constitutes another advantage since the photoinduced alkylation occurred exclusively at the imide chromophore and not at

ester or amide carbonyl groups (R_1 = CH_2CO_2Me, $CH_2CONHCH_2CO_2Me$). Mild conditions, high yields and the easy availability of all starting materials demonstrate the usefulness of these synthetic applications. The reaction is conducted in water/acetone mixtures as solvent taking advantage of the triplet-sensitizing properties of acetone. Mechanistically, this process involves the triplet state of the phthalimide **1** which acts as the oxidant in an intermolecular photoinduced electron transfer. After rapid CO_2 release from the phenylacetate radical **2´´**, recombination with the phthalimide radical anion leads to an alcoholate that is subsequently protonated.[6] Yielding in the corresponding hydroxyphthalimidines **3´**, this photodecarboxylative addition shows an important example of synthetic organic photochemistry and its contribution to green chemistry. The addition products **3** are member of and entrance cards to important classes of potential pharmacologically active molecules: hydroxy-isoindolinones, methylene-isoindolines and isoindolinones.



1 **2** **3**

2´ **2´´** **3´**

R_1 = Benzyl, Methyl, CH_2CO_2Me, $CH_2CONHCH_2CO_2Me$

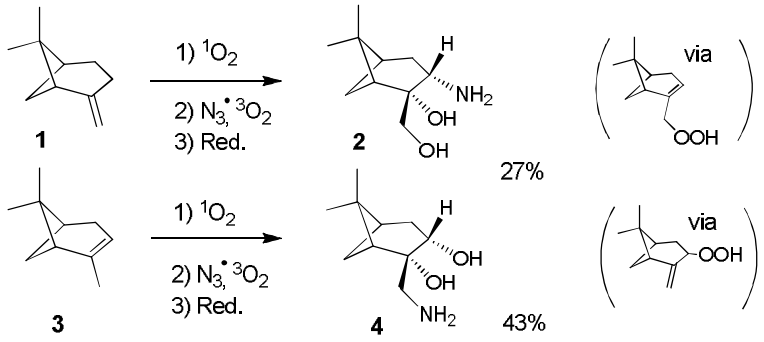
R_2 = H, Me, Ph, OMe, OH, F, Cl, Br, CF_3, NH_2

5. The photodecarboxylative addition of carboxylates to phthalimides: scope and limitations. M. Oelgemöler, P. Cygon, J. Lex and A. G. Griesbeck, *Heterocycles* **2002**, *59*, 669-689.
6. Intermolecular photodecarboxylation of electron-deficient substrates by phthalimides in water: efficiency, selectivity and online monitoring. A. G. Griesbeck, N. Nazarov, J. Neudörfl and M. Heffen, *Green Chem.* **2012**, *14*, 3004-3006.

Sabrina Molitor

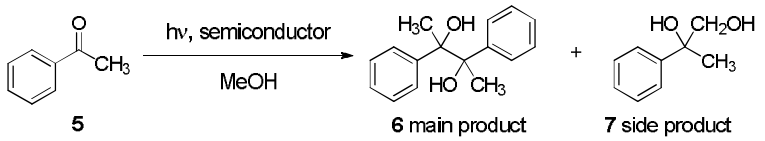
Photocatalytic hydroxymethylation of ketones

Type B photocatalysis is a term – introduced by Horst Kisch in the context of semiconductor photocatalysis[7] - that describes the complete consumption of both radical ion species that are formed in a photoredox-catalyzed step and thus the application of the reaction principle without the need of sacrificial reagents. A well-studied reaction sequence that we have developed is the 1,2-azidohydroperoxidation of alkenes. This reaction can also be coupled with another oxygenation step, the singlet oxygen ene reaction. If terpene substrates are used (like the pinene isomers), enantiomerically pure amino diols are produced from a three-step reaction involving a) singlet oxygen photooxygenation, b) Type B photocatalytic azidyl radical / triplet oxygen addition and c) reduction.[8]



Not many further applications of this type of reaction for applications in organic synthesis are known. Currently, we are studying the use of

different semiconductors in the photocatalytic hydroxymethylation of ketones, which was developed by Sato and coworkers by the end of the 1970s.[9] In the model process, acetophenone is UV-irradiated in methanol solution in the presence of different semiconductor nanoparticles and colloids, e.g. titanium dioxide P25 or hematite nanoparticles. The desired 1,2-diol **3** was hereby only formed as side product, as main product the pinacol **2** was generated, which is a product of type A photocatalysis (describing the synthetic use only of the redox-activated donor/acceptor pair). A synthetically useful method to shift the product ratio to the Type B 1,2-diol is the application of the dilution trick, i.e. slow addition of the ketone component to the reaction solution during irradiation.



5 **6** main product **7** side product

7. Type B semiconductor photocatalysis: the synthesis of homoallyl amines by cadmium sulfide – catalyzed linear photoaddition of olefins and enol/allyl ethers to N-phenylbenzophenone imine. H. Keck, W. Schindler, F. Knoch and H. Kisch, *Chem. Eur. J.* **1997**, *3*, 1638-1645.
8. Azide / oxygen photocatalysis with homogeneous and heterogeneous photocatalysts for the 1,2- aminohydroxylation of acyclic / cyclic alkenes and Michael acceptors. A. G. Griesbeck, J. Steinwascher and M. Reckenthäler, J. Uhlig, *Res. Chem. Intermed.* **2013**, *39*, 33-42.
9. Metal-catalyzed organic photoreactions. One-step synthesis of (±)-prontalin by the titanium(IV) chloride-catalyzed photoreaction of heptane-2,6-dione. T. Sato, S. Yamaguchi and H. Kaneko, *Tetrahedron Lett.* **1979**, 1863-1864.

Melissa Reckenthäler

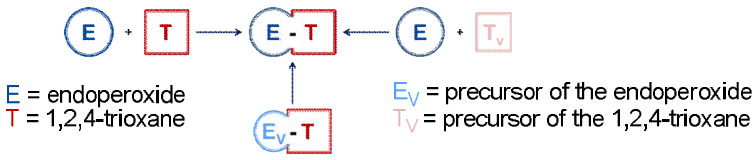
JLJLJLJLJL **Antimalarial peroxidic dyads by singlet oxygen routes**

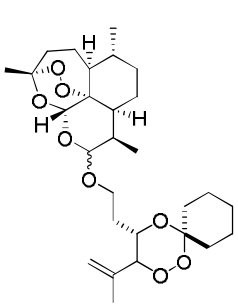
In recent years an increasing number of malaria strains that are resistant against commercial artemisinin derivatives appeared due to

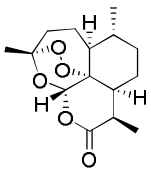
the extensive use of these relatively cheap and nearly uncontrolled available drugs. In order to further be able to take advantage of the highly active peroxide pharmacophore of the natural artemisinin, the dyade strategy has been followed by several groups, either combining the natural peroxide with other pharmacophores such as quinolines or isoquinolines. Alternatively, molecular dyads can be composed in which peroxides of different structure and of biological activity pattern are combined. We are currently investigating strategies to covalently link natural and synthetic trioxanes, the first dyads of this kind already showing high antimalarial activities.[10] Following this scientific tradition, we are trying to preserve the active principle of the anthelmintic endoperoxide ascaridol by fusion with natural or (artificial) synthetic 1,2,4-trioxanes.

The different strategies to synthesize such dyads are depicted in the scheme below. The dyads are generated either by linking two existing pharmacophores in a terminal step, or by photooxygenation of 1,2,4-trioxanes with appropriate side chains. Another possibility is the peroxyacetalization of an endoperoxide with reactive aldehydes. The key step of each route is the photo-oxygenation with singlet oxygen to obtain endoperoxides ([4+2]-addition) or hydroxy-hydroperoxides (Schenck-ene reaction).

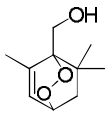
general reaction scheme of dyads (E-T):



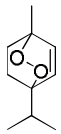




artemisinin



endoperoxide of safranol



ascaridol

10. Antimalarial Peroxide Dyads from Natural Artemisinin and Hydroxyalkylated 1,2,4-Trioxanes. A. G. Griesbeck, J. Neudörfl, A. Hörauf, S. Specht and A. Raabe*, J. Med. Chem.* **2009**, *52*, 3420-3423.

Sarah Sillner

SPECIAL REPORTS ON PHOTOCHEMISTRY OF BIOMOLECULES

Introduction

Dear EPA members,

This issue of the EPA Newsletter, December 2013, is dedicated to "Photochemistry of Biomolecules", currently a dynamic research area aimed at understanding how and why biomolecules respond to light exposure and the relevant consequences of such interaction, as well as identifying photosensitized processes. We would like to thank all the authors of the contributions to this issue of the EPA Newsletter, but most particularly Miguel Angel Miranda, who was the driving force for encouraging the authors to contribute their work and submit it on time!

An interesting bunch of contributions is here presented, many of them revealing fruitful collaborations between theoreticians and experimentalists and between experts of different research areas. From the computational approach to generating relevant information on the photochemistry of bimolecules, *ManuelaMerchán et al.* (Instituto de Ciencia Molecular, Universitat de Valencia) report on the benefit of combining photochemical reaction path approach and the multiconfigurational second-order perturbation theory method to establish photochemical and chemiluminiscent mechanisms in biomolecules. In addition, *Lluis Blancafort* (Institut de Química Computacional I Catàlisis and Departament de Química, Universitat de Girona) reports on the geometric deformations that control the photodynamics of nucleobases and their dimers and also on relevant photochemical processes involving these species. *Roberto Improta*`s (Istituto Biostrutture e Bioimmagini, Consiglio Nazionale delle Ricerche, Napoli) current investigation focuses on oligonucleotides in solution, using time-dependent density functional theory for electronic calculations and the polarizable continuum model to include solvent effects.

On the other hand, *Tetsuro Majima et al.* (The Institute of Scientific and Industrial Research, Osaka University) report on the direct observation of excess electron transfer processes in DNA and the relevance of these processes not only in the biology field but also in material science. *Miguel A. Miranda et al.* (Instituto de Tecnología Química, Universitat Politecnica de Valencia) present recent results which challenge the validity of established mechanistic paradigms related with pyrimidine photosensitized dimerization in DNA. *David Lee Phillips et al.* (Department of Chemistry, University of Hong Kong) report on time-resolved transient absorption and resonance raman investigations of nonsteroidal antiflammatory drugs aiming at understanding their phototoxicity.

In addition, *Sandra Monti et al.* (Istituto per la Sintesi Organica e la Fotoreattività. Bologna) current research focuses on cationic porphyrazines as ligands of DNA structures, envisaging the potential application of these ligands as three-modal therapeutic anticancer drugs. *Dimitra Markovitsi* (CNRS, IRAMIS, SPAM, Laboratoire Francis Perrin, Gif-sur-Yvette) reports on the activity of her group, dealing with the investigation of the primary processes which precede the UV-induced damage to DNA. The aim is to gain insight into these first steps and determine their contribution in skin cancer.

Finally, *Tito Scaiano* (Department of Chemistry and Centre for Catalysis Research and Innovation, University of Ottawa) reports on the superior antibacterial activity of AgNPs than that of silver cation, and the stabilization of the nanoparticles within a protein environment, thus resulting in a new biomaterial. These investigations are part of an on-going collaboration between Ottawa University and Linköping University/Karolinska Institute.

The EPA Newsletter Board greatly appreciates these experts' contributions to this issue.

Julia Pérez Prieto
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Computational Photochemistry and Chemiluminescence of Biomolecules

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Photochemistry and chemiluminescence of biomolecules (hereafter photobiology and bioluminescence, respectively) can be understood as exchanges between two types of energy: chemistry and light (see Fig. 1). In the first type of phenomenon, the irradiation ($h\nu$) with light provides the molecule with extra energy which can be released through different decay paths: 1) creating new compounds or photoproducts in a productive manner (photoreactivity) or 2) decaying to the original configuration of the atoms (photostability) (see details in Fig. 2). On the other hand, bioluminescence corresponds to the emission of light as the result of a thermally-activated (Δ) chemical reaction. The study of such phenomena by means of computers using specialized software tools and sophisticated strategies can be defined as Computational Photobiology and Bioluminescence (CPB). Among the strategies to characterize decay paths, one of the most accurate is the Photochemical Reaction Path Approach (PRPA),[1,2,3] which implies the determination of the most relevant channels for the transit of energy by means of the computation of Minimum Energy Paths (MEPs) and the location of the accessible minima or crossings (CIs or STCs) between the potential energy hypersurfaces of the excited and ground states. Since 2003, our group Quantum Chemistry of the Excited State of València (QCEXVAL), together with its

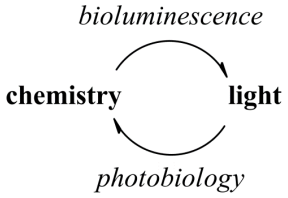

Figure 1. Scheme showing the relationship between photochemistry and chemiluminescence in biomolecules.

collaborations, has employed this computational approach and other approximations in combination with the multiconfigurational second-order perturbation theory (CASSCF/CASPT2) method[1,4,5,6] to establish photochemical and, more recently, chemiluminescent mechanisms in biomolecules.[7,8,9,10] Here, we present some of our most recent findings in the field.

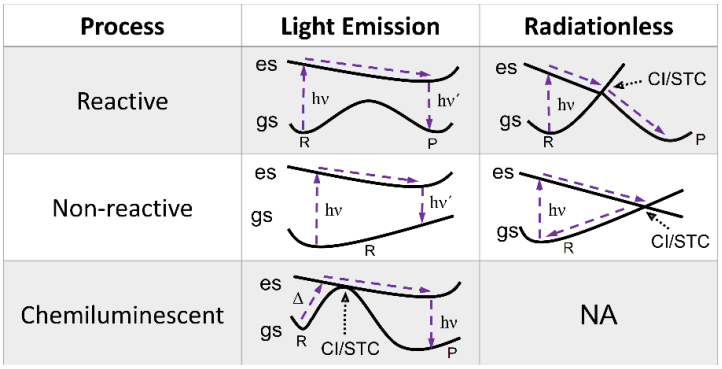

Figure 2. Classification of the photochemical and chemiluminescent processes from a mechanistic standpoint. After irradiation of a compound R and population of the excited state (es), several pathways are possible for energy deactivation to the ground state (gs). New species (P) can be formed via an adiabatic evolution on the excited state and light emission (*adiabatic photochemistry*) or via a non-adiabatic process, such as an *internal conversion* (IC) or an *intersystem crossing* (ISC), which are mediated by *conical intersections* (CIs) and *singlet-triplet crossings* (STCs), respectively (*non-adiabatic photochemistry*). Decay channels can also bring the molecule back to the original structure, R, by either an excited state vibrational relaxation followed by light emission or either a non-adiabatic process mediated by a CI or a STC. NA = Not Applicable.

The DNA molecule presents a rich variety of photochemical processes induced by direct UV irradiation.[11] Nucleobases themselves have photostable properties against UV irradiation, which have been related, on the basis of computations using the PRPA approach, to the presence of barrierless non-radiative decay pathways to the original ground state equilibrium structure.[2,3,12,13,14,15] On the other hand, UV irradiation of stacked pyrimidine dimers might give rise to

the formation of lesions in the DNA,[16] such as cyclobutane pyrimidine dimers[17,18] and pyrimidine-pyrimidone 6-4 dimers.[19] The mechanism established in this case implies a precursor excited-state dimer (excimer) formation and the participation of excited triplet states in the photoformation of the 6-4 adducts. Moreover, the hydrogen-bonding network in the base pairs has been shown to be able to provide photostable channels via a mechanism of excited-state double hydrogen transfer mediated by CIs.[20] An analogous mechanism involving the same CI structures has been also characterized giving rise to photoreactive routes for tautomer production.[20]

In addition to the direct effects of radiation to the DNA components and the molecule itself, indirect mechanisms can occur as a result of the irradiation to the environment of the biomolecule, implying serious damage to the nucleic acids. One of the harmful indirect processes is the attachment of low-energy electrons (LEEs), which can produce fragmentations in all DNA nucleobases.[21] The CPB has been recently applied to the uracil case.[22] CI crossings between the two lowest-lying anionic π^- states and the dissociative $\sigma_{N\text{-}H}^-$ states have been ascribed as the responsible structures mediating the dissociative electron attachment of N-H bond in the nucleobase. Additionally, DNA nucleobases are also susceptible to the attack of the hydroxyl radical (·OH).[23] The instability of the radicals formed in the ·OH addition to the nucleobases makes difficult its experimental study. Employing the tools of CPB, the transient absorption spectra of the ·OH adducts of pyrimidine nucleobases have been interpreted, assigning the band recorded at lower energy in the visible range of the electromagnetic spectrum to the adduct formed by attachment at the atom C6 of the nucleobase.[24]

The molecular basis of bioluminescent processes can be also understood by using the CPB. The chemical functionalities of the bioluminescent molecules have been established in collaboration with Professor Lindh at the Uppsala University and co-workers, implying the presence of a chemiluminophore, an electron-donating fragment, and substrate-enzyme interactions tuning the emission energy.[25] Remarkable differences between fluorescence and chemiluminescence, not reported in previous studies, have been found in another computational study on a small model for

coelenteramide and cypridina oxyluciferin. On the basis of the analysis performed, some recommendations have been given for future experiments aimed to study bioluminescent properties.[26] Furthermore, the correct use of fast methodologies, in particular density functional theory (DFT), to characterize chemi/bioluminescent reactions has been recently described in detail comparing with the higher accurate method CASPT2.[27,28]

Finally, it is important to keep in mind that biomolecules are embedded in a network of water molecules, which might play an important role in the photochemical and bioluminescent processes. The photochemistry of the water dimer has been studied in two configurations: the conventional H-bonded dimer[29] and a π-stacked arrangement.[30] Whereas a photoinduced dissociation mechanism is obtained for the H-bonded dimer, the π-stacked dimer has been found to possess vertical electronic excitation energies which can be related to the distinct spectroscopic properties of water in contact with charge systems, in particular the absorption at 270 nm.[31] Since the DNA/RNA nucleobases and amino acids with aromatic rings also absorb in this region, they may easily resonate with π-stacked water. We believe that certain characteristics such as the photostability properties of the material of the genetic code, which are generally ascribed to the own intrinsic properties of the molecular system, might also be intimately united to the presence *in vivo* of π-stacked water.

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Computational photophysics and photochemistry of the DNA nucleobases. There's more to it than photostability

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Introduction. The photophysics and photochemistry of the DNA nucleobases have been the subject of great attention in the last decade. The aim is understanding the interaction of these building blocks of life with visible and UV light. The development of femtosecond time-resolved spectroscopy has also made it possible to obtain an increasingly detailed picture of these phenomena. Theory and computations have been essential to interpret the spectra, and our research has aimed to provide a mechanistic description in terms of potential energy surfaces. This has allowed us to identify the nuclear coordinates - *ie* the geometric deformations - that control the photodynamics.

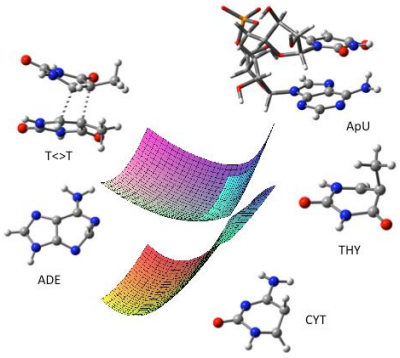

Figure 1. Examples of conical intersections relevant in the photophysics and photochemistry of the DNA nucleobases.

