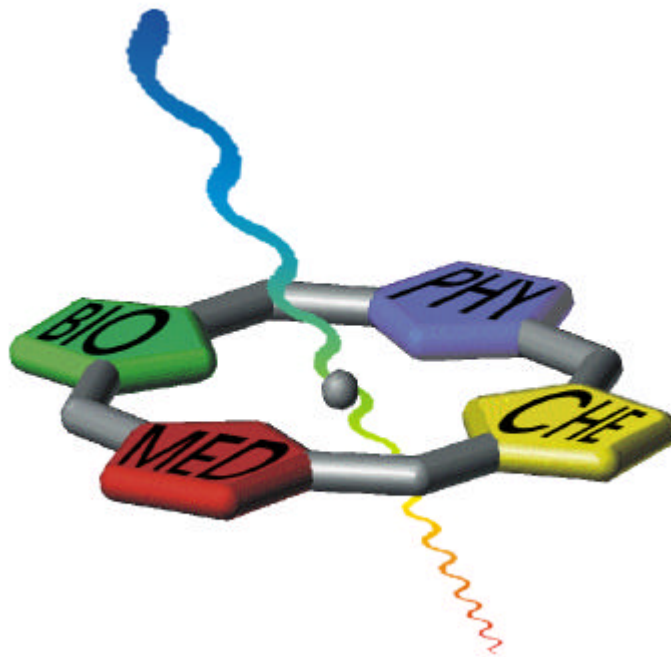


Graduate College

**SENSORY PHOTORECEPTORS
IN NATURAL AND ARTIFICIAL SYSTEMS**



**Summer Meeting
Nové Hradý/ CZ
July 20-24 2001**

University of Regensburg



Deutsche
Forschungsgemeinschaft

DFG

Deutsche Forschungs Gemeinschaft

PROGRAM

Friday, July 20th

15.00 - 16.00: Arrival to Buquoy chateau Nové Hradý

16.00 - 18.00: refreshment, welcome address of Guenter Hauska/ Ladislav Nedbal, registration and accommodation

18.00 - 19.30: free time

(concert: Latymer upper school choir, dir. David Elliot (England) in St. Peter and Paul church at Nové Hradý. Dubois: Saxophone Concerto, Byrd: Sanctus and Benedictus, Duruflé: Ubi Caritas, Spiritual arr. By R. Lloyd: Where are you?, Finzi: God is Gone Up, Barber: Adagio for Strings, Fauré: Requiem)

19.30 - 20.30: Icebreaker reception with dinner at Buquoy chateau, afterwards poster mounting

Saturday, July 21st

8.00 – 9.00 Breakfast at the accommodation place

9:00 Opening, introduction to the graduate college (Peter Hegemann)

9:30 – 10:30 Session I – QUANTUM CHEMICAL CALCULATION OF PHOTORECEPTORS

Bernhard Dick, introduction (15 min)

Christian Neiß, Peter Saalfrank and Bernhard Dick, *Quantum chemical calculations on flavins: properties of the ground and the lowest excited states*

10:30 – 11:00 coffee break

11:00 – 12:30 Session II - LASER SPECTROSCOPY OF FLUORESCENT DYES AND POLYMERS

Alfons Penzkofer: Introduction

Roland Stark, A. Bergmann, W. Holzer, H. Gratz, A. Penzkofer, F. Amat-Guerri, Costela, I. Garcia-Moreno and R. Sastre: *Photophysical characterization of pyrromethene dyes in solid matrices of acrylate copolymers*

Wolfgang Holzer, A. Penzkofer, H. Tillmann, R. Raabe and H.-H. Hörhold: *Photophysical characterization and travelling-wave lasing of some TPD-based polymers*

12:30 - 13:30 Sandwich lunch at Buquoy chateau

13:30 - 16:00 poster viewing, social interaction, demonstration of FluorCam

H. Balzer, N. Wrobel, G. Hauska and U. Bogner, *Persistent spectral holeburning in dye/matrix systems:*

Optical sensors of electric fields and probing of dynamical processes

Julie Soukupová, S. Smatanová, L. Nedbal and A. Jegorov
Fluorometric visualisation of the plant response to destruxins isolated from *Alternaria brassicae*.

Ferimazova N, Küpper H, Morse A, Maldener I, Šetlík I, Nedbal L
Chlorophyll fluorescence kinetic imaging: Cyanobacteria under a microscope and dinoflagellate algae on corals.

16:00 – 18:00 Session III – PHOTORECEPTORS WITH RETINAL AND FLAVIN

Peter Hegemann: Introduction

Tilman Kottke, Joachim Heberle, Peter Hegemann and Bernhard Dick: *Investigation of the phototropin photocycle by transient absorption measurements*

Sunil Kateriya, Markus Fuhrmann and Peter Hegemann: *Chlamyopsin-3 (COP3) as a potential photoreceptor of phototaxis in green algae*

Srinivasa Reddy, Markus Fuhrmann and Peter Hegemann: *Gene silencing in Chlamydomonas reinhardtii by using antisense technology*

18:00 - 19:00 Warm dinner at the refectory of the Serviten monastery

19:00 - 20:00 Guest lecture of **Aba Losi**/ University of Parma, Italy: *Photoacoustics of Photoreceptors*

20.15 **Organ concert by Michal Novenko:** Johann Sebastian Bach: Preludium and fuga e moll, Choral overture „Wer nur den lieben Gott lässt walten“, Ernst Pepping: Little Choral Partite, Joseph Gabriel Rheinberger: Vision, Felix Mendelssohn-Bartholdy: Sonata No.2 (Grave-Adagio-Allegro-Fuga), Bedøich Antonín Wiedermann: Pastorale mixolydico, Jehan Alain: Litanies, Michal Novenko: Improvisation.

Sunday, July 22nd

8.00 – 9.00 Breakfast at the accommodation place

9.00 – 10.00 Guest lecture -

Joachim Heberle/ FZ-Juelich, Germany, *IR-Spectroscopy of Photoreceptors*

10.00 – 10.30 Coffee break

10.30 – 12.30 Session IV – ENTRAINMENT OF THE CIRCADIAN CLOCK

Ralf Stanewsky: Introduction

Sinje Maruhn and Ralf Stanewsky: *A new role for cryptochrome in a Drosophila circadian oscillator*

Veleri Shobi and Ralf Stanewsky: *Do extra-ocular photoreceptors play a role in circadian entrainment of Drosophila melanogaster?*

12.30 – 13.30 Sandwich lunch at Buquoy chateau

13.30 – 18.00 Sightseeing bike trips (10, 20 or 40 km around Nové Hradky)

18.00 – 19.00 Warm dinner at the refectory of the Serviten monastery

20.00 – 21.00 Concert of the String quartet “Collegium Marianum” in the Serviten monastery. Johann Sebastian Bach: Fünf fugen - in modification for strings quartet by Wolfgang Amadeus Mozart - Erste fuge; Joseph Haydn: Quartetto 68 op.45; Josef Suk: Barkarola d moll; Josef Suk: Meditation on old Czech choral „Svaty Vaclave“, op. 35; Antonín Dvorák: Strings quartet F dur op. 96, „American“

Monday, July 23rd

8.00 – 9.00 Breakfast at the accommodation place

9:00 – 11:00 Session V – SYNTHETIC PHOTOCHEMICAL SWITCHES

Jörg Daub: Introduction

Zhen Shen and Jörg Daub: Modelling of photosensory processes by new synthetic flavins

Roman Procházka and Jörg Daub, *Axially coupled dye dyads as models for actuation of blue-light receptor function*

Roland Reichenbach-Klinke and Burkhard König, *Photoinduced electron transfer in a supramolecular assembly consisting of two biological redox cofactors: A model system for flavin reductase*

11:00 – 11:30 coffee break

11:30 – 13:00

Michael Büschel, M. Hölldobler and Jörg Daub, *Control of electronic interaction of electrochemically switchable oligopyridyl-benzodithiophene conjugates*

Jörg Strauss and Jörg Daub, *Optical and Chiroptical Properties of Dye Modified Cyclopeptides*

Mike Kercher, Burkhard König and Luise de Cola, *Non-covalently linked Acceptor-Donor Systems*

13:00 - 14:00 Sandwich lunch at Buquoy chateau

14:00 - 18:00 poster viewing, social interaction

18:00 – 19:00 Warm dinner at the refectory of the Serviten monastery

19:00 – 21:00 Session VI – APPLICATIONS / OPTICAL MICROSENSORS

Günter Hauska, introduction

Jens Kürner and Otto Wolfbeis, *Inert phosphorescent nanospheres as markers for optical assays*

Christoph Griesbeck and Günter Hauska, *Sulfide-Quinone Reductase, a Flavoprotein of the Glutathione Reductase Family*

Thomas Schödl and Günter Hauska, *The Development of a Fluorescent Microsensor for Sulfide with a Flavoprotein*

Closing of the meeting

Tuesday, July 24th

- Departure after breakfast by bus, sightseeing and lunch in Český Krumlov (Krumau)

Session I – Quantum Chemical Calculation of Photoreceptors

Quantum Chemical Calculations on Flavins: Properties and Reactions of the Ground and the Lowest Excited States

Christian Neiß*, Peter Saalfrank*, Bernhard Dick*

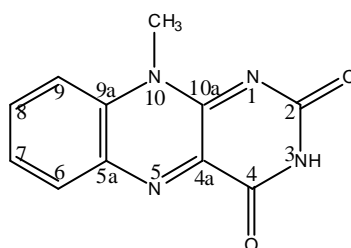
*Department of Physical and Theoretical Chemistry, University of Regensburg, D-93040 Regensburg

Flavins such as FAD and FMN play an important role in biochemistry not only as redox catalysts in many enzymes,¹ but also as blue light receptors, e. g., in photolyases, the related cryptochromes,² and phototropin.³ To get a better mechanistic understanding of the reactions initiated by the light induced excitation of the flavin, the lowest excited singlet and triplet states of some flavin-type molecules are investigated by means of quantum chemical methods.

In its oxidized form the flavin molecule is planar (C_S symmetry). In the electronic ground state, atom N5 (for numbering of the atoms see the figure below) shows a remarkable electron deficiency, consistent with the electrophilic character of this site.

Time dependent DFT excitation energies are in reasonable agreement with experimental data;⁴ the (lowest visible) $S_0 \rightarrow S_1$ excitation is a $\pi-\pi^*$ -transition being mostly of HOMO \rightarrow LUMO type. Reflecting this fact, the greatest rearrangement of electron density is observed at atoms N5 and C9a, where it increases, and at C5a and N1, where it decreases, respectively. The flavin stays planar in the S_1 and T_1 states. Spin densities for the triplet were also computed.

Furthermore, some possible intermediates of the phototropin photocycle are presented. As the excitation of flavin (probably) results in an addition of an adjacent cystein residue,⁵ we investigated the energetics of reactions of excited flavin with HSCH_3 as a model for cysteine. The results show that, for example, hydrogen abstraction from the SH-group by triplet flavin is possible, while direct electron or proton transfer from cysteine to triplet flavin seem very unfavorable.



