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II INTERNATIONAL SYMPOSIUM on PHOTOPHARMACOLOGY

Innovative therapeutics
and research tools using light

ABSTRACTS BOOK

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CONTENTS

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Photopharmacology is an emerging branch of science based in the administration of a photosensitive compound in combination with illumination to provide a high degree of local and temporal control of compound activity, which makes it useful both for therapeutic applications and as a research tool.

In the last 10 years photopharmacology, a polyhedric science involving biology, pharmacology, chemistry, physics, engineering and medicine, has flourished and achieved a critical mass of researchers and resourceful methods in chemistry and optics, raising hopes for clinical trials of the most advanced compounds.

The First International Symposium on Photopharmacology took place at the University Medical Center Groningen on February 16th, 2017. Topics covered in this First Symposium ranged from photochemistry and organic synthesis to vision restoration and brain research.

This Second Symposium is planned as a forum to gather the worldwide experts in photopharmacology to debate and present their advances, facilitate partner interaction, foster cooperativity and open discussions to delimitate problems, define solutions, delineate strategies and envision future developments.

The Symposium intends to bring together opinion leaders in the field and seeks to incorporate medical practitioners and members of the industrial sectors related to light (photonics, spectroscopy, optics), molecular therapeutics (pharma industry, biotechnology, medicinal chemistry) and medical devices.

The institutions that propose this Symposium have solid experience in the field of photopharmacology in the last 10 years and have done important research contributions to the synthesis of light-sensitive ligands and their applications to chemical biology and neurobiology, including pain research and vision restoration.

In addition, they have been involved in the organization of several scientific symposia and meetings focused on photopharmacology both at national and international levels.

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ABSTRACTS
INVITED LECTURES

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Andrew Woolley

Chemistry
University of Toronto

Andrew Woolley is a professor in the chemistry department at the University of Toronto. He has been active in design of photo-switchable small molecules and proteins for more than 20 years. He developed azobenzenes that switch with red light, methods to photo-control protein structure using intramolecular cross-linkers, and most recently, general methods to find binding partners for photoswitchable proteins.

Photo-control of affinity reagents

Photopharmaceuticals can, in principle, be created by linking photoswitchable moieties to bioactive molecules. However, a general strategy for converting a therapeutic agent into its photoswitchable version is not currently available. We are trying to develop generalizable, modular approaches for obtaining light controllable bioactive agents by modifying the scaffolds of protein affinity reagents using azobenzene photoswitches. Progress and problems will be reported.

Jae-Woong Jeong

School of Electrical Engineering
Korea Advanced Institute of Science and Technology

Dr. Jae-Woong Jeong is an Assistant Professor of Electrical Engineering at Korea Advanced Institute of Science and Technology (KAIST). He received his PhD degree in electrical engineering from Stanford University in 2012, and worked as a postdoctoral research associate at the University of Illinois at Urbana-Champaign from 2012 to 2014. Before joining KAIST, he was an Assistant Professor of Electrical, Computer & Energy Engineering at University of Colorado, Boulder from 2015 to 2017. Dr. Jeong's research focus is in the future generation bio-integrated electronics and systems. He is a senior member of IEEE and the recipient of BMES Career Development Award, Samsung Global Research Outreach Award, and University of Colorado Outstanding Research Award.

Wireless in vivo pharmacology and optogenetics

Combination of optogenetics and pharmacology represents a unique approach to dissect neural circuitry with high specificity and versatility. However, conventional tools available to perform these experiments, such as metal cannulas connected to external drug supplies for pharmacological infusions and tethered fiber optics for optogenetics, are not ideal for minimally-invasive, untethered studies on freely behaving animals. This talk summarizes our recent progress in wireless optofluidic probe systems that offer miniaturization, integration, and automation of drug delivery and photostimulation. The devices integrate microscale inorganic light-emitting diodes and microfluidic drug delivery systems with a wireless interface. This design enables compact, lightweight, soft and flexible platform, thus facilitating seamless implantation and operation in the brain without causing disturbance of naturalistic behavior. I will demonstrate wireless capabilities of these devices in freely moving animals that can deliver pharmacological agents, and provide concurrent photostimulation with drug delivery to manipulate reward-related behavior. The minimally-invasive operation of the optofluidic systems forecasts utility in *in vivo* pharmacology and optogenetics, with potential for broad application in biomedical science, engineering, and medicine.

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Burkhard König

Faculty of Chemistry and Pharmacy
University of Regensburg

Burkhard König received his Ph.D. in 1991 from the University of Hamburg. He continued his scientific education as a post-doctoral fellow with Prof. M. A. Bennett, Research School of Chemistry, Australian National University, Canberra, and Prof. B. M. Trost, Stanford University. Since 1999, he is a full professor of organic chemistry at the University of Regensburg, Germany. His current research interests are the development of synthetic methods in photoredox catalysis and the control of biological functions using photochromic molecules.

Photoswitches in Photopharmacology: The good, the bad and the ugly

Visible light is a fascinating reagent: It provides energy for chemical trans-formations, can be selectively delivered to a specific molecule and leaves no trace even if applied in large excess. The three typically classes of photo-chromic molecules in decreasing order of their use are azobenzenes,[1] dithienylethenes and fulgides. The different photoswitches have specific advantages and disadvantages for applications in photopharmacology. We discuss their properties with recent examples from our laboratory aiming at photochromic enzyme inhibitors,[2] ligands of G-protein coupled receptors,[3] redox mediators4 and ion channel modulators.

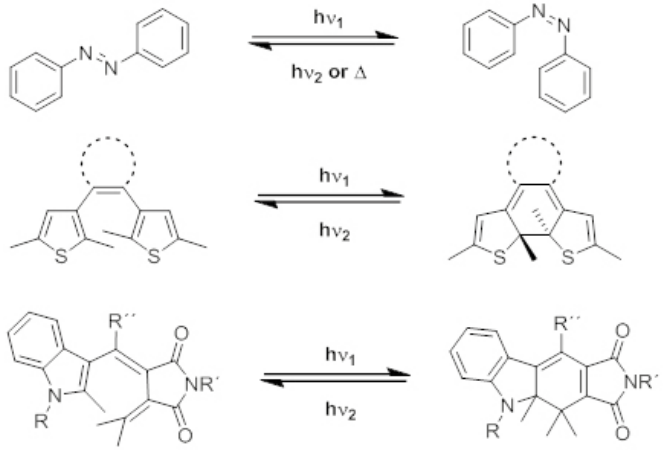

Figure 1. Typical photoswitches in photopharmacology

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Wiktor Szymanski

University Medical Center Groningen

Wiktor Szymanski received his PhD degree from The Warsaw University of Technology, Poland, in 2008, working under the supervision of Prof. Ryszard Ostaszewski. He spent two years working on the use of biotransformations in organic chemistry with Prof. Ben L. Feringa and Prof. Dick B. Janssen at the University of Groningen. Since 2010 he has been working on the construction of photoactive protein- peptide- and DNA-bioconjugates and photopharmacology in the Feringa Labs. In 2014, he joined the Department of Radiology, University Medical Center Groningen, where he was appointed in 2015 as tenure track assistant professor.

Towards theranostic applications of photopharmacology

Wiktor Szymanski,[a,b] Mark. W. H. Hoorens,[a,b] Friederike Reeßing,[a,b] Willem A. Velema,[b] Mickel J. Hansen,[b] Dusan Kolarski,[b] Piermichele Kobauri,[b] Jana Volaric,[b] Kaja Sitkowska,[b] Michael M. Lerch,[b] Ben L. Feringa[b]

[a] Medical Imaging Center, University of Groningen, University Medical Center Groningen; [b] Stratingh Institute for Chemistry, Faculty of Science and Engineering, University of Groningen

Light offers unparalled advantages in regulation of compound bioactivity (**photopharmacology**, Figure 1A)[1-3] and as an input/output signal in **medical** (mostly optical) **imaging**.[4-5] Combination of those two paradigms along the principles of theranostics ("treat what you see, see what you treat") reuires light-responsive tools that, preferably in combination, enable both therapy and imaging.

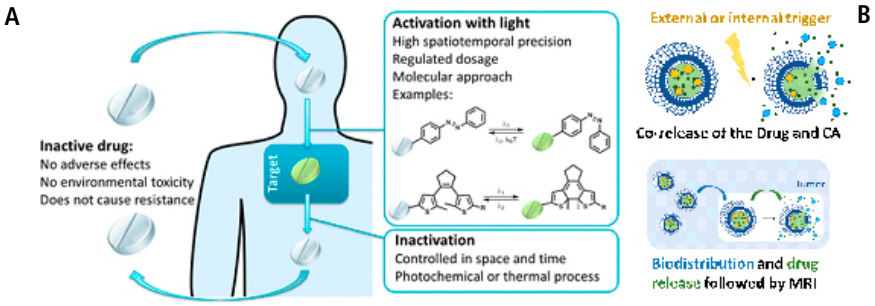

Figure 1. Photopharmacology (A) and liposomes for drug delivery and MRI imaging using light-responsive contrast agents (CA)

I will present our efforts towards the discovery of such tools, focusing on new (i) photopharmacological agents, (ii) molecular photoresponsive tools and (iii) new light-responsive, MRI-active liposomal drug delivery agents (Figure 1B).

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Edith Caroline (Phoebe) Glazer

John C. Hubbard Professor of Chemistry
University of Kentucky, Kentucky, USA

Phoebe Glazer is an Associate Professor of Chemistry at the University of Kentucky. Her research group is interested in the development of metal complexes as potential medicinal agents and reporters. Their work focuses on the synthesis and study of photoactive metal complexes, covalent drugs, metal complexes containing drug and drug like ligands, and DNA structure-selective reporters and damaging agents. The research also includes biological mechanism of action studies, phenotypic screening, cellular imaging agents, and the development of reporter assays. Long standing interests include unusual photophysical phenomena, particularly dual emission, and the biophysics and regulation of heme proteins such as cytochrome P450s and nitric oxide synthase.

Metal complexes for the protection and delivery of bioactive ligands

David K. Heidary, Dmytro Havrylyuk, Edith C. Glazer
[a]University of Kentucky, Lexington, KY, USA

Metal complexes provide a modular architecture for the development of photoresponsive biological reporters and medicinal agents. We have developed ruthenium-based coordination compounds as prodrugs by developing scaffolds that can be activated by light to transform into potent cytotoxic agents. The active species form coordinative bonds to essential biomolecules and inhibit fundamental processes, resulting in micromolar and sub-micromolar cellular cytotoxicity. Structural modifications have produced other complexes that can act as inert delivery agents for bioactive ligands. Recent efforts have focused on targeting specific enzymes involved in chemotherapeutic resistance. Various features including charge, choice of co-ligands, and complex geometry affect activity via photochemistry, subcellular localization, affinities for specific biomolecules, and biological properties within cancer cells. In order to better evaluate and understand the biological activities of the metal complexes, phenotypic screening approaches are being employed to utilize global reporters of enzyme activity and other fundamental processes in cancer cells and tumor spheroids.

Acknowledgements
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Michael Telias

University of California Berkeley

Dr. Michael Telias obtained his B.Sc.Med and M.Sc. (Neurobiology) from the Faculty of Medicine at the Hebrew University of Jerusalem and his Ph.D. (Cell & Developmental Biology) from the Faculty of Medicine at Tel Aviv University. His graduate research focused on modeling fragile x syndrome and autism through neuronal differentiation of human embryonic stem cells. Currently, as a postdoc at the lab of Prof. Richard Kramer in the University of California, Berkeley (Molecular & Cell Biology); his research focuses on physiological remodeling of the inner retina during photoreceptor degeneration, and on the development and implementation of photoswitches for vision restoration and as research tools in neuroscience.

Light-sensitive drugs that act on endogenous ion channels for reinventing phototransduction in the blind retina

Blindness can occur when photoreceptor (PR) cells including rods and cones, degenerate and die, as it happens in retinitis pigmentosa, and other types of retinal degeneration. However, the cells in the inner retinal layers survive, including retinal ganglion cells (RGCs) that maintain connectivity with the brain, providing a cellular substrate for vision restoration. Using light-sensitive intracellular blockers of endogenous ion channels ('photoswitches'), RGCs in blind mice can be photo-sensitized, restoring vision in vivo. The photoswitch BENAQ responds to wavelengths of light within the visual-spectrum and remains in the retina for many weeks after intraocular delivery. Strikingly, photoswitches do not affect healthy retinas with intact PRs, actin on degenerated retinas exclusively. PR degeneration triggers pathophysiological remodeling of RGCs, resulting in membrane hyperpermeability and spontaneous hyperactivity. Using photoswitches as research tools, we have recently uncovered that the signal triggering remodeling in RGCs of degenerated retinas is retinoic acid, acting through its canonical nuclear receptor. Our evidence shows that retinoic acid is both necessary and sufficient for inducing remodeling in degenerated retinas, and blocking its receptor reduces hyperpermeability and hyperactivity, augmenting remaining light responses in mice undergoing slow retinal degeneration. Our work highlights the use of photoswitches as both potential drugs for clinical treatment and as research tools to tackle fundamental questions in biomedicine.

Dirk Trauner

New York University

Dirk Trauner was born and raised in Linz, Austria, studied biology and chemistry at the University of Vienna, and received his undergraduate degree in chemistry from the Free University, Berlin. He then pursued a PhD in chemistry under the direction of Prof. Johann Mulzer, with whom he moved.

Photoswitches and lipids

Lipids and photoswitches are a natural match. I will show how azobenzenes can be incorporated into a variety of glycerophospholipids, sphingolipids, and lipophilic ligands to enable the control of biological pathways with light. The focus will lie on lipid receptors that function as GPCRs. If time permits, I will discuss the use of photoswitchable lipids to determine the biophysical properties of membranes and liposomes and to shuttle solutes across membranes.

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Anja Hoffmann-Röder

Ludwig-Maximilians-Universtität Munich

Prof. Dr. rer. nat. Anja Hoffmann-Röder:
Born 1972 in Bonn, Germany; 1991-1994 vocational education, Degussa AG, Bonn; 1994-1999 study of chemistry at University of Bonn; 2003 PhD degree with Prof. Dr. Norbert Krause, Technical University Dortmund, Germany; 2003-2005 Postdoctoral fellow with Prof. Dr. François Diederich, ETH Zurich, Switzerland; 2005-2006 Liebig-Fellow (VCI) at Johannes-Gutenberg University Mainz, Germany; 2006-2011 Emmy Noether-Fellow (DFG) at Johannes-Gutenberg University Mainz; 2009-2011 W1-professor Johannes-Gutenberg University Mainz; since 2011 tenure track W2-professor at LMU Munich, Germany.

Peptidomimetics for photopharmacology and structural biology applications

M. Gailer[a], T. Podewin[b], A. Hoffmann-Röder[a]
[a]Department of Organic Chemistry, Faculty for Chemistry and Pharmacy, Ludwig-Maximilians-University Munich; [b]Department for Chemical Biology, Max Planck Institute for Medical Research Heidelberg

Although synthetic photoswitches have been known for decades, their usefulness to precisely control biological and pharmacological functions in complex systems has only gathered significant attention in the past few years.[1] Thereby, the vibrant field of photopharmacology mostly relies on the use of specifically designed azobenzene photoswitches either attached to their molecular target (PTLs/PORTLs) or in form of freely diffusible photochromic ligands (PCLs).
This presentation will highlight work from our laboratory towards optical control of (i) glucagon-like peptide-1 receptor (GLP-1R) signaling via LirAzo[2], a photoswitchable peptide derived from the drug liraglutide, (ii) a receptor-linked guanylyl cyclase, the atrial natriuretic peptide (ANP) receptor,[3] and (iii) reversible SNAP-tag-directed activation and trafficking of class A and B GCPRs involved in gut hormone signaling.[4] Last but not least, recent applications of azobenzene-derived β-hairpin peptides for deciphering the molecular mechanisms of protein folding will be presented.[5,6]

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Romain Quidant

ICFO-ICREA

Quidant received a PhD in Physics in 2002 from the University of Dijon (France). Right after, he joined ICFO as a postdoctoral researcher. In 2006, he was appointed junior Professor (tenure-track) and group leader of the Plasmon NanoOptics group at ICFO. In 2009, he became tenure Professor both at ICFO and ICREA. While his core expertise is in fundamental nano-optics, he and his team are very much interested in multidisciplinary research, interfacing physics with other disciplines of science, as well as in technology transfer. The most fundamental part of his work is mainly directed towards enhanced light/matter interaction and quantum physics. From a more applied viewpoint, his team investigates news strategies to control light and heat at the nanometer scale for biomedical applications, including lab-on-a-chip technology and targeted hyperthermia.