We have considered three types of processes: First, the photophysics of the isolated nucleobases, focusing on the role of conical intersections (CI) in their photostability (Figure 1). Moving towards larger systems, we have also studied the nucleobase dimers, centring

on the role of charge transfer states and excitonic interactions. Finally, we have considered important photochemical processes such as hydrogen transfer between Watson-Crick pairs, the formation of cyclobutane pyrimidine adducts, tautomerization, and electron transfer.

For the calculations, we have relied on the CASSCF and CASPT2 methods. Their combination provides a good description of the highly distorted structures involved in the reaction paths, in particular the CIs. In our approach, the reaction paths are calculated at the CASSCF level, and the energies corrected with CASPT2 to include dynamic correlation.

1. Photophysics of the nucleobases. It is well known that the photophysics of the DNA nucleobases is dominated by excited state lifetimes that lie in the femto- and picosecond range. This has been made responsible for their photostability. In this context, we were among the first to show that the ultrafast decays are associated to CIs - points on the potential energy surface where the ground and excited states cross, and the radiationless decay is specially favoured.[1] On a series of publications on cytosine,[1,2] adenine[3] and thymine,[4,5] we identified the structures of the CIs associated to the ultrafast decay, and we showed that the reaction paths to access them from the Franck-Condon region have low barriers, which explains the short time scales found experimentally. For cytosine, we have also shown that a crossing region with strong spin-orbit coupling between the singlet $^1(\pi,\pi^*)$ and triplet $^3(n,\pi^*)$ surfaces enables population of the triplet state, which accounts for a long-lived, 'dark' species.[6] We have also studied how hydration affects the decay.[7]

Turning to more methodological work, we have shown for cytosine and thymine that it is crucial to chose the right level of theory to describe the mechanism and avoid artefacts in the calculation.[2,4] Another important concept is that the CI is not an isolated point on the potential surface. Instead, it forms extended seams of intersection. For cytosine, we have studied how these seams are linked to a three-state crossing,[8] and for thymine we have studied in detail how an extended S_2/S_1 crossing seam affects the branching between the (π,π^*) and (n,π^*) states after excitation.[5]

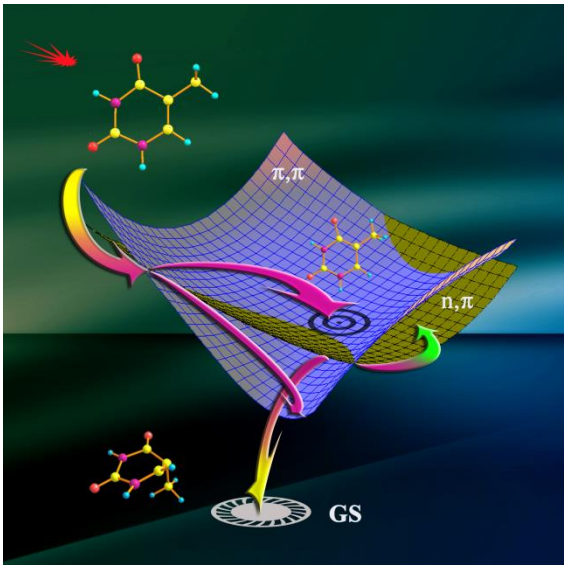

Figure 2. Sketch of the (π,π*)/(n,π*) intersection seam in thymine.

2. Nucleobase dimers. The photophysics of DNA oligomers or wild type DNA is significantly different from that of the isolated nucleobases. The nucleobase polymers show longer lifetimes than the monomers. Excitons and charge transfer states have been invoked to explain these differences. A quantum mechanical treatment of the polymer is beyond the current cmoputational capabilities, and therefore we have directed our attention more recently to the nucleobase dimers, as a first step towards more extended systems. First, we have considered the vertical excitations for the nucleobase dimers in their ideal, stacked B-DNA conformation. We have found that the 5'-purine-pyrimidine-3' sequence favours the formation of low lying charge transfer states.[9] Currently we are studying the photophysics of the ApU dinucleotide in water, which has lifetimes more than one order of magnitude longer than the A and U nucleotides. Using a hybrid quantum mechanics - molecular mechanics approach, our aim is to elucidate whether the long lifetime is due to the formation of a charge transfer state, or whether the decay of the monomer-like excited states is slowed down by the environment.[10]

3. Photochemistry of the nucleobases. Although the DNA nucleobases are well known to be photostable, they also show photochemical reactivity. The most prominent example is the dimerization of stacked pyrimidine dimers to form cyclobutane adducts. The potentially mutagenic lesions are repaired by the photolyase enzyme. The adducts are formed in less than 1 ps after excitation, and our calculations have identified the CI responsible for the ultrafast product formation.[11] We have also shown that the product state correlates with a high-lying state in the ideal B-DNA conformation. In another study, we have considered intermolecular hydrogen transfer in the GC Watson-Crick pair. For the triplet state, the preferred reaction is the transfer of a single hydrogen. This leads to a triplet diradical that can decay to the singlet state and regenerate the canonical GC pair by back hydrogen transfer, contributing to the photostability of the system.[12]

A further reaction we have considered is the UV-induced tautomerization of cytosine. This reaction seems particularly intriguing because cytosine is always considered to be photostable. According to our results, the non-canonical tautomers are formed after excitation as side products of the ultrafast radiationless decay at a CI.[13] We have also studied the sensitization of DNA with 5-bromo uracil, and we have found a mechanism where the cleavage of the Br-C bond in uracil occurs from a locally excited 5BrU state.[14] Finally, we have also studied a process that is not directly photochemical but is closely related, namely electron transfer in charged nucleobase dimers. Here we have benchmarked the coupling elements for hole and excess electron transfer at the CASPT2 level.[15,16]

Conclusions. The photophysics and photochemistry of the nucleobases are a great example of fruitful synergy between theory and experiment. Although here we have focused on our own work, it should be stressed that many other groups, several of them in Europe, have contributed to this field. Significantly, the conceptual and computational developments carried out here have been useful to rationalize a broad range of other photochemical reactions.

While the photophysics of the isolated nucleobases is nowadays quite well understood, the current challenge is to get a similar level of understanding for more extended systems. We follow a bottom up approach, modelling nucleobase dimers as a first step towards the

more complex systems. Another challenge followed by our group is going from potential energy surface calculations to directly modelling the spectra.

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Studying DNA excited states by Quantum Mechanical methods: from the isolated nucleobase in the gas phase to oligonucleotide in solution.

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The biological relevance of UV light absorption by DNA (which can lead to genetic code damages) explains the strong scientific interest in the excited state dynamics of polynucleotides and of its main constituents, i.e. the nucleobases (purine –adenine and guanine- and pyrimidine –thymine/uracil and cytosine). The main challenges in this field are describing the very complex dynamical processes triggered in DNA by UV absorption, interpreting them in terms of elementary molecular processes and, hopefully, understanding how photodamage can be limited.[1] To these aims, Quantum Mechanical calculations have been fundamental, since they have provided important insights on the static and dynamical properties of nucleobases' electronic excited states, on their crossing and on their decay.[1,2] For example, ultrafast time-resolved spectroscopic experiments have shown that photoexcited nucleobases return to the ground electronic state on the ps and sub-ps time scale (likely a key features for their selection as DNA building blocks and for the DNA photostability) and theoretical studies have been able to explain these findings. For all the bases barrierless paths exist on the Potential Energy Surface of the lowest energy $\pi\pi^*$ states connecting the Franck-Condon region to Conical Intersections with the ground electronic state, thus allowing a fast and effective non-radiative ground state recovery. Although several intriguing questions are still open and some mechanistic details still the object of a very lively debate, several basic issues concerning the excited state decay of isolated nucleobases in the gas phase can be considered well assessed.[2]

The picture is less clear when dealing with oligonucleotides in solution: in this case, the large size of the species to be considered and the complexity of the processes involved in the excited state decay of a strongly coupled multi-chromophore system (with characteristic times spanning a time range between < 100 fs and several ns) pose formidable challenges to quantum mechanical calculations. This has been the focus of the efforts we made in the last years: studying *in silico* systems as close as possible to those studied '*in vitro*' by experimentalists and to those involved '*in vivo*' in the biological processes. In order to tackle this task at least two ingredients are necessary: (i) a quantum mechanical method able to treat fairly large systems (i.e. with more than 100 atoms) with a sufficient degree of accuracy (ii) a model able to give account of solvent effect on excited state properties. On the ground of our experience, and being aware that each method has always its advantages and its limitations, we have selected the methods rooted in the Time-Dependent Density Functional Theory (TD-DFT)[3] as our reference method for electronic calculations and the Polarizable Continuum Model (PCM)[4] as our reference model to include solvent effects. In this respect, the introduction of long-range corrected density functionals, able to overcome the traditional failures of TD-DFT in describing excited states with Charge Transfer (CT) character and the development of effective PCM/TD-DFT implementations,[3] allowing fast excited state geometry optimizations (LR-PCM/TD-DFT methods) and accurate calculation of absorption and emission energies (SS-PCM/TD-DFT), have been fundamental methodological advances.[5]

On the other hand, the quality of the insights gained by calculations hugely increases when the researches are performed in tight collaboration with experimentalists. This has always been a cornerstone of our approach: when dealing with dynamical processes involving a complex system as DNA the integration between different computational approaches and, especially, between experiments and calculations is fundamental.

On this ground, we succeed in extending the use of QM calculations from the static study of isolated nucleobases in the gas phase to that of small oligonucleotides in solution, also including quantum dynamical effects. It has thus been possible not only to get a direct

comparison with the experimental results (mainly performed in solution) but also to propose new interpretative mechanisms for several basic, lively debated, processes.

For what concerns isolated nucleobases, for example,[6-10] while the most common view until some years ago was that solvent does not significantly affect the excited state decay mechanisms, in some papers (incidentally representing the first computations of emission spectra in solution) we studied the photophysics of uracil derivatives in different solvents predicting that, in some solvents, a population transfer between the spectroscopic bright states and an underlying dark state can take place.[6-8] More in general those studies, for the first time, showed that solvent can deeply affect the excited state decay mechanism, rationalizing a large amount of experimental data.

For what concerns oligonucleotides, QM calculations were applied for the first time to single and double strand sequences in solution, including up to four residues and the phospho-deoxyribose backbone, tackling both photophysical[11-14] and photochemical (as thymine-thymine photodimerization, the most common photolesion within DNA)[15,16] processes. Those studies highlight the fundamental role played by excited states with Charge Transfer character, which is instead often overlooked by calculations performed in the gas phase, and also provided a very useful interpretative key for the excited state decay of a complex system as DNA. While it is often tempting trying to define which is the single excited state responsible of all the spectral features, the behavior of strongly coupled multichromophore species is often ruled by the dynamic equilibrium of different states, whose interplay is modulated by environmental effects and molecular fluctuations, each one with its own spectral signature. For example, a study of the excited states of $(dA)_4$ polynucleotide, including the phosphor-deoxyribose backbone, shows that after absorption to exciton states delocalized over multiple Ade bases, the excited state behavior is shown to be ruled by the interplay among a number of species: (i) monomer like excited state localized over a single Ade, (ii) 'neutral' excimers and (iii) charge transfer exciplexes involving stacked Ade dimers, representing the most stable minimum. This study provided the first comprehensive model for interpreting the excited state decay, and its wavelength dependence, in Ade based polynucleotides.[12,13]

A final challenge concerns the development and the application to these processes of suitable quantum dynamical approaches, which could provide data (lifetime, branching ratios etc.) to be directly compared with Time-resolved experiments. In this respect, promising results have been obtained isolated nucleobase (by performing the first quantum dynamical study of the excited state decay in solution)[17] and effective vibronic Hamiltonian was developed, which provides insights on the interplay between bright exciton and CT states in polyAde.[18]

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Direct Observation of Excess Electron Transfer Dynamics in DNA

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Oxidation and reduction of biological compounds are key processes of various biological phenomena. For example, oxidation of DNA by naturally generated oxidation agent causes DNA damage, which promotes cancer and so on.[1,2] On the other hand, reduction process is known to be included in the DNA repair process by photolyase.[3] It is important that damage and/or repair of DNA occur also with nucleobases apart from the initially oxidized and/or recued cites due to the charge transport in DNA. From this point, charge transfer in DNA attracts wide attentions of scientists. In addition, possibility of nanowire application also makes DNA an attractive material for researchers in the field of material science.

Charge transfer in DNA has been investigated by various ways, including electric current measurement, product analysis, EPR, time-resolved spectroscopic methods, and so on. Among them, transient absorption spectroscopy using ultrashort pulse laser has provided various kinetic information on charge transfer in DNA. Direct observation of hole transfer (HT) process has been reported by the research group of Lewis.[4] They have evaluated the A to A and G to G hole-hopping rates among consecutive A and G sequences, respectively, to be on the order of 10^9 s^{-1}. On the other hand, our research group evaluated the hole hopping rates in various DNA sequences by means of laser flash photolysis and indicated that the hole hopping rate varied depending on kinds and number of intervening nucleobases between nucleobases which act as step stones.[5] Furthermore, hole hopping over 100 angstrom was confirmed by means of the transient absorption spectroscopy during the laser flash photolysis.[6] Thus, detailed information on HT in DNA has been obtained until now. On the other hand, information on the dynamics of negative charge transfer, namely, excess electron transfer (EET), in DNA is rather limited even now. Investigations based on the product analysis suggested that EET occurs by multiple hopping mechanism.

For studies on EET in DNA, our research group has employed oligothiophenes as photosensitizing electron donor to DNA. Their donor ability was investigated using dyad compounds of oligothiophene and nucleobase systematically. In the case of bithiophene (2T), it was revealed that the singlet excited 2T can donate excess electron to T, C, and A, but not to G.[7,8] Furthermore, electron transfer rate was in the order of T > C > A in accordance with the electron acceptor ability of nucleobase. On the other hand, recombination rate was in the order of T > C > A, indicating that the charge recombination process is in the inverted region of the Marcus theory.[9,10] For further understanding of the observed tendency, the evaluated electron transfer rates were plotted against driving force (Fig. 1). It is clear that the evaluated rates can be reproduced by Marcus theory using parameters listed in caption (solid line). It is interesting to note that the obtained curve is similar to that reported for the hole injection process to DNA (dashed line).[11] These results are important information for the design of efficient hole or excess electron injector to DNA.

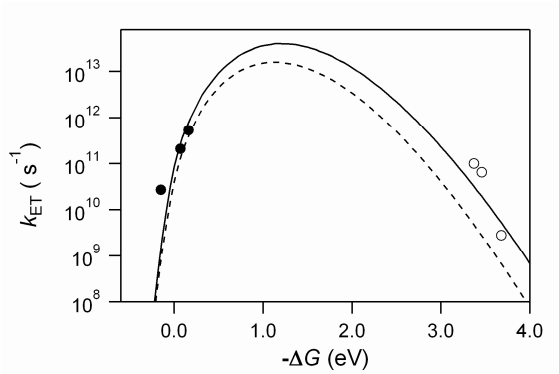

Figure 1. ΔG (ΔG_{CS} and ΔG_{CR}) dependence of k_{ET} (k_{CS} (filled circles) and k_{CR} (opened circles)). The solid line was estimated using 0.20, 1.10, 0.070, and 0.19 eV of λ_S, λ_V, V, and $\hbar\langle\omega\rangle$, respectively. The dashed line was calculated using 0.23, 0.99, 0.043, and 0.19 eV, respectively.[11]

In order to study EET dynamics in DNA, our research group synthesized a series of nicked dumbbell DNA (consecutive A:T base pairs) conjugated with tetrathiophene (4T) and diphenylacetylene (DPA), which act as a photosensitizing electron donor to T and an acceptor of excess electron in DNA, respectively (Fig. 2).[12] The transient absorption spectra during the laser flash photolysis using femtosecond laser which excited 4T selectively showed formation of 4T radical cation with decay of singlet excited 4T within ~10 ps after excitation, indicating effective excess electron injection from singlet excited 4T to adjacent T. The absorption band of DPA radical anion showed gradual rising profile over 200 ps in the case of T_3, indicating the excess electron capture by DPA after hopping of excess electron among consecutive Ts. The multiple hopping mechanism was supported by the fact that the formation rate of DPA radical anion largely depends on the number of intervening T between 4T and DPA. From the formation kinetics of DPA radical anion, the T to T

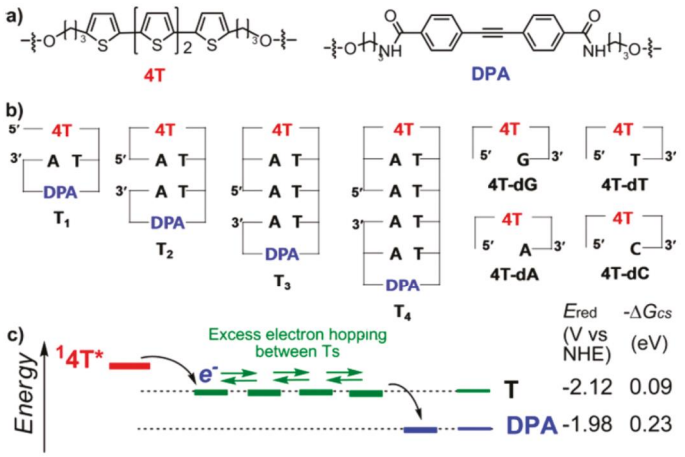

Figure 2. (a) Molecular structures of tetrathiophene derivative (4T) and diphenylacetylene (DPA) conjugated to DNA. (b) Structures of nicked dumbbell DNA conjugated with 4T and DPA. (c) Simplified illustration of EET mechanism in the present DNA systems.

hopping rate of excess electron in consecutive T sequence was evaluated to be 4.4×10^{10} s^{-1} on the basis of random walk model. It should be noted that the evaluated T to T hopping rate of excess

electron is faster than those of A to A and G to G hole hopping rates in DNA.

In addition, we studied EET in DNA hairpins, another B-type DNA, conjugated with *N,N*-dimethylaminopyrene (APy) and DPA.[13] In these DNA hairpins, APy was conjugated to 5' position through an alkyl linker and DPA was placed at loop of hairpin, because DPA is known to form a stable hairpin structure.[14,15] EET dynamics in DNA hairpins were investigated by transient absorption spectroscopy under the condition where APy was selectively excited by femtosecond laser pulse. EET dynamics in DNA were confirmed by generation of the absorption band of DPA radical anion. Because the generation dynamics of DPA radical anion in the DNA hairpin in which APy and DPA were separated by two A:T base pairs was the same as that of DNA hairpin with one A:T base pair, it was indicated that APy did not behave as an end-capping group but was intercalated between two terminal base pairs.[16] By assuming intercalation of APy, T to T hopping rate of excess electron in consecutive T was estimated to be 6.1×10^{10} s^{-1}, which is a similar value to that estimated with the nicked dumbbell DNA conjugated with 4T and DPA.[12] Slightly larger hopping rate of DNA hairpins probably comes from stable DNA hairpin structure without nick and/or intercalation of APy in DNA. The present experiment results reasonably confirmed that the rate constant of EET through consecutive T's is on the order of 10^{10} s^{-1}, which is faster than HT in DNA.