¹ J. Kyte: *Mechanism in Protein Chemistry*, Garland Publishing, New York 1995

² A. Sancar: *Annu. Rev. Biochem.*, 69 (2000), 31-67; A. R. Cashmore et al.: *Science*, 284 (1999), 760-765

³ J. M. Christie et al.: *Proc. Natl. Acad. Sci. USA*, 96 (1999), 8779-8783

⁴ M. Sun, T. A. Moore, P.-S. Song: *J. Am. Chem. Soc.*, 94 (1972), 1730-1740; K.-H. Dudley et al.: *Helv. Chim. Acta*, 47 (1964), 1354-1383; M. Sakai, H. Tahahashi: *J. Mol. Struct.*, 379 (1996), 9-18

⁵ S. Crosson, K. Moffat: *Biochemistry*, 98 (2001), 2995-3000

Session II – Laser Spectroscopy of Fluorescent Dyes and Polymers

Introduction

Laser Spectroscopy of Fluorescent Dyes and Polymers

A. Penzkofer, W. Holzer, R. Stark,

Institut II - Experimentelle und Angewandte Physik, Universität Regensburg, D-93040 Regensburg

Fluorescent dyes play an important role for staining and sensing in analytical chemistry, environmental science, biology and medicine [1]. Some fluorescent dyes are laser active and are used in dye lasers [2]. In recent years the research interests changed from liquid dye lasers [3] to solid-state dye lasers [4]. Often organic polymers are used as solid matrices for the organic dyes. A characterisation of dye doped polymers by measuring absorption and emission cross-section spectra, fluorescence quantum yields, fluorescence lifetime, excited-state absorption cross-sections, and quantum yields of photo-degradation is necessary to evaluate their performance in solid-state laser devices [5]

Conjugated organic polymers are semiconductors and behave like molecular exciton systems [6]. Some conjugated organic polymers are fluorescent even in the solid state [7]. The electro-luminescence of the fluorescent polymers finds increased application in polymeric light emitting diodes (PLEDs) [8,9]. Some of the fluorescent polymers are laser active ([10] and references therein). Research on optically pumped solid-state polymer lasers is very active [11]. One goal is the realisation of electrically pumped polymer lasers [12]. The laser action of an electrically pumped organic crystal has already been achieved [13]. Detailed photo-physical characterisations and lasing tests are carried out on luminescent polymers in solutions, neat films, and blends for their evaluation in laser devices.

Reference

1. R. P. Haugland, Handbook of Fluorescent Probes and Research Chemicals, 7th Edition (Molecular Probes, Eugene, OR, 1999).
2. U. Brackmann, Lambdachrome laser Dyes, (Lambda Physik, Göttingen, 1997).
3. F. J. Duarte and L. W. Hillman, Dye Laser Principles with Applications (Academic Press, Boston, 1990).
4. A. Costela, I. García-Moreno, and R. Sastre, in *Handbook of Advanced Electronic and Photonic Materials and Devices*, edited by H. S. Nalva, Vol. 7 (Academic Press, San Diego, 2001), pp. 161.

5. W. Holzer, T. Schmitt, H. Gratz, A. Penzkofer, A. Costela, I. Garcia-Moreno, R. Sastre, and F. J. Duarte, *Chem. Phys.* 256 (2000) 125-136.
6. N. S. Sariciftci, Editor, *Primary Photoexcitations in Conjugated Polymers: Molecular Exciton versus Semiconductor Band Model*, (World Scientific, Singapore, 1997).
7. N. N. Barashkov and O. A. Gunder, *Fluorescent Polymers*, (Ellis Horwood, New York, 1994).
8. M. T. Bernius, M. Inbasekaran, J. O'Brien, and W. Wu, *Adv. Mater.* 12 (2000) 1737.
9. D. Y. Kim, H. N. Cho, and C. Y. Kim, *Prog. Polym. Sci.* 25 (2000) 1029.
10. A. Penzkofer, W. Holzer, T. Schmitt, A. Hartmann, C. Bader, H. Tillmann, D. Raabe, and H.-H. Hörhold, *Proc. SPIE* 4087 (2000) 635.
11. D. D. McGehee and A. J. Heeger, *Advanced Mater.* 22 (2000) 1655.
12. N. Tessler, D. J. Pinner, V. Cleave, P.K.H. Ho, R. H. Friend, G. Yahioghlu, P. Le Bary, J. Gray, M. de Souza, and G. Rumbles, *Synth. Met.* 115 (2000) 57.
13. J. H. Schön, Ch. Kloc, A. Dodabalapur, and B. Batlogg, *Science* 289 (2000) 519.

Photophysical Characterization of Pyrromethene Dyes in Solid Matrices of Acrylic Copolymers

A. Bergmann, W. Holzer, R. Stark, H. Gratz, and A. Penzkofer

Institut II - Experimentelle und Angewandte Physik, Universität Regensburg, Universitätsstraße 31,
93053 Regensburg, Germany.

F. Amat - Guerri

Instituto de Química Orgánica, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain.

A. Costela and I. García - Moreno

Instituto de Química Física "Rocasolano" CSIC, Serrano 119, 28006 Madrid, Spain

R. Sastre

Instituto de Ciencia y Tecnología de Polímeros, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain.

Some photophysical parameters of the pyrromethene laser dye PM567 [1] and of two newly synthesized pyrromethene laser dyes, P5Ac and P10Ac, in solid matrices of different acrylic copolymers and in corresponding mimetic liquid solutions are determined [2]. They include absorption cross-section and stimulated emission cross-section spectra, fluorescence quantum distributions, fluorescence quantum yields [3], and fluorescence lifetimes. The saturable absorption behavior due to picosecond laser pulse excitation is studied [4]. The photo-degradation under cw laser excitation [5] and under pulsed picosecond pulse excitation [6] is investigated.

Laser action of the investigated pyrromethene dye doped acrylic copolymers has been achieved [7]. The photophysical parameters and the laser action of the dye doped polymer samples studied here compare well with rhodamine 6G doped polymers investigated earlier [8].

References

1. M. Shah, K. Thangaraj, M. L. Soong, L. T. Wolford, J. H. Boyer, I. R. Politzer, and T. G. Pavlopoulos, *Heteroatom. Chem.* **1** (1990) 389.
2. A. Bergmann, W. Holzer, R. Stark, H. Gratz, A. Penzkofer, F. Amat-Guerri, A. Costela, I. Garcia-Moreno, and R. Sastre, *Chem. Phys.*, (2001), to be published.
3. A. Penzkofer and W. Leupacher, *J. Luminesc.* **37** (1987) 61.
4. A. Penzkofer, *Appl. Phys. B* **46** (1988) 43.
5. S. Reindl and A. Penzkofer, *Opt. Quant. Electron.* **30** (1998) 49.
6. W. Holzer, M. Mauerer, A. Penzkofer, R.-M. Szeimies, C. Abels, M. Landthaler, and W. Bäuml, *J. Photochem. Photobiol. B: Biol.* **47** (1998) 155.
7. A: Costela, I. García-Moreno, R. Sastre, D. W. Coutts, and C. E. Webb, *Appl. Phys. Lett.*, (2001), to be published.
8. W. Holzer, T. Schmitt, H. Gratz, A. Penzkofer, A. Costela, I. Garcia-Moreno, R. Sastre, and F. J. Duarte *Chem. Phys.* **256** (2000) 125-136.

Photophysical Characterization and Travelling-Wave Lasing of some TPD-based Polymers

W. Holzer and A. Penzkofer

Institut II - Experimentelle und Angewandte Physik, Universität Regensburg,
Universitätsstraße 31, D-93053 Regensburg, Germany

H. Tillmann, D. Raabe, and H.-H. Hörhold

Innovent Technologieentwicklung e.V., D-07745 Jena, Germany and
Institut für Organische Chemie und Makromolekulare Chemie der Universität Jena,
Humboldtstrasse 10, D-07743 Jena, Germany

Travelling-wave lasing (amplification of spontaneous emission) is reported for neat films of a series of five red, green and blue emitting TPD-based polymers, the TPD-phenylenevinylene and the TPD-xylylene copolymers [1]. Previously laser action was also achieved on neat films of TPD (triphenylamine dimer) molecules [2]. Thin samples on glass substrates were fabricated by spin-coating and transversally pumped with picosecond excitation pulses (wavelength 347.15 nm, duration 35 ps). Lasing occurs around 421 nm, 536 nm, 540 nm, 571 nm, and 618 nm with a linewidth smaller than 10 nm. The threshold pump pulse energy densities are determined and found to be $60 \mu\text{J}/\text{cm}^2$ for the blue emitting non-conjugated polymer (Poly-TPD(4M)-DPX) and $6 - 8 \mu\text{J}/\text{cm}^2$ for the green and red emitting conjugated polymers TPD(4M)-MEH-PPV and TPD(4M)-MEH-M3EH-PPV. The laser output saturation at high excitation energy densities is studied. The length of effective amplification of spontaneous emission is approximately 1 mm. Effective stimulated emission cross-sections are derived from the pump pulse energy density dependent spectral narrowing of the amplified emission signals [3].

The optical constants (absorption spectrum and refractive index spectrum) of the neat films are determined by reflection and transmission measurements [4]. The absorption cross-section spectra are extracted [4]. The fluorescence quantum efficiencies [5] and the fluorescence lifetimes are measured.

References

1. W. Holzer, A. Penzkofer, H. Tillmann, D. Raabe, and H.-H. Hörhold, *Opt. Mater.* (2001), to be published.
2. W. Holzer, A. Penzkofer, and H.-H. Hörhold, *Synth. Metals* 113 (2000) 281-287.
3. W. Holzer, A. Penzkofer, T. Schmitt, A. Hartmann, C. Bader, H. Tillmann, D. Raabe, R. Stockmann, and H.-H. Hörhold, *Opt. Quantum Electron.* 33 (2001) 121-150.
4. W. Holzer, M. Pichlmaier, E. Drotleff, A. Penzkofer, D. D. C. Bradley, and W. J. Blau, *Opt. Commun* 163 (1999) 24-32.
5. 180. W. Holzer, M. Pichlmaier, A. Penzkofer, D. D. C. Bradley, and W. J. Blau, *Chem. Phys.* 246 (1999) 445-462.