Light-induced Hyperthermia for oncology and disinfection

Jordi Morales [a], Clara Vilches [a], Miguel Mireles [a], Turgut Durduran [a,b], Oriol Casanovas [c], Irene Prieto, Christine Weis, Pau Turon and Romain Quidant [a,b]

[a] ICFO-Institut de Ciències Fotòniques, The Barcelona Institute of Science and Technology, 08860 Castelldefels (Barcelona)
[b] ICREA- Institució Catalana de Recerca I Estudis Avançats, 08010 Barcelona
[c] Tumour Angiogenesis Group, ProCURE, ICO - IDIBELL, 08908 Barcelona
[d]Bbraun Surgical, S. A. 08191 Rubí, Barcelona

Recent years have witnessed a growing interest in controlling temperature on the nanoscale motivated by applications to different fields, including information technology, chemistry and medicine. Under illumination at its plasmon resonance, a metal nanoparticle features enhanced light absorption, acting as an ideal nano-source of heat, remotely controllable by light. Such a powerful and flexible photothermal scheme sets the basis of the emerging and fast-growing field of *thermoplasmonics*. In this talk, we first briefly present the specificities of heat generation in metal nanoparticles compared to standard macroscopic heating. We then focus on two different biomedical applications, namely less-invasive cancer treatment and disinfection of surgical implants.

In the first application, PEG-coated gold nanorods (PEG-GNRs) are tail-injected into an orthoxenograph mouse model of clear cell renal cell carcinoma. Due to their small size, PEG-GNRs can penetrate through the leaky tumor neovasculatures and eventually accumulate in the cancer tissue. This accumulation is non-invasively monitored over time

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using diffuse optics. Local hyperthermia is then locally induced upon a suitable NIR laser illumination. We study the nature of the cancer tissue damage and demonstrate tumor shrinking.

The second application relates to the prevention of biofilm formation at the surface of surgical implants. In our experiment, a surgical mesh, used for hernia surgery, is coated with a high density of GNRs. We demonstrate that under suitable illumination parameters, bacteria adhesion is reduced preventing the biofilm to form.

Vasilis Ntziachristos

Technische Universität München

Vasilis Ntziachristos PhD is a Professor of Medicine and Electrical Engineering, the Director of the Chair for Biological Imaging (CBI) and of the Munich School of Bioengineering (MSB) at the Technical University of Munich and the director of the Institute for Biological and Medical Imaging (IBMI) at the Helmholtz Zentrum München. He received a Diploma in Electrical Engineering and Computer Science from the Aristotle University of Thessaloniki, Greece, and MSc and PhD degrees in Bioengineering from the University of Pennsylvania in Philadelphia PA and served as faculty at Harvard University and the Director of the Laboratory for Bio-optics and Molecular imaging at the Massachusetts General Hospital. Professor Ntziachristos regularly serves as chair in international meetings and councils and on the editorial boards of several scientific journals and has received numerous awards and distinctions, including the Gold Medal from the Society for Molecular Imaging (2015), the Gottfried Wilhelm Leibniz prize (2013), and the Erwin Schrödinger Award (2012) and was named one of the world's top innovators by the Massachusetts Institute of Technology (MIT) Technology Review in 2004.

Optoacoustic imaging of tissue dynamics

Optical imaging is unequivocally the most versatile and widely used visualization modality in the life sciences. Yet it has been significantly limited by photon scattering, which complicates the visualization of tissue beyond a few hundred microns. For the past few years, there has been an emergence of powerful new optical and optoacoustic imaging methods that offer high resolution imaging beyond the penetration limits of microscopic methods. The talk discusses progress in multi-spectral opto-acoustic tomography (MSOT) and mesoscopy (MSOM) that bring unprecedented optical imaging performance in visualizing anatomical, physiological and molecular biomarkers. Advances in light technology, detection methods and algorithms allow for highly-performing visualization in biology and medicine through several millimetres to centimetres of tissue and real-time imaging. The talk demonstrates implementations in the time and frequency domain, showcase how it is possible to accurately solve fluence and spectral coloring issues for yielding quantitative measurements of tissue oxygenation and hypoxia and demonstrate quantitative in-vivo measurements of inflammation, metabolism, angiogenesis in label free mode. In parallel, progress with clinical systems and the complementarity with ultrasound imaging, fluorescence molecular imaging and other modalities is discussed. Finally the talk offers insights into new miniaturized detection methods based on ultrasound detection using optical fibers, which could be used for minimally invasive applications.

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Ferruccio Pisanello

Istituto Italiano di Tecnologia

Ferruccio Pisanello has a Master Degree in Telecommunication Engineering and a PhD in Physics, obtained at the Université Piere et Marie Curie in Paris. After his PhD he moved at the Italian Institute of Technology (IIT) and since 2016 he coordinates the research line on "Multifunctional Neural Interfaces with deep-brain regions" at the IIT Center for Biomolecular Nanotechnologies in Lecce. The main main aim of his research activity is to exploit advanced micro- and nano-fabrication techniques to develop new tools to investigate deep brain regions

Tapered optical fibres for multifunctional optical neural interfaces

F. Pisanello.[a], F. Pisano[a], M. Pisanello[a], A. Rizzo[a,b], A. Balena[a], L. Sileoa, R. Peixoto[c], E. Lemma[d], E. Maglie[a,b], A. Qualtieri[a], B. Spagnolo[a], E. Bellistri[a], B. L. Sabatini[c], M. De Vittorio[a,b]

[a] Center for Biomolecular Nanotechnologies @UNILE, Istituto Italiano di Tecnologia, Via Barsanti, I-73010 Arnesano (LE), Italy.
[b] Dipartimento di Ingegneria Dell'Innovazione, Università del Salento, Via Provinciale Lecce-Monteroni, I-73100 Lecce, Italy.
[c] Department of Neurobiology, Howard Hughes Medical Institute, Harvard Medical School, 02115, MA, Boston, USA.

In vivo optical interfaces with in deep structures of the mouse brain are still limited by the use of flat-cleaved optical fibers, whose illumination and light collection performances is restricted to a small and fixed volume close to fiber facet [1,2]. If two-photon microscopy has allowed multipoint stimulation and monitoring of neural activity for almost the whole cortical depth, last 5 years have seen the development of several technological approaches to reach sub-cortical regions with improved spatial resolution, including µLEDs, GRIN lenses, Indium Thin Oxide-based probes and tapered optical fibers [3-10].
After a review of the state-of-the-art in this field, this presentation will focus on the engineering and use of tapered optical fibers to control and monitor neural activity using only one optical waveguide with reduced invasiveness. The technology exploits mode-division demultiplexing operated by a millimeters-long taper that allows to redirect and/or collect light over different brain regions and subregions. Exploting micro and nanotechnologies to structure the highgly curve surface of the fiber taper, it is possible to engineer the stimulation and the collection volume, as well as to realize multiple electrodes for extracellular electrophysiology along the taper.

The simplicity of this technique, together with its versatility, reduced invasiveness and compatibility with both laser and LED sources, indicate this approach can greatly complement the set of existing methods for optical neural interfaces with deep brain regions.

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Ben Feringa

University of Groningen

Ben L. Feringa obtained his PhD degree at the University of Groningen in the Netherlands under the guidance of Professor Hans Wynberg. After working as a research scientist at Shell in the Netherlands and at the Shell Biosciences Centre in the UK, he was appointed lecturer and in 1988 full professor at the University of Groningen and named the Jacobus H. van't Hoff Distinguished Professor of Molecular Sciences in 2004. He was elected Foreign Honory member of the American Academy of Arts and Sciences and is member and vice-president of the Royal Netherlands Academy of Sciences. In 2008 he was appointed Academy Professor and was knighted by Her Majesty the Queen of the Netherlands.

Feringa's research has been recognized with a number of awards including the Koerber European Science Award (2003), the Spinoza Award (2004), the Prelog gold medal (2005), the Norrish Award of the ACS (2007), the Paracelsus medal (2008), the Chirality medal (2009),the RSC Organic Stereochemistry Award (2011), Humboldt Award (2012), the Grand Prix Scientifique Cino del Duca (French Academy 2012), the Marie Curie medal (2013) and the Nagoya Gold Medal (2013). The research interest includes stereochemistry, organic synthesis, asymmetric catalysis, optopharma, molecular switches and motors, self-assembly and molecular nanosystems.

Photoresponsive Biomolecular Systems

Kristian Berg

Department of Radiation Biology, Institute for Cancer Research - Norwegian Radium Hospital, Oslo University Hospital

Kristian Berg has been Head of the Department of Radiation Biology at the Oslo University Hospital since 2010 and professor at School of Pharmacy, University of Oslo, since 2009. He is trained as a biochemist and worked in the basic to translational fields of photomedicine since 1985. This has resulted in more than 200 peer reviewed publications and 10 patents that have contributed to establish two biotech companies registered on Oslo Stock Exchange. He has been appointed as Chair or member of a large number of Boards and Program Committees, including President of the European Society of Photobiology. His main scientific interest the last 20 years has been the technology photochemical internalization (PCI) that has been developed from basic research to clinical trials.

Photodynamic processes for treatment of cancer

Photodynamic therapy (PDT) is a treatment modality, which has been approved for several cancer indications. The therapeutic effect is based on the formation of reactive oxygen species (ROS) of which singlet oxygen is the dominating species. ROS's are formed through light-activation of a photosensitizer in an oxygen dependent manner. The photosensitizers used in PDT are in most cases related to the porphyrin structure although there are examples of non-porphyrin-based photosensitizers that may be utilized for clinical purposes. The fluorescence from such photosensitizers is also utilized for fluorescence-guided tumor resection. Singlet oxygen is highly reactive and oxidises several biomolecules and thereby inactive the target cells, the vasculature and stimulate inflammatory responses. Photochemical internalisation (PCI) is a novel technology based on PDT for release of endocytosed macromolecules into the cytosol. The technology is based on the use of photosensitizers located in endocytic vesicles that upon activation by light induce rupture of the endocytic vesicles and thereby release of the macromolecules into the cytosol. PCI has been shown to enhance the biological activity of a large variety of macromolecules and other molecules that do not readily penetrate the plasma membrane. PCI has also been shown to enhance cross-presentation of tumor antigens in dendritic cells and thereby stimulating cancer vaccination. For clinical utilization a novel photosensitizer has been developed and evaluated for PCI of bleomycin. Early phase clinical trials have shown promising results on several advanced cancers. The background and mechanisms involved in PDT and PCI as well as preclinical and clinical examples will be presented.

Production of this booklet has been kindly supported by ESTEVE

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*Both authors have equally contributed to this work
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Michael Decker

University of Würzburg

Michael Decker studied Chemistry at Bonn and Cambridge University. He received his PhD in Pharmaceutical Chemistry from Bonn University in 2001, and pursued his postdoctoral research qualification ('Habilitation') in Pharmacy at Jena University. After that, he worked as a Visiting Scientist at McLean Hospital of Harvard Medical School as a Leopoldina Scholar. He was appointed a Lecturer at Queen's University Belfast, followed by a position at Regensburg University. Since 2012 he is Professor of Pharmaceutical and Medicinal Chemistry at Würzburg University, where his group works on various GPCRs and enzymes developing hybrid molecules, PET radiotracers, fluorescent and photoswitchable probes.

Photopharmacology in Alzheimer research: Chemical tools to investigate the functions of enzymes and GPCRs

Luca Agnetta[a], Dominik Dolles[a], Andrea Strasser[b], Michael Kauk[c], Massimo Nabissi[d], Roger G. Pertwee[e], Ulrike Holzgrabe[a], Carsten Hoffmann[c], Michael Decker[a]

[a]Pharmaceutical and Medicinal Chemistry, Julius Maximilian University of Würzburg; [b]Institute of Pharmacy, University of Regensburg; [c]Institute for Molecular Cell Biology, University Hospital Jena; [d]Department of Experimental Medicine, University of Camerino; [e]Institute of Medical Sciences, University of Aberdeen

Until now Alzheimer's disease (AD) can only be treated symptomatically and in early stages of the disease. The role of novel and innovative targets remains to be elucidated in the complex pathophysiological processes of neurodegenerative disorders. Photochromic ligands can help in these task.[1] Herein, we describe the development of photochromic compounds to investigate the role of receptor targets in AD. First, the development of a highly selective human cannabinoid receptor type 2 agonist as "affinity on-switch" determined in binding and functional studies.[2] Second, a dualsteric agonist at the muscarinic acetylcholine receptor type 1 (M_1), which represents a dimmable "efficacy off-switch" in FRET studies.[3] Furthermore, we applied tetra-fluoro-substitution to a photochromic M-ligand ("photoiperoxo") to optimize photophysical properties, and observed unexpected biological differences between substituted and unsubstituted azobenzenes.

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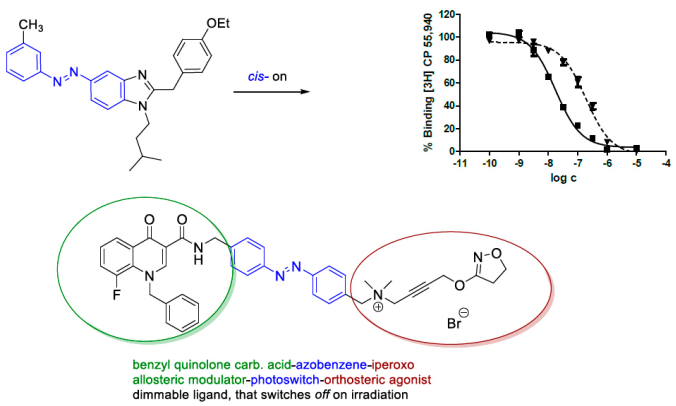

Figure. A photoswitchable affinity-on hCB_2R-selective ligand[2] and a "dimmable" dualsteric efficacy-off ligand at the M_1R[3]

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Cyril Goudet

Institut de Génomique Fonctionnelle, Montpellier, France

Dr. Cyril GOUDET (PhD) is a research director at French National Centre of Scientific Research (CNRS) who is working at the Institut de Génomique Fonctionnelle in Montpellier, France. He obtained his PhD from the University of Bourgogne in 1999, under the supervision of Dr. Marie-Louise Milat. Then, he moved to the University of Leuven in 2000 where he studied the pharmacology and toxicology of voltage-gated sodium channels, under the supervision of Pr. Jan Tytgat. He continued his postdoctoral studies in Montpellier in 2002, under the supervision of Dr. Jean-Philippe Pin, and started to work on the pharmacology of G-protein coupled receptors activated by the neurotransmitter glutamate, also called metabotropic glutamate receptors (mGluRs). His current research focuses on the discovery of new ligands, understanding their mode of action, photopharmacology and pain neuromodulation by mGluRs. He is the president of the NC-IUPHAR committee of mGluRs and the vice-president of the Scientific Council of the French Society of Pharmacology and Therapeutics (SFPT). He is also a member of the French Neuroscience Society. He has published 54 scientific articles in peer-reviewed journals and 3 patents (http://orcid.org/0000-0002-8255-3535).