In the report previous to these two papers, we studied EET process in DNA hairpins conjugated with *N*-methyl-*N*-pyrenylglycine (^{A}Py) and DPA by means of nanosecond laser flash photolysis.[17] In the transient absorption spectra during the nanosecond laser flash photolysis of DNA hairpins conjugated with ^{A}Py and DPA, the formation dynamics of DPA radical anion could not be confirmed and superposition of ^{A}Py radical cation and DPA radical anion was observed, even when ^{A}Py and DPA were separated by nine base pairs, because of fast EET dynamics and poor time resolution of nanosecond laser flash photolysis system. But the result indicates that excess electron can tranport even when photosensitizing electron donor and acceptor were separated by 34 angstrom. Since the initial absorbance of DPA radical anion estimated by nanosecond laser flash photolysis reflects the yield of EET, we examined efficiency of EET in DNA hairpins with various

sequences such as consecutive Cs and so on. From this study, we confirmed following tendencies. Mismatch sequence in DNA diminished EET yield. Especially, small EET yields were observed with DNA hairpins with the T:C and T:T mismatches, while more stable T:G mismatch made EET possible to some extent. Replacement of A:T base pairs by G:C also decreased EET yield, although the EET yield was similar to that of consecutive G:C sequence, indicating that the C is not a severe EET trap like G in HT. On the other hand, replacement of A:T by C:G decreased the EET yield due to the slower T-G-T hopping rate, in which G acts as a barrier for EET. The observed tendencies in sequence dependence of EET yield seem to be reasonable on the basis of the results provided by other research groups studying EET using product analysis. The study based on the EET yield helps our understanding on EET in qualitative manner. For further understanding, we are planning to examine these issues in more quantitative manner by means of ultrafast laser spectroscopy. These will be useful information in the fields of physical chemistry, biology, material science and so on.

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Photosensitized pyrimidine dimerization in DNA

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The incidence of skin cancer in humans has steadily increased over the last few decades, with melanoma reaching last year the 5th position among the most commonly diagnosed types of cancer in Europe.[1] It has been unambiguously established that exposure to solar ultraviolet radiation is involved in the pathogenesis of most types of skin cancer. Hence, investigation of the photochemical mechanisms is essential to understand most of the key processes involved in DNA photodamage. Over the last years, this area of research has attracted a renewed interest, owing to the application of recent spectroscopic advances, which have challenged the validity of

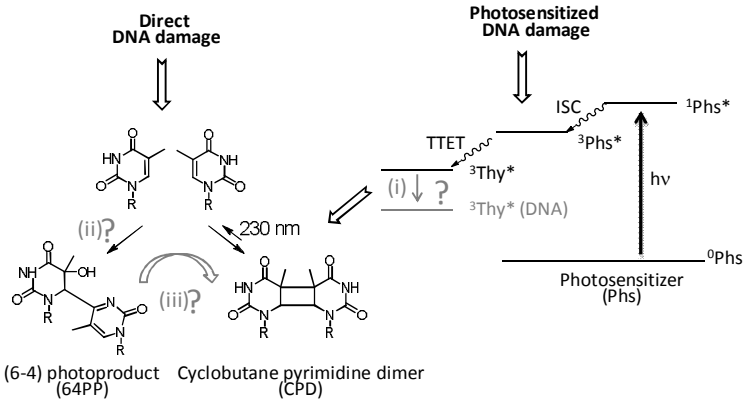

Figure 1. Structure of bipyrimidine DNA photoproduct formation and processes addressed in this report.

established mechanistic paradigms. Thus, a complete picture of the chemistry involved in DNA photodamage is still missing.

In this context, the DNA photosensitizing ability of nonsteroidal antiinflammatory drugs (NSAIDs) and antibacterial fluoroquinolones, has been investigated. Both dimerization of pyrimidines and oxidation of purine bases are reported.[2-4]

Recently, our interest has been focused on the photochemistry of pyrimidine bases. Indeed, cyclobutane (CPD) and pyrimidine (6-4) pyrimidone (64PP) photodimers represent by far the most prevalent DNA photolesions under direct UVB-irradiation (Fig. 1).[5] The former are obtained by a formal [2+2] photocycloaddition between two adjacent pyrimidines, giving rise to the *cis-syn* CPD as the main isomer. The 64PP are formed by the so-called Paterno-Büchi reaction, which involves a [2+2] photoaddition between the C4 carbonyl (or imino) group of the 3´ pyrimidine and the C5-C6 double bond of the adjacent 5´ pyrimidine. The resulting unstable oxetane (or azetidine) undergoes ring opening by C4-O bond cleavage, accompanied by a proton shift from N3, affording the 64PP. In the case of CPD, in addition to the direct irradiation, an indirect process involving triplet-tripet energy transfer (TTET) from the photosensitizer to the thymine moiety has also been evidenced.[2]

With this background, we have addressed three important issues related to bipyrimidine dimers formation: (*i*) the triplet state energy of thymine in DNA (*ii*) the presence of intrinsic DNA photosensitizers, (*iii*) the photoreactivity of higher triplet excited state of thymine in connection with 64PP formation.

Triplet state energy of thymine in DNA:

In the literature, few compounds have been reported as CPD photosensitizers. Indeed, they are generally aromatic ketones, carbazoles, psoralens, or fluoroquinolone antibacterial agents, with a sufficiently high triplet state energy to trigger the photodimerization mechanism.[2] As the feasibility of this process is linked to a favorable thermodynamics for energy transfer, the thymine triplet state energy in DNA is a key parameter.

An interesting example relies on the dual photoreactivity of ketoprofen (KP), a NSAID bearing a benzophenone chromophore. On the one hand, when thymine is part of an oligonucleotide or DNA sequence, its triplet excited state lies below that of KP giving rise to CPD formation through TETT.[6-7] By contrast, for free thymidine in solution (Thd), a Paternó-Büchi reaction is favored giving rise to mixed oxetanes between KP and Thd; CPD are

obtained only at high nucleoside concentration.[8] Interestingly, the Paterno-Büchi reaction occurs when the triplet energy of the alkene (here, Thd) is comparable to (or higher than) that of the carbonyl compound (KP). It is noteworhty that, in spite of its biological importance, an accurate value for energy of the thymine triplet excited state (^{3}Thy*) in DNA has only recently been determined by our group.[9-10] This problem has been tackled combining laser flash photolysis experiments, to determine the photosensitizer triplet state energy, with agarose gel electrophoresis to quantify photoinduced CPD formation in plasmid DNA. In this context, the ability of a panel of fluoroquinolone antibacterial drugs with different triplet state energy has allowed establishing a precise threshold of 267 kJ mol^{-1} for ^{3}Thy* energy in plasmid DNA.[9-10] This result provides the basis for an alert rule as any xenobiotic with a triplet excited state higher than this threshold value should be considered as a potential photogenotoxic agent.

Intrinsic DNA-photosensitizer, the concept of Trojan horse:

The harmful effect of UVA radiation has attracted considerable attention in the last years in relation with the classification of sunlamps and sunbeds as carcinogenic to humans by the International Agency of Research on Cancer (IARC). Indeed, UVA-mediated photoreactions triggered by endogenous or exogenous chromophores lead to an extension of the active fraction of the solar spectrum with photocarcinogenic potential. In this context, an appealing point relies on the UVA absorption of 64PP associated with their 5-methyl-2-pyrimidone chromophore. Thus, it appears feasible that photogeneration of this DNA damage within the double helix constitutes the insertion of a potential intrinsic UVA-sensitizer. To test this concept, we have investigated the photosensitizing properties of 1-(β-D-2'-deoxyribosyl)-5-methyl-2- pyrimidone (Pyo) as a model of 6-4PP.[11] Agarose gel electrophoresis experiments performed with supercoiled circular DNA show that Pyo is able to photoinduce oxidative DNA damage (observed through single strand break formation) together with CPD formation (revealed after treatment with T4 endonuclease V, a specific enzyme for CPD detection). Furthermore, photophysical experiments have demonstrated that Pyo gathers the properties of an efficient DNA damaging agent. On the one hand, the Pyo triplet excited state energy of ca. 291 kJ mol^{-1}, determined by phosphorescence, is higher than

that of ^{3}Thy* in DNA. Thus, the TTET at the origin of CPD formation is a thermodynamically favored process. On the other hand, detection of oxidative DNA damage could be attributed to the generation of reactive oxygen species, namely singlet oxygen and hydroxyl radical, evidenced by EPR during the photolysis of Pyo.[11]
As a consequence, it appears feasible that the 64PP lesion can act as a Trojan horse promoting CPD formation and oxidative damage in its neighboring. Furthermore, this result is of particular importance in relation with clustered DNA damage formation.

Photoreactivity of higher triplet excited state of thymine:
By contrast with CPD, no report exists on photosensitization of the 64PP lesions. Thus, it has generally been accepted that their formation occurs upon direct irradiation, from an excited singlet state. However, this paradigm contrasts with the fact that the Paternó-Buchi photocycloaddition is a characteristic nπ* triplet process. Therefore, on the basis of Thy energetic diagram (Fig. 2), it appears feasible that the nucleobase photoreactivity proceeds from an upper T_2 triplet excited state with the appropriate nπ* electronic configuration. In a first approach to prove this concept, we selected the Norrish-Yang photocyclization as a typical example of nπ* triplet photochemistry, and 5-*tert*butyluracil (*t*Ura) as Thy model.[12] This compound contains γ-hydrogens that can be abstracted by the neighboring C4 carbonyl group; after dehydration of a cyclobutanol intermediate, formation of 1,2-dihydrocyclobuta[d]-pyrimidine-2-one should be observed. Selective population of T_2(nπ*) has been accomplished by biphotonic excitation of benzophenone (BP) with high energy laser pulses at 355 nm or using a two laser/two color setup (308 nm/532 nm). In this way, a BP higher triplet excited state is reached capable to transfer its energy to the T_2(nπ*) Thy triplet state. This process has been optimized by the design of the *t*-Ura/Thy dyad shown in Figure 2, which allows circumventing diffusional restrictions. The viability of this concept has been confirmed by analysis of the irradiation mixture by UPLC coupled with tandem mass spectrometry and comparison of the retention time and fragmentation pattern with an authentic sample of the Norrish-Yang photoproduct (NY/BP, Fig. 2). Therefore, these results open the channel to the potential formation of 64PP through a higher T_2(nπ*) of Thy.

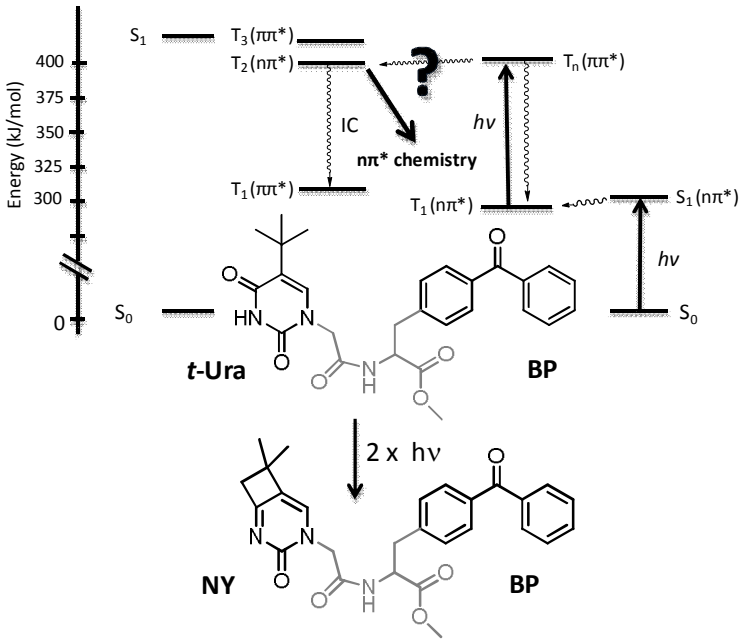

Figure 2. Energetic diagram of *t*-Ura/BP dyad and Norrish Yang photoreaction.

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Time-Resolved Resonance Raman Studies of Nonsteroidal Antiflammatory Drugs

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Nonsteroidal anti-inflammatory drugs (NSAIDs) like ketoprofen when exposed to light can exhibit phototoxicity effects that limit their application in medical treatments.[1-4] This has stimulated investigations of the photochemistry of NSAIDs to better understand the phototoxicity associated with these types of drugs.[2,3] We have used a combination of several time-resolved spectroscopic methods like femtosecond and nanosecond time-resolved transient absorption (fs-TA and ns-TA) and time-resolved resonance Raman (TR^3) spectroscopy to directly observe and characterize the electronic excited states and intermediate species involved in the photochemistry for NSAIDs of interest.[5-9] Time-resolved vibrational spectroscopic methods like TR^3 spectroscopy are particularly useful in obtaining structural and fingerprint information for reactive intermediates. In addition, density functional theory (DFT) calculations were done to explore reaction pathways and predict the structures and absorption and Raman spectra of probable intermediate species observed in the time-resolved experiments. The combined time-resolved spectroscopy results and DFT computational results were used to deduce the reaction mechanisms for the photochemistry of the NSAIDs of interest.[5-9] Here we provide two examples of this approach to study the photochemistry, intermediates and reaction mechanisms associated with the ketoprofen (KP) and tiaprofenic acid (TPA) drugs.

1 Direct observation of triplet state mediated decarboxylation of the neutral and anion forms of ketoprofen (KP) in water-rich, acidic, and PBS solutions.

Nanosecond time-resolved resonance Raman (ns-TR^3) and femtosecond transient absorption (fs-TA) were used to study the photochemical reactions of KP in acetonitrile (MeCN) and in aqueous solutions of varying conditions. Fs-TA spectra show that the singlet state KP transforms into the triplet state KP by intersystem

crossing (ISC). In isopropanol, the $n\pi^*$ triplet state KP undergoes hydrogen abstraction to generate a KP ketyl radical intermediate. Figure 1 E shows a comparison of the experimental ns-TR[3] spectrum of KP in isopropanol solvent acquired at 400 ns (top spectrum) with the DFT calculated spectrum of the KP ketyl radical species (bottom spectrum). The good agreement between the vibrational frequency patterns of these spectra indicates that the intermediate observed in the isopropanol solvent is the ketyl radical species. In a strong acidic aqueous solution, the triplet state of KP was firstly detected by ns-TR[3]. The fs-TA study found that the transient absorption (524 nm) of the triplet state KP gradually decreases in intensity while two new bands appear at 324 nm and 496 nm (see Figure 1 B). Figure 1 D shows that the triplet state KP can be observed at 5 ns along with a characteristic Raman band associated with a key biradical intermediate species (1577 cm^{-1}). This indicates that the triplet state KP can undergo an acid catalyzed photodecarboxylation reaction to generate a biradical intermediate. Figure 1 F shows a comparison of the experimental ns-TR[3] spectrum of KP in pH=0 acidic solution acquired at 5 ns (top spectrum) with a DFT calculated spectrum of the triplet protonated KP carbanion biradical intermediate (bottom spectrum). The excellent agreement between the vibrational frequency patterns of the experimental Raman spectrum with the DFT predicted Raman spectrum of the biradical convincingly demonstrates that the transient absorptions observed at 324 nm and 496 nm can be assigned to the biradical intermediate. The DFT computational study also found that an excited triplet state intramolecular proton transfer (ESIPT) is involved in the photodecarboxylation reaction. Further work found that KP will undergo a water mediated ESIPT induced photodecarboxylation reaction to generate a biradical species even in a water-rich solution (90%). In a phosphate buffered aqueous solution (PBS), the singlet state KP anion also undergoes an ISC process to convert into the triplet state KP anion. The photodecarboxylation reaction proceeds from the triplet state KP anion and a carbanion intermediate appears at 590 nm (see Figure 1 C). Ns-TR[3] spectra confirmed that a biradical intermediate was observed at 5 ns.

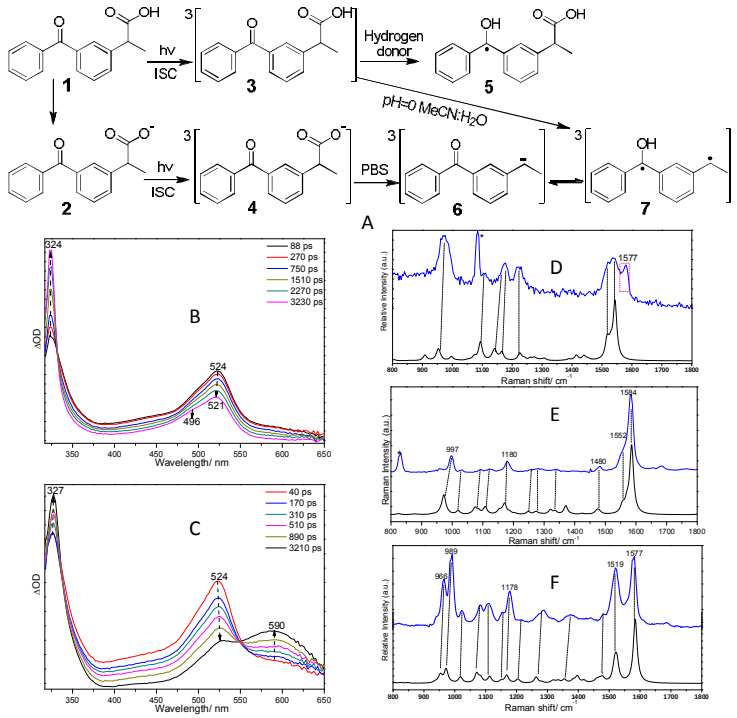

Figure 1. A) Proposed photochemical mechanism of KP in different solvents. B) Fs-TA of KP obtained in pH=0 MeCN:H_2O (1:1) solution. C) Fs-TA of KP obtained in PBS:MeCN (1:1) solution. D) Comparison of the experimental ns-TR^3 spectrum of KP in pH=0 acidic solution acquired at 5 ns (top) with DFT calculation spectrum of ^{3}KP (bottom). E) Comparison of the experimental ns-TR^3 spectrum of KP in isopropanol solvent acquired at 400 ns (top) with DFT calculation spectrum of KP ketyl radical species (bottom). F) Comparison of the experimental ns-TR^3 spectrum of KP in pH=0 acidic solution acquired at 5 ns (top) with DFT calculation spectrum of triplet protonated KP carbanion biradical intermediate (bottom).

In summary, Figure 1 A shows the scheme of the proposed photochemical mechanism of KP in different solvents, all of the significant intermediates involved in the photochemistry were characterized by the ns-TR^3 and fs-TA experiments, which provides convincing evidence to make assignments of the intermediate species

involved in the photochemistry and to determine the photochemical pathways of KP after excitation by ultraviolet light.