Photoacoustics of Photoreceptors

Aba Losi, University of Parma

In the photocycle of a photosensor the understanding of the early thermodynamics is of key importance, because it drives the rest of the photocycle and reflects mechanisms by which chromophore structural changes are transmitted to the protein moiety. Unfortunately the measure of the free energy of the various transitions, ΔG_i , is impaired by the impossibility to determine entropy variations (ΔS_i) in a time resolved way, without introducing assumptions. This difficulty can be by-passed, in some cases, by the use of time-resolved, photocalorimetric methods (1). In particular, pulsed Photoacoustics (PA) is suitable to investigate the ns-to- μ s time region in light-initiated reactions, at ordinary temperature and pressure. PA (also called LIOAS: Laser Induced Optoacoustic Spectroscopy) can monitor enthalpy variations (ΔH_i) and structural volume changes (ΔV_i), with a time resolution between 20 ns and 5 μ s (1). When a linear correlation between ΔH_i and ΔV_i is the result of an enthalpy-entropy compensation effect (*i.e.*, ΔG_i does not vary within a particular series of changes, and reaction enthalpy and entropy variations are due to strong coupling of the reaction with the medium), it is possible to calculate the ΔS_i from the measured ΔV_i . Following this approach a time-resolved method to measure ΔS_i could be applied to electron transfer reactions in water, and to the decay of the early, red-shifted intermediate K for *Natronobacterium pharaonis* sensory rhodopsin II (2) and halorhodopsin. In this last case also the ΔS for K formation from the parent state could be determined. Collectively, the results showed that entropy variation have a relatively large importance already in these early stages of the photocycle. Recently PA was applied to the photoactive domains of the blue-light phototropins. Different to rhodopsins, these domains showed very small, albeit clearly detectable, ΔV_i in this time domain while a considerable amount of the excitation energy is stored in long-lived species.

1. Braslavsky, S.E. and G.E. Heibel (1992) Time-resolved photothermal and photoacoustic methods applied to photoinduced processes in solution. *Chem. Rev.* **92**, 1381-1410.
2. Losi, A., A.A. Wegener, M. Engelhardt and S.E. Braslavsky (2001) Enthalpy-entropy compensation in a photocycle: The K to L transition in sensory rhodopsin II from *Natronobacterium pharaonis*. *J. Am. Chem. Soc.* **123**, 1766-1767 (and references therein).
3. Losi, A. and S.E. Braslavsky (2001) Time-resolved Optoacoustic studies of the photochemically active LOV2 domain of *Arabidopsis* phototropin, presented at the 29th meeting of the American Society for Photobiology, Chicago (USA), July 7-12.

MULTIPLE SENSORY PHOTORECEPTORS OF THE GREEN MICROALGA
CHLAMYDOMONAS REINHARDTII

Peter Hegemann
Institut für Biochemie, Universität Regensburg,

In order to fully exploit environmental light as a sensory stimulus, higher plants, macro- and microalgae have developed sophisticated networks of photoreceptors and interacting sensory pathways. *Chlamydomonas reinhardtii* exhibits two different photobehavioural reactions upon light stimuli: positive or negative phototaxis and a photoshock response upon sudden changes in light intensity. These responses are mediated by one or possibly two rhodopsin photoreceptors with *at*-retinal chromophore (Type I rhodopsin) that trigger a set of rapid photoreceptor and flagellar currents. From the electrophysiological data we have suggested that at least the rhodopsin responsible for the photophobic responses (high light intensity responses) acts as a light-gated passive ion transporter in analogy to bacteriorhodopsin from *Halobacterium halobium*.

One single retinal binding protein was identified in *Chlamydomonas* and purified during the recent years. However, the primary sequence is related to the type II rhodopsins from higher animals, photoreceptors that contain *11-cis* retinal chromophores. This protein was localized within the eyespot region by fluorescence microscopy and by expression of a GFP-tagged recombinant protein. The mRNA of this chlomyopsin is processed into three different mRNA variants, encoding two opsin-related proteins Cop1 and Cop2 and two smaller proteins. Whereas Cop1 and Cop2 were detected by specific antibodies, the small proteins have not been found *in vivo*. Since the proteins do not fit to the suggested chromophoric properties, their function was examined *in vivo* by modulation of their expression levels with RNAi-like approaches (antisense technology). Physiological experiments including electrophysiology, motion analysis and light scattering indicate that the chlomyopsin variants Cop1 and Cop2 are not the responsible receptors for the light induced movement responses of *Chlamydomonas*. These results implicate that another recently identified retinal binding protein, Cop3, serves as the photoreceptor for behavioural responses. This protein will be discussed by S. Kateriya.

C.reinhardtii also performs several blue-light responses, which are chloroplast development, and gametogenesis, i.e. development from a vegetative state (growth of a culture by cell division) into gametes (male and female), which sexually reproduce. In addition, photosynthetic activity, phototactic activity, and cell division are under rhythmic control, the *Zeitgeber* of which is blue light. Two genes, *cph1* and *nph1*, were discovered by G.Small or appeared from a genome sequencing project and are hypothetical blue light receptors. The encoded proteins are relatives of the plant blue light receptors *cryptochrome* and *phototropin*. The cryptochrome homologue Cph1 is under investigation by S.Reddy, whereas the phototropin homologue Nph1 (now named Phot1) is studied by J. Heberle, T. Kottke, and W. Holzer.

INVESTIGATION OF THE PHOTOTROPIN PHOTOCYCLE BY TRANSIENT ABSORPTION MEASUREMENTS

Tilman Kottke¹, Joachim Heberle², Peter Hegemann³, Bernhard Dick¹; ¹Institut für Physikalische Chemie, Universität Regensburg, ²Institut für biologische Informations-verarbeitung, Forschungszentrum Jülich, ³Institut für Biochemie, Universität Regensburg

Phototropin is a 120 kDa blue light receptor protein which plays a key role in phototropism of higher and lower plants. Its structure includes two light receiving domains named LOV1 and LOV2 and a protein kinase domain. The chromophore incorporated after expression is a flavin mononucleotide (FMN)^[1].

Up to now most work has been done on the LOV domains of *Arabidopsis thaliana*. Two intermediates in the photocycle of the protein domain have been observed, but were not characterized in detail^[2]. Our work concentrates on the LOV1 domain of the algae *Chlamydomonas reinhardtii* the sequence and structure of which is similar to LOV1 from higher plants. In the *C. reinhardtii* LOV1 immediately after blue light excitation a species with 650 and 710 nm absorption is formed which closely resembles the triplet spectrum of free FMN^[3]. The LOV1_{650/710} species decays within 3µs into a 390 nm absorbing intermediate. LOV1₃₉₀ is a ground state species which has been assumed to be a flavin C(4a) - thiol adduct (see Fig.1)^[4]. This adduct decays monoexponentially with a pH-dependant half time of 170s (pH=7) leading to recovery of the dark form LOV1₄₄₆.

Further details about the reaction mechanism can be derived from the behaviour of a C57S mutant of the LOV1 domain. The mutant cannot form the wild type flavin C(4a) - adduct with the cystein thiol group and shows direct recovery of LOV1₄₄₆ from LOV1_{650/710}. On first view this process shows a monoexponential decay with a half time of 20µs. A more detailed analysis reveals a multiexponential dependence.

Using laser flash photolysis and varying temperature, pH and excitation conditions we expect to get more information regarding the branching reactions in the photocycle (see Fig.2) and possible radical intermediates in the LOV1₃₉₀ formation process.

- [1] J. M. Christie, M. Salomon, K. Nozue, M. Wada, W. R. Briggs, *Proc. Natl. Acad. Sci. USA* **96** (1999), 8779-8783.
- [2] T. E. Swartz, S. B. Corchnoy, J. M. Christie, W. R. Briggs, R. A. Bogomolni, *Biophys. J.* **80** (2001), 428a.
- [3] M. Sakai, H. Takahashi, *J. Mol. Struct.* **379** (1996), 9-18.
- [4] M. Salomon, J. M. Christie, E. Knieb, U. Lempert, W. R. Briggs, *Biochemistry* **39**, (2000), 9401-9410.

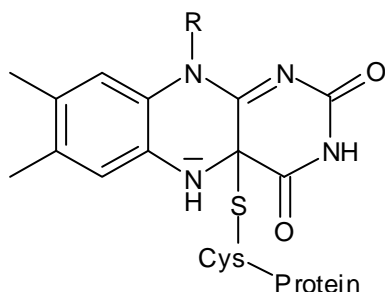


Fig.1

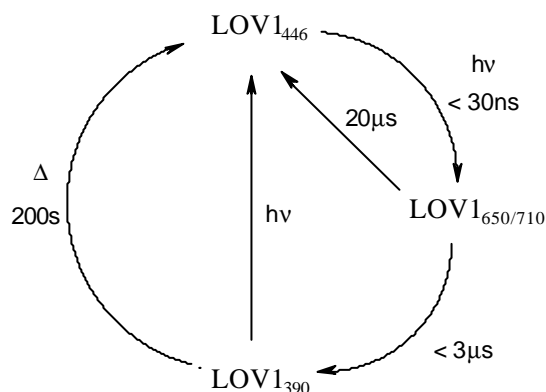


Fig.2

Chlamyopsin-3 (COP-3) is a potential photoreceptor for behavioral responses in *Chlamydomonas reinhardtii*

Suneel Kateriya, Markus Fuhrmann and Peter Hegemann

In order to find optimal light conditions for photosynthetic growth, the green alga *Chlamydomonas reinhardtii* uses a simple visual system including an optical system and a rhodopsin photoreceptor that triggers a set of photoreceptor currents. *In vivo* experiments showed that the algal rhodopsin/s contain an *all-trans* retinal chromophore (archaeal or type I rhodopsin). A retinal binding protein was isolated from eyespot membranes and sequenced. It is a mixture of two splicing variants of almost equal size but different hypothetical retinal binding sites (Cop1 and Cop2). Both proteins are related to animal rhodopsins (type II rhodopsins) and are hardly compatible to the proposed retinal chromophore. Recently, it was shown on the bases of biophysical studies on antisense transformants with reduced Cop1 and Cop2 content that both are not the photoreceptors for phototaxis and photophobic responses (Fuhrmann et al. 2001).

The only green alga in which two retinal proteins of different size (29 and 46 kDa) were identified is *Dunaliella salina* (Hegemann et al 2001).

As consequences we tried to find a sequence coding for a larger opsin from the cDNA data bank resulting from a *C.reinhardtii* genome project. We have identified four overlapping cDNA sequences which all together encode for a 56 kDa protein (Accession No. AF 385448).

For the mentioned purpose we have done *in silico* cloning followed by cloning and sequencing of the real cDNAs. The protein is by 22% identical to sensory rhodopsin II and to 20% to bacteriorhodopsin from *halobacterium salinarium*.

The homology appears quite small but most amino acids that define the retinal binding pocket and/or are involved in ion transport process are conserved. The hydropathy plot identifies 6 to 8 transmembrane segments which is a good criterium for 7 transmembrane spanning rhodopsin. This protein is a highly interesting candidate for serving as a photoreceptor (Cop3) in *C.reinhardtii* which triggers photocurrent as well as subsequent photophobic responses and phototaxis.