Photodynamic processes for treatment of cancer

Chronic pain is among the most debilitating and costly afflictions in Europe, seriously affecting the quality of life of about 20% of adult Europeans. However, there is a clear need for new analgesics against chronic pain. Indeed, while acute pain can be correctly managed, chronic pain is not efficiently alleviated by current treatments. Glutamate is the main neurotransmitter involved in the transmission of pain throughout the pain pathway. A loss of the balance between excitatory glutamatergic transmission and inhibitory GABAergic transmission is involved in the development of central sensitization of the pain neuraxis, and leads to the development of the symptoms observed in patients with chronic pain. Herein, we used photopharmacology to study regulatory mechanisms involved in persistent and chronic pain. Metabotropic glutamate receptors (mGluRs) are G protein-coupled receptors responsible of the neuromodulatory effect of glutamate. Taking advantage of novel selective photoswitchable allosteric modulators that we recently developed, we took control of mGluRs by light in the brain of freely moving animals and studied the role of these receptors in chronic pain. We demonstrated that mGluRs expressed in the amygdala, a key brain region linking pain sensation with negative emotions, can rapidly and reversibly inhibit behavioral symptoms associated to chronic pain. These findings could help to define novel and more precise therapeutic interventions for chronic pain, and exemplify the potential of in vivo photopharmacology.

David Hodson

University of Birmingham

David is Professor of Cellular Metabolism, Professorial Research Fellow and leads the Islet Biology Group at the University of Birmingham, UK. David has particular interest in using optical approaches to tackle challenging questions in metabolism research. The overall objective of his work is to identify new mechanisms through which insulin-secreting pancreatic beta cells fail during diabetes.

Shining light on pancreatic beta cell function using optical approaches

Institute of Metabolism and Systems Research, University of Birmingham, UK; Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners; COMPARE University of Birmingham and University of Nottingham

Type 2 diabetes (T2DM) is now recognised by the World Health Organization as the third foremost non-communicable disease state. This complex metabolic disorder can be described as a failure of the pancreatic beta cell mass to secrete sufficient insulin to counteract elevated blood glucose levels. Despite this, T2DM incidence continues to incease and new treatments for the restoration of functional β-cell mass are urgently required. Key to this is the use of optical tools to open up new mechanisms underlying insulin release. The current presentation will discuss how photopharmacology has increased our understanding of β-cell function, as well as helped to identify a new class of insulin secretagogues.

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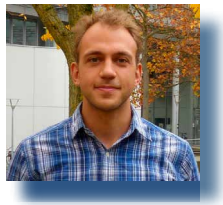

Oliver Thorn-Seshold

Ludwig-Maximilians-Universtität Munich

Oliver Thorn-Seshold was born in Sydney, Australia in 1985. He studied chemistry at the University of Sydney then did his PhD under Prof. Jens Hasserodt at the Ecole Normale Superieure de Lyon, France, working on enzyme-responsive fluorogenic probes.
In his postdoc he started working on microtubule inhibitors and in 2013 moved to Prof. Dirk Trauner's lab at LMU Munich, where he also worked on several other classes of azobenzene-based cytoskeleton inhibitors.
In 2016 he started his independent group at the LMU Munich, working on inhibitors of critical cellular processes and their adaptation into photopharmaceutical probes, where he is now an Emmy Noether junior group leader.

Non-azobenzene photopharmaceuticals: cell biology potential

Li Gao, Yvonne Kraus, Alexander Sailer, Carsten Donau, Ferdinand Lutter, Andrea Stegner, Max Bremerich, Oliver Thorn-Seshold

LMU Munich, Germany

Reversibly, bidirectionally photoswitchable scaffolds other than azobenzenes are barely studied as pharmacophores applied to *in cellulo* biology, and are practically unknown *in vivo*. This talk presents ongoing work studying the scope of photoswitch scaffolds for reagents that are reliably photoswitched *in situ & in cellulo*, using proof-of-concept *in cellulo* studies addressing photocontrol of tubulin polymerization and of membrane perturbation. These alternative scaffolds can find niche applications where the inherent geometric, synthetic, or photophysical properties of "ordinary" azobenzenes are unfavourable. They may also provide reagents with increased photopharmacological control for systems where azologuisation would yield *trans*-active reagents.[1]

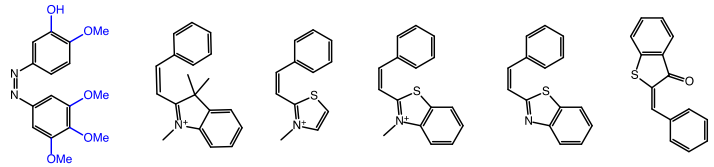

Figure 1. Metastable state geometries of bioactive azobenzene photoswitch **PST-1**, and of scaffolds that have yet to be exploited as pharmacophore photoswitches in biology.

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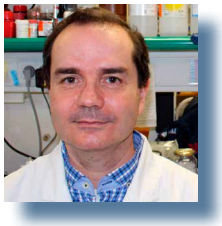

Francisco Ciruela

UB-IDIBELL

Francisco Ciruela graduated in Biology at the Universitat de Barcelona. He performed his Ph.D. at the Department of Biochemistry and Molecular Biology, Faculty of Chemistry, Universitat de Barcelona (1995). During this period he focused on the study of the mechanisms by which adenosine regulates cell functioning through adenosine receptors. He completed a post-doctoral stage first at the MRC Anatomical Neuropharmacology Unit, University of Oxford (1996-1999) with an EMBO Long Term Fellowship, and then at the Department of Pharmacology, University of Oxford (1999-2000) as a Reader in Pharmacology. In early 2000 he enrolled back to the Universitat de Barcelona as a "Ramón y Cajal" researcher. He focused on the study of GPCRs oligomerization and developed several fluorescence-based methods for the analysis of receptor oligomerization. In December 2007 he joined the Department of Pathology and Experimental Therapeutics at the Universitat de Barcelona as an Associate Professor of Pharmacology; there he established the Neuropharmacology and Pain Research Group. Currently, his research work focuses on neuropathological conditions involving GPCRs. He received the "ICREA Acadèmia" award from the Catalan Institution for Research and Advanced Studies, and became Full Professor of Pharmacology in 2017.

Illuminating adenosine receptors in movement disorders

[a]Unitat de Farmacologia, Departament Patologia i Terapéutica Experimental, Facultat de Medicina, IDIBELL, Universitat de Barcelona, L'Hospitalet de Llobregat, Spain. [b]Institut de Neurociències, Universitat de Barcelona, Spain.

G protein-coupled adenosine receptors are promising therapeutic targets for a wide range of pathological conditions, including movement disorders. However, the ubiquity of adenosine receptors and the ultimate lack of selectivity of certain adenosine-based drugs have frequently diminished their therapeutic use. Thus, we implemented a photopharmacological approach to control adenosine receptor function with high spatiotemporal resolution to circumvent some of these limitations. Accordingly, we developed several light-sensitive adenosine receptor-based drugs to photocontrol receptor's function both *in vitro* and *in vivo*. First, we synthesized an orthosteric, photoisomerizable, and nonselective adenosine receptor agonist, nucleoside derivative MRS5543 containing an aryl diazo linkage on the N(6) substituent, which in the dark (relaxed isomer) behaved as a full adenosine A_3 receptor (A_3R) and partial adenosine A_{2A} receptor ($A_{2A}R$) agonist[1]. Interestingly, upon photoisomerization it remained a full A_3R agonist but became an $A_{2A}R$ antagonist[1]. Indeed, the ability to photoswitch MRS5543 intrinsic activity through A2ARs opens new pharmacotherapeutic opportunities revolving movement disorder management, however the fast MRS5543 *cis* to *trans* relaxation time make it difficult *in vivo* studies. Subsequently, we synthesized MRS7145, the first photocaged $A_{2A}R$ antagonist[2], which bound and blocked $A_{2A}R$ in a light-dependent fashion in living cells. Interestingly, upon local brain irradiation, MRS7145 allowed the fine control of spontaneous locomotor activity and reversed pharmacologically-induced Parkinsonian-like behaviour. Thus, we demonstrated that this compound can be effectively photo-delivered in the striatum of rodents, increasing locomotor activity while reverting pharmacologically-induced parkinsonian-like symptoms. Collectively, we show here a proof of concept to design novel photopharmacological approaches for the management of adenosine receptor related disorders.

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Goitzen van Dam

TRACER Europe BV

- Professor of Surgery, Nuclear Medicine and Molecular Imaging and Intensive Care
- Certified surgeon oncologist / abdominal surgeon
- Head Optical Molecular Imaging Groningen research group (www.omig.nl)
- World-leader in the field of clinical translation of fluorescence imaging for image-guided surgery, pathology and endoscopy.
- More than 130 high-ranked scientific publications (i.e. Nature Medicine, Nature Communications, Science Translational Medicine, Clinical Cancer Research, Cancer Research, Gut, Theranostistic etc)

Current and future aspects of fluorescence guided healthcare for surgery, pathology, endoscopy - the next frontier activatable therapeutics

Conflict of interest: CEO / Founder TRACER Europe BV

In recent years, significant progress has been made in both optical imaging systems and fluorescent contrast agents for clinical applications. NIRF imaging with a free-floating imaging device mounted on the ceiling of the operating theatre or on a microscope articulating arm during surgery for cancer will enable visualization of tumor delineation, locoregional metastases, remnant disease as well as e.g. tumor-containing lymph nodes. Hereby, the surgeon can both detect (diagnostic) and excise (therapeutic) malignant tissue and possible residual disease at the same time. The use of NIRF optical imaging has a range of advantages. Most prominent among these is the fact that it is very safe technology, simple to operate, fast, high resolution (as low as 10 µm), relatively inexpensive and makes use of non-ionizing radiation. Based on the above, it is clear that intra-operative imaging is on the verge of entering standard clinical practice for surgery. Not only the imaging system but also the availability of clinical grade tumor-targeted probes is of the utmost importance for a successful introduction into clinical practice. This talk will give an overview of the current concepts and future perspectives of intraoperative fluorescence image-guided surgery using non-targeted and targeted optical contrast agents related to the first-time ever used in patients with ovarian cancer 10 years ago and the anticipated developments within the next 10 years. It will become clear that agencies like the FDA and EMA will play a major role in bringing this technology into the clinic and the necessity of standardization of methodology when applied in multinational multicenter studies.

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ABSTRACTS
SHORT PRESENTATIONS
*in order of appearance in the programme

Towards photocontrol of the cell entry of cell-penetrating peptides

Tim Schober[a], Oleg Babii[b], Sergii Afonin[b], Igor V. Komarov[c], Anne S. Ulrich[a,b]

[a]Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry (IOC); [b]KIT, Institute of Biological Interfaces 2 (IBG2); [c]Taras Shevchenko National University of Kyiv, Institute of High Technologies; email: tim.schober@kit.edu

Cell-penetrating peptides are a powerful tool to transport otherwise impermeable cargos into cells.[1] Herein we combine this delivery concept to in-cell transport with photopharmacology, by enabling photocontrol of peptide cell-penetrating ability. We designed several cyclic cell-penetrating peptides, each of having a diarylethene-based photoswitch (DAE)[2] in the backbone. The DAE building blocks were synthesized as Fmoc-amino acids and were directly employed in standard solid phase peptide synthesis.[3] The peptides differed in the macrocycle ring size (10 – 14mers) and in amino acid composition, hence allowing a structure-activity relationship study.
Pure photoisomers were independently evaluated for their uptake efficiency in HeLa cells. Mean fluorescence intensity and flow cytometry were used to quantify the differences. Cytotoxicity was assessed through MTT-assays. For some amphipathic 10-mer peptides, we observed well-pronounced differences in uptake between the two photoforms. The ring-open forms in all cases showed higher uptake than the ring-closed counterparts. For arginine-only analogues, however, the effect was much less prominent if at all present. We explain the differences by the changes in rigidity/flexibility and in backbone exposure upon photoswitching, which differentially affect both, the entry-enabling preorganized charge display and peptide CPP-activity deteriorating intramolecular aggregation.

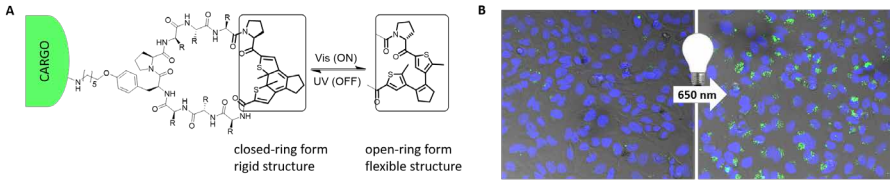

Figure 1. Overview of the photocontrol of the cell entry of cell-penetrating peptides. A) General scaffold with indicated photoswitching of the DAE-building block. B) Intracellular fluorescence (green) of different photophorms in HeLa cells after 3 h incubation at 5 µM.

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Studies on the sensitization phenomenon of P2X7 receptors by use of photoisomerisable tools.

Kate Dunning[a,b], Thomas Grutter[a,b]

[a]Laboratoire de Conception et Application de Molécules Bioactives, Équipe de Chimie et Neurobiologie Moléculaire, Faculté de Pharmacie, Centre National de la Recherche Scientifique (CNRS), UMR 7199, F-67400 Illkirch, France; [b]Université de Strasbourg, F-67000 Strasbourg, France; email: kdunning@unistra.fr

P2X receptors, found exclusively in eukaryotes, are a sub-family of ligand-gated ion channels (LGIC), which open an ion-permeable pore in response to the extracellular binding of ATP. These receptors, expressed in a wide range of cell types throughout the cardiovascular, immune and nervous systems, have been shown to play a role in numerous pathologies, and as such are considered as promising therapeutic targets. Seven P2X genes have been identified thus far in mammals, corresponding to seven subunit variants, named P2X1 – P2X7, which assemble in either a homo or heterotrimeric manner. Each P2X subunit has a common architecture comprising intracellular N- and C-termini, two transmembrane domains and a large ectodomain where the ATP binding site is found[1].
Amongst the P2X subtypes, P2X7 is set apart by a number of particularities. Not only is it structurally unique, featuring an elongated C-terminus, but it also exhibits functional differences with regard to other P2X variants, most notably the phenomenon of sensitization, a remarkable increase in current with prolonged or repeated applications of ATP[2]. This unusual process is poorly understood, and the molecular mechanism remains unclear. Previous studies have shown the importance of lipidic composition on P2X7 receptor function[3], in particular the effects of cholesterol, which acts as an inhibitor by binding to the transmembrane domains. Given the therapeutic interest of P2X7, due to its role in diseases such as neuropathic and chronic pain[4], a detailed understanding of its functionality, including the sensitization phenomenon, is of utmost importance. Here, we use a combination of patch clamp electrophysiological single channel recordings and photoactivatable molecular tools[5] (Figure 1) to probe further the molecular characteristics of P2X7 sensitisation.

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Synthesis and Pharmacological Evaluation of Photoswitchable Histamine H3 Receptor Agonists

N.J. Hauwert, T.A.M. Mocking, D. Da Costa Perreira, K. Lion, H.F. Vischer, I.J.P. de Esch, M. Wijtmans and R. Leurs

Division of Medicinal Chemistry, Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), Faculty of Sciences, VU University Amsterdam, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands. email: N.J.Hauwert@vu.nl

Histamine receptors are G protein-coupled receptors which are key mediators in a plethora of pathophysiological processes ranging from inflammation and itching to obesity. The family of histamine receptors consists of four receptors with moderate homology. The histamine H_3 receptor (H_3R) is highly expressed in the central nervous system (CNS). Its main function is the modulation of neurotransmitters release, acting on acetylcholine, GABA, dopamine, serotonin, and noradrenaline. It also modulates the release of histamine and has therefore an auto-regulatory function.[1] H_3R has been identified as a potential target in diseases such as obesity, narcolepsy, Alzheimer's and ADHD.[1] A wide range of ligands with diverse chemotypes have been published, but sofar only one molecule targeting the H_3R has reached the market having narcolepsy as its disease indication.[2]

Despite these advances, the pharmacology of H_3R signaling is still poorly understood and molecules enabling temporal and spatial control of signaling would be desirable. Therefore we aim to discover molecules which have the ability to switch under influence of light from an inactive to an active isomer. Using azobenzene-containing ligands, this aimed isomerization may be achieved using illumination at specific wavelengths. A variety of azobenzene-containing ligands has been synthesized, and photochemically and pharmacologically characterized in our labs. We have identified new ligands which are able to fully activate H_3R, that have over 20-fold differences in H_3R pEC_{50} between *trans* and *cis* isomers.