2 **Time-Resolved Spectroscopic Study of the Photochemistry of Tiaprofenic Acid (TPA) in a Neutral Phosphate Buffered Aqueous Solution From Femtoseconds to Final Products**

The fs-TA experimental data indicated that the lowest lying excited singlet state S_1 underwent an efficient intersystem crossing process (ISC) to quickly transform into the lowest lying excited triplet state T_1. Then the triplet state T_1 undergoes decarboxylation to generate a triplet biradical species (TB^3) (**10**) (see Figure 2 B) and this correlates with the transient absorption at 360 and 600 nm decreasing in intensity and a new band at 415 nm appearing on the sub-nanosecond time scale. In the ns-TR^3 experiments, the TB^3 species can be observed at 10 ns and Figure 2 D shows a comparison of the experimental ns-TR^3 spectrum of TPA in PBS:MeCN (7:3) solution acquired at 10 ns (top spectrum) with the DFT calculated spectrum of ^{3}TB (bottom spectrum) and the good agreement between the vibrational frequency patterns of the two spectra indicates that the first intermediate observed by ns-TR^3 is the TB^3 species. Then TB^3 undergoes a protonation process to produce a neutral biradical species (TBP^3) (**11**). The ns-TA experiments observed a transient absorption at 420 nm and 600 nm decreases in intensity that correlates with the appearance of a new band at 369 nm. This further demonstrates that the TB^3 species undergoes the protonation reaction to generate the biradical species (TBP^3). Figure 2 E shows a comparison of the experimental ns-TR^3 spectrum of TPA in PBS:MeCN (7:3) solution acquired at 400 ns (top spectrum) with a DFT calculated spectrum of ^{3}TBP (bottom spectrum). The good correspondence between the vibrational frequencies of the ns-TR^3 experimental spectrum and the DFT calculated spectrum implies that the second intermediate observed is the biradical intermediate species. Subsequently, the TBP^3 species

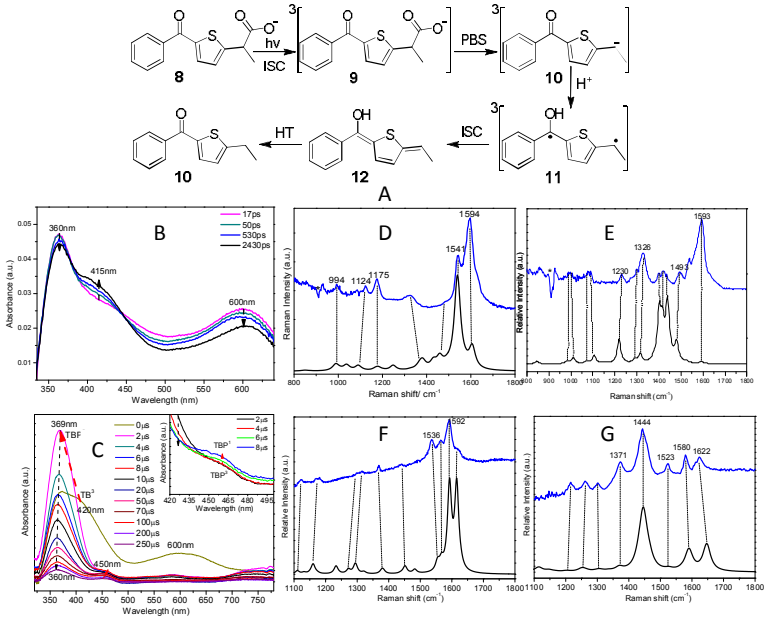

Figure 2. A) Shown is a proposed photodecarboxylation mechanism of TPA in PBS:MeCN (7:3) solution based on our experimental results. B) Fs-TA of TPA obtained in PBS:MeCN (7:3) solution. C) Ns-TA of TPA obtained in PBS:MeCN (7:3) solution. D) Comparison of the experimental ns-TR^3 spectrum of TPA in PBS:MeCN (7:3) solution acquired at 10 ns (top) with a DFT calculated spectrum of ^{3}TB (bottom). E) Comparison of the experimental ns-TR^3 spectrum of TPA in PBS:MeCN (7:3) solution acquired at 400 ns (top) with a DFT calculated spectrum of the ^{3}TBP intermediate (bottom). F) Comparison of the experimental ns-TR^3 spectrum of TPA in PBS:MeCN (7:3) solution acquired at 120 μs (top) with a DFT calculated spectrum of the TBP intermediate (bottom). G) Comparison of the experimental ns-TR^3 spectrum of TPA in PBS:MeCN (7:3) solution acquired at 500 μs (top) with a DFT calculated spectrum of the DTPA final product.

decayed via ISC to produce a singlet TBP (**12**) species that further reacted to make the final product (DTPA) (**13**) (see Figure 2 F and G). In this study, all of the photochemical processes and the intermediates involved were firmly confirmed by the transient

absorption and ns-TR^3 spectra from femtoseconds to final product. Comparison of the present results for the TPA^- anion with similar results for the deprotonated form of ketoprofen (KP^-) in the literature can investigate how the thiophene moiety in TPA^- anion affects the reaction mechanism and photochemistry of these non-steroidal anti-inflammatory drugs (NSAIDs). The combined use several time-resolved spectroscopic methods and in particular time-resolved vibrational spectroscopy techniques like time-resolved resonance Raman (TR3) spectroscopy that can obtain structural and fingerprint information for short-lived excited states and intermediates are very powerful tools to directly observe and characterize the excited states and intermediate species involved in the photochemistry and phototoxicity of a variety of NSAIDs and can be readily extended to study other classes of drugs and biolmolecules.

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Close up on cationic porphyrazines as ligands of ds DNA and G-quadruplex DNA, a promising target for cancer treatment

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In nature we encounter DNA in different forms each playing a crucial role in distinct biological processes. The best known DNA structure is the so-called double-stranded helix described by Watson and Crick.[1] DNA consists of polymers of nucleotides linked to a backbone of alternating deoxyribose molecules and phosphate groups. Each sugar molecule is covalently linked to one of four possible bases (Adenine, A, and Guanine, G, the two purins and Cytosine, C, and Thymine, T, the two pyrimidines). In doubled stranded (ds) DNA consisting of two polymeric chains base A always pairs with T and G always pairs with C *via* two and three hydrogen bonds, respectively (see Fig. 1). The aromatic planes of the bases are oriented perpendicularly to the helix axis. Hydrophobic sites locate between adjacent planes of base pairs. The backbone of the polynucleotide chain is highly charged with one negative charge per phosphate group balanced by positive counterions structurally essential for the double helix. The structural features of DNA allow two main types of binding modes, intercalative binding between adjacent base pairs and minor and major groove association.

A less known DNA structure is the G-quadruplex (G4).[2] G4 structures of G-rich DNA sequences consist of stacked planes of four guanosines, cyclically bound to each other via eight hydrogen bonds according to the Hoogsteen motif (see Fig. 1). Formation of the G4 is favored by the presence of monovalent cations like Na^+ and K^+ between the planes. G-rich sequences are present in important regions of the human genome, like the telomeres at the end of the chromosomes and the promoter regions of several oncogenes.[2] The human telomere possesses a single stranded overhang at the 3' end consisting prevalently of repeats of the

GGGTTA sequence. Telomere shortening during cell replication is responsible for cellular senescence. The enzyme telomerase mediates a mechanism of telomere maintenance. It is overexpressed in about 85% of rapidly replicating cancer cells, thereby guaranteeing their "immortality".[3] The activity of this enzyme critically depends on the structural organization of these guanine-rich sequences. In fact ligands able to stabilize G4 in solution also interfere with the biological processes involving guanine-rich sequences.[3, 4] This fact has been observed not only for the telomeric sequences but also for guanine-rich sequences present in the promoter regions of some oncogenes.[5] Consequently guanine-rich sequences have become a very promising target for the development of new anticancer drugs.[6]

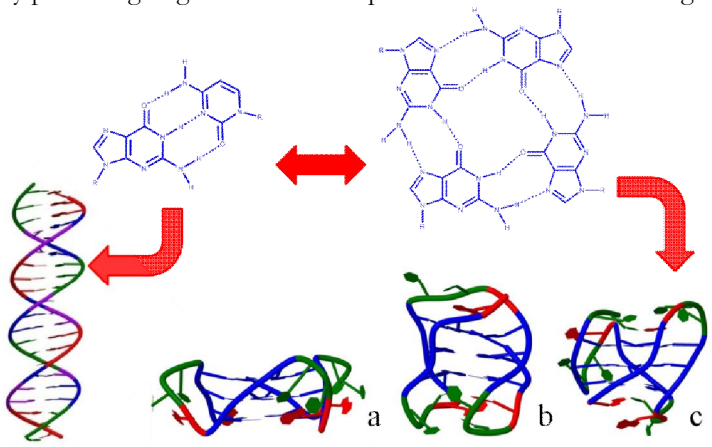
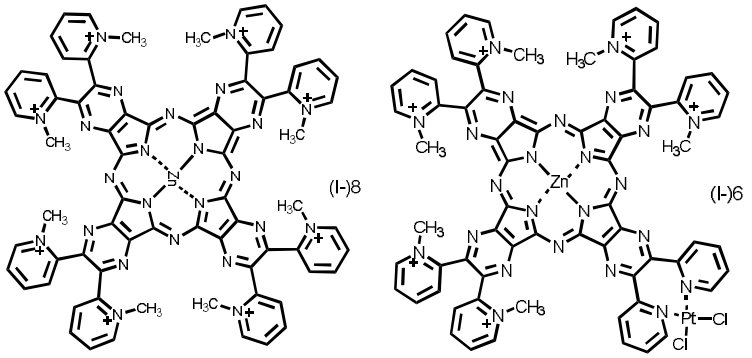


Figure 1. Cartoon showing the base pair GC participating in ds DNA helix formation and the Hoogsteen tetrad giving rise to different conformations of G4 structures, parallel (a), basket (b) and hybrid (c). G, blue, T, green, A, red.

We studied the ability of two cationic Zn(II) compounds both soluble in water, $[LZn]^{8+}$ and $[LZnPt]^{6+}$ (see scheme 1), to form complexes with telomeric DNA in ds and G4 form.[7-9] The presence of the exocyclic Pt(II) unit confers lower symmetry and larger rigidity to the molecule and reduces the number of positive charges, possibly influencing affinity and/or binding modes for negatively charged ds DNA and G4 structures. These compounds both show good singlet oxygen photosensitizing properties and thus hold promises for application in PDT.[9] The aromatic planar core of $[LZn]^{8+}$ and

$[LZnPt]^{6+}$ has a dimension similar to that of the G4 tetrad and the presence of the peripheral positive charges is expected to favor binding due to the electrostatic interaction with the negatively charged backbone. This proned us to investigate the interaction of LZn^{8+} and $LZnPt^{6+}$ with the telomeric 22-mer sequence 5'-d[AGGG(TTAGGG)$_3$]-3' in K^+ and Na^+ rich solution. The latter sequence adopts a conformation that strongly depends on the nature of the cation: in Na^+ rich solution a "basket" G4 structure is formed, with one diagonal and two lateral TTA loops (Fig. 1b); in a K^+ containing crystal a parallel G4 folding is assumed, with three TTA loops of the double-chain-reversal type (Fig. 1a); NMR showed that the same 22-mer in K^+ rich solutions adopts a hybrid structure, as major conformer, featuring one double-chain-reversal loop and two lateral loops (see Fig. 1c).



Scheme 1. The octacation $[LZn]^{8+}$ and the hexacation $[LZnPt]^{6+}$ both neutralized by I^- ions (L = tetrakis-2,3-[5,6-di(2-pyridyl)pyrazino]porphyrazinato dianion).[10]

The interaction between the compounds $[LZn]^{8+}$ and $[LZnPt]^{6+}$ and the 22-mer in G4 conformation was monitored by means of absorption, fluorescence, and circular dichroism (CD). Global analysis of the multiwavelength data of spectroscopic titrations afforded the binding model, the binding constants as well as the spectra of the complexes. Both $[LZn]^{8+}$ and $[LZnPt]^{6+}$ form dimers in solution. These can be disrupted by addition of SDS micelles (Fig. 2a). The dimerization constants obtained from dilution (absorption) experiments have log values of 7.12 and 6.99 for $[LZn]^{8+}$ and $[LZnPt]^{6+}$, respectively.

Some very interesting properties emerged for the G4 complexes in K^+ rich solution from an accurate analysis of the spectroscopic data in the UV as well as the visible region. Both ligands exhibit high affinity for G-quadruplex DNA and form complexes of 1:1 and 2:1 stoichiometry. The remarkable thermal stability of the complexes is revealed by a significant increase in the melting temperature of complexed G4 compared to free G4. The UV CD spectra provide structural information relevant to the G4 conformation in the bound state. Exclusive parallel G-quadruplex is observed for the 2:1 complex in K^+ rich solution (Fig. 2d). As to the 1:1 complex a mixture of at least two conformers likely exists, especially in the case of $[LZnPt]^{6+}$ as ligand. Further, the absorption spectra (Fig. 2b) clearly show that complexation results in progressive monomerization of the porphyrazine, largely present in its aggregated form in the absence of DNA.

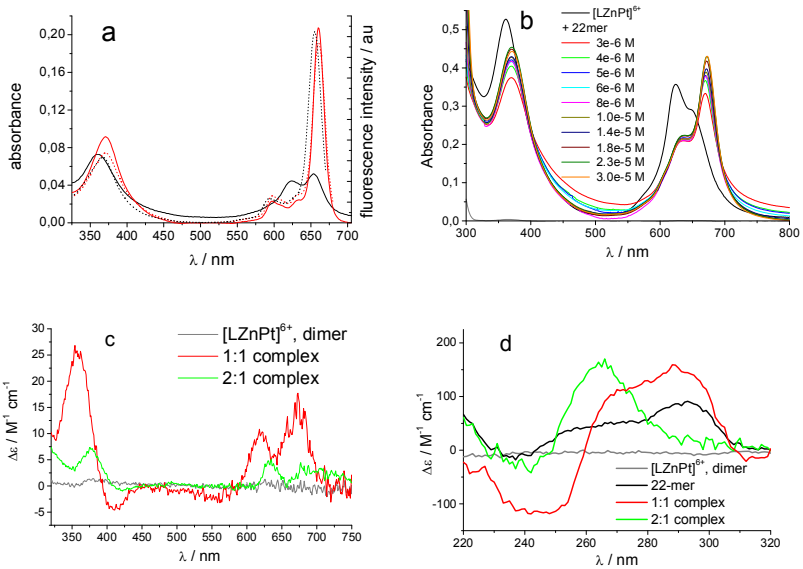


Fig. 2. (a) Absorption (line) and corrected fluorescence excitation (dotted) spectra of 1×10^{-6} M solution of $[LZnPt]^{6+}$ in pure water (black) and with 20 mM SDS (red); d = 1.0 cm, 22 °C. Excitation spectra were measured at emission wavelength of 690 nm for solution absorbing less than 0.1; (b) Absorption spectra of 1.0×10^{-5} M $[LZnPt]^{6+}$ solutions with increasing 22mer concentration (range 0.3-3.0)$\times10^{-5}$ M in TRIS/KCl buffer, pH 7.4, d = 1.0 cm; (c) Calculated CD spectra ($\Delta\varepsilon$) of the free compound and the complexes $[LZnPt]^{6+}$,

$\log(K_{11}/M^{-1}) = 5.5 \pm 0.4$ and $\log(K_{21}/M^{-1}) = 12.3 \pm 0.4$; (d) calculated UV CD spectrum ($\Delta\varepsilon$) of the free compounds and the complexes, $\log(K_{11}/M^{-1}) = 5.5$ and $\log(K_{21}/M^{-2}) = 11.9 \pm 0.2$.

The CD signal of the complexes in the visible is attributable to the ligand, lacking intrinsic chirality, but experiencing the asymmetric G4 DNA environment (Fig. 2c). A strong CD signal manifests in the 1:1 complex, whereas it is weak in the 2:1 complex. This may be due to a cancellation effect in a configuration where the two porphyrazine ligands stack as monomers on different diastereotopic faces of the top and bottom tetrads, thereby reasonably contributing to a CD of opposite sign.

Dilute solutions of $[PzZn]^{8+}$ and $[PzZnPt]^{6+}$ in 0.01 M TRIS buffer of pH 7.4 with 0.1 M KCl exhibit a weak fluorescence band with maximum at 662 nm and a shoulder at 726 nm. This emission is completely quenched upon addition of 22mer. The quenching mechanism may be an electron transfer process, most likely from the guanosine residues, to the excited porphyrazine.

A similar series of experiments were performed in Na^+ rich buffer where the 22mer adopts exclusively the basket structure, thereby allowing us to examine the interaction of the hexacation with this sole conformer. A completely different complexation behaviour towards the G4 basket conformer was observed, where neither formation of the parallel G4 complex nor stabilisation of the complexed basket structure occurred.

In conclusion induction of the parallel G4 form upon ligand binding seems to require the presence of K^+ as a co-factor and/or the presence of the hybrid G4 conformer as a prerequisite for promoting initial association and subsequent relaxation to the final complex geometry. In the case of the parallel complex we are likely observing binding to top and bottom tetrads *via* π-π stacking. Actually in such geometry the three propeller loops do not confer substantial steric hindrance to ligand accomodation. Moreover the presence of the peripheral positive charges allows optimization of the electrostatic interactions with the G4 phosphate groups present at the tetrad borders. In the basket case we reasonably have binding at the grooves since the TTA loops at top and bottom provide steric hindrance to ligand access to the tetrad. From the biological point of view the most relevant results are those obtained in the presence of excess K^+. Considering the G4 binding behaviour in the presence of K^+ these compounds have promising features as G4 stabilizing ligands.

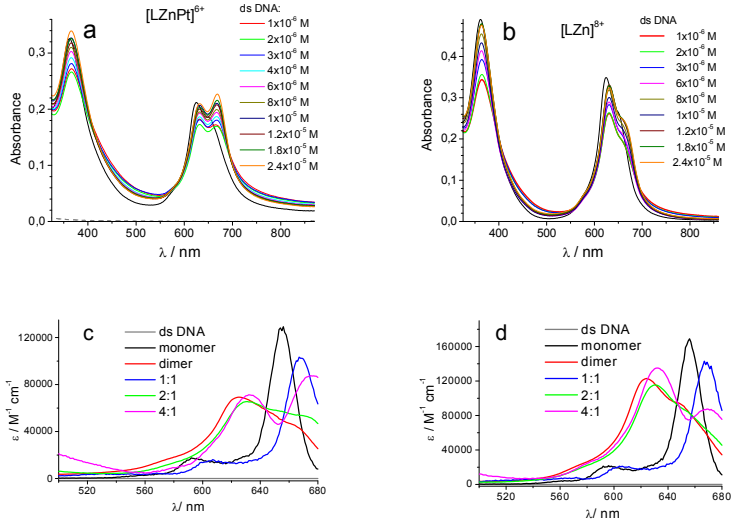


Fig. 3 (a) Absorption spectra of a 6×10^{-6} M $[LZnPt]^{6+}$ solutions with increasing ds DNA concentration (range 0.1-2.4)$\times10^{-5}$ M; (b) Absorption spectra of a 6.0×10^{-6} M $[LZn]^{8+}$ solutions with increasing ds DNA concentration (range 0.1-2.4)$\times10^{-5}$ M; Note: (a-b) in 10 mM TRIS buffer, 0.1 M KCl, pH 7.4, d = 1.0 cm, 22 °C and spectra of $[LZnPt]^{6+}$ and $[LZn]^{8+}$ alone are in black. (c) Calculated absorption spectra (ε) in correspondence of the Q band of monomeric and dimeric $[LZnPt]^{6+}$ and the hexacation:dsDNA complexes, $\log(K_{11}/M^{-1}) = 5.85 \pm 0.03$, $\log(K_{21}/M^{-1}) = 14.0 \pm 0.05$ and $\log(K_{41}/M^{-1}) = 27.5 \pm 0.14$; (d) Calculated absorption spectra (ε) of monomeric and dimeric $[LZn]^{8+}$ and the octacation:dsDNA complexes, $\log(K_{11}/M^{-1}) = 5.84 \pm 0.08$, $\log(K_{21}/M^{-1}) = 14.3 \pm 0.14$ and $\log(K_{41}/M^{-1}) = 28.2 \pm 0.35$.

The study of $[LZnPt]^{6+}$ and $[LZn]^{8+}$ as G4 DNA ligands has been completed with an investigation of binding to ds DNA. In particular, we explored the association of $[LZnPt]^{6+}$ and $[LZn]^{8+}$ to the ds 21mer 5'-d[GGG(TTAGGG)$_3$]-3'/3'-d[CCC(AATCCC)$_3$]-5', as model for B-DNA.[7] The exocyclic $N_{2(pyr)}PtCl_2$ coordination site in $[LZnPt]^{6+}$ (Scheme 1) resembles cis-platin, $(NH_3)_2PtCl_2$, an extensively used chemotherapeutic anticancer drug. The latter drug is known for its ability to covalently bind to guanine residues of ds DNA inhibiting crucial biological processes. In this frame the non covalent binding of $[LZnPt]^{6+}$ was compared to that of $[LZn]^{8+}$

lacking the cis-Pt functionality (Scheme 1). The study was performed monitoring absorption, fluorescence and CD. The behaviour observed in the presence of ds DNA is different from that in presence of G4-DNA. Higher order binding stoichiometries were evidenced. The results for $[LZnPt]^{6+}$ and $[LZn]^{8+}$ exhibit both similarities and differences. The binding constants are of the same order in both systems; the absorption spectra of the complexed species (Figure 3a-d) exhibit a typical red shift as well as a hypochromic effect compared to those of the free ligands; the absorption profiles of the 1:1 and 2:1 complexes are similar in both compounds; on the contrary the absorption spectrum of the 4:1 complex is different, showing in the case of $[LZnPt]^{6+}$ a more intense peak on the red side of the Q-band, typical of monomeric species. Reasonably in the 4:1 complexes the macrocyle is associated both as dimer and monomer at different sites of the ds DNA and the relative weight of the two forms is different for the two compounds, with the monomeric form more favoured in the 4:1 complex of the hexacation. In general, ds DNA has a lower ligand monomerization potential than G4 DNA,[8, 9] the latter being able to bind both compounds mainly as monomers in the parallel conformation. Apparently the surface of the top and bottom base pairs in ds DNA, smaller than that of a tetrad in G4 DNA, does not favour stacking of the porphyrazine macrocycle.