Our goal is to functionally express the protein in *E.coli*, the yeast *Sacharomyces pombe* or in baculovirus infected SF9 cells as a prerequisite for any spectroscopical measurement. We also intend to localise the protein in the alga by expression Cop3 as a GFP fusion construct (Fuhrmann et al. 1999) and to study the function *in vivo* in a mutant with reduced Cop3 content. The latter will be achieved by expression of an antisense DNA-construct (Fuhrmann et al. 2001).

References:

1. Hegemann, P. Fuhrmann, M. and Kateriya, S. (2001) Algal sensory photoreceptors. *J. Phycol.* (accepted).
2. Fuhrmann, et al. (2001) The most abundant retinal protein in the eye of *Chlamydomonas* is not the photoreceptor for phototaxis and photophobic responses. *J. Cell Sci* (accepted)
3. Fuhrmann, et al. (1999) A synthetic gene coding for the green fluorescent protein (GFP) is a versatile reporter in *Chlamydomonas reinhardtii*. *Plant J* 19(3):353-61

Gene silencing in *Chlamydomonas reinhardtii* by using antisense technology

P. Srinivasa Reddy, Markus Fuhrmann and Peter Hegemann

Light is an important environmental factor controlling growth and different plant responses like de-etiolation, circadian clock, floral initiation, phototropic curvature, chloroplast relocation and stomatal opening. Molecular genetic studies have shown that the effects of blue light on plant development are mediated by at least four different blue light receptors i.e. cry1, cry2, nph1, npl1 (Tatsuya *et al.*, 2001). Cryptochromes show significant homology to microbial photolyases but lack photorepair activity and are likely to function by mediating light dependent redox reactions. There is an evidence in favour of a nuclear localization of cryptochromes. In green alga cph1 deficient mutants are not available and targeted disruption of non-selectable nuclear genes is not yet possible. So we used antisense technology with intron-containing gene fragments directly linked to their intron-less antisense counterparts, that have been shown to provide efficient post transcriptional gene silencing (Fuhrmann *et al.*, 2001). Antisense technology allows efficient reduction of a specific gene product in green alga. Antisense construct was obtained by starting from a plasmid vector, which contains the whole CPH gene. In order to get a small fragment (860bp) of this gene, we have digested the vector with NcoI and ApaI. We have ligated the back bone of pHSP CPH with 860bp in antisense orientation of cDNA and confirmed by sequencing of the construct. Transformation of plasmid which contains antisense construct was carried out according to the glass bead method with pArg 7.8 as selectable marker in *Chlamydomonas*. Transformants were selected on standard medium lacking arginine and were confirmed by the DNA-blotting. We will be measuring photocurrents with this construct and gametogenesis, circadian rhythm and compare physiological responses with wild type in *Chlamydomonas reinhardtii*.

References

Fuhrmann, M., Alke, S., Simone, R., Elena, G. and Hegemann, P. (2001). Abundant retinal protein of the *Chlamydomonas reinhardtii* eye is not the photoreceptor for phototaxis and photophobic responses. *J. Cell. Sci.*, (accepted).

Tatsuya, S. et al. (2001) Arabidopsis nph1 and npl1: Blue light receptors that mediate both phototropism and chloroplast relocation. *Proc.Natl.Acad.Sci.USAS*, 98, 6969-74.

Guest Lecture

**The contribution of vibrational spectroscopy to the elucidation
of the primary reaction steps in photoreceptors**

***Joachim Heberle, Forschungszentrum Jülich,
IBI-2: Biologische Strukturforschung, D-52425 Jülich***

Vibrational Spectroscopy is a powerful tool in investigating protein dynamics on an atomic level and infrared as well as Raman spectroscopy are the most prominent techniques. Over the past three decades, both methods contributed significantly to the molecular understanding of the light-induced reactions in photoreceptors. Vibrational spectroscopy of large molecules like proteins, delivers only limited spatial information. Yet, it is very sensitive for minute conformational changes which can be recorded with high temporal resolution. Therefore, enzymatic dynamics are observable across the whole time-regime with high local resolution¹.

The principles and applications of modern vibrational techniques will be introduced with special emphasis on FT-IR and resonance-Raman spectroscopy. It will be demonstrated with the photon-driven proton pump bacteriorhodopsin at hand, how details of the catalytic mechanism are elucidated by static and time-resolved vibrational spectroscopy². The application of the methodology to other membrane proteins (halorhodopsin und cytochrom c oxidase) will also be included. Finally, our latest results on the LOV1 domain of phototropin will be presented and discussed.

References:

1. Vogel, R. & Siebert, F. (2000), *Vibrational spectroscopy as a tool for probing protein function*. **Curr. Opin. Chem. Biol.** 4, 518-523.
2. Heberle, J. (2000), *Proton transfer reactions across bacteriorhodopsin and along the membrane*. **Biochim Biophys Acta** 1458, 135-147.

Session IV – Entrainment of the Circadian Clock

Is the neuropeptide PDF involved in a molecular feedback loop of the *Drosophila* circadian oscillator?

Sinje Maruhn and Alois Hofbauer

University of Regensburg, NWF III, Biologie und Vorklinische Medizin, Institut fuer Entwicklungsbiologie

Drosophila shows a circadian pattern in locomotor activity which can be entrained by light-dark-cycles (LD) and continues in constant darkness (DD), typical for endogenous rhythmic behaviour.

The circadian system of the *Drosophila* clock is composed of three components and organized in three levels.

First there is the **input**, where information about daytime given by light is transduced by photoreceptive structures and photoreceptors like cryptochrome (Stanewsky *et al.*, 1998) from several components of the visual system (Helfrich-Foerster *et al.*, 2001) to the central clock.

The second component is the oscillating **pacemaker**, the endogenous clock itself with its oscillating system of rhythmically expressed clock genes, regulated by feedbackloops.

At last in the **output**-pathway the measured "time"-information is given over to effector organs which generates locomotor activity and physiological processes like cell proliferation (overview: Zulley and Knab, 2000).

One candidate gene for the output pathway regulated by clock genes is the neuropeptide *pigment dispersing factor* (PDF) that might play a role in the regulation of insect biological rhythms. The *pdf*-gene is co-expressed with the clock genes *period* and *timeless* in individual neurons known to be part of the pacemaking system (sLN_v, lLN_v) and the PDF-protein shows an oscillating distribution.

The *pdf⁰¹*-mutant and PDF cell-ablated animals become arrhythmic after several days in constant darkness (Renn *et al.*, 1999) and additional ectopic expression of PDF results in increased activity and in destabilisation of rhythm in DD (Helfrich-Foerster *et al.*, 2000).

These observations suggest that PDF is involved in the coupling of molecular clock to behaviour and physiological processes and that there might be a feedback influence onto the endogenous pacemaker. To test this we will manipulate the PDF-expression and monitor its influence on the cyclic expression of the clock genes *period* and *timeless* in individual pacemaker neurons.

References:

Helfrich-Foerster *et al.* (2000): Ectopic Expression of the Neuropeptide Pigment-dispersing-Factor Alters Behavioral Rhythms in *Drosophila melanogaster*. *J. Neurosci.* 20 (9): 3339-53

Helfrich-Foerster *et al.* (2001): The Circadian Clock of Fruit Flies Is Blind after Elimination of All Known Photoreceptors. *Neuron* (30): 249-261

Renn *et al.* (1999): A *pdf* neuropeptide gene mutation and ablation of PDF neurons each cause severe abnormalities of behavioral circadian rhythms in *Drosophila melanogaster*. *Cell* 99: 791-802

Stanewsky *et al.* (1998): The *cry^b* mutation identifies cryptochrome as a circadian photoreceptor in *Drosophila*. *Cell* 95: 681-92

Zulley and Knab (2000): Unsere innere Uhr – Natuerliche Rhythmen nutzen und der Non-Stop-Belastung entgehen. *Herder/Spektrum*, Heidelberg

Do extra-retinal photoreceptors play a role in circadian entrainment of *Drosophila melanogaster* ?

Shobi V, Helfrich-Forster C, Hofbauer A and Stanewsky R

Institute für Zoologie, Universität Regensburg, Universitätsstr. 31, 93040 Regensburg, Germany.

Circadian rhythms are oscillations in the biochemical, physiological, and behavioural functions of organisms with a periodicity of approximately 24 hr. They exist in organisms ranging from cyanobacteria to humans, and this conservation over evolution underlines their importance, implying that circadian clocks generating those rhythms confer a selective advantage (Sancar, 2000).

A circadian rhythm is the external expression of an internal timing mechanism that measures daily time. Circadian clocks are normally set by periodic environmental cues, with the light-dark cycle being the most pervasive and potent entraining stimulus. This enables the organisms to keep their behaviour in tune with the 24 hr solar day (Reppert and Weaver, 2001). The photoreceptors function as the conduit for light stimuli to central pacemaker (circadian clock). So far 2 circadian photoreceptors are identified, first the well defined compound eyes, mediated through the rhodopsin receptors; second the recently discovered cryptochrome (Stanewsky *et al.*, 1998). Mutants for both, functional eyes and cryptochrome are extremely crippled in terms of circadian entrainment; *albeit* subjected to long exposures of light-dark (12 h L:D 12 h) cycles still could achieve circadian entrainment. Furthermore, a set of "pacemaker neurons" in the brain still continues to express the major clock components PERIOD and TIMELESS rhythmically, in synchrony with the external environmental L:D cycle. This points toward a still unidentified novel photoreceptor, which brings input to the central clock to entrain the behavioural and molecular rhythms. A promising candidate is the so called "Hofbauer-Buchner" (H-B) eyelet, because the only axonal projections emanating from these extra-retinal cells project to the pacemaker. Therefore, we investigated the role of H-B eyelet in circadian entrainment of *D. melanogaster*, employing molecular genetics and immunohistochemical techniques. The prospective results will be discussed.

References:

- 1). Aziz Sancar: *Annu. Rev. Biochem.* 69:31-67 (2000).
- 2). Steven M Reppert & David R Weaver. *Annu. Rev. Physiol.* 63: 647-76 (2001).
- 3). Stanewsky R *et al.*, *Cell* Nov 25;95(5):681-92 (1998).

Session V – Synthetic Photochemical Switches

Introduction

Jörg Daub

Institut für Organische Chemie, Universität Regensburg,
Universitätsstr. 31, D-93040 Regensburg (Germany)

e-mail: joerg.daub@chemie.uni-regensburg.de

Artificial Sensory Photoreceptors: A Quest for the Designing and Making of Molecular Machines.

From a chemical point of view sensory photoreceptors constitute highly specialized molecular systems which trade, administer and even store information and beyond it are stimulated by action from outside. As a consequence sensory photoreceptors fit into molecular electronics, which encompass all processes dealing with photons, electrons and thermal energy (phonons). Sensory photoreceptors are multifunctional with size beyond the molecular level. Therefore the notation „molecular machines“ is used at times.