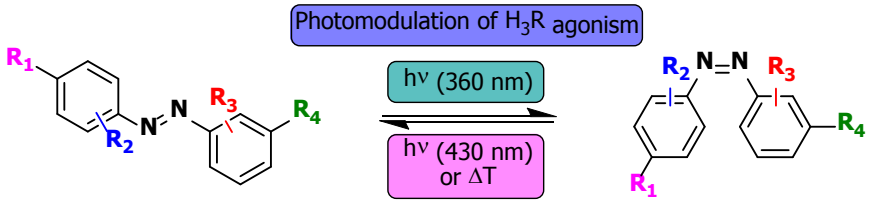

Figure 1. Characteristics of a photoswitchable H_3R agonist

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Photo-tuned Compounds for the Optical Control of the muscarinic ACh Receptor M1

Luca Agnetta[a], Timo Littmann[b], Ulrike Holzgrabe, Michael Decker

[a]*Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy and Food Chemistry, Julius-Maximilians-University of Würzburg, Am Hubland, D-97074 Würzburg, Germany;* [b]*Institute of Pharmacy, University of Regensburg, 93053 Regensburg, Germany. Email: Michael.Decker@uni-wuerzburg.de*

To expand the application of photopharmacological tool compounds to therapeutic applications, frequently the need for redshifted azobenzenes for the design of light-responsive systems is emphasized. This is due to the tissue damaging and cell toxic properties of UV light, necessary to achieve photoisomerization. In addition, low photostationary states (PSSs), poor thermal stability and potential interference with the commonly used optical readout methods, especially in GPCR research, are serious restrictions. Generally, azobenzenes that can be switched with visible light are preferred over their blue shifted counterparts, as they overcome the mentioned drawbacks. We investigated a set of mono- and bivalent photoswitchable compounds based on the highly potent muscarinic acetylcholine receptor (mAChR) agonist iperoxo with both, the unsubstituted azobenzene core and redshifted *bis-ortho*-fluorinated analogs in a novel luciferase complementation assay, reflecting Gq activation. Remarkably, *ortho*-fluorination does not only positively affect the photochromic behaviour but also enhances both efficacy and activity at the mAChR M1 receptor: Uni- and bivalent iperoxo ligands act as "efficacy"-switches, whereas the bis-*ortho*-fluorinated analogs act as potent "affinity"-switches with the desired PSS improvement and advantageous photophysical properties (right shift). These findings also demonstrate, that substituted azobenzenes in photopharmacological compounds not just represent analogs with other photophysical properties but can exhibit a considerably different biological profile that has to be carefully investigated.

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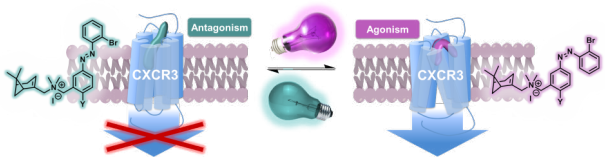
The second generation of GPCR photochromic ligands: switching efficacy from antagonism to agonism

Xavier Gómez-Santacana[a§], Sabrina M. de Munnik, Prashanna Vijayachandran, Daniel Da Costa Pereira, Jan Paul M. Bebelman, Iwan J. P. de Esch, Henry F. Vischer, Maikel Wijtmans, Rob Leurs

[a]*Division of Medicinal Chemistry, Amsterdam Institute for Molecules Medicines and Systems (AIMMS), Vrije Universiteit Amsterdam, The Netherlands;* [§]*Present address: Institute of Functional Genomics, Université de Montpellier, Unité 5302 CNRS and Unité U1191, INSERM, Montpellier, France; email: xavi.gomez-santacana@igf.cnrs.fr*

Photopharmacology is an emerging discipline that makes use of photoswitchable ligands as pharmacological tool compounds to yield temporal and spatial control of protein function[1]. However, the development of photopharmacology to target G protein-coupled receptors (GPCRs) is still in its infancy, despite their high therapeutic relevance. Since 2014, several photoswitchable ligands have been reported for GPCRs. Although it is not always straightforward to discern contributions by affinity or potency shift from the contribution by any efficacy shift, most reported photoswitchable GPCR ligands display light-induced alterations in affinity or potency.
In order to obtain an improved optical control of GPCR function, we set out to develop small-molecule ligands with photoswitchable efficacy in which both configurations bind the target protein but exert distinct pharmacological effects, i.e., stimulate or antagonise GPCR activation. Our design was based on a previously identified efficacy hotspot for the peptidergic chemokine receptor CXCR3[2] and resulted in the synthesis and characterization of five new azobenzene-containing CXCR3 ligands.[3] G protein activation assay and real-time electrophysiology experiments demonstrated a photoswitching from antagonism to partial agonism and even to full agonism (VUF16216).[3] SAR evaluation suggests that the size and electron-donating properties of the inner aromatic ring substituents are important for the efficacy photoswitching. These azo compounds are the first GPCR ligands with a nearly full efficacy photoswitch that enable a reversible photoswitching from antagonism to agonism and thus may become valuable pharmacological tools for the optical control of peptidergic GPCR signaling.



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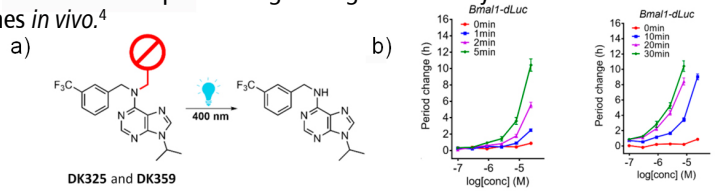
Light Dosimetry: A Method for Conditional Adjustment of Circadian Period

Dušan Kolarski[a], Albert Schulte[a], Wiktor Szymanski[a,b], Ghisalin Breton[c], Kenichiro Itami[d], Tsuyoshi Hirota[d], and Ben L. Feringa[a]

[a] *Centre for Systems Chemistry, Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands*
[b] *Department of Radiology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands*
[c] *Department of Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, 6431 Fannin St, MSB 4.216, 77030 Houston, US*
[d] *Institute of Transformative Bio-Molecules (WPI-ITbM), Nagoya University, Chikusa, Nagoya 464-8602, Japan*

Inspired by the crucial role of circadian clock disruption in disease development,[1] during the last decade chemical biology studied how to adjust cellular clocks using small molecule modifiers[2]. Unfortunately, these modifiers are still facing a big drawback when in vivo application is needed: due to the similarity in cellular regulation of clocks, besides curing the disrupted biological rhythm they would affect all the others, healthy rhythms in other cells. To overcome this problem, a potential strategy would be photo-uncaging - based on the regulation of a compound`s bioactivity with light, which can be delivered precisely in space and time.[3]
Here, we show for the first time the possibility to lengthen the circadian period in mammalian cells, tissues, and zebrafish just by choosing an interval of visible light irradiation (400 nm) in order to release Longdaysin - a compound that exhibits a drastic effect on the circadian period lengthening in a variety of mammalian cells but also zebrafishes *in vivo.*[4]



Scheme 1. a) Photocleavage of the protected Longdaysin; b) correlation diagram of period lengthening, concentration, and light exposure time.

The kinetics of photo-deprotection was studied by UPLC-MS and NMR analysis. These results correlate well with CKIα and CKIδ inhibition, as well as with cellular, tissue, and zebrafish period change. The cellular time dosing was investigated by a cell-based luminescence assay using *Bmal1–d*Luc reporter U2OS cells, and employing visible light (400 nm). In the tissue assay, explants of spleen were dissected from m*Per2*^{Luc} knockin mice and used to follow period change. And as an *in vivo* model, the *per3*-luc transgenic zebrafish line was chosen to demonstrate a correlation between photocleavage and period lengthening.

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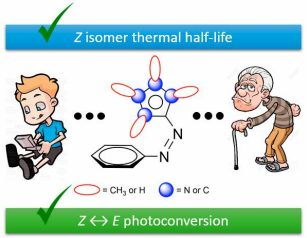
Azoheteroarenes: Novel Photoswitches with Tunable Properties and Multiple Functional Opportunities

Matthew J. Fuchter[1]

[1]*Department of Chemistry, Imperial College London, London SW7 2AZ. U.K*

Photoswitchable compounds, which can be reversibly switched between two isomers by light, continue to attract significant attention for a wide array of applications. Azoheteroarenes represent a relatively new but understudied type of photoswitch, where one or both of the aryl rings from the conventional azobenzene class has been replaced with a five-membered heteroaromatic ring. This poster will give an overview of our work in this area, particularly focusing on our discovery of the arylazopyrazoles[1], which offer quantitative photoswitching and high thermal stability of the Z isomer (half-lives of up to ~1000 days). It will describe our recent studies to elucidate the origin of the long thermal half-lives and excellent addressability of the arylazopyrazoles, applying this understanding to determine important structure-property relationships for a wide array of comparable azoheteroaryl photoswitches[2]. Through this, we have identified compounds with Z isomer half-lives ranging from seconds to hours, to days and to years, and variable absorption characteristics; all through tuning of the heteraromatic ring.

Given the large tunability of their properties, the predictive nature of their performance, and the other potential functional opportunities afforded by usage of a heteroaromatic system, we believe the azoheteroaryl photoswitches to have huge potential in a wide range of optically addressable applications. Our initial studies will be highlighted towards this end, including the development of photoswitchable bases[3] and photopharmacological agents[4].



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Adhesive Photoswitch for Modulation of Protein Functions

Rina Mogaki, Kou Okuro, and Takuzo Aida

Department of Chemistry and Biotechnology, School of Engineering, The University of Tokyo;; email: mogaki@macro.t.u-tokyo.ac.jp

In situ modulation of biomolecular functions by light is an important issue that leads to the elucidation of biological events and the treatment of diseases with fewer side effects. An effective approach is to incorporate photoresponsive moieties to the target biomolecule. However, the applicability of this approach is limited due to the requirement of a proper functional group for covalent tethering, which is mostly incorporated by genetic mutation. We have developed "molecular glues"[1–12] that bear multiple guanidinium ion (Gu^+) pendants and therefore tightly adhere to various biomacromolecular surfaces by forming salt bridges with oxyanions (Figure 1). With an aim to develop a universal method to modulate biomolecular functions without genetic engineering, we designed an "adhesive photoswitch", a molecular glue conjugated with an inhibitor for the target enzyme via a photoresponsive spacer. The adhesive photoswitch selectively adheres to the target wild-type enzyme and can reversibly modulate the enzyme activity (Figure 1). The molecular design strategy and the application of this adhesive photoswitch will be discussed.

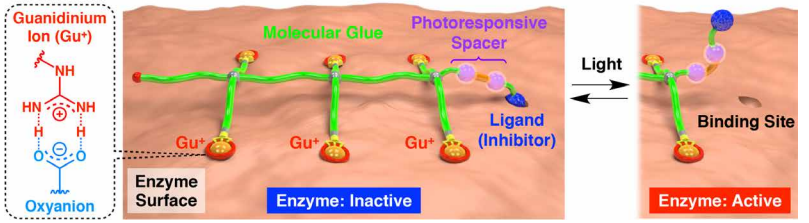

Figure 1. Modulation of protein functions with an adhesive photowitch bearing an inhibitor moiety for the target enzyme.

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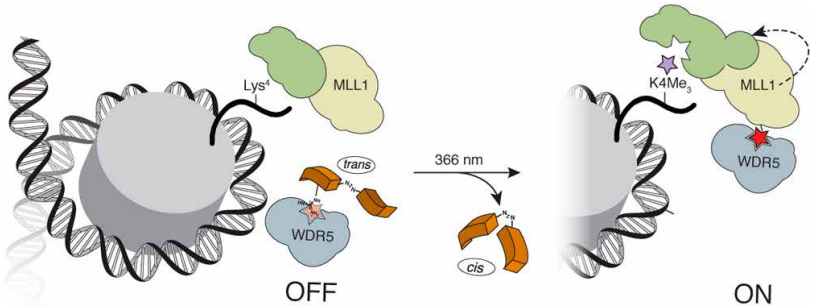
OPTOEPIGENETICS: Light-controlled Modulation of Gene Expression

Lea Albert[a], Jing Xu[b], Vasundara Srinivasan[a], Yali Dou[b] and Olalla Vázquez[a*]

[a]*Fachbereich Chemie, Philipps-Universität Marburg, Germany;* [b]*University of Michigan, USA; email: vazquezv@staff.uni-marburg.de*

Epigenetics is the collection of highly dynamic processes involving multiple chromatin modifying and remodelling enzymes that control the accessibility to genes and their expression through a complex signalling network of protein-protein interactions.[1] In this context, our young group has recently described a cell-permeable photoswitchable probe capable of modulating epigenetic cellular states by disruption of an essential protein–protein interaction within the MLL1 methyltransferase core complex. Our azobenzene-containing peptides selectively block the WDR5-MLL1 interaction by binding to WDR5 with high affinity (Ki = 1.25 nM). We determined the co-crystal structure of this photoswitchable peptiomimetic with WDR5 to understand the interaction at the atomic level. Importantly, the photoswitchable *trans* and *cis* conformers of the probe display a clear difference in their inhibition of MLL1.[2]



We further demonstrate that the designed photo-controllable azo-peptidomimetics affect the transcription of the MLL1-target gene Deptor, which regulates hematopoiesis and leukemogenesis, and inhibit the growth of leukemia cells. This strategy confirms the potential of photopharmacological inhibition of methyltransferase protein–protein interactions as a novel method for external epigenetic control, providing a new toolbox for controlling epigenetic states.

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ABSTRACTS
POSTERS
*in alphabetical order

P01- Photo-tuned Compounds for the Optical Control of the muscarinic ACh Receptor M1

Luca Agnetta[a], Timo Littmann[b], Ulrike Holzgrabe, Michael Decker

[a]Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy and Food Chemistry, Julius-Maximilians-University of Würzburg, Am Hubland, D-97074 Würzburg, Germany; [b]Institute of Pharmacy, University of Regensburg, 93053 Regensburg, Germany. Email: Michael.Decker@uni-wuerzburg.de

To expand the application of photopharmacological tool compounds to therapeutic applications, frequently the need for redshifted azobenzenes for the design of light-responsive systems is emphasized. This is due to the tissue damaging and cell toxic properties of UV light, necessary to achieve photoisomerization. In addition, low photostationary states (PSSs), poor thermal stability and potential interference with the commonly used optical readout methods, especially in GPCR research, are serious restrictions. Generally, azobenzenes that can be switched with visible light are preferred over their blue shifted counterparts, as they overcome the mentioned drawbacks. We investigated a set of mono- and bivalent photoswitchable compounds based on the highly potent muscarinic acetylcholine receptor (mAChR) agonist iperoxo with both, the unsubstituted azobenzene core and redshifted *bis-ortho*-fluorinated analogs in a novel luciferase complementation assay, reflecting Gq activation. Remarkably, *ortho*-fluorination does not only positively affect the photochromic behaviour but also enhances both efficacy and activity at the mAChR M1 receptor: Uni- and bivalent iperoxo ligands act as "efficacy"-switches, whereas the bis-*ortho*-fluorinated analogs act as potent "affinity"-switches with the desired PSS improvement and advantageous photophysical properties (right shift). These findings also demonstrate, that substituted azobenzenes in photopharmacological compounds not just represent analogs with other photophysical properties but can exhibit a considerably different biological profile that has to be carefully investigated.Synthesis and

P02- Light-controlled inhibitors in studies of glutamate transporters

Arkhipova V[ab], Hoorens MWH[bc], Fu H[d], Trinco G[a], Poelarends GJ[d], Guskov A[a], Szymanski W[bc], Slotboom DJ[a]

[a]Department of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands; [b]Department of Radiology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands; [c]Centre for Systems Chemistry, Stratingh Institute for Chemistry, Faculty of Science and Engineering, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands; [d]Department of Chemical and Pharmaceutical Biology, Groningen Research Institute of Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV, Groningen, The Netherlands; email: valentina.arkhipova@rug.nl

Glutamate transporters are membrane proteins that transport amino acid substrates using energy stored in gradients of cations over the membrane. Mammalian glutamate transporters (so-called excitatory amino acid transporters, EAATs) support neuronal signaling by clearing excess neurotransmitter glutamate from the synaptic cleft. Dysfunctions of EAATs cause neurotoxicity that is linked to multiple human diseases, including Alzheimer's disease and amyotrophic lateral sclerosis.
Prokaryotic homologues of EAATs catalyse uptake of glutamate and aspartate as nutrients. Structural studies of archaeal homologues Glt_{Ph} and Glt_{Tk} provided insight in structural organization and transport mechanism of glutamate transporters.
We study the archaeal glutamate transporter homologue Glt_{Tk} using azobenzene-based photoswitchable substrate derivatives[1]. We measured binding affinities (Kd) of these compounds to Glt_{Tk} by Isothermal titration calorimetry, and their inhibition efficiency using radiolabelled aspartate transport by the protein reconstituted in proteoliposomes. The protein activity was inhibited to different extents by the *cis* and *trans* forms of the compounds. The compound with the highest difference in inhibition efficiency of isomeric forms was crystallized with the protein. The crystal structure revealed conformational changes in the protein molecule upon binding of the photoswitchable inhibitor showing the competitive inhibitory mechanism.
The posibility to manipulate the protein structure using light-controlled compounds opens exciting opportunities for time-resolved structural studies of glutamate transporters.