The fluorescence spectra in Fig. 4 show an important decrease of the emission intensity at high porphyrazine excess while a partial recovery of the intensity, accompanied by a ca. 10 nm red shift, occurs on increasing the ds DNA concentration. The corrected excitation spectra measured at 690 nm of $[LZnPt]^{6+}$ and $[LZn]^{8+}$ are attributable to monomeric species, either free or complexed. These features are consistent with negligible fluorescence from higher order DNA complexes (4:1 and 2:1). Singular Value Decomposition of the spectral data in Fig. 4 confirm the presence of only two species with differentiated spectra, thereby supporting that free monomer and complexed monomer exclusively contribute to the total steady state emission.

The overall findings of the ds and G4 DNA binding study of $[LZnPt]^{6+}$ and $[LZn]^{8+}$ allow to envisage for the hexacation three-modal therapeutic anticancer potentialities: as singlet oxygen photosensitizer, as G4 stabilizer and as DNA ligand.

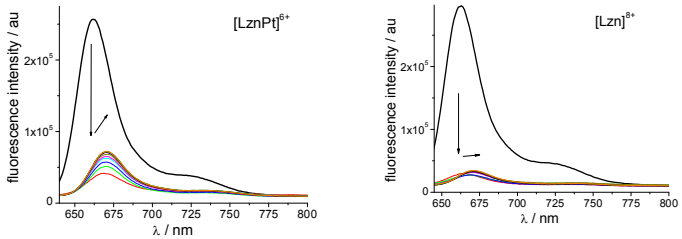


Fig. 4 Titration of a 6×10^{-6} M $[LZnPt]^{6+}$ (left) or $[LZn]^{8+}$ (right) solutions with increasing ds DNA concentration (range 0.06-2)$\times10^{-5}$ M in TRIS 10 mM buffer with KCl 0.1 M, pH 7.4 monitoring fluorescence spectra (corrected) upon exc. at 595 nm. All the solutions absorb less than 0.1 at the excitation wavelength.

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The activities of the group "Excited Biomolecules"

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The group "Excited Biomolecules (BME) carries out photophysical and photochemical studies of biologically relevant molecular systems. The main tools are optical spectroscopic techniques (absorption, fluorescence) from the femtosecond to the millisecond time-scale. In addition to the author, four other permanent research scientists (A. Banyasz, J. Brazard, P. Changenet-Barret, T. Gustavsson) and a technician (M. Perron-Poisson) work at BME.

The major project of the group concerns the interaction of UV radiation with DNA. The underlying motivation for this project is twofold. On one hand, to describe the primary processes preceding the UV-induced damage of DNA and provide a rational basis for the understanding of the first steps leading to the appearance of skin cancer. On the other hand, to determine the factors which govern the optical properties of DNA-inspired materials in relation with their potential application in the emerging field of bioelectronics.

Our methodology consists in (i) studying systems of increasing complexity (isolated bases, single, double and quadruple strands; model systems with simple and repetitive base sequence and highly disordered systems such as natural DNA), (ii) following their behaviour on a very large time-scale, which is crucial for systems characterized by slow movements (multi-scale effects) and (iii) combining experimental observations with theoretical modelling in collaboration with theoreticians.

In parallel with the work on DNA, a new research activity dealing with the interaction of drugs with biomolecules (DNA and proteins) started recently. In this case, light is used to probe the structural dynamics of the drug-biomolecule complex. Another goal is to understand specific reactions responsible for the phototoxicity of drugs.

The most important results obtained during the past five years are highlighted below:

UVA spectroscopy and reactivity: It was widely accepted that the UVA-induced damage of DNA takes place only via an indirect mechanism, involving energy or charge transfer from photosensitisers present in the cell. This view was supported by the fact that DNA bases do not absorb UVA radiation. We demonstrated that, in contrast to isolated bases, UVA light can be absorbed by DNA helices (Fig. 1) and provoke photochemical reactions (collaboration with T. Douki, CEA Grenoble, France).[1,2] Subsequently, we performed the first photophysical study in the UVA spectral domain, characterizing the DNA excited states. We showed that base-pairing enhances UVA absorption and favours cyclobutane dimer formation.[2] Theoretical studies performed by R. Improta (CNR, Napoli, Italy) correlated the UVA absorption to low-lying excited charge transfer states.[3,4]

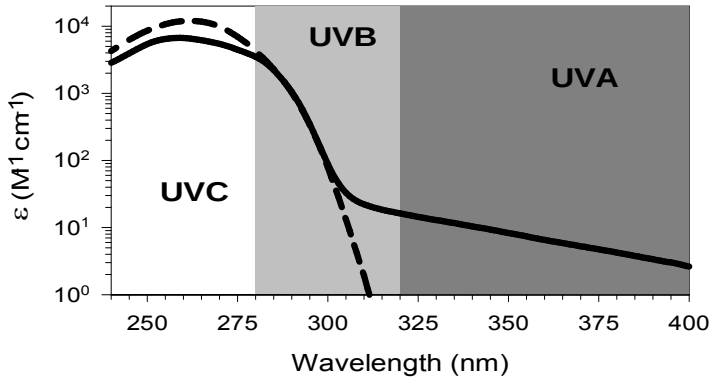

Figure 1. UV absorption spectrum of a DNA double helix composed of twenty adenine-thymine pairs (solid line) in aqueous solution compared to that of an equimolar mixture of its monomeric components (dashed line).

Energy transfer in natural DNA. The UV-induced DNA lesions at mutational hotspots are not randomly distributed but depend on the base sequence around them. This effect could be related to the redistribution of the electronic excitation energy among the bases.

We had evidenced that ultrafast energy transfer takes place in model duplexes with repetitive base sequence; this process is possible because the initially populated states are delocalized on more than one base (excitons).[5] It was predicted that this phenomenon should not occur in natural DNA since it is a highly disordered system. Studying the fluorescence of natural DNA, we showed that this prediction was not correct. In this case, the energy transfer process can be mediated by interconversion between ππ* states and charge transfer states. As a result ππ* excitations exhibit multiscale decay, from the femtosecond to the nanosecond time-scale (collaboration with Yuri Berlin, Northwestern University, USA).[6,7]

High energy long-lived excited states in DNA. Long-lived excited states with substantial excess energy may trigger photochemical reactions. So far, the only known long-lived excited states in DNA were considered to be excimers/exciplexes whose energy is lower than that of the ππ* states. By studying the fluorescence of alternating guanine-cytosine duplexes and hairpins (collaboration with F. D. Lewis, Northwestern University, USA), we revealed the existence of charge transfer states decaying on the nanosecond time domain whose energy can exceed that of the ππ* states by as much as 0.5 eV.[8,9] The existence of such states could also explain the complex mechanism responsible for energy transfer in natural DNA.

Tuning excited state of guanine nanostructures by metal ions. Metal ions located in the internal cavity of guanine quadruplexes play a key role in their structure and stability. Yet, their effect on the excited states had not been studied so far. We showed that the presence of Na^+ ions in the quadruplexes favours trapping of ππ* excitations by charge transfer states, in contrast with the K^+ ions, which are larger and less mobile.[10,11] This effect could have important consequences for the UV-induced damage of telomeric sequences and also inspire the design of guanine nanostructures for opto-electronics.

Uracils as fluorescent probes. Designing new fluorescent analogues of the DNA bases emitting in the visible spectral domain is an important issue for analytical applications, such as cellular (*in vivo*) fluorescence-based imaging. We showed for the first time that certain uracil derivatives, which can form Watson-Crick base pairs without perturbing the helical structure, may serve this purpose. In particular,

5-dimethylaminouracil has a fluorescence quantum yield and lifetime that exceed by more than three orders of magnitude those of the natural base. These results were rationalized by high-level quantum chemistry calculations including solvent effects, in collaboration with Roberto Improta.[12,13]

Unravelling the fluorescence properties of Doxorubicin. Doxorubicin (DOX) is a potent anti-tumoral agent widely used for cancer therapy. The fluorescence properties of DOX, widely exploited for the characterization of its interaction with biological media has led to controversial interpretations due to the self-association of the drug in aqueous solution. In collaboration with Ilse Manet and Sandra Monti (CNR, Bologna, Italy), we managed to discriminate the fluorescence properties of DOX dimers whose quantum yield (10^{-5}) is three orders of magnitude lower than that of monomers.[14] Under these conditions, acquisition of their fluorescence spectra, fluorescence decays and anisotropy decays on the femtosecond time scale constitutes a major achievement.

Stereoselectivity in the dynamics of protein-drug interaction. Many drugs are chiral compounds with only one of the stereo-isomers being active. While the stereo-selectivity is crucial for the ground state interactions, it is equally relevant for photo-induced reactions, leading to drug-connected phototoxicity. Experiments on flurbiprofen and human serum albumin, performed in the frame of a collaboration with M. C. Jiménez and M.A. Miranda (Universitat Politécnica de València, Spain) showed for the first time that the excited state interaction is stereo-selective already on the picosecond time scale.[15] Moreover, we found that the reorientational relaxation of the drug, when complexed with the protein, is more restricted for the (R)-enantiomer, a fact that may be related to its pharmacological activity.

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Antibacterial Properties of Silver Nanoparticles: More than Just Silver?

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The antibacterial properties of metal nanoparticles have interested us over the last few years, and have led us into a partnership across the Atlantic Ocean to collaborate with Linköping University and with Karolinska Institute in Sweden. Our group has developed new photochemical strategies for the synthesis of stable metal nanoparticles, starting with a facile strategy to make gold nanoparticles in minutes.[1] This strategy is based on the reducing power of some free radicals, notably ketyl radicals and is the basis of a method that works with many elements, including the synthesis of silver nanoparticles (AgNP), Figure 1.[2]

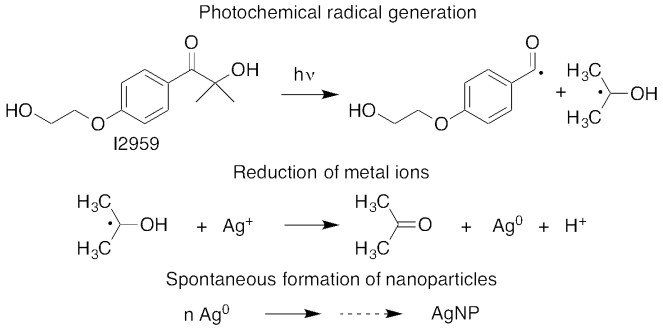

Figure 1. Mechanism for the synthesis of silver nanoparticles using Irgacure-2959® (I2959), a commercial water-soluble benzoin.

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Photochemistry has also proven extremely useful for the synthesis and modification of ultraclean nanoparticles,[3] and for the control of the morphology and dimensions of structures, such as AgNP, using simple methodologies based on LED irradiation.[4]

Our interest on nanomaterials focuses on their applications in several fields, including spectroscopy, catalysis and biomedicine. When it comes to performance, a very common question is whether the action of metal nanostructures is due to the nanomaterial itself, or to metal ions leaching into the surrounding media. In the case of catalysis the question is whether heterogeneous catalysts are actually responsible for homogeneous catalysis. For the pharmaceutical industry, such secondary homogeneous catalysis would largely offset the benefits of easily separated heterogeneous catalysts.[5] A similar question can be asked in the case of therapeutic applications of metal nanostructures: is their action due to the nanostructure or to metal ions leaching from it? In the latter case the nanostructure would effectively be just a tool for slow release of the active metal ion.

In the case of silver ions, their antibacterial properties have been known for centuries and normally attributed to silver ions,[6] including in the case of widely used materials such as silver sulfadiazine.[7] It is thus not surprising that the first assumption in the case of silver nanoparticles as antimicrobials is that their action is due to silver ion release. Numerous commercial products including diverse fields such as medicine, cosmetics and textiles take advantage of the antibacterial properties of silver and the commercial opportunities that these create.

Our work, which does not question the beneficial action of silver ions, demonstrates that silver nanoparticles have intrinsic antibacterial properties and that these are superior to those of the ions themselves.[8] Silver nanoparticles can be synthesized and stabilized within a protein environment;[8-10] Of particular interest is the case of collagen, where a new composite material AgNP@collagen has potential applications in tissue engineering and in the treatment of a range of cutaneous medical conditions.

Our Sweden-Canada collaboration has led to the fabrication of new biomaterials where the silver activity exceeds that that could be achieved with the same total concentration of silver ions. Figure 2 compares the '*Minimal Inhibitory Concentration*' or MIC for various silver sources against three types of bacteria. In this type of graph the

shorter bar indicates a better antimicrobial agent, and it is clear that AgNP@collagen is the most effective in all there examples studied.

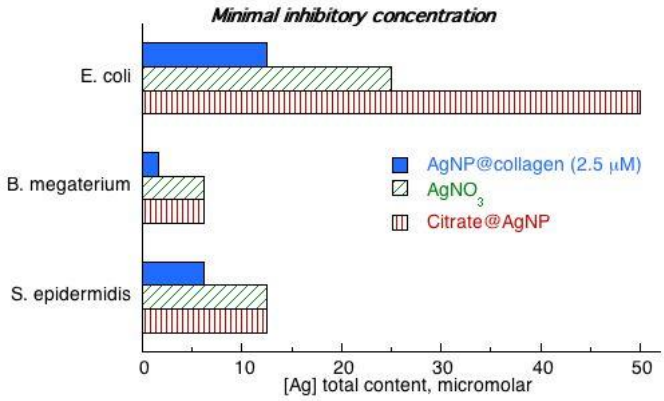

Figure 2. Bar plot showing the antibacterial activity of silver in various forms against three bacterial strains. Adapted from data in a recent publication.[8]

Figure 2 clearly shows that the action of AgNP goes beyond the delivery of silver ions and the potential applications of this technology are the subject of our on-going collaboration. The development of novel hydrogels with anti-infective properties, the stabilization of AgNP with small peptides and the fabrication of bimetallic particles (Au/Ag) suitable for photodynamic therapy while retaining antibacterial activity promise to keep us busy with nanotechnology research for the foreseeable future. Beyond our collaboration with Sweden, research in this and other topics involve partnerships with scientists in Spain, Brazil, Argentina and Chile.

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PILLS OF HISTORY

At the origin of photochemistry. The photochemical behaviour of santonin. Some documents in this field.

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Santonin is an anthelminthic compound (its formula is depicted in the Figure 1), isolated in *Artemisia maritima* and *Artemisia cina* (Compositae).

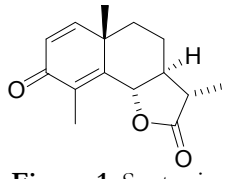
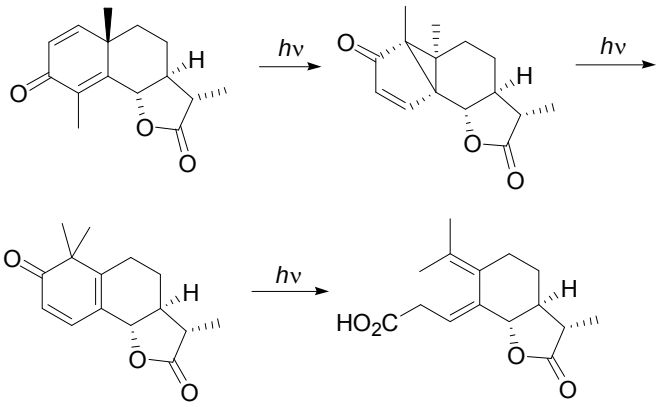


Figure 1. Santonin



Scheme 1. The photoisomerization of Santonin

The compound is not stable to sunlight and allowed the formation of the photosantonic acid (Scheme 1).[1]

In this article we want to collect the most significant contributions given in a pioneering period of photochemistry, contributions that can be considered the basis to explain the above described complex photochemical behavior.

The first report on the photochemical behavior of santonin is the observation of the apothecary Kahler. He obtained santonin from *A. cina* and noted: "Im Sonnenlichte nehmen sie eine gelbe Farbe an."[2] Some years later the same observation allowed the author to perform some important considerations. "Ich habe schon oben angelführt, dass das Santonin die Eigenschaft besitzt, den Sonnenstrahlen ausgesetzt, gelb zu werden. Um sowohl die Bedingungen, unter denen diese Umwandlung vor sich geht, als such das gelbe Santonin selbst nach seinem chemischen Verhalten noch näher kennen zu lernen, stellte ich eine Reihe von Versuchen an (deren Resultate ich nachstehend mittheile), die jedoch leider, aus Mangel an Stoff, bis jetzt noch nicht so weit ausgedehnt werden konnten, als es nöthig ist, um über das Wesen jener interessanten Erscheinung genügenden Ausschluss zu erhalten. Das Santonin wird sowohl durch den unzerlegten, als durch den blauen und violetten Strahl gefärbt (ersterer wirkt stärker als die gefärbten Strahlen); der gelbe, grüne und rothe bringen nicht die mindeste Veränderung hervor. Am Tageslicht, den directen Strahlen der Sonne entzogen, wird das Santonin ebenfalls, doch weit langsamer gefärbt. Die Färbung geht ferner gleich gut von Statten in atmosphärischer Luft, im luftleeren Raum (Toricelli'sche Leere), unter Wasser, Weingeist, Aether, ätherischen und fetten Oelen, Säuren und Alkalien. Die Farbe erscheint anfangs schwefelgelb, und steigert sich allmählig bis zum Goldgleb. Während dem Gelbwerden zerspringen die Krystalle in kleine Stücke, die dabei oft weit umhergeschleudert werden. Geschmolzenes Santonin wird ebenfalls gelb, und erhält dabei eine Menge kleine Risse. - Wird dieses gelbe Santonin mit Weingeist und einem Alkali erhitzt, um die Verbindung herzustellen, so tritt, während dieselbe vor sich geht, nicht jene intensiv *rothe*, sondern eine rein *gelbe* Farbe ein, die ebenfalls, jedoch sehr langsam wieder verschwindet. (Auch ohne Zusatz von Weingeist tritt diese gelbe Farbe bei der Verbindung mit Alkalien ein.) War das Santonin nicht lange genug den Sonnenstrahlen ausgesetzt, so färbt sich die Flüssigkeit röthlichgelb, geht aber bald durch schnelles Verschwinden

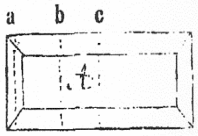
der rothen, in die rein gelbe Farbe über. - Nach völliger Entfärbung der Flüssigkeit kommt die erzeugte Verbindung ganz mit jener überein, die mit weißem Santonin dargestellt ist, und scheidet auf Zusatz einer Säure Krystalle aus, die sich wieder mit Alkalien unter rother Färbung verbinden, und sich ganz wie das ursprüngliche weiße Santonin verhalten. Das gelbe Santonin, wenn es bis zur völligen Umwandlung der Sonne ausgesetzt war, gibt auch mit Alkalien erhitzt nicht jene rothe, sondern nur eine bräunliche Färbung, die jedoch, wenn das Santonin nicht vollkommen gelb geworden, stets mit roth gemischt erscheint. - Wird das gelbe Santonin in Alkohol (oder irgend einem andern Lösungsmittel) gelöst, so entfärbt sich die anfangs gelbe Lösung in Kurzem, und das Santonin krystallisirt beim Erkalten oder Abrauchen in farblosen Prismen, die im Äußern ganz dem ursprünglichen Stoffe gleichkommen. Keineswegs sind aber diese beiden Stoffe identisch, sondern das durch Umkrystallisiren entfärbte Santonin ist seinem chemischen Verhalten nach ganz dem gelben gleich; es vereinigt sich mit Alkalien unter gelber Farbenerscheinung, und gibt mit Alkalien erhitzt keine rothe, sondern braune oder rothbraune Farbe. Aus seinen Verbindungen durch Säuren geschieden, tritt es jedoch wieder mit ursprünglichen Eigenschaften auf. Zur Bezeichnung dieser drei Modificationen desselben Stoffes halte ich, so lange bis ihre innere Beschaffenheit näher erforscht ist, für zweckmäßig, sich (nach Berzelius Vorschlag, s. Poggend. XXVIII. S. 396) der Anfangsbuchstaben des Alphabets zu bedienen, so dass Modificat. A das weiße sich mit Alkalien unter rother Färbung verbindende, Modificat. B das am Sonnenlicht gelb gewordene, und Modificat. C das weiße bei Verbindung mit Basen eine gelbe Farbe hervorbringende bezeichnet."[3]

Heldt reported a comprehensive work on santonin and described the photochemical behaviour of the crystals:[4] "Das Santonin wird durch das Licht gelb gefärbt, weshalb alle Operationen mit demselben bei Abschluss des Tageslichts vorgenommen werden müssen.