The following topics will be addressed:

- * „Beyond-molecular-level“ multifunctional systems associated with molecular electronics^[1].
- * Mechanisms of coupling between molecular subunits^[2,3].
- * Molecular switching: Basic process of signalling^[4].
- * Chemistry of „molecular level logic functions“^[5].

References:

- [1] J. Jortner and M. Ratner (Eds), 'Molecular Electronics A 'Chemistry for the 21st Century' monograph', Blackwell Science Ltd, **1997**.
- [2] M. D. Newton and R. J. Cave, in 'Molecular Control of Electron and Hole Transfer Processes: Electronic Structure Theory and Application', ed. J. Jortner and M. Ratner, Oxford, **1997**, p. 73.
- [3] J. R. Lakowicz, 'Principles of Fluorescence Spectroscopy', Kluwer Academic/Plenum Publ., **1999**, Chapter 13.
- [4] B. L. Feringa (Ed.), 'Molecular Switches', Weinheim, **2001**.
- [5] Logische Schaltungen mit leuchtenden Molekülen, A. P. deSilva, G. D. McClean, N. D. McClenaghan, T. S. Moody, and S. M. Weir, Nachrichten aus der Chemie, **2001**, 49, 602.

*Modelling the photonic and electronic parts of signal cascading of
blue light receptors
Supervisor : Jörg Daub*

Part 1 - Zhen Shen : „Oligad“ approach

Part 2 - Roman Prochazka: Stereocontrol of efficiency by biaryl binding

Light is the most important factor that plants use to optimize growth and development and which triggers behavioral circadian rhythms of mammals.

Photoreceptors are sensitive either towards blue and ultraviolet-A light (cryptochrome^[1] or phototropin^[2]), or red and far-red irradiation (phytochrome). The functional units that absorb and transduce the light signals are either of photochromic^[3] or redox-active nature^[4]. The blue-light processes are based on flavins as redox mediators and pterins or deazaflavins as antenna.

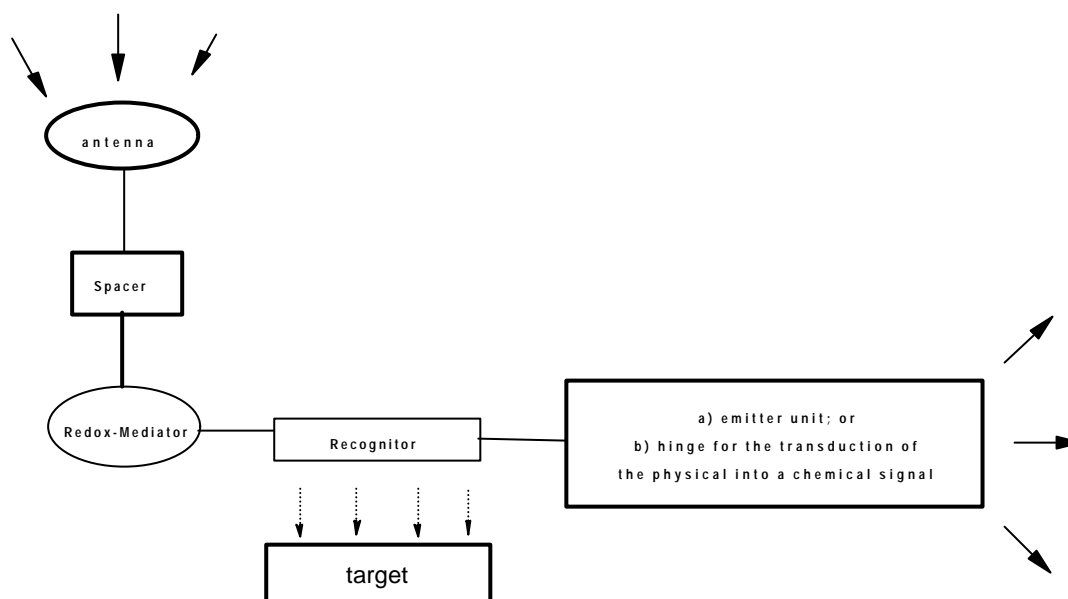
We are interested in the synthesis and study of chemically modified flavins, pterins, deazaflavins, and their potential application as actuators in genetically expressed natural photoreceptors.

In a second approach multifunctional dye systems are being synthesized and investigated by spectroscopic, photophysical, electrochemical, and spectroelectrochemical methods^[5] in order to find out the potential of signal transduction and signal transfer. Interests are towards a systematical investigation of the mechanisms of coupling^[6] between the dye units (wire or not^[7]) and their relevance towards expression of signaling effects. The flavin chromophore is used as central redox-mediator unit^[8]

Present work comprises two main fields:

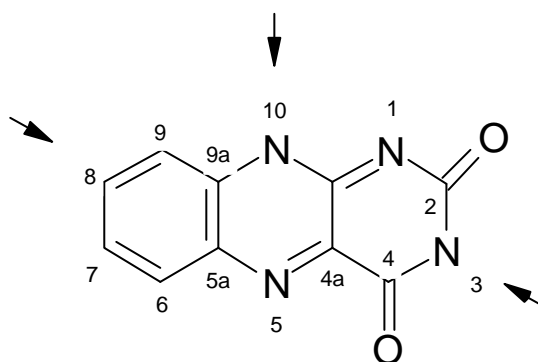
Dr. Zhen Shen: According to scheme 1 multi-array systems are under investigation comprising antenna, redox mediator (consisting of a redox active unit, inclusive electron donor), site for recognition and emitter or transducer unit (dye unit for the chemical or physical signal expression).

Scheme 1: Architecture of an artificial sensory photoreceptor



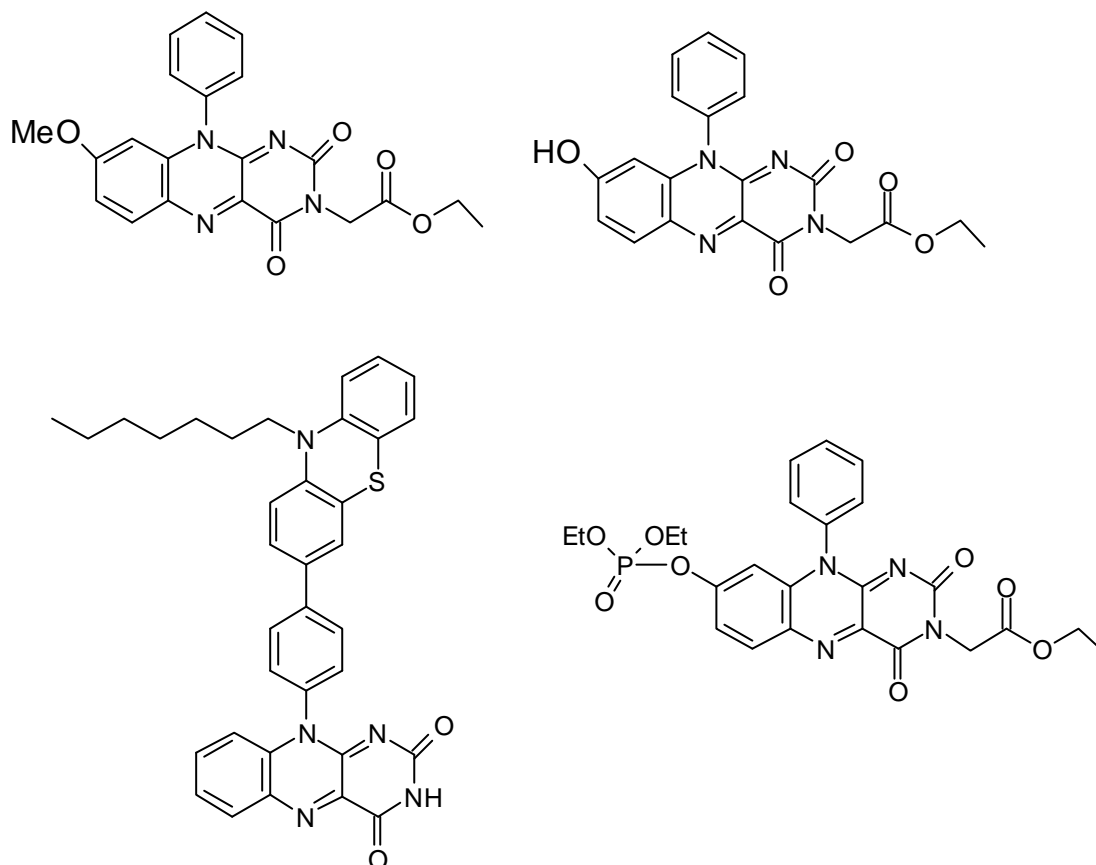
In the first part of the project the subunits are synthesized and their physical properties investigated. Scheme 2 shows the strategy of functionalization of the flavin chromophore.

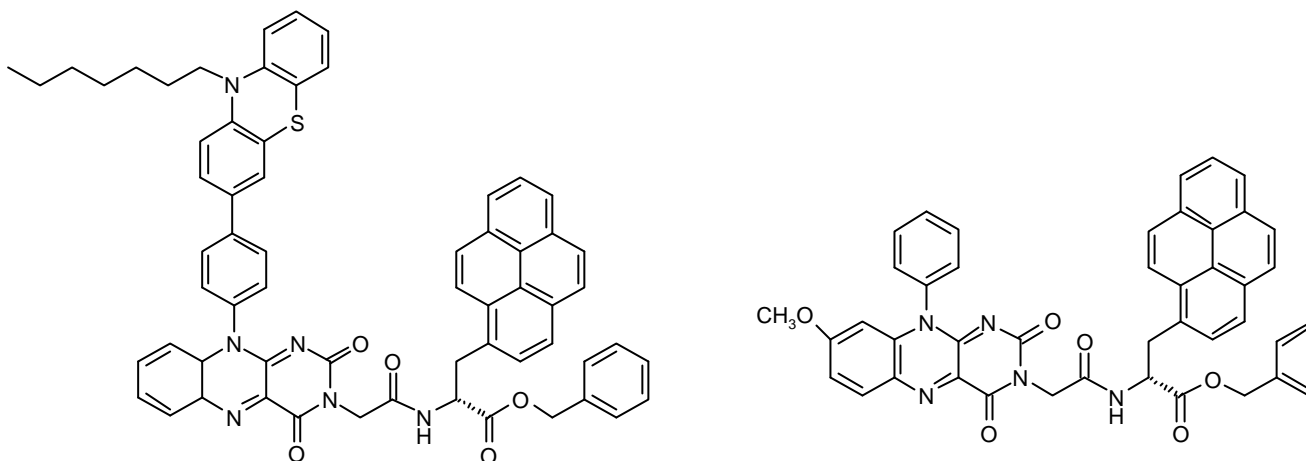
Scheme 2 Different substitute positions of the isoalloxazine ring that we employ in the model system.



Newly synthesized compounds are shown in scheme 3. The chemical and physical properties of the compounds will be discussed including electrochemistry and intermolecular recognition.