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P03- Construction of a knock-in mouse model to study G-protein coupled receptor signaling using tethered pharmacology

Julia Ast[a], Johannes Broichhagen[b], Tom Podewin[b], Andrea Bacon[c], David J. Hodson[a]

[a]*Institute of Metabolism and Systems Research, University of Birmingham, UK; Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners; COMPARE University of Birmingham and University of Nottingham;* [b]*Max Planck Institute for Medical Research, Department of Chemical Biology, Heidelberg, Germany;* [c]*Transgenic Mouse Facility, University of Birmingham, Birmingham, UK; email: j.ast@bham.ac.uk*

The glucagon-like peptide 1 receptor (GLP-1R) is a key drug target for the treatment of type 2 diabetes (T2D) and belongs to the large protein family of G-protein coupled receptors (GPCRs). These receptors translate extracellular stimuli into intracellular responses by activation of signal transduction pathways. Activation of the GLP-1R enhances glucose-stimulated insulin secretion and therefore regulates glucose homeostasis. After activation and signaling, the GLP-1R gets internalized, followed by recycling to the plasma membrane or lysosomal degradation. The GLP-1R still signals while internalized but whether and how this may influence blood glucose concentration and T2D risk is unknown. Tethered pharmacology allows selective and prolonged activation of receptors. By fusing a SNAP-tag onto the GLP-1R and a benzyl-guanine (BG) onto a peptide agonist, we were able to continuously activate the receptor[1]. The use of a reductively-cleavable linker between BG and the agonist also allows dissocation of the ligand from the receptor and therefore reversible control over receptor activation, as well as recycling and degradation[1]. To extend the Reductively Cleavable agONists (RECON) concept in vivo, we aimed to express endogenously SNAP-tagged GLP-1R (SNAP_GLP-1R) in mice. Using CRISPR/Cas9 genome editing, we were able to generate a novel knock-in mouse line expressing SNAP_GLP-1R. Single-guide RNA targeting the endogenous *Glp1r* locus and a single-stranded repair template encoding the SNAP-tag and harboring 55 bp homology arms were injected into Cas9 endonuclease-expressing one-cell stage embryos. After reaching the 2-cell stage, embryos were transferred into surrogate mothers. The SNAP-tag could be detected in two out of six offspring. Besides the targeted insertion, in two out of six offspring we found small deletions in the *Glp1r* gene resulting in a frame-shift and a GLP1R knock-out. SNAP_GLP-1R expressing animals together with the RECON ExONatide, a tethered version of the GLP-1R agonist Ex4, might allow the role of internalized GLP-1R signaling to be specifically studied in homoestatic tissues.

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P04- Optimised Azobenzene Photoswitches For Reliable Two-Photon Neuronal Excitation

G. Cabré[a], A. Garrido-Charles[b], M. Moreno[a], M. Bosch[b], R. Gelabert[a], J. M. Lluch[a], F. Busqué[a], J. Hernando[a], P. Gorostiza[b,c,d], and R. Alibés[a]

[a] *Departament de Química, Universitat Autònoma de Barcelona (UAB), Cerdanyola del Vallès, Spain;* [b] *Institut de Bioenginyeria de Catalunya (IBEC), Barcelona Institute of Science and Technology, Barcelona, Spain;* [c] *Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain;* [d] *Centro de Investigación Biomédica en Red en Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Zaragoza, Spain; email: gisela.cabre@uab.cat*

Ionotropic glutamate receptors (iGluRs), the main responsible of excitatory currents in the central nervous system, can be remotely controlled by means of light-responsive molecules. This is the case of MAG ligands, which are composed of a **maleimide** moiety for receptor binding, an **azobenzene photoswitch** and a **glutamate agonist** (Figure 1a).1 Trans-cis photoisomerisation of these compounds allows modulation of glutamate-receptor interaction, thus resulting in light-induced operation of the cell membrane ionic channels governed by iGluRs.

In our group we have rationally developed new MAG switches capable to trigger iGluRs upon 2-photon excitation with near infrared light, which should enable larger penetration depths in biological tissues with minimal biological degradation.[2] This requires high 2-photon absorption cross-sections (σ_2) as well as long *cis* state lifetime (τ_{cis}). In this presentation, new MAG compounds synthesised along these design principles are presented MAG^{slow}_{2P} and $MAG^{slow}_{2P\_F}$, Figure 1b), with which we have achieved up to 6-fold enhancement of the 2-photon response of iGluRs with near infrared light (Figure 1c-d).

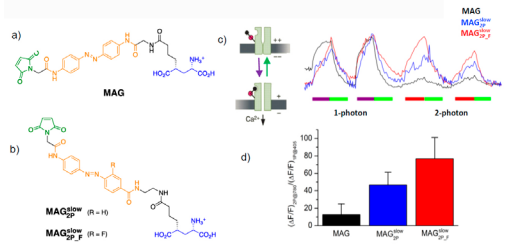

Figure 1. a-b) Structure of **MAG** and MAG-derivatives MAG^{slow}_{2P} and $MAG^{slow}_{2P\_F}$ with enhanced 2-photon absorption. c) Reversible light-induced activity of MAG-tethered iGluRs under 1-photon (405 nm) and 2-photon (780 nm) excitation. d) Average 2-photon/1-photon response of the different MAG-derivatives.

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P05- Photoswitchable dynasore analogs to control endocytosis with light

N. Camarero[1], A. Trapero[1,2], A. Pérez-Jiménez[1], E. Macia[3], Alexandre Gomila-Juaneda[1], A. Martín-Quirós[1], L. Nevola[4], A. Llobet[5], J. Hernando[6], A. Llebaria[2], E. Giralt[6,7] and P. Gorostiza[1,8,9]

[1]Institute for Bioengineering of Catalonia (IBEC); [2]Institute for Advanced Chemistry of Catalonia (IQAC-CSIC); [3]Institut de Pharmacologie Cellulaire et Moléculaire (IPMC), Université Nice; [4]Institute for Research in Biomedicine (IRB); [5]Bellvitge Biomedical Research Institute (IDIBELL); [6]Universitat Autònoma de Barcelona (UAB); [7]University of Barcelona; [8]CIBER-BBN; [9]ICREAa email: ncamarero@ibecbarcelona.eu

The spatiotemporal control of cellular dynamic processes has great fundamental interest but lacks versatile molecular tools. Dynamin is a key protein in endocytosis and an appealing target to manipulate cell trafficking using patterns of light. We have developed the first photoswitchable small-molecule inhibitors of endocytosis, by a stepwise design of the photo-chromic and pharmacological properties of dynasore, a dynamin inhibitor. We characterized their photochromism with UV-visible and transient absorption spectroscopy and their biological activity with transferrin uptake assays in live cells using confocal microscopy and flow cytometry. They are water-soluble, cell permeable, and photostable, and enable fast, single-wavelength photoswitchable inhibition of clathrin-mediated endocytosis at micromolar concentration.

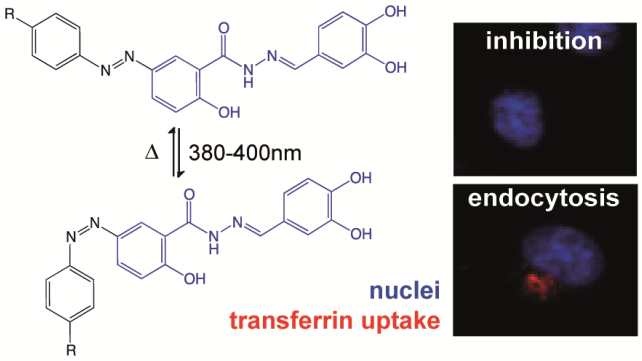

Figure 1. Controlling dynamic cellular processes using spatiotemporal patterns would be very useful for fundamental studies, but the available molecular tools are currently limited. Dynamin is a key protein in endocytosis, and an appealing target to manipulate cell trafficking with light. We have developed photoswitchable derivatives of dynasore, a dynamin inhibitor, that enable photocontrolling clathrin-mediated endocytosis.

Acknowledgements
We acknowledge financial support from the European Research Council (OpticalBullet and TheraLight grants), the ERANET-SynBio programme (Modulightor grant), and the Human Brain Project (WaveScalES grant) of the Europan Union, from the Catalan Government (CERCA Programme and 2014SGR-1251 grant); 2017SGR-0998 grants), from the Spanish Government (CTQ2016-80066R, BIO 2016-75327-R grants CTQ2015-65439-R grants), from FEDER funds and from the Ramón Areces foundation.

P06- Use of genetically encoded biosensors to delineate GPCR signaling

Meritxell Canals

The Centre Of Membrane Proteins And REceptors, University of Nottingham, Nottingham, United Kingdom; email: mcanalsbuj@gmail.com

Genetically encoded biosensors have been instrumental to our understanding of how intracellular signaling is organized and regulated within cells. In the last decade, these biosensors have shed light onto the mechanisms that control the intracellular organization of G protein-coupled receptor (GPCR) signaling and have allowed the visualization of signaling events with unprecedented temporal and spatial resolution. Our work with ERK, PKC and cAMP FRET and BRET sensors[1,2] specifically targeted to subcellular compartments within the cell, has highlighted the relevance of GPCR localization for the generation of specific signals. I will present data that shows that endocytosis of receptors for the neuropeptides neurokynin and CGRP is required to elicit specific signaling responses that are essential for sustained pain transmission[3,4]. Specific targeting of these endocytic signals may provide a novel therapeutic strategy for the treatment of pain. I will also show how the translocation of the mu-opioid receptor (MOR) across the plasma membrane dictates the signaling outcomes of receptor activation and can explain the differential signaling elicited by morphine[5].

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P07- Studies on the sensitization phenomenon of P2X7 receptors by use of photoisomerisable tools

Kate Dunning[a,b], Thomas Grutter[a,b]

[a]*Laboratoire de Conception et Application de Molécules Bioactives, Équipe de Chimie et Neurobiologie Moléculaire, Faculté de Pharmacie, Centre National de la Recherche Scientifique (CNRS), UMR 7199, F-67400 Illkirch, France;* [b]*Université de Strasbourg, F-67000 Strasbourg, France; email: kdunning@unistra.fr*

P2X receptors, found exclusively in eukaryotes, are a sub-family of ligand-gated ion channels (LGIC), which open an ion-permeable pore in response to the extracellular binding of ATP. These receptors, expressed in a wide range of cell types throughout the cardiovascular, immune and nervous systems, have been shown to play a role in numerous pathologies, and as such are considered as promising therapeutic targets. Seven P2X genes have been identified thus far in mammals, corresponding to seven subunit variants, named P2X1 – P2X7, which assemble in either a homo or heterotrimeric manner. Each P2X subunit has a common architecture comprising intracellular N- and C-termini, two transmembrane domains and a large ectodomain where the ATP binding site is found[1].
Amongst the P2X subtypes, P2X7 is set apart by a number of particularities. Not only is it structurally unique, featuring an elongated C-terminus, but it also exhibits functional differences with regard to other P2X variants, most notably the phenomenon of sensitization, a remarkable increase in current with prolonged or repeated applications of ATP[2]. This unusual process is poorly understood, and the molecular mechanism remains unclear. Previous studies have shown the importance of lipidic composition on P2X7 receptor function[3], in particular the effects of cholesterol, which acts as an inhibitor by binding to the transmembrane domains. Given the therapeutic interest of P2X7, due to its role in diseases such as neuropathic and chronic pain[4], a detailed understanding of its functionality, including the sensitization phenomenon, is of utmost importance. Here, we use a combination of patch clamp electrophysiological single channel recordings and photoactivatable molecular tools[5] (Figure 1) to probe further the molecular characteristics of P2X7 sensitisation.

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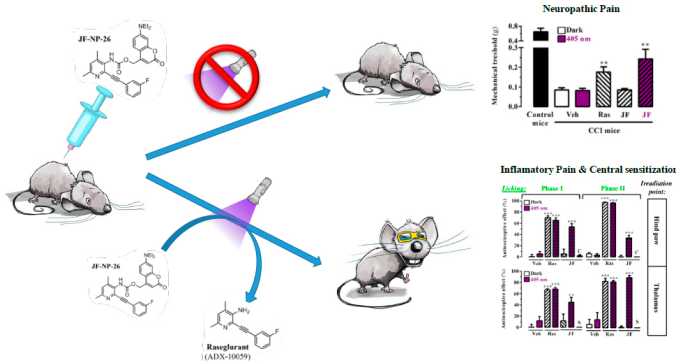
P08- Optical control of pain *in vivo* with a photoactive $mGlu_5$ receptor negative allosteric modulator

Joan Font[1,2,3], Marc Lopez-Cano[4], Serena Notartomaso[5], Pamela Scarselli[5], Paola Di Pietro[5], Roger Bresoli-Obach[6], Giuseppe Battaglia[5], Fanny Malhaire[3], Xavier Rovira[3], Juanlo Catena[1], Jesus Giraldo[2], Jean-Philippe Pin[3], Victor Fernandez-Duenas[4], Cyril Goudet[3], Santi Nonell[6], Ferdinando Nicoletti[5], Amadeu Llebaria[1], Francisco Ciruela[4]. E-mail: joan.font-ingles@igf.cnrs.fr

[1]*MCS, Laboratory of Medicinal Chemistry, Institute for Advanced Chemistry of Catalonia (IQAC-CSIC);* [2]*Institut de Neurosciències, Unitat de Bioestadistica, Universitat Autonoma de Barcelona;* [3]*IGF, CNRS, INSERM, Univ. Montpellier.* [4]*Departament de Patologia i Terapèutica Experimental, IDIBELL, Institut de Neurociències, Universitat de Barcelona;* [5]*Department of Physiology and Pharmacology, University Sapienza;* [6]*Institut Quimic de Sarrià.*

Light-operated drugs constitute an emerging trend in drug discovery, since they may provide spatiotemporal resolution for the treatment of complex diseases. Here we applied this strategy to alleviate pain in preclinical models of chronic pain. JF-NP-026 is an inactive photocaged derivative of Raseglurant (ADX 10059), which is a negative allosteric modulator of the metabotropic glutamate type 5 ($mGlu_5$) receptor. Violet light illumination of JF-NP-26 induces a photochemical reaction promoting the active-drug's release, which effectively controls mGlu5 receptor activity both in ectopic expressing systems and in striatal primary neurons. Systemic administration in mice followed by local lightemitting diode (LED)-based illumination, either of the thalamus or the peripheral tissues, induced JF-NP-026-mediated light-dependent analgesia both in neuropathic and in acute/tonic inflammatory pain models. These data offer the first example of optical control of analgesia in vivo using photocaged mGlu5 receptor negative allosteric modulator[1].