Setzt man Santoninkrystalle der Einwirkung der Sonnenstrahlen aus, so bemerkt man schon nach 10 Minuten einen Farbenwechsel; sie erhalten einen Stich ins Gelbliche, werden mit der Zeit immer dunkler gelb und zerspringen dabei mit Lebhaftigkeit, zuletzt nehmen sie eine goldgelbe Farbe an.

Tromsdorf hat nachgewiesen, dass sowohl das unzerlegte Sonnenlicht, als auch der violette und blaue Strahl diese Veränderungen hervorbringen, während der gelbe, grüne und rothe

Lichstrahl ohne Wirkung darauf sind. Die Farbenumwandlung ist unabhängig von dem Medium, sie findet sowohl in der atmosphärischen Luft, als unter Flüssigkeiten und Gasen statt.
Ich habe die durch das Licht in der Krystallform bewirkten Veränderungen unter dem Mikroscop beobachtet.
Die Santoninkrystalle zerspringen zuerst nach Schnitten, welche normal auf die Längenaxe zugehen; die zugeschärften Endflachen werden gleichfalls durch Schnitte abgetrennt, welche die Längenaxe rechtwinklich schneiden. Die Schnittflächen sind keine Ebenen, sie haben sehr unregelmäßige Begrenzungen.
Ist A die Oberansicht eines Krystalls, so zeigen die Linien a, b, c die Richtung der Spaltungsflächen an.



Die abgelösten Stücke zerfallen dann weiter in kleinere, an welchen eine regelmäßige Form nicht mehr erkennbar ist.
Der gebildete gelbe Körper unterscheidet sich vom weißen Santonin durch sein Verhalten gegen Kali und Weingeist, wie schon Tromsdorf beobachtete, indem er damit nicht eine karminrothe, sondern eine rein gelbe Auflösung hervorbringt."
Yellow santonin has been studied by Montemartini.[5] He did not confirm the observation of Heldt: " Per mio conto avendo osservato, con un ingrandimento di 80 diametri, dei piccoli cristalli incolori e degli altri ingialliti (...) non vi potei riscontrare differenze; in entrambi i casi si avevano delle strie di sfaldatura normali all'asse longitudinale. Anche esaminando uno stesso cristallo prima e dopo l'ingiallimento non notai variazioni di sorta; neppure le strie di sfaldatura erano aumentate". He found that: "Il punto di fusione si abbassa continuamente; il rammollimento che precede la fusione credo sia dovuto principalmente al fatto che sul principio la santonina ingiallisce (...); il potere rotatorio della santonina diminuisce per ingiallimento (...); la soluzione di santonina ingiallita non dava (...) bande di assorbimento, limitava però l'estensione dello spettro, esso era visibile solo dal rosso al verde, dopo il verde più nulla si osservava. (...) La santonina ingiallita è più solubile. (...) E' (...) indiscutibile che la composizione centesimale del prodotto giallo è

identica a quella della santonina. (...) La santonina ingiallendo conserva (...) la stessa grandezza molecolare. (...) Quando la santonina gialla è disciolta in qualunque solvente a caldo, e la soluzione è lasciata raffreddare allo scuro, i cristalli che si depositano sono di santonina incolora, come mi accertai esaminandone la forma cristallina, il punto di fusione, ed anche facendone la combustione. (...) La santonina inalterata (...) è molto stabile in presenza del permanganato (...). Invece ripetendo la stessa esperienza colla santonina ingiallita il permanganato potassico è immediatamente distrutto, e la soluzione rimane decolorata dopo qualche secondo. E non solo la velocità della reazione è diversa, sono, oltre a questa, pure diversi i prodotti da essa derivanti, giacchè limitando l'ossidazione della santonina gialla non si trova tra questi prodotti l'acido ossalico che è il prodotto predominante, si può dire il principale offerto dalla santonina inalterata. (...) Appare che la cromosantonina può solo differire dalla santonina per la posizione dei legami che legano fra di loro gli atomi di carbonio del gruppo idronaftalico che ne costituisce il nucleo".

In 1865 Sestini reported the first photochemical synthesis of photosantonin: "Sei mesi or sono (...) feci conoscere che la luce solare, agendo per lo spazio di un mese sopra la soluzione alcolica della sostanza predetta, trasforma la Santonina in altra, che io più per comodità che per altro chiamai Acido Fotosantonico; (...) Onde averla in questo stato ho dovuto isolare la fotosantonina dalla sua soluzione alcolica, diluendo la soluzione stessa ottenuta per l'azione del sole, con un volume di acqua stillata 15 volte maggiore al proprio. L'aggiunta dell'acqua rende lattescente il liquido, sul quale vengono tosto a galleggiare delle goccie oleose, che dopo uno, due, o tre giorni si trovano consolidate in bianche lamine cristalline; delle quali al fondo del liquido se ne trovano in grande quantità".[6]

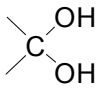
Sestini in 1876 showed the best way to obtain photosantonin: "Si sciolgono 40 parti di santonina in 600 di acido acetico contenente dal 70 all'80 p.% di $C_2H_4O_2$, e si espone il soluto in bottiglia a smeriglio alla diretta azione del sole.

Scorsi 30 o 40 giorni d'insolazione, secondo che è estate o no, o meglio quando nel liquido acetico non si può riscontrare più santonina si aggiunge ad esso un volume di acqua stillata 5 o 6 volte maggiore del soluto acetico, con che si depone l'acido fotosantonico, che cristallizzato più volte nell'alcoole, o nell'etere misto ad alcoole, si purifica."[7]

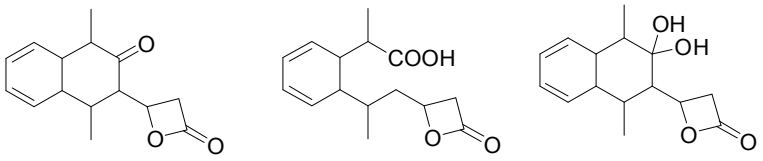
The procedure was implemented some years later by Villavecchia: "Eine Lösung von 20 g Santonin im Liter Alkohol von 90° wird während dreier Monate der Wirkung des directen Lichtes ausgesetzt. Die kaum gelb gefärbte Flüssigkeit wird zur Entfernung des Lösungsmittels im Vacuum auf dem Wasserbade destillirt; zum Rückstand, einem dicken, gelbbraun gefärbten Oel, fügt man eine lauwarme Sodalösung und erwärmt gelinde. Den in der alkalischen Flüssigkeit gelösten Theil des Productes erhält man beim Neutralisiren mit Salzsäure in Form eines flockigen Niederschlages, aus Alkohol umkrystallisirt giebt er bei 154° schmelzende Krystalle der Photosantonsäure."[8] This procedure was followed some years later when Francesconi and Maggi studied the synthesis of some derivatives of photosantonin.[9]

In 1886 Cannizzaro found a second product in the photochemistry of santonin: "Das Santonin, d. i. ein Lacton der Santoninsäure, giebt unter dem fortgesetzten Einfluss des Lichtes, indem es die Elemente eines Moleküls Wasser fixirt, die Lactone zweier verschiedener Säuren; die eine ist zweibasisch, die Photosantonsäure, die andere einbasisch, wird von uns Isophotosantonsäure genannt. Es scheint demnach, dass in dem einen wie in dem andern Lacton die Lactongruppe des Santonins unverändert geblieben sei. In dem Photosantonlacton hat sich überdies eine Carboxylgruppe gebildet, welche es als einbasische Säure befähigt, den entsprechenden Monoäthyläther, das Photosantonin, zu bilden. In dem Isophotosantonlacton ist dagegen keine Carboxylgruppe vorhanden. Im einem wie im andern verhält sich die Lactongruppe wie diejenige des Santonins, d. h. sie keinen entsprechenden Aether.

Die wahrscheinlichste Hypothese, um die verschiedene Constitution der beiden Lactone zu erklären, dass sich einer der Ringe des Dimethylhydronaphtalins öffnet, wenn das Santonin Photosantonlacton wird, d. h. die Gruppe CO wird COOH und trennt sich vom anderen Kohlenstoffatom, an welchem die Lactongruppe gebunden ist und an welches sich das Wasserstoffatom des Wassers anfügt, während im Isophotosantonlacton beide Ringe des Naphtalins geschlossen bleiben; wahrscheinlich wird die Gruppe CO, mit den beiden benachbarten Kohlenstoffatomen verbunden bleibend, zu



Die beiden Acetylverbindungen des Isophotosantonlactons würden diese Annahme bestätigen, während die leichte Umwandlung der Photosantonsäure durch Kohlensäureabspaltung in die einbasische Säure C_{14} ..... die Öffnung eines der Naphtalinringe bestätigt."[10]



Santonin Photosantolacton Isophotosantonlacton

These unrealistic structures were corrected some years later. In 1893 Cannizzaro, on the basis of his previous work[11] and that of Gucci and Grassi Cristaldi,[12] gave its structure of santonin, a structure not so different as that accepted now, and that of photosantonin (Figure 2).[13]

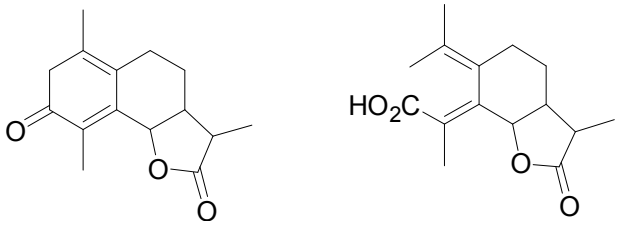


Figure 2. Santonin (left) and photosantonin (right) structure proposed by Cannizzaro.

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ABSTRACTS OF THESIS IN PHOTOCHEMISTRY

Photooxygenations in domino processes and in organocatalysis; Luminol derivatives as ion pair fluorescence sensors.

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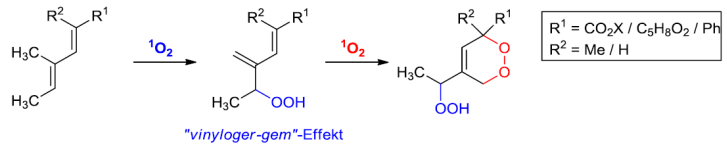
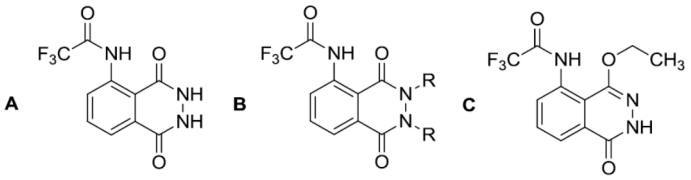
Ph.D. Thesis (in German), 2013; Research Adviser: Axel G. Griesbeck

In this Thesis, a new directing effect for singlet oxygen ene reactions is described. The "vinylogous-gem" effect leads in a highly chemo- and regioselective fashion to (ω-2)- hydroperoxy-substituted compounds from Schenck-type ene reactions of polyunsaturated donor- as well as acceptor-substituted substrates. These primary reactions enable the addition of a second equivalent of singlet oxygen yielding hydroperoxy-endoperoxides as products of these domino ene/[4+2] processes. The primary formed hydroperoxides as well as the secondary formed hydroperoxy-endoperoxides can be used for further synthetic applications. Accessible product classes are alcohols, furanes, epoxides and triazoles (from thermal Diels-Alder reactions). Furthermore, ester-based starting materials can be linked to alcohols like dihydroartemisinin and reacted with singlet oxygen.

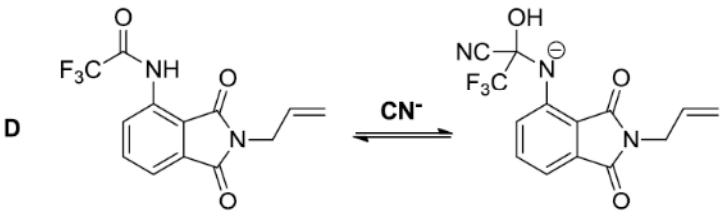


In a second part of this work, cyclic conjugated dienales were formed via organocatalytic induced cycloaddition reactions of α,β–unsaturated aldehyde compounds. These so formed molecules can be reacted with singlet oxygen furnishing bicyclic endoperoxides. In addition, the reactivity of organocatalytic formed dienamines towards singlet oxygen was explored. A comparative experimental and

computational study concerning the singlet oxygen ene reactions of cyclic versus acyclic β,γ–unsaturated ketones is presented.
In the third part of the Thesis, luminol derivatives **A**, **B** and a luminol isomer **C** were analyzed as fluorescent probes for the detection of ion pairs. In all sensors, the trifluoracetamide receptor unit is directly linked to the fluorophor luminol via amidation.



Compound **C** represents the first compound of a new class of fluorescent probes and is the only probe of all sensors described herein which yields satisfying results in the detection of ion pairs. Strong intramolecular hydrogen bonding in the luminol derivatives of type **A** and **B** inhibit the trifluoracetamide receptor unit from being efficient in the detection of analytes; whereas probe **C** in its neutral form allows a selective detection of the halide ions fluoride and bromide. In its deprotonated form, the selective detection of lithium ions is possible. For the detection of halides different mechanisms are responsible: fluoride acts as a base towards compound **C** and deprotonation of the amidic proton results in enhanced fluorescence intensity. Bromide acts as a nucleophile towards compound **C** resulting in enhanced fluorescence intensity accompanied by a blue shift of the fluorescence maximum.



Furthermore, sensor **D** was developed for the selective detection of cyanide anions in water is presented. The trifluoracetamide receptor

unit acts as electrophile towards cyanide ions and the addition of cyanide results in a strong enhancement of fluorescence intensity accompanied by a strong blue shift.

Publication:
"Comparison of the singlet oxygen ene reactions of cyclic versus acyclic ß,γ-unsaturated ketones: an experimental and computational study" Axel G. Griesbeck, Bernd Goldfuss, Matthias Leven und Alan de Kiff, Tetrahedron Lett. 2013, 54, 2938-2941.
"A new directing mode for singlet oxygen ene reactions: the vinylogous gem effect enables a $^1O_2$ domino ene/[4+2] process" Axel G. Griesbeck und Alan de Kiff, *Org. Lett.* **2013**, *15*, 2073-2075.

Photodecarboxylation of aryl acetates and polyfluorinated carboxylic acids. Degradation studies on PFT and polyfluorinated carboxylic acids in water under UV- and VUV-photolysis conditions: mechanistic course, efficacy and selectivity

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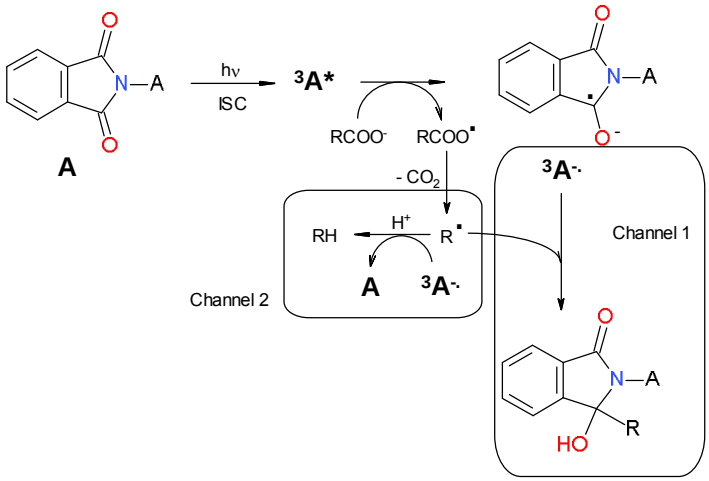
Ph.D. Thesis (in German), 2013; Research Adviser: Axel G. Griesbeck

In the first part of this Thesis, perfluorinated carboxylic acids with chain lengths of C2 to C8 and perfluorooctane sulfonic acid (PFOS) were photolyzed at 172 nm (VUV range) in water. The degradation rate as well as the degradation mechanism were examined, based on 19F-NMR spectroscopy. It was found that with increasing chain length the decomposition rate increases. Perfluorooctanoic acid (PFOA) could be degraded to 100 % in comparison to perfluoropropionic acid (PFPA) which is still present to 70 % after photolysis. Trifluoro acetic acid (TFA) and PFOS are special substrates because of the lacking CF2 groups in TFA and no carboxylic group in PFOS and do not follow the general degradation mechanism. They are decomposed only to a small percent.
In the second part of the Thesis, benzyl acetates as well as poly- or perfluorinated carboxylates were irradiated in the presence of phthalimides. For the reaction process two pathways could be established depending on the substrate: on one hand the stoichiometric addition/reduction (channel 1) and on the other hand the phthalimide-induced photodecarboxylation (channel 2 in the reaction scheme).



Publication:
"Intermolecular photodecarboxylation of electron-deficient substrates by phthalimides in water: efficiency, selectivity and online monitoring" Axel G. Griesbeck, Nestor Nazarov, Jörg Neudörfl und Maria Heffen, *Green Chem.* **2012**, *14*, 3004-3006.