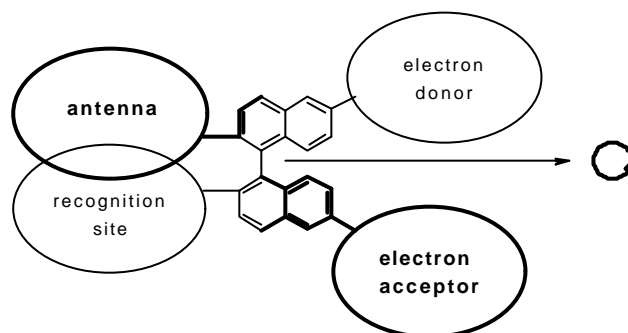
Scheme 3:





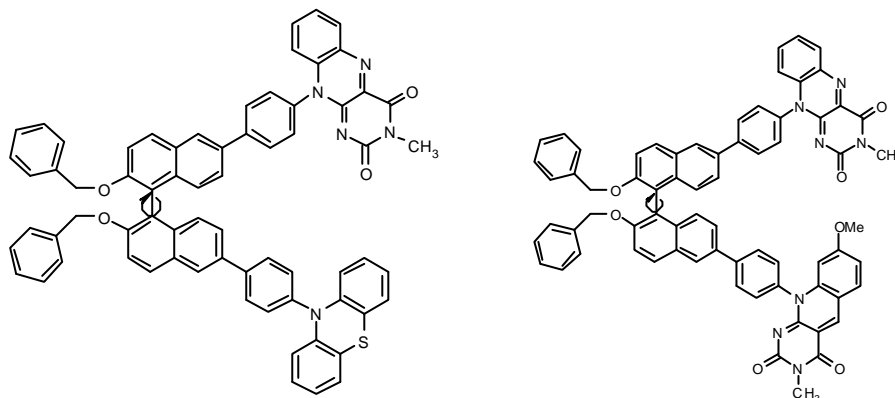
Dipl. Chem. R. Prochazka: Biaryls^[9] are found to be useful for tuning the coupling between chromophoric subunits^[10]. Hence, functionalized biaryls (scheme 4) are expected to be good candidates for modelling signalling.

Scheme 4:



A first generation of target molecules (scheme 5) will be presented: Synthesis, spectroscopic and electrochemical properties.

Scheme 5:



References

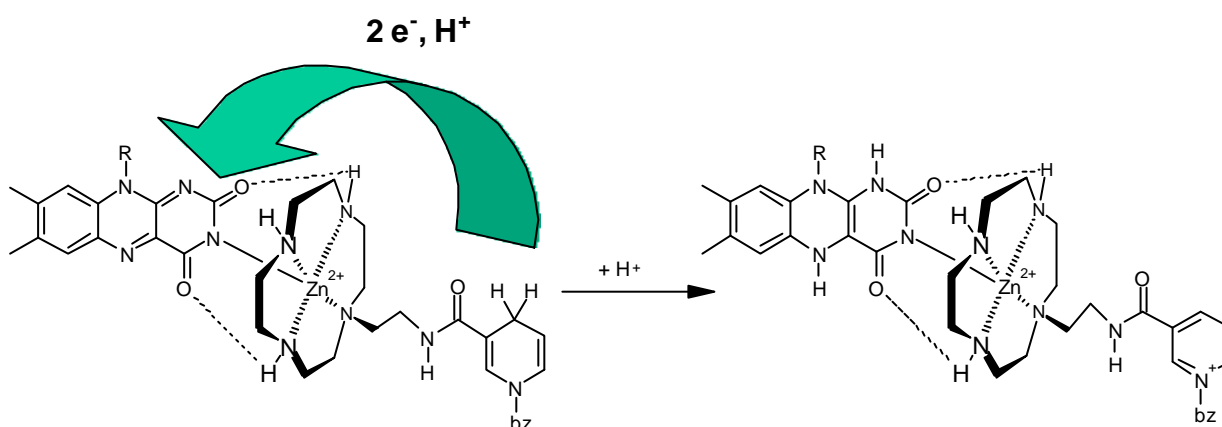
- [1] A. R. Cashmore, J.A. Jarillo, Y-J. Wu, D. Liu, *Science* **1999**, 284, 760.
- [2] M. Salomon, J. M. Christie, E. Knieb, U. Lempert, W.R. Briggs, *Biochemistry* **2000**, 39, 9401.
- [3] T. Mrozek, J. Daub, and A. Ajayaghosh, in 'Optoelectronic Molecular Switches Based on Dihydroazulene-Vinylheptafulvene (DHA-VHF)', ed. B. L. Feringa, Weinheim, **2001**, p. 63.
- [4] J. Salbeck, U. Schöberl, K. M. Rapp, J. Daub, *Z. Phys. Chem. (Munich)*, **1991**, 171, 191.
- [5] M. Büschel, C. Stadler, C. Lambert, M. Beck, J. Daub, *J. Electroanal. Chem.* **2000**, 484, 24-32.
- [6] For recent work on donor/acceptor diads: J. Daub, R. Engl, J. Kurzawa, S.E. Miller, S. Schneider, A. Stockmann, M.R. Wasielewski, *J. Phys. Chem. A*, **2001**, 105, 5655.
- [7] M. Mayor, M. Büschel, K. M. Fromm, J.-M. Lehn, and J. Daub, *Chem. Eur. J.*, **2001**, 7, 1266.
- [8] B. König, M. Pelka, R. Reichenbach-Klinke, J. Schelter, J. Daub, *Eur. J. Org. Chem.*, **2001**, 2297.
- [9] L. Pu, *Chem. Rev.*, **1998**, 98, 2405.
- [10] G. Beer, C. Niederalt, S. Grimme, and J. Daub, *Angew. Chem. Int. Ed.*, **2000**, 39, 3252.

Electron Transfer in a Supramolecular Assembly Consisting of Two Biological Redox Cofactors: A Model System for Flavin Reductase

Roland Reichenbach-Klinke, Burkhard König

*Institut für Organische Chemie, Universität Regensburg
Universitätsstr. 31, 93040 Regensburg*

Flavin coenzymes (FAD, FMN) and pyridine nucleotides (NAD⁺, NADP⁺) are both important biological redox cofactors in various enzymes particularly in dehydrogenases. Flavins can also serve as substrates of a class of enzymes named flavin reductases.[1] These enzymes are defined by their ability to catalyse the reduction of free flavins by reduced pyridine nucleotides. To mimic the processes in flavin reductase we linked together in our model system analogues of these two cofactors through metal-ligand coordination in a supramolecular assembly.



The supramolecular assembly between riboflavintetraacetate and 1-benzyl-3-[2-(1,4,7,10-tetraaza-cyclododec-1-yl)-ethyl]carbamoyl]-1,4-dihydropyridin **1** is formed through coordinative zinc cyclen - imide binding.[2,3] Even in water under physiological conditions this binding motif shows very high association constants. The binding constant of riboflavintetraacetate and the oxidized form of **1** was determined by potentiometric pH titration as $\log K = 6.5$ and by UV-titration as 5000 l/mol (pH 7.4, 20°C). The electron transfer in this enzyme model system is studied by UV and fluorescence methods.

- [1] F. Fieschi, V. Niviere, C. Frier, J. Decout, M. Fontecave, *J. Biol. Chem.* **1995**, 270, 30392-30400.
- [2] M. Shionoya, E. Kimura, M. Shiro, *J. Am. Chem. Soc.* **1993**, 115, 6730-6737.
- [3] B. König, M. Pelka, H. Zieg, T. Ritter, H. Bouas-Laurent, R. Bonneau, J.-P. Desvergne, *J. Am. Chem. Soc.* **1999**, 121, 1681-1687.

Control of electronic interaction in electrochemically switchable Oligopyridyl/Benzodithiophene Conjugates

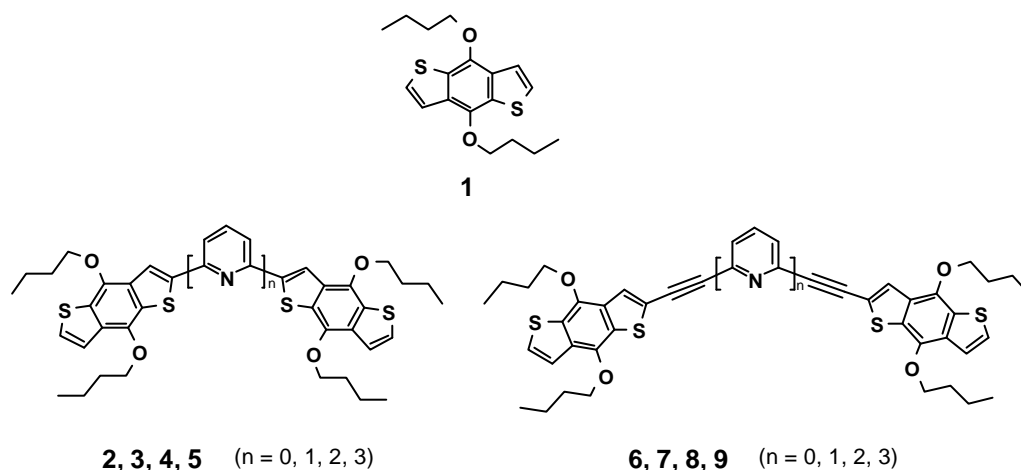
M. Büschel, M. Helldobler, J. Daub

Universität Regensburg, Universitätsstr. 31, D-93053 Regensburg, Germany

There is a great deal of interest concerning electronic coupling in the field of molecular electronics¹.

On the basis of recent investigations² a modular concept is employed to design multifold switchable systems based on hydroquinone **1**. The present report deals with compounds **1-9**.

Synthesis of compounds **1-9** is achieved by Hagihara or Stille cross-coupling using



monohalogenated or monostannylated derivatives of **1** and the appropriate bromo-substituted oligopyridyl systems. Good solubility of all compounds is guaranteed by the alkyl groups in subunit **1**.

The cyclic voltammogram of compound **1** shows a reversible wave for the radical cation formation. The bishydroquinone systems exhibit two separated waves, indicating strong coupling between the subunits.

By UV/Vis/NIR spectroelectrochemistry of **2-9** the spectral changes upon oxidation are obtained. The radical cations of the bishydroquinone systems (mixed valence states) are analysed with regard to electronic coupling according to Hush³.

FT-IR spectroelectrochemistry⁴ provides the vibrational spectra depending on the redox state. The positive charges are localised in the hydroquinone moieties. Calculations at the DFT level were undertaken.

The electronic coupling between the subunits sensitively depends both on protonation and metal ion complexation. Interactions through bonds or through space are discussed.