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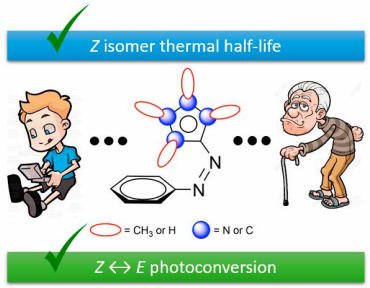
P09 - Azoheteroarenes: Novel Photoswitches with Tunable Properties and Multiple Functional Opportunities

Matthew J. Fuchter[1]

[1]*Department of Chemistry, Imperial College London, London SW7 2AZ. U.K*

Photoswitchable compounds, which can be reversibly switched between two isomers by light, continue to attract significant attention for a wide array of applications. Azoheteroarenes represent a relatively new but understudied type of photoswitch, where one or both of the aryl rings from the conventional azobenzene class has been replaced with a five-membered heteroaromatic ring. This poster will give an overview of our work in this area, particularly focusing on our discovery of the arylazopyrazoles[1], which offer quantitative
photoswitching and high thermal stability of the Z isomer (half-lives of up to ~1000 days). It will describe our recent studies to elucidate the origin of the long thermal half-lives and excellent addressability of the arylazopyrazoles, applying this understanding to determine important structure-property relationships for a wide array of comparable azoheteroaryl photoswitches[2]. Through this, we have identified compounds with Z isomer half-lives ranging from seconds to hours, to days and to years, and variable absorption characteristics; all through tuning of the heteraromatic ring.
Given the large tunability of their properties, the predictive nature of their performance, and the other potential functional opportunities afforded by usage of a heteroaromatic system, we believe the azoheteroaryl photoswitches to have huge potential in a wide range of optically addressable applications. Our initial studies will be highlighted towards this end, including the development of photoswitchable bases[3] and photopharmacological agents[4].



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P10- Extending the Toolbox of Photochromic Ligands

M. Gailer[a], I. Karpenko[b], T. Podewin[c], D. Trauner[d], A. Hoffmann-Röder[a]

[a]*Department of Organic Chemistry, Faculty for Chemistry and Pharmacy, Ludwig-Maximilians-University Munich;*
[b]*Laboratory of Therapeutic Innovation, Faculty for Pharmacy, University Strasbourg;*
[c]*Department for Chemical Biology, Max Planck Institute for Medical Research Heidelberg;*
[d] *Janice Cutler Chair in Chemistry, Adjunct Professor of Neuroscience and Physiology, Department of Chemistry, New York University;*
email: maaich@cup.uni-muenccahen.de

One of the most interesting peptidic hormones is oxytocin[1], which got a lot of public attention in the last decades. The neurohypophysial hormone oxytocin plays a role in food intake and energy expenditure, social bonding, sexual reproduction and during and after childbirth. Its dysfunction is also related to psychiatric disorders like autism or schizophrenia.[2] Due to the low bioavailibility and half-life of oxytocin, there is a growing interest in new peptidic and non-peptidic agonists and antagonists of the oxytocin-receptor. A peptidomimetic with higher bioavailability is carbetocin which is used as an antihemorragic and uterotonic drug in the peripheral nervous system.[3]

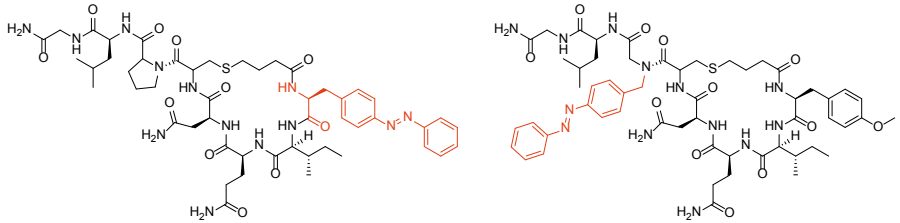

Figure 1. Photoswitchable mimetics of Carbetocin

Photopharmacology opens doors for new spatiotemporal controllable analogous. In our group, we synthesized photoswitchable oxytocin and carbetocin mimetics by introducing different AzoPhe derivatives into the backbone, either by incorporation of photoswitchable SPPS building blocks or via *N*-alkylation of backbone amides. After functional evaluation by intracellular Ca^{2+}-measurements we were able to determine agonstic and antagonistic behavior towards the oxytocin and vasopressin receptors.

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P11- Azo-BODIPY Photoswitches for Amino Acids, Peptides and Proteins

Ouldouz Ghashghaei[a], Mathew Horrocks[b], Marc Vendrell[c], Rodolfo Lavilla[a]

[a]*Laboratory of Medicinal Chemistry, Faculty of Pharmacy and Institute of Biomedicine (IBUB), University of Barcelona, Barcelona 08028, Spain;* [b]*School of Chemistry, University of Edinburg, UK;* [c] *MRC/UoE Centre for Inflammation Research, The University of Edinburgh, 47 Little France Crescent, Edinburgh EH16 4TJ, UK; email: rlavilla@ub.edu*

The BODIPY fluorophore is found in a wide range of useful bioimaging probes, because of its chemical and photophysical properties.[1] In this context, we imagined the inclusion of this privileged structure in the aromatic ring moiety of a standard azo photoswicht.[2] To this end, we synthesized diazonium-BODIPY salts **1**, through a short sequence from a known aniline precursor (Figure1). The resulting diazonium salts were suitably prepared and characterized and then used to tag a range of activated aromatic amino acids. Fmoc-Tyrosine was coupled with salts **1** to conveniently yield the corresponding azo derivatives **2**, ready for direct use in solid phase peptide synthesis. Tagging of Tyr-containing peptides and proteins is also feasible with these reagents, leading to the photoswitchable entities **3** and **4**. Details on this experimentation will be disclosed.

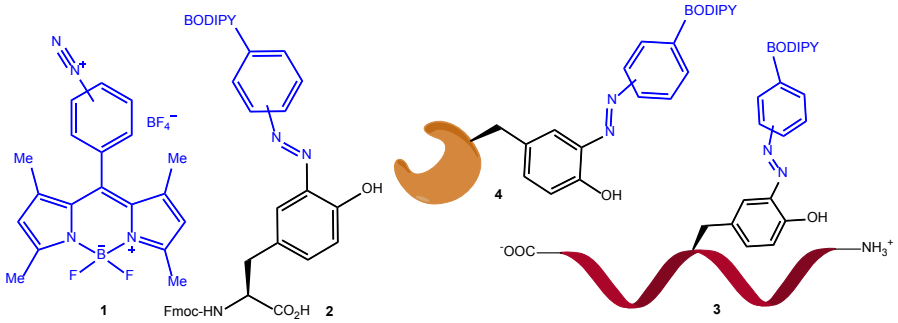

Figure 1. Structures of BODIPY diazonium salts **1**, Azo-BODIPY Tyrosine derivative **2** and labelled peptides (**3**) and proteins (**4**).

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P12- Allostery, oligomerization and biased signaling drive mechanism-based GPCR drug discovery

Agustín Bruzzese, Adrià Ricarte, Óscar Díaz, María Tena, Bin Zhou, James A.R. Dalton, Jesús Giraldo

Laboratory of Molecular Neuropharmacology and Bioinformatics, Unitat de Bioestadística, Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
Unitat de Neurociència Traslacional, Parc Taulí Hospital Universitari, Institutd'Investigació i Innovació Parc Taulí (I3PT), Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain. email: Jesus.Giraldo@uab.es

Structural and mathematical models are being developed in our group to assess the growing knowledge on the complexity of receptor function, which include allosteric compounds[1], membrane lipid modulation[2], receptor homo- and heteromerization[3-6], and biased signaling[7]. Long-timescale molecular dynamics (MD) simulations are run to identify the molecular determinants of receptor function and ligand action. We are working on selected G protein-coupled receptors (GPCRs) such as the β-adrenergic, adenosine A2A, μ-opioid receptor, dopamine D2, CB1 and mGlu receptors and their potential heteromers. Importantly, we have developed a new scale for the analysis of biased signaling, which is able to account for inverse agonists, in contrast with currently available scales. Inclusion of molecular environment is fundamental in any area of pharmacology, and, consequently, in photopharmacology. The construction of a conceptual framework for structural and functional receptor complexities is key for mechanism-based drug discovery.

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Acknowledgements

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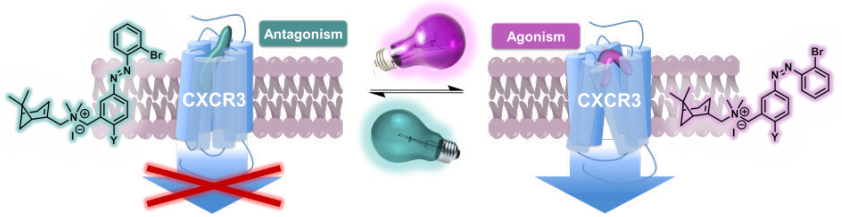
P13- The second generation of GPCR photochromic ligands: switching efficacy from antagonism to agonism

Xavier Gómez-Santacana[a§], Sabrina M. de Munnik, Prashanna Vijayachandran, Daniel Da Costa Pereira, Jan Paul M. Bebelman, Iwan J. P. de Esch, Henry F. Vischer, Maikel Wijtmans, Rob Leurs

[a]*Division of Medicinal Chemistry, Amsterdam Institute for Molecules Medicines and Systems (AIMMS), Vrije Universiteit Amsterdam, The Netherlands;*
[§]*Present address: Institute of Functional Genomics, Université de Montpellier, Unité 5302 CNRS and Unité U1191, INSERM, Montpellier, France; email: xavi.gomez-santacana@igf.cnrs.fr*

Photopharmacology is an emerging discipline that makes use of photoswitchable ligands as pharmacological tool compounds to yield temporal and spatial control of protein function[1]. However, the development of photopharmacology to target G protein-coupled receptors (GPCRs) is still in its infancy, despite their high therapeutic relevance. Since 2014, several photoswitchable ligands have been reported for GPCRs. Although it is not always straightforward to discern contributions by affinity or potency shift from the contribution by any efficacy shift, most reported photoswitchable GPCR ligands display light-induced alterations in affinity or potency.
In order to obtain an improved optical control of GPCR function, we set out to develop small-molecule ligands with photoswitchable efficacy in which both configurations bind the target protein but exert distinct pharmacological effects, i.e., stimulate or antagonise GPCR activation. Our design was based on a previously identified efficacy hotspot for the peptidergic chemokine receptor CXCR3[2] and resulted in the synthesis and characterization of five new azobenzene-containing CXCR3 ligands.[3] G protein activation assay and real-time electrophysiology experiments demonstrated a photoswitching from antagonism to partial agonism and even to full agonism (VUF16216).[3] SAR evaluation suggests that the size and electron-donating properties of the inner aromatic ring substituents are important for the efficacy photoswitching. These azo compounds are the first GPCR ligands with a nearly full efficacy photoswitch that enable a reversible photoswitching from antagonism to agonism and thus may become valuable pharmacological tools for the optical control of peptidergic GPCR signaling.



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P14- Photoactivation of GPCRs

Eugene Grushevskyi[a, b], Ali Işbilir[a, b], Jan Möller[a, b], Katarina Nemec[a, b], Jean-Yves Tano[b], Ruth Pareja[b], Ulrike Zabel[a], Martin Lohse[a, b]

[a]*Institute of Pharmacology and Toxicology, University of Würzburg, Germany;* [b]*Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany; email: yevgenii.grushevskyi@mdc-berlin.de*

G-protein coupled receptors (GPCRs) bind neurotransmitters or hormones (e.g. glutamate, adrenaline), pheromones and drugs (e.g. trimethylamine and beta-blockers) at specific sites of a receptor. Binding triggers a conformational change in the receptor, which enables it to bind to and activate G-proteins.
We have developed optimized systems to monitor activation of GPCRs and downstream messengers using photoactivatable ligands. While combining them with widefield and confocal microscopy, we study where and when in a cell signal transduction occurs. We use aqueous solutions of caged and photoswitchable agonists to activate receptors by light, and to monitor signaling with sub-millisecond resolution.
Characterization of photoswitchable compounds designed for GPCRs could be also done in pharmacological assays with wildtype receptors. We measure G-protein activation and cAMP accumulation with up-to-date FRET- and BRET-based sensors. We are also working on techniques to adapt such measurements to high throughput screening in order to search for compounds with unusual signalling properties, which might make them new classes of drugs with only a subset of effects compared to conventional drugs.

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P15- Photoswitchable hydrophobic and amphiphilic peptides for membrane insertion studies

Gutiérrez-Salazar MV.[a], Sampedro D[b], Santamaría E [b]; Lórenz-Fonfría VA [a].

[a] *Instituto de Ciencia Molecular, Universitat de València, España.;* [b] *Centro de Investigación en Síntesis Química, Universidad de la Rioja, España. email: movicgu@uv.es*

Mechanics and kinetics of folding and insertion of hydrophobic and amphiphilic peptides in lipidic membranes is a process not fully understood. This is mainly due to the lack of sensitive experimental procedures that allow to follow the process with sufficient temporal and structural resolution[1]. A practical approach to achieve this sensitivity is photocontrol of folding, using molecular photoswitches linked to the peptide. These photoswitches isomerize when irradiated with light of specific wavelengths and can induce changes in the structure of the peptide, as folding or unfolding of the peptide and, consequently, modulate the insertion in the membrane[2,3]. This work aims to characterize two model peptides (hydrophobic and amphiphilic) linked to a derivate of the molecular photoswitch azobenzene, by circular dichroism, infrared and ultraviolet/visible spectroscopies, to use them in future folding and membrane insertion studies. We designed different lighting setups to achieve photoisomerization with precision and in controlled environment. We characterized the azobenzene derivative (BCA) and concluded that it is a suitable candidate for photoswitching. The photoswitchable peptides were characterized in membrane-mimicking environments and solvents, to know in which conditions their structure could be controlled with photoswitching. The hydrophobic peptide showed good photoisomerization of BCA, but poor control of structure in most solvents. We managed to photocontrol the structure of the amphiphilic peptide in solvents, and promising results were obtained in membrane environments. This work aims to be taken as a starting point for finding suitable photoswitchable membrane peptides.

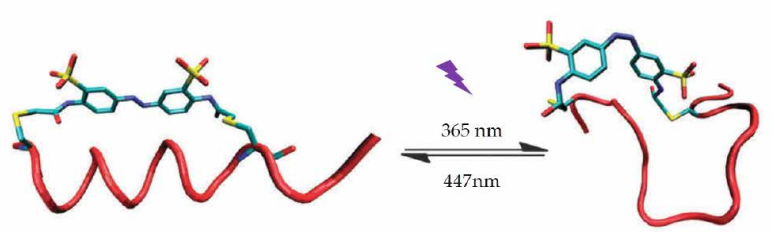

Figure 1. Molecular model of peptide cross-linked to a modified azobenzene[4]. Upon irradiation, azobenzene switches between cis and trans isomers, modifying the secondary structure of the peptide.

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P16- Synthesis and Pharmacological Evaluation of Photoswitchable Histamine H3 Receptor Agonists

N.J. Hauwert, T.A.M. Mocking, D. Da Costa Perreira, K. Lion, H.F. Vischer, I.J.P. de Esch, M. Wijtmans and R. Leurs

Division of Medicinal Chemistry, Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), Faculty of Sciences, VU University Amsterdam, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands. email: N.J.Hauwert@vu.nl

Histamine receptors are G protein-coupled receptors which are key mediators in a plethora of pathophysiological processes ranging from inflammation and itching to obesity. The family of histamine receptors consists of four receptors with moderate homology. The histamine H_3 receptor (H_3R) is highly expressed in the central nervous system (CNS). Its main function is the modulation of neurotransmitters release, acting on acetylcholine, GABA, dopamine, serotonin, and noradrenaline. It also modulates the release of histamine and has therefore an auto-regulatory function.[1] H_3R has been identified as a potential target in diseases such as obesity, narcolepsy, Alzheimer's and ADHD.[1] A wide range of ligands with diverse chemotypes have been published, but sofar only one molecule targeting the H_3R has reached the market having narcolepsy as its disease indication.[2]

Despite these advances, the pharmacology of H_3R signaling is still poorly understood and molecules enabling temporal and spatial control of signaling would be desirable. Therefore we aim to discover molecules which have the ability to switch under influence of light from an inactive to an active isomer. Using azobenzene-containing ligands, this aimed isomerization may be achieved using illumination at specific wavelengths. A variety of azobenzene-containing ligands has been synthesized, and photochemically and pharmacologically characterized in our labs. We have identified new ligands which are able to fully activate H_3R, that have over 20-fold differences in H_3R pEC_{50} between *trans* and *cis* isomers.