Singlet oxygen reactions for the synthesis of new biologically active 1,2,4-trioxane acids, amides, and ester and further cyclic peroxides as well as "photocage" compounds

Viktor Schlundt
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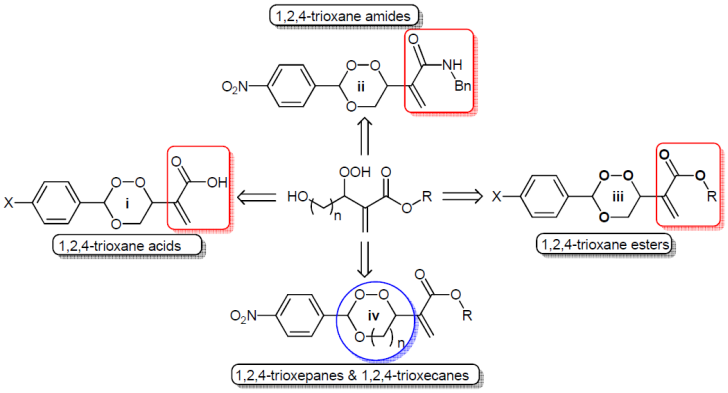
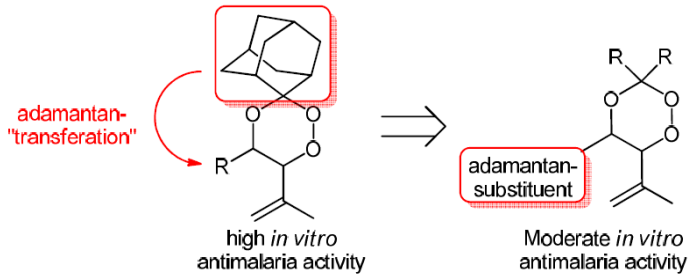
Ph.D. Thesis (in German), 2013; Research Adviser: Axel G. Griesbeck

In this Thesis, the synthesis of ten new 1,2,4-trioxane acids (**i**), amides (**ii**) and esters (**iii**) from 4-hydroxy tiglic acid (n = 1) was realized. This task was achieved by photooxygenation of 4-hydroxy tiglic acid with subsequent peroxyacetalisation to give 1,2,4-trioxanes using various carbonyl compounds. These 1,2,4-trioxane acids are of interest considering derivatization to esters, which have already showed high GST-inhibition activities. Furthermore, new syntheses of several 7- and 8-membered rings with endoperoxide structures (**iv**, n = 2,3) as test structures GST-inhibition activities were realized.



Additionally, ten new 5'-adamantanyl-substituted or annulated 1,2,4-trioxanes were synthesized. The synthetic route proceeded via photooxygenation of adamantylated allylic alkohols with subsequent peroxyacetalizations to the desired products. In this part, the relation between the position of adamantyl substituent and antimalarial

activity was investigated. While 3',3'-spiroadamantylated 1,2,4-trioxans show *in vitro* activity against malaria strains (left), the new 5'-adamantylated 1,2,4-trioxanes showed only moderate activities.



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"5-Adamantylated 1,2,4-Trioxanes: Adamantane Position is Crucial for Antiparastic Activity" Axel G. Griesbeck und Viktor Schlundt, *Synlett* **2011**, 2430-2432.

"Functionalized polar 1,2,4-trioxanes as bulding blocks by singlet oxygenation of 4-hydroxy tiglic acid using the deuterium isotope trick" Axel G. Griesbeck, Viktor Schlundt und Jörg M. Neudörfl, *RSC Adv.* **2013**, *3*, 7265-7270.

The Porter Medal 2014 – Call for Nominations

The Porter Medal is awarded every two years to the scientist who, in the opinion of the European Photochemistry Association, the Inter-American Photochemistry Society, and the Asian and Oceanian Photochemistry Association, has contributed most to the subject of Photochemistry. The Porter Medal, named for the late George Porter FRS, Nobel Laureate, is awarded biannually to the scientist who in the opinion of the judges, has contributed most to the science of photochemistry with particular emphasis on more physical aspects, reflecting George Porter's own interests.

To nominate European candidates for The Porter Medal 2014, candidate's details should preferably be sent directly to the President of the European Photochemistry Association, Professor Werner Nau (w.nau@jacobs-university.de). For nomination of candidates from other continents, see the Porter Medal webpage: http://www.portermedal.com. Nominations may also be sent to the Chair of the Porter Medal Committee, Professor David Klug. The nomination package should include:

- Curriculum Vitae of the candidate
- A list of publications
- A citation for the award, not exceeding five pages
- Two letters of reference

Provisional closing date for the receipt of nominations (based on the guidelines from previous years) will be 31 January 2014.

Previous winners:

1988 Lord Porter (George Porter), UK (Founding medal)

1990 Michael Kasha, USA

1992 Kinichi Honda, Japan

1994 Nicholas J.Turro, USA

1995 J.C."Tito" Scaiano, Canada (Special Medal for London ICP)
1996 Noboru Mataga, Japan
1998 Frans de Schryver, Belgium
2000 Vincenzo Balzani, Italy
2002 Josef Michl, USA
2004 Graham R.Fleming, USA
2006 Howard E. Zimmerman, USA
Hiroshi Masuhara, Japan
2008 Michael R. Wasielewski, USA
2010 David Philips, UK
2012 Thomas J. Meyer, USA

EPA Prize for Best PhD Thesis in Photochemistry
Call for Nominations

The EPA Prize for the best PhD thesis in photochemistry will be attributed during the XXVth IUPAC Symposium on Photochemistry which will be held in Bordeaux, France, in 2014. The awardee will present his/her work at the Symposium. The Prize is 1000 Euros, plus travel costs to Bordeaux (within the limit of 300 €) and one free year of EPA membership. The candidate must have defended his/her PhD thesis in 2012/2013 and be nominated by an EPA member. Nominations should be sent (electronically only) to Werner Nau (w.nau@jacobs-university.de). The nomination package should include:

• Curriculum Vitae of the candidate

• Copy of the thesis

• Abstract of thesis in English, no more than five pages

• List of publications arising from the thesis

• A letter of support.

Closing date for the receipt of nominations has been extended until 15 January 2014.

Previous winners:

2008 Maria Abrahamsson (thesis supervisor: Leif Hammarström), Sweden, Alexandre Fürstenberg (thesis supervisior: Eric Vauthey), Switzerland

2010 Anne Kotiaho (thesis supervisior: Helge Lemmetyinen), Finland

2012 Karl Börjesson (thesis supervisior: Bo Albinsson), Sweden

TECHNICAL NOTES

Sample Chambers, Optical Interconnections, and Measurement Systems

An optical spectrometer relies on optics to image the radiant energy of the experiment from the source onto the sample and the resulting radiant power from the sample onto the entrance slit of the analyzing monochromator or spectrograph.

The goal of the sample compartment is to present the sample to the excitation and analysing optical channels in a simple and reproducible way. Since we are dealing with optical signals and often very weak signals then it is important that the access lid, the construction of the sample compartment and the connection to monochromators and light sources are perfectly light tight, often requiring light tightness to "single photon detection level".

Suitable holders for sample temperature, position and for stirring the contents might be needed. In all cases these need to be designed, along with the sample compartment, for minimum stray light as a fraction of the radiation passing through the sample or incident upon the sample might be reflected or scatted inside the sample holder or sample compartment and hence contribute to the signal measured by the detector.

An arrangement of programmable shutters for excitation and emission channels is often also very useful to minimize photo-bleaching effects in samples and is only opened during the measurement time such that sample irradiation is minimised.

Different types of sample cells can be used and, for example, one of the most common used in fluorescence spectroscopy is the 1cm path length square cuvette, which is polished on all sides. These cells are often made from fused synthetic silica and provide the best optical transmission, especially in the UV excitation range and also the lowest intrinsic fluorescence from the cell sides. Other cell arrangements can be micro-cells, down to 100uL sample volumes and

also EPR cooled sample rods for freezing samples. In all cases the imaging of monochromator to sample is particularly important to ensure maximum light coupling and minimum stray light generation.

Lenses and mirrors are the most frequently used optics, with optical fibers now playing an increasingly important role but these also need correct coupling to the wavelength selection devices and detectors. The choice of component is dictated by spectral range and cost requirements.

For lenses, the spectral range will determine the material and thus the cost. There are thousands of transmission materials to choose from for lenses. Glass (BK 7) and quartz (of fused silica) make up the bulk of lenses used in the 190 nm to nearly 3μm range. The choice of material decreases above 3μm decrease dramatically and beyond 20μm and below 180 nm, reflective optics is more the norm.

Aside from the shape of a mirror, the coating is the most important parameter for a mirror. Over-coated gold and platinum mirrors are available for the VUV wavelength range (10 to 180 nm). These coatings are very costly and difficult to produce. Most mirrors are coated with aluminum or gold. Aluminum-coated mirrors may also be over-coated with MgF_2 or SiO_2 to prevent oxidation. The reflectance range between these two materials is 150 nm to more than 50μm. These coatings are also very durable and non-hygroscopic. Glass is the main substrate because of its ease of fabrication, rigidity and low thermal expansion; other materials include nickel, brass and aluminum.

Optical fibers are being used more frequently in instrument designs for sample delivery and sample collection. Fibers are used in the 200 nm to 3.0μm range. A limited selection of fiber materials with different acceptance, numerical apertures and diameters are available. These can be single fibers or can be arranged in bundles, depending on the application.

The common comment is that mirrors are better for instruments because they focus of the light does not change with wavelength, where as a lens has a refractive index that is wavelength dependent

and hence the focus position for blue light is different from that for red light.

In the example of a fluorimeter the job of the excitation and emission optical systems is to image light from the excitation source to the sample, and then to collect the luminescence generated in the sample and image that to the analysing detector and this needs a knowledge of all of the aberrations and the limiting factors that produce "the circle of least confusion" or the best optical spot. Both excitation and emission channels need to be designed to get the maximum light transferred at each stage. What is important is the best image quality considering all aberrations in the system not just one aberration out of many.

The question therefore is – does it make any practical difference to fluorescence measurements? The short answer is generally not if the optical system is correctly designed in the first place. There are several reasons that we can say this, but first let us examine the problem.

Aberrations in an optical system: the Circle of Least Confusion

There are many possible causes of aberration in an optical system and these can be defined by monochromatic and chromatic aberrations. The former is not wavelength dependent while the later is. What is important is to minimise all aberrations from an imaging point of view to provide best optical coupling and best transfer of light from one place to another. The limiting image spot size is determined by the combination of all of these aberrations, not just one on its own.

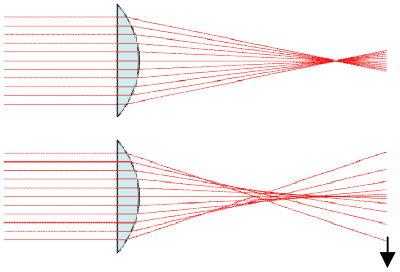
Recognising that real lenses or mirrors do not focus all rays perfectly under even the best of conditions, the circle of least confusion is a characterisation of the best optical spot possible. The term circle of least confusion is often used for the smallest optical spot a lens or mirror can make, for example by picking a best focus position that makes a good compromise between the varying effective focal lengths of different lens or mirror zones due to spherical or other aberrations. Diffraction effects from wave optics and the finite aperture of a lens can be included in the circle of least confusion, or the term can be applied in pure ray (geometric) optics.

Monochromatic Aberrations

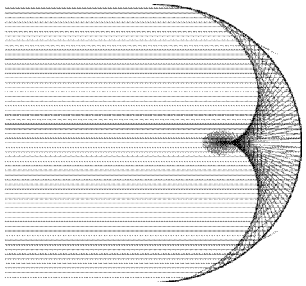
Piston, Tilt, Defocus, Spherical aberration, Coma, Astigmatism, Field curvature, and Image distortion are all types of error that an imaging system can be subject to. Each play an important role in determining the performance of an imaging system whether it is lens or mirror based. And each could be subject to a technical note in its own right beyond the scope of this document.

Spherical Aberration

This is a monochromatic aberration that is due to the spherical nature of lenses and mirrors and also the size (or diameter) of that optic. Basically the bigger the aperture, or the smaller the f/#, then the more prominent the problem of spherical aberration will be. Spherical aberration produces a circle of least confusion that is at a shorter distance than the paraxial focus. .



A perfect lens (top) focuses all incoming rays to a point on the optic axis. A real lens with spherical surfaces (bottom) suffers from spherical aberration: it focuses rays more tightly if they enter it far from the optic axis than if they enter closer to the axis. It therefore does not produce a perfect focal point. CLC position marked with arrow (Drawing is exaggerated.)



Spherical aberration from on-axis spherical mirror causing significant image blur and loss of light at focus. (Drawing is exaggerated.)

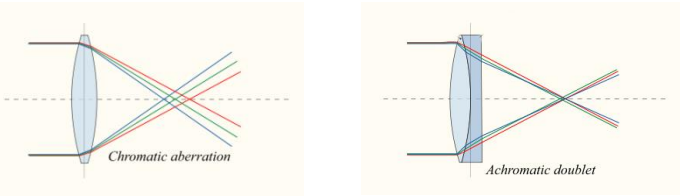
Basically this circle defines the best spot size that is possible to image too and hence it provides the best optical power density possible; that is power / unit area The effect is proportional to the fourth power of the diameter and inversely proportional to the third power of the focal length, so it is much more pronounced at short focal ratios, i.e., "fast" lenses. So as a result it is often one of the dominant monochromatic errors in the system

Chromatic aberration of a single element lens

In optics, chromatic aberration is the failure of a lens to focus all colours to the same point. It occurs because lenses have a different refractive index for different wavelengths of light (the dispersion of the lens). The refractive index decreases with increasing wavelength.

Chromatic aberration manifests itself as "fringes" of colour along boundaries that separate dark and bright parts of the image, because each colour in the optical spectrum cannot be focused at a single common point on the optical axis.

Since the focal length of a lens is dependent on the refractive index, different wavelengths of light will be focused on different positions. Chromatic aberration can be both longitudinal, in that different wavelengths are focused at a different distance from the lens; and transverse or lateral, in that different wavelengths are focused at different positions in the focal plane (because the magnification of the lens also varies with wavelength).



(Diagram Source: Wikipedia)

Chromatic aberration can be corrected by appropriate optical design. Instruments should be designed to minimise the effect of chromatic

aberration throughout the optical path and hence should be optimised for best focal length control and efficient image transfer.

Astigmatism and Coma of using a spherical mirror off-axis

In an instrument that uses spherical mirrors the mirrors are used in an off-axis configuration, this means that they are naturally subject to astigmatism and in some instances also to coma distortions. These two distortions degrade the image position and most importantly the image shape. This is often why a system that uses an all mirror configuration can have an asymmetric spectral response – clearly not representative of the sample behaviour. Clearly, the optical design of the mirror system is of critical importance and also very accurate alignment of that system to ensure best practical control of the image quality and minimisation astigmatism and coma. Small misalignments can have big effects. It is sometimes quite difficult and very time consuming in adjustment to remove these alignment distortions from a mirror system, even by well-trained technicians.

As an aside to this, the fact that off-axis spherical mirror produce astigmatism is very well known and has been utilised to advantage in a number of monochromator designs, such as the Czerny-Turner design. However to ensure best optical transmission an imaging monochromator with toroidal optics is often used to control the extent of the astigmatism by utilising a mirror with two focal lengths at axes right angle to each other.

A final thought on the optical issues

The practical reality is that it should make no difference to a system performance if using a lens or mirror based optical arrangement so long as the optical system is designed well in the first place, assuming the basic requirements of optical transmission and spectral range are satisfied. Monochromatic aberrations play an equally important role, or sometimes more so, than chromatic aberrations so the idea that a mirror based system is intrinsically better than a lens based instrument is flawed. If could be that the mirror based system is good in chromatic aberration and very poor in monochromatic aberration because of the difficulty to maintain surface qualities and alignment issues resulting in a rather poor performance – spectrally

and also in terms of sensitivity. In many ways a lens-based system is significantly better solution both in terms of image performance, stability, ease of manufacture and for many other reasons. It is for these reasons that they are the dominant components used for photographic optics in cameras, microscopes, and many other optical instruments and are often the preferred component over mirrors.

In all cases its important that the instrument optical design is thoroughly optimised to produce the best image quality across the operational spectral range required of it. If this is done then the result is an instrument with an excellent optical and image performance as a function of wavelength and hence best light gathering and transfer capabilities.

Measurement systems

Once the optical signal is relayed to the detector for measurement the output signal from the detector must be monitored, stored and analyzed to make a spectroscopic measurement. As such, the signal-processing and readout system is extremely important to the overall performance of the system. The particular type of signal processing depends on the form of the output signal, the noise sources expected and the signal level itself. The signal-processing step can perform many conversions such as current-to-voltage, analog-to- digital conversion, amplification, or some mathematical operation designed to improve the measurement of signal-to-noise ratio.

The outputs of most detectors are used in the analogue mode. The exception to this is the photomultiplier, which may be used in the photon-counting mode. Nearly all measurements are made by using an analog-to-digital converter to convert the output of a detector to the digital domain for future processing, analysis and display. Even in digital measurements some conditioning, such as filtering or amplifying the analog signal, is usually necessary to make it suitable for recording by the converter. In photon counting systems, the recorded signal is the number of pulses observed or counted. Photon counting is particularly useful in applications where the signal irradiance is relatively low, such as Raman spectroscopy and some photoluminescence measurements.

In the infrared, the use of a mechanical chopper and a lock-in amplifier can be very effective. These allow the signal processing system to measure alternately the optical and dark noise signals and subtract the dark noise automatically. There can be significant advantages to the measured signal-to-noise ratio and signal stability by using such a technique. In fact, the use of such a synchronous demodulation system can literally recover signals that would otherwise be buried in the noise. Lock-in amplifiers cannot be used with transient signals or signals with very high repetition rates.

If a signal is repetitive, particularly those from pulsed experiments, then a boxcar integrator can be an effective data acquisition tool. The boxcar integrator allows the measurement of signals related to a trigger event and the rejection of all others.

Lock-in amplifiers and boxcar integrators provide analogue processing of the signal. Such methodologies can also be implemented in the digital domain using either hardware or software techniques. Photon-counting systems can be constructed where a digital lock-in approach is used. Thus, during the irradiated cycle the counter counts up and, during the dark cycle, counts down to remove the background signal.

In the next article we will review in brief the different types of detectors and the method of use in optical spectroscopy to ensure a best signal-to-noise consideration.

Analysis software

At the most basic level, spectroscopic software must allow for reliable, efficient collection and storage of data with minimal sacrifice to hardware performance. As spectroscopy systems increase in complexity and importance in everyday activities, software is becoming a more important, even critical, components of the system. Today, software often needs to be sufficiently flexible to adapt to varied applications, yet simple enough for the increasing number of users with limited spectroscopic backgrounds. More sophisticated software packages provide the user with several options in data

display and analysis, as well as comprehensive export functions to allow the user to transfer data to their favourite analysis program.

It is the successful combination of the light sources, light discriminators, coupling optics, and sampling and detection methods that provide an optimal system. This hardware package, coupled with a complete robust software analysis package, is the path to a successful instrument.

Dr. John R Gilchrist,
Gilden Photonics Ltd

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A novel, alternative method for quantitative detection of photogenerated molecular oxygen in photoinduced water oxidation

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Introduction and motivation

In natural photosynthesis, absorption of light by photosynthetic pigments initiates a series of energy transfer and charge separation processes that lead to the oxidation of water to molecular oxygen (eq. 1), while the reducing equivalents are used to drive the reduction of CO_2 into carbohydrates (eq. 2). In an artificial photosynthetic system, similar lines can be followed as far as water oxidation is concerned, but the reducing equivalents will be eventually used to drive the production of high energetic fuels such as hydrogen (eq. 3), or various reduced forms of carbon (eq. 4-8).

$$2\ H_2O \rightarrow O_2 + 4\ H^+ + 4\ e^- \quad (1)$$
$$n\ CO_2 + 2\ ne^- + 2\ H^+ \rightarrow (CH_2O)_n \quad (2)$$
$$2\ H_2O + 2\ e^- \rightarrow H_2 + 2\ OH^- \quad (3)$$
$$CO_2 + 2\ H^+ + 2\ e^- \rightarrow HCOOH \quad (4)$$
$$CO_2 + 2\ H^+ + 2\ e^- \rightarrow CO + H_2O \quad (5)$$
$$CO_2 + 4\ H^+ + 4\ e^- \rightarrow HCHO + H_2O \quad (6)$$
$$CO_2 + 6\ H^+ + 6\ e^- \rightarrow CH_3OH + H_2O \quad (7)$$
$$CO_2 + 8\ H^+ + 8\ e^- \rightarrow CH_4 + H_2O \quad (8)$$

An artificial photosynthetic system capable of mimicking the natural one on large scale should contain the following basic components: (i) light-harvesting antennae; (ii) charge separation units; (iii) light-activated multi-electron transfer catalysts (Figure 1).[1] Although practical systems do not exist yet, considerable progress has been made in the development of the required components, and the

strategies to functionally linking these components.[2,3] Whereas the design of light-harvesting antennae systems as well as of charge separation units have been extensively pursued in the last decades, with quite remarkable results,[1,2,4-12] the degree of success in this research field is likely to depend strongly on the development of efficient water oxidation catalysts (WOCs), which is therefore the real bottleneck of research on artificial photosynthesis, at the present.