¹ J. Jortner, M. Ratner (Eds.), *Molecular Electronics*, Blackwell, Oxford, **1997**; M. Mayor, M. Büschel, K.M. Fromm, M. Beck, A. Knorr, H. Spreitzer, J. Daub, *Chem.Eur.J.* **7**(6), 1266-1272

² J. Daub, M. Beck, A. Knorr, H. Spreitzer, *Pure Appl.Chem.* **1996**, **68**, 1399-1404

³ N.S. Hush, *Coord.Chem.Rev.* **1985**, **64**, 135-157

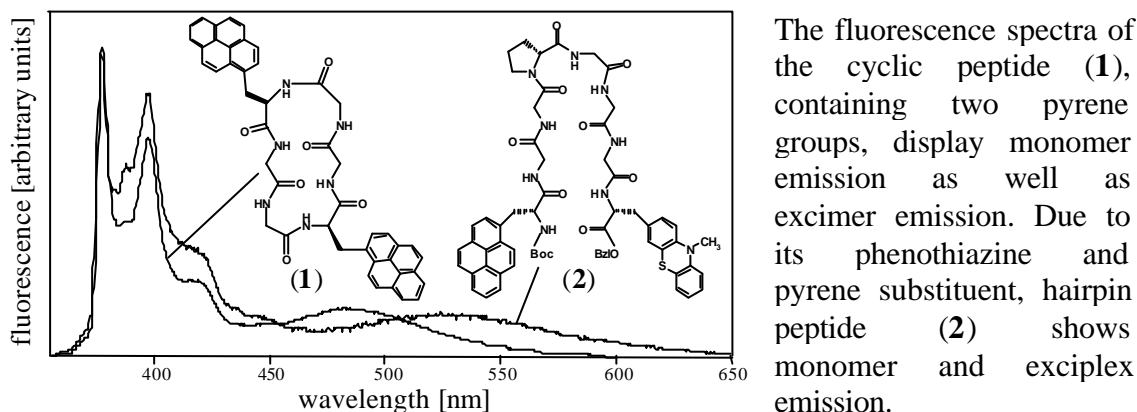
⁴ M. Büschel, C. Stadler, C. Lambert, M. Beck, J. Daub, *J.Electroanal.Chem.* **2000**, **484**, 24-32

Photoactive cyclic and hairpin peptides as optoelectronic probes and molecular activators

Jörg Strauss, Rolf Hörger, Markus Komma, Jörg Daub

Rigid and conformationally stable peptides are known to play important roles in medicinal chemistry and biochemistry. The linkage of such biomolecules with chiral fluorescent dyes allows the detection of biochemical properties by simple spectroscopic methods (e.g. fluorescence or circular dichroism)¹. The ultimate goal of this work is to study dye modified compounds like cyclic peptides and hairpin peptides.

Dye substituted amino acids were synthesized in enantiomeric pure form. Linear peptides and hairpin peptides were synthesized by solid phase methods using a conventional Fmoc-strategy. Peptide cyclizations were carried out in solution under high dilution conditions.



The fluorescence spectra of the cyclic peptide (1), containing two pyrene groups, display monomer emission as well as excimer emission. Due to its phenthiazine and pyrene substituent, hairpin peptide (2) shows monomer and exciplex emission.

This indicates that the respective chromophores are stacked to form the excimer or the exciplex.

The peptides (1) and (2) form complexes with metal cations, amino acids or small molecules like urea. This strongly affects the excimer or exciplex formation. These results demonstrate, that the behavior of the chromophore probe is coincident with the complexation properties of the peptides.

Donor and acceptor substituted species are also known to show electrogenerated chemiluminescence (ECL)². Thus, the ECL-properties of hairpin peptide (2) were investigated.

[1] H. Mihara, J. Hayashida, H. Hasegawa, H. I. Ogawa, T. Fujimoto, N. Nishino, *J. Chem. Soc., Perkin Trans. 2* **1997**, 517-522.

[2] A. Knorr, J. Daub, *Angew. Chem., Int. Ed. Engl.* **1996**, 34, 2664-2666.

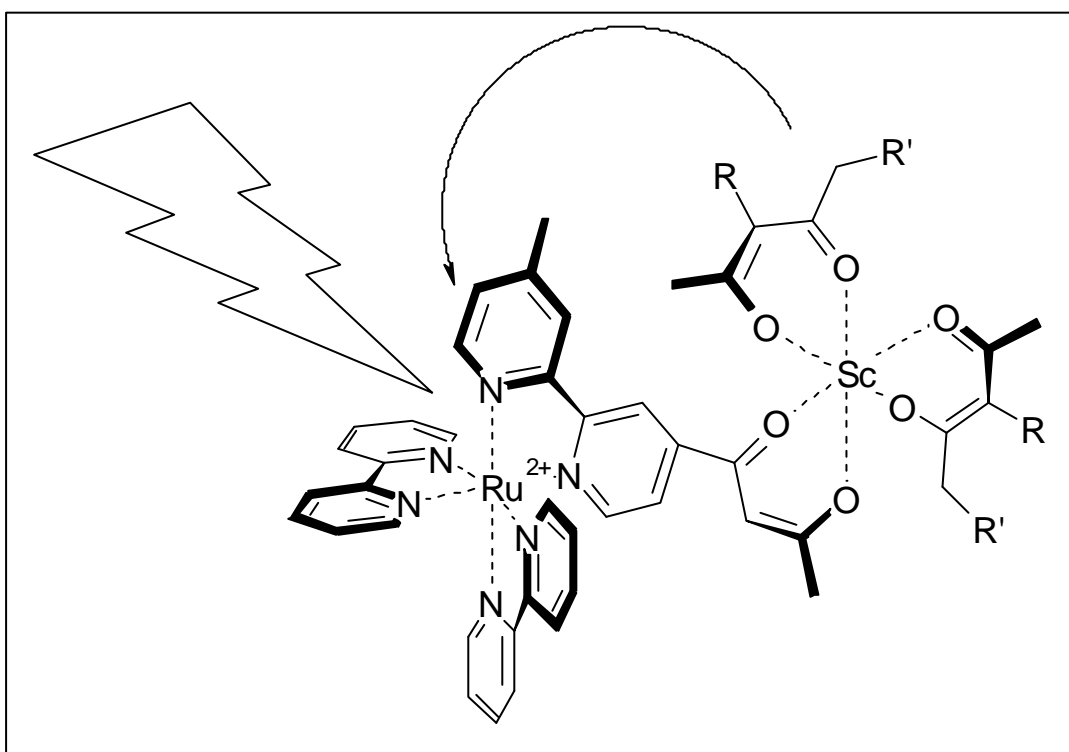
NON-COVALENTLY LINKED DONOR-ACCEPTOR SYSTEMS

Michael Kercher, Burkhard König and Luisa de Cola

Institut für Organische Chemie der Universität Regensburg,
Universitätsstr. 31, D-93040 Regensburg, GERMANY
University of Amsterdam, Instituut voor Moleculaire Chemie
Nieuwe Achtergracht 166, 1018 WV Amsterdam, THE NETHERLANDS

Several electron donor-acceptor systems tethered by hydrogen bonds, salt bridges or charge-transfer interactions can be found in the recent literature. For coordination compounds very few examples have been reported.¹ Such assemblies do offer some advantages in the study of photoinduced electron transfer (PET): high association constants are achieved even in solvents like water with a suitable choice of ligand and metal ion, the geometrical arrangement of electron donor and acceptor groups is readily determined by the coordination geometry of the metal ion and a variety of PET-active assemblies is easily available by combining various substituted ligands.

We have used scandium tris(acetylacetonate) as kinetically labile, but thermodynamically very stable coordination compounds to build up a system on which photoinduced electron transfer and photoinduced energy transfer can be studied. The simplicity of the method of assembly can open up a library of different systems and allows the fine tuning of properties.



¹M. Di Casa, L. Fabbrizzi, M. Licchelli, A. Poggi, A. Russo A. Taglietti, *Chem. Commun.* **2001**, 825 – 826; C. A. Hunter, R. K. Hyde, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1936 – 1939; B. Alpha, J.-M. Lehn, G. Mathis, *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 266 – 267; S. I. Weissman, *J. Chem. Phys.* **1950**, *18*, 1258

INERT PHOSPHORESCENT NANOSPHERES

AS MARKERS FOR OPTICAL ASSAYS

Jens M. Kürner, Otto S. Wolfbeis

*University of Regensburg, Institute of Analytical Chemistry, Chemo- and Biosensors,
D-93040 Regensburg, Germany*

A simple encapsulation technique is presented to produce highly phosphorescent, inert nanospheres which are suitable luminescent markers. It is based on the co-precipitation of phosphorescent ruthenium(II)-tris(polypyridyl) complexes and polyacrylonitrile (PAN) derivatives from a solution in N,N-dimethylformamide. The beads precipitate in the form of very small aggregates of spherical shape and a typical particle diameter of less than 50 nm. This process allows the encapsulation of phosphorescent and fluorescent dyes in an individual nanosphere provided that they are sufficiently lipophilic. Quenching by oxygen is negligible due to the use of PAN.

The nanospheres were characterized with respect to their spectral properties (quantum yields of the luminophores, brightness, luminescence decay time), stability in aqueous buffered suspensions, and in terms of size, shape and surface charge of the particles, as well as storage stability, quenching by oxygen, and dye leaching.

Furthermore, a new concept to create a set of encoded phosphorescent nanospheres for use as label for biomolecules is presented. Their individual luminescence decay behaviour and the spectral properties of the emitted phosphorescence can distinguish the spheres. They contain a coimmobilized donor / acceptor couple with a highly luminescent ruthenium(II)-polypyridyl complex as donor with a decay in the microsecond range and a longwave emitting strongly fluorescent cyanine dye.

References:

- Kürner, J. M., Klimant, I., Krause, C., Preu, H., Kunz, W., Wolfbeis, O. S. (2001) Inert phosphorescent nanospheres as markers for optical assays. *Bioconjug. Chem.*, in press.
- Kürner, J. M., Klimant, I., Krause, C., Pringsheim, E., Wolfbeis, O. S. (2001) A new type of phosphorescent nanospheres for use in advanced time-resolved multiplexed bioassays. *Anal. Biochem.*, in press.

Sulfide-Quinone Reductase, a Flavoprotein of the Glutathione Reductase Family

Christoph Griesbeck and Günter Hauska

Lehrstuhl für Zellbiologie und Pflanzenphysiologie, Universitätsstraße 31, 93040 Regensburg

Inorganic reduced sulfur compounds as sulfide (HS^- and H_2S at biologically relevant pH) can serve as electron donors for phototrophic and lithotrophic growth of Bacteria and Archaea. Sulfide-quinone reductase (SQR), an ancient flavoprotein, is obligatory for the initial step of growth on sulfide, the enzymatic sulfide oxidation, in many bacteria¹. Up to now, SQR activity has been characterized in six phototrophic and chemotrophic organisms, eight genes encoding SQR are known. SQR from the purple bacterium *Rhodobacter capsulatus* has been purified, and its gene has been cloned, sequenced and heterologously expressed in *E. coli*. The membrane-associated protein with a molecular weight of 48 kDa consists of 427 amino acids and exhibits sequence homology to the glutathione reductase family of flavoproteins, particularly within three FAD-binding sites. The isolated protein shows absorption and fluorescence properties that are typically for flavoproteins. The sole cofactor FAD is non-covalently bound to the protein. Further characterizing data with respect to SQRs kinetic and redox properties are presented.