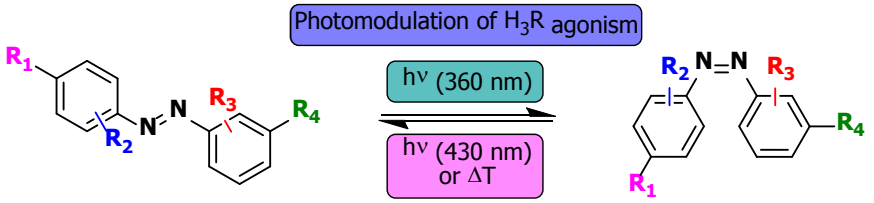

Figure 1. Characteristics of a photoswitchable H_3R agonist

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P17- Axitinib – A Photoswitchable Approved Kinase Inhibitor

Linda Heintze[a], Theo Rodat[a], Dorian Schmidt[a], Jantje Weber[a], Rebecca Horbert[a], Lara Bußmann[b], Malte Kriegs[b] and Christian Peifer[a,*]

[a]*Institute of Pharmacy, University of Kiel, Gutenbergstraße 76, 24116 Kiel, Germany;*
[b]*Laboratory of Radiobiology & Experimental Radiooncology and UCCH Kinomics Core Facility, University Medical Center Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg, Germany;*
email: lheintze@pharmazie.uni-kiel.de, trodat@pharmazie.uni-kiel.de

Kinases have become important drug targets due to their essential role in the regulation of signal transduction cascades in cells and the association of dysfunctions with several diseases, especially cancer. However, treatment with kinase inhibitors often leads to severe side effects or the emergence of resistances. Photopharmacology can help to reduce these adverse effects by spatially and temporally controlled drug activation mediated by irradiation. Here, we investigated the photoinduced *E/Z* isomerization of the approved kinase inhibitor axitinib to explore if its inhibitory effect can be turned "on" and "off", triggered by light.

Figure 1. (Right): (*E*)-axitinib in the ATP binding pocket of VEGFR2 (pdb code 4AG8). (Left): Superposition with (*Z*)-axitinib (steric clashes with the protein are indicated as red dotted lines).

Axitinib targets tyrosine kinases including the vascular endothelial growth factor receptor 2 (VEGFR2) and is licensed for second-line therapy of renal cell carcinoma. Interestingly, axitinib contains a stilbene-like double bond allowing for *EZ* isomerization. Under controlled light conditions, we could demonstrate that the isolated (*Z*)isomer is 43 times less active compared to (*E*)axitinib in an *in vitro* VEGFR2 kinase assays. In addition, proliferation of human umbilical cells (HUVECs) was inhibited 31 times less by (*Z*)axitinib compared to the (*E*)-isomer. By irradiating (*Z*)axitinib *in vitro* with UV light (385 nm) it is possible to switch it almost quantitatively to the (*E*)-isomer and to completely restore the biological activity of (*E*)axitinib. However, *vice versa* switching the biological activity "off" from (*E*)- to (*Z*)-axitinib was not possible in aqueous solution due to a competing irreversible [2+2]-photocycloaddition yielding a biologically inactive axitinib-dimer.

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D. Schmidt, T. Rodat *et al., ChemMedChem* **2018**, ASAP, doi: 10.1002/cmdc.201800531.

Acknowledgement

This research project was supported by DFG (German Research Society) grant Collaborative Research Centre 677 "*Function by Switching*".

P18- Photo-controlled inhibition of $BRAF_{V600E}$

Mark W.H. Hoorens[a,b], Petra E. Ettema[c], Ben L. Feringa[b], Frank J. Dekker[c], Wiktor Szymanski[a,b]

[a]*Department of Radiology,University of Groningen University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands.*
[b]*Centre for Systems Chemistry, Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands.*
[c]*Department of Pharmaceutical Gene Modulation, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands.*
email: m.w.h.hoorens@rug.nl

Of all melanomas, approximately 40 to 50% harbors the V600E mutation in the B-RAF kinase, which drives the formation of cancer through the RAS/RAF/MEK/ERK pathway [1]. Inhibition of this pathway by $BRAF_{V600E}$ inhibitor Vemurafenib has been successfully used in the treatment of melanomas, however – paradoxally - inhibition of wtBRAF in healthy tissue drives the formation of new cancers through a not fully elucidated mechanism [2,3]. Here we show the design, synthesis and evaluation of $BRAF_{V600E}$ kinase inhibitors with light-controlled activity, which would ultimately facilitates local treatment [4] of melanoma and form a strong research tool for mechanistic studies [5] of the BRAFV600E kinase and associated proteins in the RAS/RAF/MEK/ERK pathway.

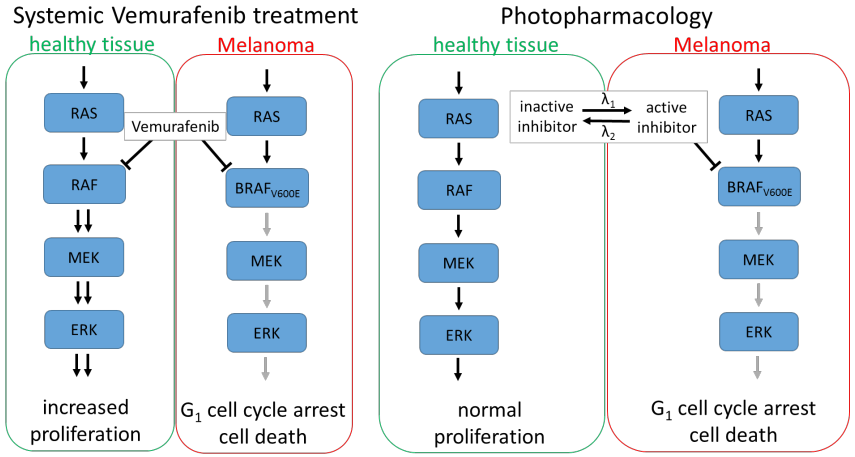

Figure 1. Systemic treatment of melanoma by $BRAF_{V600E}$ inhibitor Vemurafenib and the proposed photopharmacology approach in which the $BRAF_{V600E}$ inhibitor is only active in melanoma tissue and inactive in healthy tissue.

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P19- Vision Restoration Using Photopharmacology Reduces Rhythmic Field Potentials in Blind Mouse Retina

Katharina Hüll,[1,2] Tyler Benster,[3] Dirk Trauner,[1] Russell N. Van Gelder,[3] and Laura Laprell[2,3,4]

[1] *New York University, Department of Chemistry, New York, New York, USA; kh2785@nyu.edu*
[2] *Ludwig Maximilian University, Department of Chemistry, Munich, Germany*
[3] *University of Washington, Department of Biological Structure and Department of Pathology, School of Medicine, Seattle, Washington, USA*
[4] *Institute for Synaptic Physiology, Center for Molecular Neurobiology, Hamburg, Germany; laura.laprell@zmnh.uni-hamburg.de*

Vision is probably the most important sensual perception in humans. Its loss can be devastating. Various approaches to restore light sensitivity to the blind retina have been developed to date, including retinal implants, microchips, gene therapy and photopharmacology.[1–3] From a therapeutic point of view, photopharmacology provides a less invasive, but modular and adjustable approach for future patients. Using small molecule photoswitches enables optical control of ion channels, like sodium and potassium channels.[4,5] The azobenzene molecule we use, can be reversibly isomerized from a thermodynamically stable *trans* state to a thermodynamically unstable *cis* state with light.[6]
In degenerating retinae, local field potentials with a 5-10 Hz frequency can overlay with the artificially created signal, therefore decreasing the signal to noise ratio.[7] We have developed photoswitches which in the retina, either mainly target retinal ganglion cells or bipolar cells, depending on their molecular structure. Molecules targeting upstream of retinal ganglion cells significantly reduce local field potentials, resulting in an improved signal to noise ratio. Especially in the context of future therapeutic applications, suppressing these undesired oscillations is advantageous, and has not been described before.

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P20- Structure-based photopharmacology for smart antibiotics

Piermichele Kobauri[a], Nicole Galenkamp[b], Giovanni Maglia[b], Wiktor Szymanski[a,c], Ben L. Feringa[a]

[a]*Centre for Systems Chemistry, Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands*
[b]*Groningen Biomolecular Sciences & Biotechnology (GBB) Institute, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands*
[c]*Department of Radiology,University of Groningen University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands*
Email: p.kobauri@rug.nl

Antibiotic resistance is a serious threat to human health for which coordinated actions are required. Multidrug-resistant bacteria evolve at an alarming rate and our last-resort antibiotics are starting to be inadequate[1]. Photopharmacology[2] appears to be an intriguing approach to counteract this phenomenon: photo-switchable antiobitics[3] could allow to selectively target harmful bacteria as well as to avoid the build-up of resistance in the environment. Inspired by a recently published[4] propargyl-linked antifolates, a first prototype of a novel series of compounds targeting *E. coli* Dihydrofolate reductase (DHFR) was designed and synthesized. Changes in specific protein-ligand interactions were predicted through molecular docking and MM-GBSA calculations, and single-molecule enzymology studies were performed to investigate binding affinities and kinetics. The preliminary results are here presented and discussed.

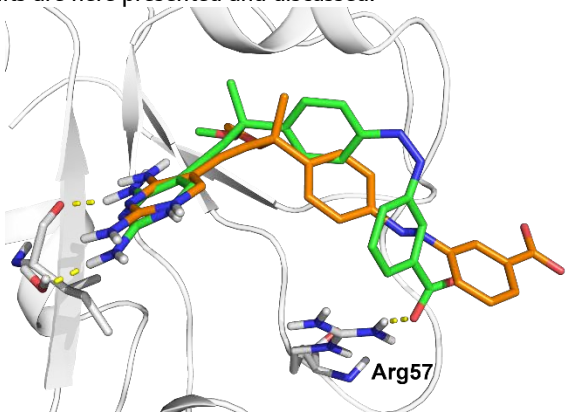

Figure 1. Docking poses of the two isomers of the photo-switchable antifolate. While the *trans* isomer sticks out of the binding pocket, the *cis* is able to form the desired salt bridge with Arg57.

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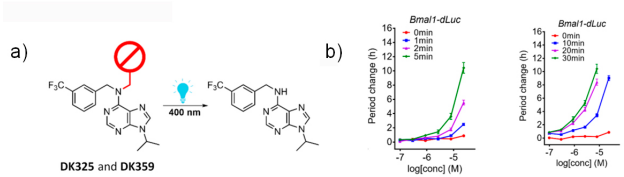
P21- Light Dosimetry: A Method for Conditional Adjustment of Circadian Period

Dušan Kolarski[a], Albert Schulte[a], Wiktor Szymanski[a,b], Ghisalin Breton[c], Kenichiro Itami[d], Tsuyoshi Hirota[d], and Ben L. Feringa[a]

[a] *Centre for Systems Chemistry, Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands*
[b] *Department of Radiology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands*
[c] *Department of Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, 6431 Fannin St, MSB 4.216, 77030 Houston, US*
[d] *Institute of Transformative Bio-Molecules (WPI-ITbM), Nagoya University, Chikusa, Nagoya 464-8602, Japan*

Inspired by the crucial role of circadian clock disruption in disease development,[1] during the last decade chemical biology studied how to adjust cellular clocks using small molecule modifiers[2]. Unfortunately, these modifiers are still facing a big drawback when in vivo application is needed: due to the similarity in cellular regulation of clocks, besides curing the disrupted biological rhythm they would affect all the others, healthy rhythms in other cells. To overcome this problem, a potential strategy would be photo-uncaging - based on the regulation of a compound`s bioactivity with light, which can be delivered precisely in space and time.[3]
Here, we show for the first time the possibility to lengthen the circadian period in mammalian cells, tissues, and zebrafish just by choosing an interval of visible light irradiation (400 nm) in order to release Longdaysin - a compound that exhibits a drastic effect on the circadian period lengthening in a variety of mammalian cells but also zebrafishes *in vivo*.[4]



Scheme 1. a) Photocleavage of the protected Longdaysin; b) correlation diagram of period lengthening, concentration, and light exposure time.

The kinetics of photo-deprotection was studied by UPLC-MS and NMR analysis. These results correlate well with CKIα and CKIδ inhibition, as well as with cellular, tissue, and zebrafish period change. The cellular time dosing was investigated by a cell-based luminescence assay using *Bmal1–d*Luc reporter U2OS cells, and employing visible light (400 nm). In the tissue assay, explants of spleen were dissected from m*Per2*^{Luc} knockin mice and used to follow period change. And as an *in vivo* model, the *per3*-luc transgenic zebrafish line was chosen to demonstrate a correlation between photocleavage and period lengthening.

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P22- Photochromic Peptidic NPY Y_4-Receptor Ligands

D.Lachmann[1], A. Konieczny[2], M. Skiba[3], J. Wegener[3], A. Buschauer[2], B. König[1]

University of Regensburg,[1]Insitute of Organic Chemistry, [2]Institute of Analytical Chemistry, [3]Institute of Pharmacy, Universitätsstr. 31, 93053 Regensburg; email: burkhard.koenig@ur.de

Recently, high potent peptidic Neuropeptide-Y (NPY) receptor agonists were synthesized which comprise two pentapeptides connected by an aliphatic linker.[1] The linker moiety was replaced by four different photochromic scaffolds: Dithienylethene (DTE), fulgimide, azobenzene and an azopyrazole (Figure 1). The different changes in flexibility, rigidity and geometry of the photochromic peptides should affect the efficacy and were studied in different functional assays (G-protein activation proximal and distal; ß-arrestin-assays; impedance measurements).[2] Those photoresponsive peptides can be controlled by light and could support the investigation of the receptors function more precisely.

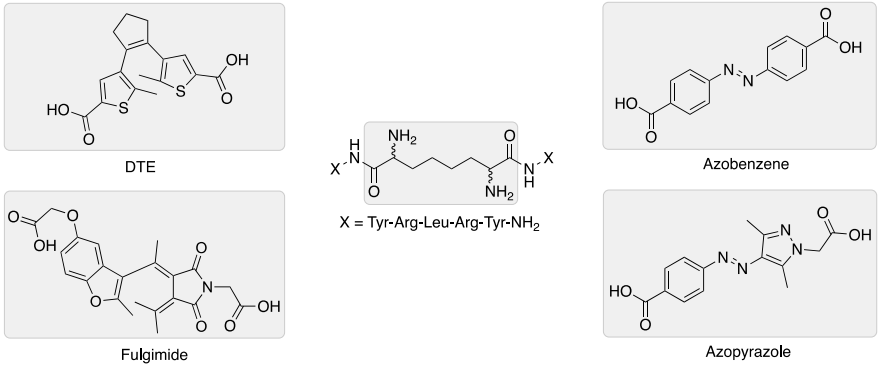

Figure 1. Photochromic scaffolds replacing an aliphatic linker.

As there is a high demand for potent Y_4R-antagonists, relating to dimeric peptidic NPY Y_4R-ligands, it is known that only slightly structural modifications are necessary to change the efficacy from agonism to antagonism.[1]

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P23- Covalent Photoswitchable Ligands to Investigate G Protein Coupled Receptor Dynamics

Ranit Lahmy[a], Daniel Lachmann[a], Jonas Kaindl[b], Harald Hübner[b], Peter Gmeiner[b], and Burkhard König[a]

[a]*Institute of Organic Chemistry, University of Regensburg, 93053 Regensburg, Germany;* [b]*Department of Chemistry and Pharmacy, Friedrich Alexander University Erlangen-Nürnburg, 91058 Erlangen, Germany.*
Email: burkhard.koenig@chemie.uni-regensburg.de

There is a current need to further understand the kinetics and dynamics of G Protein Coupled Receptors (GPCRs) for novel modes of disease treatment. Enhanced tools are required to facilitate these investigations.[1] Photoswitchable ligands exemplify these advanced tools due to their ability to be activated and deactivated when desired.[2] Generation of covalent photoswitchable ligands may enhance these mechanistic investigations.[3] In this developing project, covalent photoswitchable ligands that target the clinically significant μ-opioid receptor will be designed and synthesized. Following generation, affinity and activation screens of the photoswitchable ligands will be carried out using radioligand-based binding assays and beta-arrestin recruitment assays, respectively. Covalent binding will be evaluated, as the ability of ligands to covalently bind their cognate receptor is central to this investigation. In order to attain dynamic information on receptor interactions, Single Molecule Fluorescence Spectroscopy will be employed. The development of novel photoswitchable ligands will expand the biological 'toolbox' to study the functional significance of GPCRs.