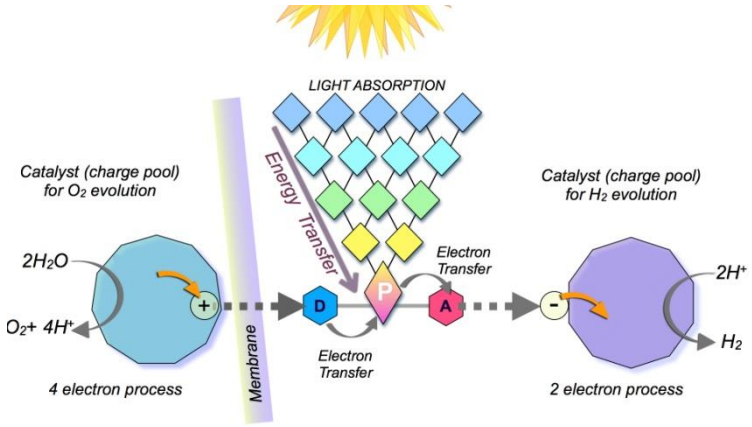

Figure 1. Schematic representation of an artificial photosynthetic system

Efficient photo-induced water splitting is a matter of balance, according to a separation of tasks and modular assembly architecture. Photon absorption, charge separation and electron transfer events should be coupled and concerted against several concurrent facts: (i) the lifetime of photo-excited states, (ii) the inactive recombination/decay, (iii) the sensitizer/environment stability, (iv) the multi-redox evolution and reorganization of the catalytic cores, and (v) the time domain of catalytic turnover.[13] These problems are far from being solved, and much effort has been devoted to the design, synthesis, spectroscopic and computational study of these systems. However, if one wish to concentrate on the problem of photoinduced water oxidation, sacrificial systems can be investigated.

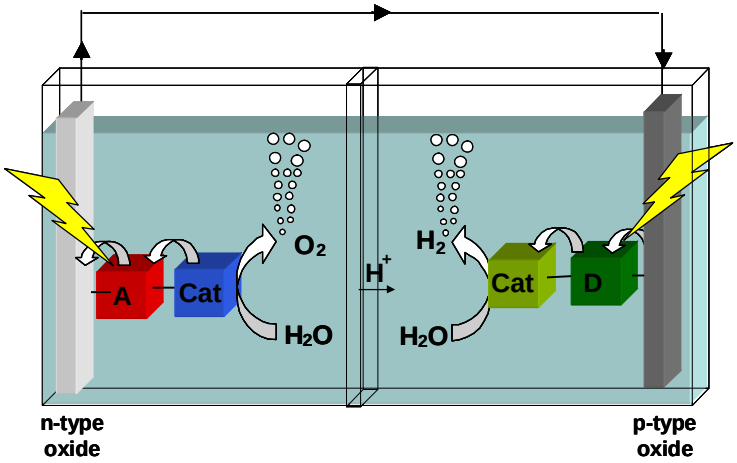

Figure 2. A proposed device that realizes photo-induced water splitting. With permission from S. Campagna

Indeed, in order to focus on photoinduced water oxidation, photosensitizers and catalysts can be tested in sacrificial systems, for example a photosensitizer-persulfate-catalyst system. In fact, it should be noted that electrons ideally transferred to the reduction side of a complete artificial water splitting apparatus have to be removed from the system. This role in the natural system is played by a series of electron acceptors embedded in the membrane and, in artificial systems, could be played by electrodes (see Figure 2). In a photosensitizer-persulfate-catayst system it is common to use sacrificial acceptors, namely persulfate anions, to accomplish this function.

In order to understand the role of each component in the water oxidation cycle, it is important to remember the reactions involved in it, For a general photo-induced water oxidation by photosensitizer-persulfate-catalyst system, the eqs 9-14 must be considered.

$$P + h\nu \rightarrow {}^*P \quad (9)$$

$${}^*P + S_2O_8^{2-} \rightarrow P^+ + SO_4^{2-} + SO_4^{-\cdot} \quad (10)$$

$$P + SO_4^{-\cdot} \rightarrow P^+ + SO_4^{2-} \quad (11)$$

$$P^+ + C \rightarrow P + C^+ \quad (12)$$

$$C + SO_4^{-\cdot} \rightarrow C^+ + SO_4^{2-} \quad (13)$$

$$C^{4+} + 2\ H_2O \rightarrow C + O_2 + 4\ H^+ \qquad (14)$$

In these equations, P is the photosensitizer, generally a Ru(II) complex, and C is the oxygen evolving catalyst. After light excitation (eq 9), oxidative electron transfer quenching of the metal-to-ligand charge transfer (MLCT) excited state of the Ru(II) photosensitizer (*P) by persulfate takes place (eq 10), with formation of the oxidized photosensitizer P^+. As the sulfate radical anion formed by eq 10 is also a very strong oxidant, a further equivalent of P^+ is formed by eq 11. Then the oxidized sensitizer P^+ reacts with C leading to hole transfer and preparation of the oxidized catalyst (eq 12). Note that the radical anion $SO_4^{-\cdot}$ could directly react with C (eq 13) in competition with eq 3. The sequence of eqs 9-12 has to be repeated twice (so two photons are required) before four holes are accumulated in the WOC, and finally eq 14 can take place.[14]

The titration of photogenerated molecular oxygen – the proposed method

A general scheme of photoinduced water oxidation requires the presence of a photosensitizer (quite often a Ru(II) polypyridine complex, but also porphyrin dyes have been used), persulfate anions as sacrificial electron acceptor, and catalysts. The most important parameter to measure is molecular oxygen concentration. This allows to obtain quantum yield of photoinduced process, as well as the kinetic profile of the photoreaction. The most used way to measure photogenerated oxygen is via gas chromatography (GC) or oxygen sensors electrodes (for example, Clark electrode). Here we report on a novel approach we have recently developed for detecting photogenerated oxygen.[i,ii,iii,iv] The method is based on the Stern-Volmer equation and requires the use of a time-correlated single-photon-counting (TCSPC) spectrometer for emission lifetime. Incidently, some of the commercial oxygen sensors electrodes use the same principle, although they are based on emission intensity and not lifetime.

We wish to stress that the method we developed does not pretend to be superior to gas chromatographic methods; it is just an alternative, that can be profitably used if one has a TCDPC spectrometer available instead of GC equipments.

The photo-driven oxygen evolving experiments in the water oxidation systems we studied[15-18] were performed by irradiating (halogen lamp of 50 W, cut-off filter) 2 mL of a deoxygenated solution containing the photosensitizer, the catalyst and the sacrificial agent in a sealed cell (*photoreactor*). When the photosensitizer was $[Ru(bpy)_3]Cl_2$ (bpy = 2,2'-bipyridine), we used a $\lambda > 400$ nm cut-off filter, whereas in the case of a red-absorbing Ru(II) tetranuclear dendrimer as photosensitizer,[16] the cut-off filter was at $\lambda > 515$ nm. Oxygen evolution was monitored in a second compartment (*reading cuvette*), following the O_2-dependent emission lifetime of a deoxygenated diluted solution (acetonitrile, 2.5 mL) of $[Ru(bpy)_3]Cl_2$ by a time-correlated single photon counting spectrometer, after quantitative calibration of the time-resolved spectral response. Experimentally, we took a specific gas volume from the head of the photoreactor by using a gas-tight syringe and injected it in the reading cuvette, at various irradiation times.

Before each experiment, we made a calibration for the instrumental response that consists in a series of preliminary experiments. The first one is needed to quantify the effective number of moles of molecular oxygen contained in a specific volume of gas at the operating pressure. Basically, we put 25 μL (for each experiment we used a 50 μL gas-tight syringe) of pure oxygen from a tank into the reading cuvette, situated in the time-correlated single photon counting spectrometer, containing 2.5 mL of a deoxygenated solution of $[Ru(bpy)_3]Cl_2$ in acetonitrile. By using Stern-Volmer equation (eq 15) we obtained the moles of oxygen acting as quencher for the luminescence of the ruthenium complex. The results showed that 100 μL of the used pure oxygen (Aldrich, 99.6%), in the operating conditions, act as 1.76 μmol of quencher for the luminescence of $[Ru(bpy)_3]Cl_2$.

$$[O_2] = [(\tau_0 / \tau_t) - 1] / k_q \tau_0 \qquad (15)$$

The second experiment consists on the calibration of the setup in the condition of photocatalys experiments. We construct a curve of μmol of oxygen detected versus μmol of oxygen added in the reaction vessel containing water maintained stirred under illumination. Basically, it was simulated a typical oxygen evolving experiment by irradiating 2 mL of buffer solution in the reactor and

injecting 25 μL of pure oxygen from a tank in the head space; after a few minutes (maintaining the solution under continuous magnetic stirring) we took 25 μL of gas from the headspace of the reactor and we injected it in the reading cuvette. By using Stern-volmer equation we calculated the μmol of oxygen measured by this calibration experiments. By considering that 100 μL of pure oxygen quenched the luminescence of $[Ru(bpy)_3]Cl_2$ as 1.76 μmol we obtained a calibration line of μmol of oxygen measured vs μmol of oxygen put in the photoreactor.

An example of calibration line obtained by irradiation (λ > 400 nm) of 2 mL of buffer phosphate is showed in Figure 3. The slope of this line is used to correct the μmol of oxygen measured in each experiment of oxygen evolution in time or experiments for quantum yield determination.

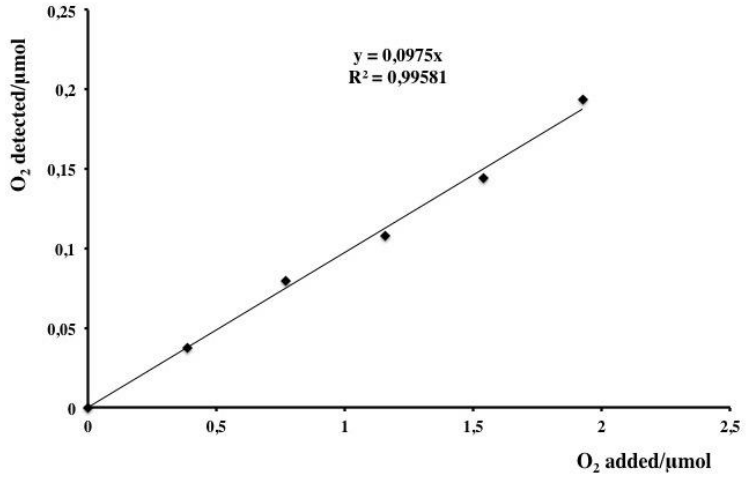

Figure 3 Calibration line reporting μmol of oxygen detected *versus* μmol of oxygen added into 2 mL of buffer phosphate in photoreactor irradiated at λ > 400 nm (cut-off filter)

A final correction have also to be considered. In each photocatalitic experiment, the quantitative oxygen evolution was determinated by

taking into account not only the calibration curve previously described, but also the stoichiometric contribution at the molecular oxygen measured at each injection by the previously added volumes. The moles of oxygen effectively added (μmol_n) considering the injection of gas in the reading cuvette was done by eq. 16, where, μmol_{Vn} are the moles of molecular oxygen obtained by the Stern-Volmer equation at the volume n injected (μL_{Vn}).

$$\mu mol_n = \mu mol_{Vn} - [(\mu mol_{Vn-1}) \times (\mu L_{Vn-1}) / \mu L_{Vn}] \qquad (16)$$

μL_{Vn-1} stands for the microliter of gases injected in the reading cuvette before the n injection.

In conclusion it was demonstrated that, after an opportune quantitative calibration, it is possible to quantify the oxygen evolved in photo-induced water oxidation catalysis trials by Stern Volmer quenching experiments avoiding the use of specific instrumentation for the molecular oxygen detection like GC or Clark electrode.

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PHOTOCHEMICAL AND PHOTOBIOLOGICAL SCIENCES

CONFERENCE REPORTS

II Autumn Meeting of the Polish Photochemistry Group "Molecules and Light 2013" 23-27 September, Zakopane, Poland

The onset of the autumn, a season called in Poland the *golden Polish autumn* for its beautiful colours of Nature, is an especially suitable time to look for stimulation and inspiration with new concepts and ideas on *molecules and light*. This was one of the motivations why two years ago we chose the fourth week of September to organise the first Autumn Meeting of the Polish Photochemistry Group "Molecules and Light 2011" (ML2011) in Zakopane. Organised under the auspices of the EPA, the conference gathered 55 participants (including one present and two past EPA presidents), with a number of invited international speakers, and comprised an opening and 6 plenary lectures, 7 invited lectures, 15 short oral communications and 18 posters. A particularly well-remembered (and appreciated) part of that meeting was the panel discussion devoted to a long-standing dispute on the photoinduced charge transfer and conformational changes.

Following a very positive response to ML2011, we decided to continue this year with the II Autumn Meeting of the Polish Photochemistry Group "Molecules and Light 2013". ML2013 was held in Zakopane in the same place, in the "Pod Berlami" Conference Centre of the Jagiellonian University, on 23-27 September 2013, was based on the same proven organisational formula, and gathered 59 participants. Though the site capacity limits the total number of participants to about 60, it has one absolutely unbeatable advantage - a small wine bar offering an excellent selection of wines and providing uniquely stimulating atmosphere for creative exchange of thoughts till late at night.

The ML2013 meeting was a joint effort of four institutions: two institutes of the Polish Academy of Sciences, the Institute of Physical

Chemistry (Warsaw) and the Institute of Physics (Warsaw), the Jagiellonian University (Cracow), and the Adam Mickiewicz University (Poznań). The conference was financially supported by our exhibitors: Comef, Eurotek International, Gilden Photonics, Princeton Instruments, and Scitec Instruments Polska, and by the Leading National Research Centre KNOW. The meeting was organised under the auspices of the European Photochemistry Association, by the Polish Section of the EPA, as we felt it was an excellent platform to combine a local review of our field with an international perspective.

The overall goal of the ML2013 was to review current research conducted in Poland in the field of photochemistry and photophysics, and to provide an opportunity for establishing and strengthening scientific and personal contacts between the members of our community. Inviting senior scientists to give plenary lectures (7) we asked them not only to present their own research work, but to combine it with some review elements as we expected the majority of participants of the meeting to be young researchers. On the other hand, the invited lectures (5) were delivered by younger scientists, on one hand just about to start their independent research activity, but on the other - already with a strong research record. Also those younger ones were offered to chair scientific sessions during the conference. The lectures were complemented by short oral communications (17) and posters (22). The scientific sessions were completed with short presentations of our exhibitors.

The scientific program started on Monday evening with the opening lecture by **Andrzej Sobolewski** (Warsaw, Poland) who inspiringly introduced into the excited state proton transfer (ESPT) phenomena focusing on the control of proton motion with the aid of light fields ("juggling with protons"). Starting the Ultrafast Spectroscopy session, **Eberhard Riedle** (Munich, Germany) offered a detailed picture of complex benzhydryl photochemistry, indicating the importance of solvation dynamics for the course of ultrafast photochemical reactions. In his voyage across time and spectral domains, he also reviewed recent developments of the transient spectrometers from his laboratory that allow measurements with fully tunable excitation from few fs to sub-ms and a probe coverage of 225 – 1700 nm. Referring to her husband's experimental work, **Regina de Vivie-Riedle** (Munich, Germany) provided a deep theoretical insight into

the nature of conical intersections and their role in the excited state processes in benzhydryl derivatives. Molecular approach to artificial photosynthesis were comprehensively addressed by **Sebastiano Campagna** (Messina, Italy), who reviewed the design of various structurally organized and functionally integrated supramolecular systems for light-harvesting, charge separation and multielectron transfer catalysis. Kinetic aspects of diffusion-enhanced Förster energy transfer in peptidic biopolymers were critically analysed by **Werner Nau** (Bremen, Germany), who showed that the phenomenon is not controlled by the experimentally observed but by the radiative lifetime of the energy donor, and indicated significant practical importance for extracting the dynamic and structural information from more and more popular FRET studies. **Mark Maroncelli** (University Park, USA) reviewed the progress in exploration of solvation dynamics in ionic liquids and presented a recently completed benchmark study of the complete solvation response in a representative collection of ionic liquids, confirming the presence of significant inertial dynamics on the sub-ps time scale together with a broadly distributed structural component acting up to10 ns. **Jan Najbar** (Cracow, Poland) showed how time-dependent diffusion in polarization medium translates into the time-dependent electron transfer kinetics.

In invited lectures, five young Polish researchers presented their studies and discussed prospects of their research areas. **Gotard Burdziński** (Poznań, Poland) briefly reviewed the techniques of transient absorption spectroscopy, demonstrating the importance of bleaching bands in ultrafast UV-Vis and mid-IR transient absorption studies for a broad range of organic molecules. **Marcin Ziółek** (Poznań, Poland) talked on ultrafast time-resolved studies on charge transfer processes in dye-sensitized solar cells. Processing information with light and molecules provided for **Konrad Szaciłowski** (Cracow, Poland) an opportunity to introduce the emerging field of infochemistry. **Wojciech Macyk** (Cracow, Poland) presented studies on the effect of NIR irradiation on singlet oxygen generation in TiO_2 colloids. Casting the spotlight on the dipole-bound anions, **Sylwia Freza** (Gdańsk, Poland) summarised briefly what is known about these unusual species (unfortunately largely unknown in the spectroscopic community), and discussed representative examples of such anions, their properties and the role they play in various physicochemical processes. Practical aspects of

fluorescence spectroscopy were presented by **John Gilchrist** (Clydebank, UK), whose contagious enthusiasm for photoluminescence impressed all the participants of the meeting.

Thursday afternoon session was entirely dedicated to panel discussion. Like two years ago, the purpose of the discussion was to freely exchange views on a scientifically important topic, controversy or dispute, to discuss concepts, ideas, and to indicate open questions, and all that in a casual and informal atmosphere. This time we enjoyed the presence of Mark Maroncelli and devoted the discussion to solvation dynamics in polar liquids. Generous support from Kompania Piwowarska bear company, allowed us to put the discussion in a really liquid context. Three introductory contributions (**Jerzy Karpiuk**, **Mark Maroncelli**, and **Eberhard Riedle**) were

presented to "heat up" the audience by proposing more specific topics for discussion. In particular, **Mark Maroncelli** presented a broad, in part historically oriented, tutorial overview of ultrafast solvation dynamics, splendidly illuminating its various aspects not only for the students but also for the more advanced researchers. The fact that the alive discussion that followed the introduction generated more questions than answers was not only a sign that there's still a 'plenty of room at the bottom', but also again indicated that such an open-forum type discussion is a very much needed element of scientific meetings.

At the end of and after the meeting we received very positive comments and remarks from many participants who also expressed their wish to meet again at ML2015...

Jerzy Karpiuk (*Institute of Physical Chemistry, PAS, Warsaw*), Marek Mac (*Jagiellonian University, Cracow*) - Co-Chairmen of the ML2013 Organising Committee

ML2013 web site: http://ichf.edu.pl/ml2013

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