In order to elucidate the catalytic mechanism of SQR, several amino acids conserved among all known sequences have been exchanged, and the resulting proteins have been studied. Three cysteine residues have been shown to be essential for SQR activity. Replacement of valine-300 by aspartate resulted in a substantial decrease in substrate affinity for sulfide, and SQR-His131Ala showed a shift of the pH optimum of two pH units towards the acidic range.

On the basis of the experimental results conceptions about a reaction mechanism are presented.

Reference:

1. Griesbeck C., Hauska G. and Schütz M. (2000): Biological Sulfide Oxidation: Sulfide-Quinone Reductase (SQR), the Primary Reaction. In: Pandalai S.G. (ed): Recent Research Developments in Microbiology, Vol. 4, pp 179-203. Research Signpost, Trivandrum, India

*The Development of a Fluorescent Microsensor
for Sulfide with a Flavoprotein*

Thomas Schödl, Otto S. Wolfbeis and Günter Hauska

Lehrstuhl für Zellbiologie und Pflanzenphysiologie, Universitätsstraße 31, 93040 Regensburg, Germany

Sulfur is one of the six main elements of the biosphere. Its compounds are involved in assimilatory processes for the synthesis of biological matter but also in dissimilatory processes for generation of energy equivalents. One of the compounds which is involved in both processes is hydrogen sulfide a common electron donor in biological systems. Sources of hydrogen sulfide in biosphere are putrefaction and dissimilatory sulfate or sulfur reduction reactions.

Due to the fact that hydrogen sulfide is a toxic compound, measurement of hydrogen sulfide is important in various biological systems, e.g. in wastewater. Until now there is no satisfactory micro-method known for sensing hydrogen sulfide. The situation is further complicated by the fact that hydrogen sulfide is partially present as the hydrogen sulfide mono anion (HS^-) at $\text{pH} > 7$. Existing methods for measurement of hydrogen sulfide are based on irreversible redox reactions, are insensitive or even not applicable to biological systems. Established methods for quantitative hydrogen sulfide determination depend on spectroscopic measurements, ion-selective electrodes, and iodometric titrations. Besides bulky and expensive spectroscopic methods, like infrared absorbance based hydrogen sulfide determination, some optical sensor approaches have been described in the past few years.

The purified SQR protein can be used as a fluorescent single-shot or sensory probe for the determination of hydrogen sulfide. The decrease of fluorescence light intensity of the cofactor FAD is proportional to the concentration of hydrogen sulfide in the low micromolar range. Although the reproducibility of the fluorescence intensity signals after one cycle of hydrogen sulfide addition and recovery with quinone is poor, the system could at least be used as a single assay sensor.

References

Griesbeck C., Hauska G. and Schütz M. (2000): Biological Sulfide Oxidation: Sulfide-Quinone Reductase (SQR), the Primary Reaction. In: Pandalai S.G. (ed): Recent Research Developments in Microbiology, Vol.4, pp 179-203. Research Signpost, Trivandrum, India

Additional Poster Presentations

Persistent spectral holeburning in dye/matrix systems: Optical sensors of electric fields and probing of dynamical processes

H.Balzer, N.Wrobel, G.Hauska and U.Bogner

Electric-field induced refilling in the centre of persistent spectral holes (PSH) provides a new method for imaging the inhomogeneous electric-field-distribution in electronic devices. We demonstrate this method by mapping the field-distribution on metallized ceramic substrates, which are part of high power electronic devices. Moreover it is especially interesting to determine the field strength and the inhomogeneity at the sharp edge between the metal and the ceramic (Al_2O_3 or AlN) with high spatial resolution. In this device the field distribution cannot be determined by other experimental methods or by computing. With our method the field-distribution was recorded as a complete image, by using a CCD- camera, with a resolution of $< 30 \mu\text{m}$. As sensor film we used perylene doped PVB, in which the PSHs were burned.

Application of hole-burning spectroscopy in the topic of biological dye/matrix systems eg. chromophors in proteins provides information about internal electric fields and dynamical processes in the immediate neighbourhood of the π – electron system of the chromophor. First results are presented concerning bacteriochlorophyll – a in the FMO – protein.

Reference:

U.Bogner, *Electric Field Effects on Persistent Spectral Holes: Applications in Photonics* in “Molecular Electronics: Properties, Dynamics and Applications”, Eds.:G.Mahler, V.May, M.Schreiber (Marcel Dekker Inc., New York, 1996),pp.233-255.

Fluorometric visualisation of the plant response to destruxins isolated from *Alternaria brassicae*.

Julie Soukupová^{1,2}, Silvia Smatanová^{1,2}, Ladislav Nedbal^{1,2} and Alexander Jegorov³

Photosynthesis Research Center at the ¹Institute of Landscape and at the ²University of South Bohemia, Zámek 136, 37333 Nové Hradky, Czech Republic;
soukupova@greentech.cz

Brassica blackspot (*Alternaria brassicae*, Berkley, Saccardo) is one of the most damaging and economically significant fungal diseases of *Brassica* crops as canola, cabbage or mustard crop (Nacamura et al. 1995). The fungus is producing phytotoxic cyclodepsipeptides called destruxins. Destruxins cause chlorotic and necrotic foliar lesions on diverse *Brassica* species and other cruciferous host plants. Destruxin B, the major phytotoxin of *A. brassicae*, appears to be the virulence factor, contributing most to the aggressiveness of *A. brassicae* by conditioning the host tissue. It inhibits vacuolar-type ATPase that is present in the membranes of many organelles where it generates a low pH environment.

Plant defense mechanisms include biosynthesis of defense compounds phytoalexins. Plants may also be resistant to a particular pathogen if they produce an enzyme that catalyses detoxification of the phytotoxin (Pedras et al. 2001). Unfortunately, the present *Brassica* crops are highly vulnerable to the infection and, thus, the efforts to produce resistant varieties of crops are of a high importance (Pedras et al. 2001, Pedras et al. 2001).

The aim of our study was to develop fluorescence imaging technique for screening of destruxin-resistant plants. In the experiments, we used detached first and second leaves of the highly vulnerable *Brassica oleracea* arranged in Petri dish on moist gauze. The leaf surface was rinsed with 24% ethanol before an exposure to a drop of destruxin mixture dissolved in 1% DMSO. The Petri dishes were incubated in 22°C and illuminated for 60 hours by 100 micromol(photons).m⁻².s⁻¹ of white light. The FluorCam instrument developed in our laboratory (Nedbal et al. 2000) was used to measure the fluorescence emission images of F₀, F₀' , F_M, F_M' , F_S and image arithmetic combinations of the leaves. The F₀/F_M ratio image was giving the highest contrast between the destruxin-exposed and untreated leaf surface. With a gradual lowering of the destruxin concentration we found that the fluorescence imaging is nearly 40 times more sensitive compared to optical microscopy. Here, a sufficient contrast between the destruxin-treated and destruxin-untreated leaf surface was observed with a concentration of 0.1 µg/ml of the destruxin mixture whereas the lowest concentration detectable with optical microscopy was 3.8 µg/ml for *Brassica oleracea* using identical exposure protocols (Buchwaldt and Green 1992).

References:

- Buchwaldt L, Green H (1992) *Plant Pathology* **41**, 55-63.
- Nacamura R, Mitchell-Olds T, Manasse R, D L (1995) *Oecologia* **102**, 324-328.
- Nedbal L, Soukupova J, Kaftan D, Whitmarsh J, Trilek M (2000) *Photosynthesis Research* **66**, 3-12.
- Pedras M, Zaharia I, Gai Y, Zhou Y, Ward D (2001) *Proceedings of the National Academy of Sciences USA* **98**, 747-752.

Chlorophyll fluorescence kinetic imaging: Cyanobacteria under a microscope and dinoflagellate algae on corals.

Ferimazova N^{1,2}, Küpper H³, Morse A⁴, Maldener I⁵, Šetlík I², Nedbal L^{1,2}

*Photosynthesis Research Center at the*¹*Institute of Landscape and at the*²*University of South Bohemia, Zamek 136, 37333 Nove Hrad, Czech Republic;*

ferimazova@greentech.cz

³*Universität Konstanz, Fachbereich Biologie, 78457 Konstanz, Germany*

⁴*Marine Research Institute, 3111 Marine Biotechnology Lab, Santa Barbara, 93106, CA, USA.*

⁵*Lehrstuhl für Zellbiologie und Pflanzenphysiologie, Universität Regensburg, 93040 Regensburg, Germany*

The potential of the chlorophyll fluorescence kinetic imaging is demonstrated using two widely differing samples of cyanobacteria and dinoflagellate algae and two experimental systems.

In the first experiment, images of fluorescence emission of filamentous cyanobacterium *Anabaena PCC 7120* were captured under a microscope to reveal changes in the photosynthetic activities on the single-cell level that are accompanying the heterocyst formation during nitrogen starvation. The microscopic time-resolved fluorescence imaging was performed using instrument designed by Küpper et al. 2000. The photosynthetic activities of the individual cells in the filament were investigated by measuring of parameters variable fluorescence (F_V) and maximum fluorescence (F_M) in the saturating irradiance. The ratio F_V/F_M approximating the maximum quantum yield of Photosystem II photochemistry was used as a measure of the Photosystem II activity. We were comparing the Photosystem II activity in vegetative cells and in cells transformed into heterocysts. Nitrogen starvation of *Anabaena* filaments for 24 hours is considered to result in a formation of fully functional heterocysts. At this point we could see cells displaying the appropriate cytological markers. Surprisingly, the young heterocysts exhibited a significant residual Photosystem II activity in response to saturating light. It took another 12 hours of nitrogen starvation to have heterocysts exhibiting no Photosystem II activity.

In the second experiment, we investigated the temperature-induced bleaching of reef-building coral *Nemanzophyllia turbida* using macroscopic kinetic fluorescence imaging as described in Nedbal et al. 2000. The system allowed the selective measurements of photosynthetic activity of endosymbiotic dinoflagellate algae (zooxanthellae) undisturbed by the signal from epiphytic algae. Fluorescence measurements showed that the heat stress was primarily affecting the photosynthetic activity of the endosymbionts. A closer analysis of the extensive data will be necessary to estimate the role played by the decrease in the number of symbiotic dinoflagellates and by the photoinhibition of their photosynthesis.

References

Küpper H, Setlik I, Trtilek M, Nedbal L (2000) *Photosynthetica* **38**, 553-570.

Nedbal L, Soukupova J, Kaftan D, Whitmarsh J, Trtilek M (2000) *Photosynthesis Research* **66**, 3-12.