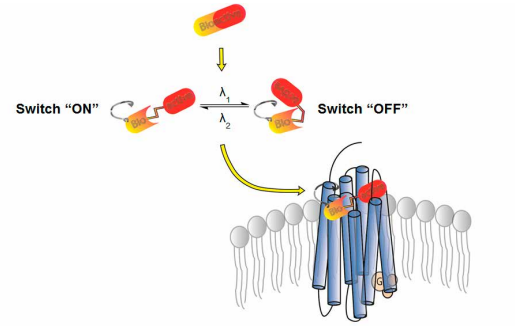

Figure 1. Concept of covalent GPCR photoswitchable ligands. A photoswitch and a covalent moiety can be inserted into a bioactive ligand. Upon exposure to light, the photoswitchable functionality (i.e. azobenzene) undergoes a reversible change in structure and properties. This may result in a change in GPCR receptor affinity, resulting in compounds that have a biologically active and inactive state. The covalent nature of these ligands ensures their localization to the receptor binding site.

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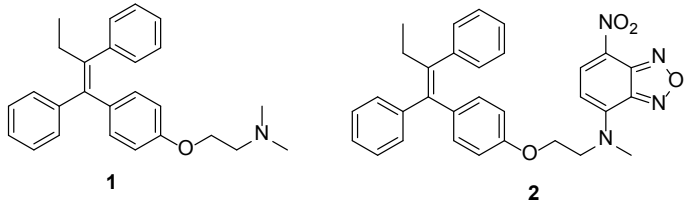
P24- Fluorescent Tamoxifen derivatives. From fluorescent probes to phototerapeutical agents.

Fernando Lobo,[a-c] Dácil Hernández,[a,b] Laura E.Scholz,[b,d] Alicia Boto,[a,b] Raquel Marín,[b,d] Jorge Marrero-Alonso,[b,d] Angel Amesty,[d] Ana Estévez,[d] David Quinto-Alemany,[d] Ricardo Puertas,[d] Fernando Lahoz,[b,d]* and Mario Díaz[b,d] *

[a] *Instituto de Productos Naturales y Agrobiología del CSIC, Avda. Astrofisico Fco. Sánchez 3, 38202, La Laguna, Tenerife, Spain.* [b]*Unidad Asociada ULL-CSIC "Fisiología y Biofísica de la Membrana Celular en Enfermedades Neurodegenetarivas y Tumorales"* [c]*Biosigma S.L. 38003 Santa Cruz de Tenerife, Tenerife, Spain.* [d]*Universidad de La Laguna. Avda. Astrofisico Fco. Sánchez s/n, 38200, La Laguna, Tenerife, Spain*

Tamoxifen (**1**) is a widely used as coadjutant therapy against breast cancer due to its activity as Selective Estrogen Receptor Modulator (SERM); although it presents several side effects as womb cancer, endometrial cancer or thromboembolism. A fluorescent derivative of tamoxifen, FLTX-1 (**2**), has been prepared and used as fluorescent probe, giving evidence of its interaction with molecular targets other than the estrogen receptor.

FLTX-1 displayed a pharmacological profile similar to tamoxifen, but lacked most of the secondary effects of the commercial drug [1,2]. FLTX-1 also presented interesting optical properties, behaving as a laser dye under different conditions [3-5]. Remarkably, it also displayed some activity as photosensitizer, generating the highly reactive specie $^1O_2$ (ROS) when irradiated at 425 nm. Due to this photoactivity FLTX-1 is a promising phototherapy drug candidate.



Tamoxifen (1) and FLTX-1 (2)

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P25- Caracterization of the seconday photocycle responsible for the desensitization of channelrhodopsin-2

M. Saita,[a] F. Pranga-Sellnau,[a] T. Resler,[a] R. Schlesinger,[a] J. Heberle,[a] V.A. Lorenz-Fonfria[b]
[a]*Freie Universität Berlin, Germnay;* [b]*Universitat de València, Spain; email: victor.lorenz@uv.es*

Channelrhodopsins (ChRs) are light-gated ion channels containing a retinal as a chromophore, predominantly in all-*trans* conformation. Amoung them, ChR2 from *C. reinhardtii* (*Cr*ChR2) has been widely used to activate neurons with light.[1] But the photocurrents of *Cr*ChR2 can be notably reduced (desensitized) under continuous and repetitive illumination, challenging its applicability.[1]
Here, we report on recent time-resolved UV-Vis experiments on *Cr*ChR2.[2] We observed and characterized a secondary photocycle that originates from photoexcitation of the P_4^{480} intermediate, a late intermediate present in the primary all-*trans* photocycle. We conclude that the photochemical and functional properties of the P_4^{480} photocycle explain the desensitization of the photocurrents of CrChR2 under continuous and repetitive illumination.

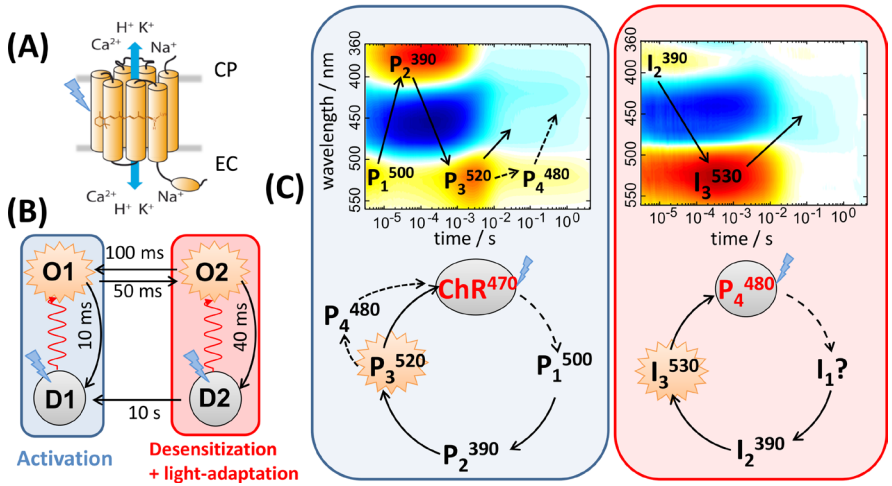

Figure 1. (A) *Cr*ChR2 is a light-gated cation channel. (B) The photocurrents of *Cr*ChR2 under continuous illumination are explained by two photocycles, with the secondary photocycle accounting for desensitization and ligh-adaptation of the channel. (C) We have characterized these two photocycles by time-resolved UV/vis spectroscopy.

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P26- Photoactivable and photoswitchable ligands to explore the function of metabotropic glutamate receptors

Fanny Malhaire [a], Charleine Zussy [a], Joan Font [b], Xavier Gómez-Santacana [b], Xavier Rovira [a], Marc López-Cano [c,d], Serena Notartomaso [e], Jesús Giraldo [f], Giuseppe Battaglia [e], Ferdinando Nicoletti [e,g], Francisco Ciruela [3,4], Jean-Philippe Pin [a], Amadeu Llebaria [b], Cyril Goudet [a]

[a] *IGF, CNRS, INSERM, Univ. de Montpellier, Montpellier, F-34094 Montpellier, France;* [b] *Laboratory of Medicinal Chemistry, Institute for Advanced Chemistry of Catalonia (IQAC-CSIC), Barcelona, Spain;* [c] *Departament de Patologia i Terapeutica Experimental, Facultat de Medicina i Ciencies de la Salut, IDIBELL, Universitat de Barcelona, Barcelona, Spain;* [d] *Institut de Neurociencies, Universitat de Barcelona, Barcelona, Spain;* [e] *I.R.C.C.S. Neuromed, Pozzilli, Italy;* [f] *Laboratory of Molecular Neuropharmacology and Bioinformatics, Institut de Neurociències and Unitat de Bioestadística, Universitat Autònoma de Barcelona, Bellaterra, Spain;* [g] *Department of Physiology and Pharmacology, University Sapienza, Rome, Italy; email: fanny.malhaire@igf.cnrs.fr*

Photopharmacology is a novel technique to control by light the function of endogenous receptors in their native environment with high spatial and temporal precision. Contrary to optogenetics which uses exogenous expression of light-sensitive channels to take control of specific neurons, the control of endogenous targets is achieved through the use of small diffusible, drug-like, photo-regulated ligands. Two different types of photo-regulated drugs can be discerned: i) inactive photo-caged ligands that can be turned ON by light thus allowing the precise onset of drug activity and ii) photo-switchable ligands containing an azobenzene moiety that can reversibly photo-isomerize thus allowing to rapidly turned ON and OFF drug activity by light. Metabotropic glutamate receptors 5 (mGlu5) are class C G protein-coupled receptors activated by glutamate, the major excitatory neurotransmitter of the central nervous system (CNS). These receptors are involved in the modulation of synaptic activity and are considered as potential therapeutic targets for many CNS disorders, such as chronic pain. Here we present two light-regulated negative allosteric modulators (NAM) that can control the activity of mGlu5 both in vitro and in vivo in the brain of freely moving animals. First, JF-NP-26 is an inactive photo-caged derivative of the mGlu5 NAM raseglurant. Violet light illumination of JF-NP-26 induces a photochemical reaction prompting the active-drug's release, which effectively controls mGlu5 activity in HEK293 cells expressing the receptor. Systemic administration in mice followed by local illumination in peripheral tissues or in the thalamus induced JF-NP-26-mediated light-dependent analgesia in inflammatory or neuropathic pain models. Second, Alloswitch-1 is an mGlu5 NAM containing an azobenzene. Illumination by violet or green light induces a reversible photo-isomerization of the molecule, inducing a change of activity of the ligand on HEK293 cells expressing the mGlu5 receptor. Local injection of Alloswitch-1 in the amygdala induced analgesia that can be dynamically controlled by light in an inflammatory pain model. These results are illustrating that light-operated drugs constitute powerful tools to manipulate and explore the function and therapeutic potential of endogenous receptors in living animals.

P27- COUPY coumarins as privileged scaffolds for the development of novel ligand-targeted imaging agents and caging groups

Anna Rovira[a], Albert Gandioso[a], Alex Galindo[a], Marta López[a], Manel Bosch[b], Vicente Marchán[a]

[a]*Department of Inorganic and Organic Chemistry, IBUB, University of Barcelona, Martí i Franquès 1-11, E-08028 Barcelona (Spain);* [b]*Unitat de Microscòpia Òptica Avançada, CCiTUB, University of Barcelona; email: vmarchan@ub.edu*

In recent years, we are witnessing a resurgence in the use of fluorophores based on organic molecules in advanced biological imaging techniques.[1] Current research efforts are focused on (i) the development of cell-permeable, low molecular-weight fluorescent dyes operating in the optical window of the tissues, and (ii) fine-tuning and improving their photophysical and physicochemical properties, which are all together required for *in vivo* applications such as fluorescence-guided surgery. Very recently, we have developed a new family of coumarin-based fluorophores, nicknamed COUPYs, with many attractive features such as large Stokes' shifts, brightness, high photostability and emission in the far-red/NIR region, as well as aqueous solubility and good cell permeability.[2] Owing to their privileged structure, COUPY scaffolds can be easily transformed in suitable conjugatable derivatives for labelling targeting ligands or metal-based anticancer drugs, as well as in novel caging groups. We will discuss our recent achievements on these topics.

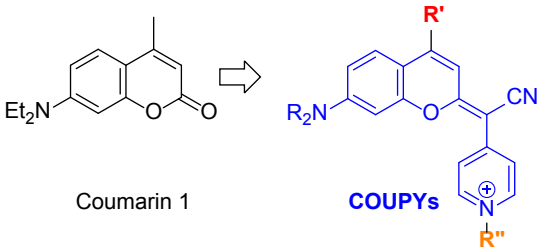

Figure 1. Schematic representation of the general structure of COUPY scaffolds.

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Acknowledgements

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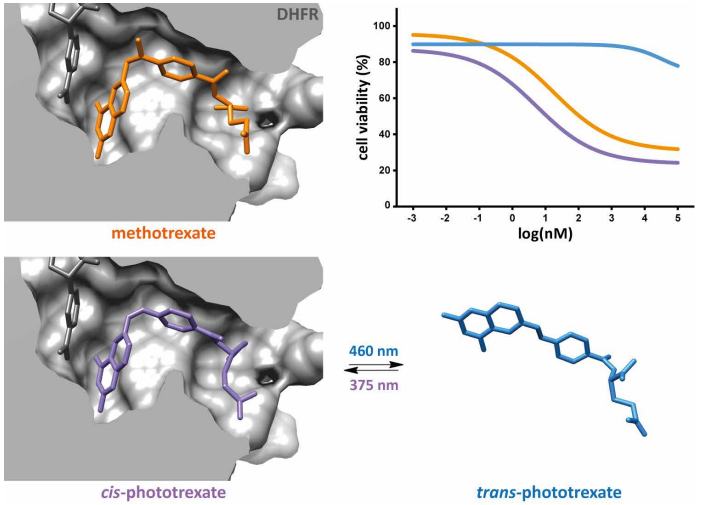
P28- A photoswitchable antimetabolite for targeted photoactivated chemotherapy

Carlo Matera[†,§], Alexandre Gomila-Juaneda[†,§,‡], Núria Camarero[†,§,‡], Michela Libergoli[†], Concepció Soler[#], and Pau Gorostiza[†,§,¶,*]

[†]*Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute for Science and Technology (BIST);* [§]*Biomedical Research Networking Center in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN);* [#]*Departament de Patologia i Terapèutica Experimental, Facultat de Medicina i Ciències de la Salut, IDIBELL, Universitat de Barcelona;* [¶]*Catalan Institution for Research and Advanced Studies (ICREA); email: pau@icrea.cat*

The efficacy and tolerability of systemically administered anticancer agents are limited by their off-target effects. Precise spatiotemporal control over their cytotoxic activity would allow improving chemotherapy treatments, and light-regulated drugs are well suited to this purpose. We have developed phototrexate, the first photoswitchable inhibitor of the human dihydrofolate reductase (DHFR), as a photochromic analog of methotrexate, a widely prescribed chemotherapeutic drug to treat cancer and psoriasis. Quantification of the light-regulated DHFR enzymatic activity, cell proliferation, and in vivo effects in zebrafish show that phototrexate behaves as a potent antifolate in its photoactivated cis configuration, and that it is nearly inactive in its dark-relaxed trans form. Thus, phototrexate constitutes a proof-of-concept to design light-regulated cytotoxic small molecules, and a step forward to develop targeted anticancer photochemotherapies with localized efficacy and reduced adverse effects.[1]



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P29- Characterization of photoswitchable histamine H_3 receptor antagonists VUF14738 and VUF14862

Tamara Mocking[a], Niels Hauwert[a], Albert Kooistra[a], Marek Staszewski[b], Yara Huppelschoten[a], Chris de Graaf[a], Krysztof Walczyński[b], Maikel Wijtmans[a], Henry Vischer[a], Rob Leurs[a]

[a]*Department of Medicinal Chemisty, Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), Vrije Universiteit Amsterdam, The Netherlands.*
[b]*Department of Synthesis and Technology of Drugs, Medical University of Łódź, ul. Muszyńskiego 1, 90-145 Łódź, Poland.*
Email: t.a.m.mocking@vu.nl

Temporal and spatial control of drug effect can be induced utilizing photoswitchable ligands. Photoswitchable ligands can reversibly photoisomerize from the *trans* to *cis* isomer upon illumination with specific wavelengths. This conformational change in the ligand may result in a distinct binding affinity for the receptor. The histamine H_3 receptor (H_3R) is a class A G protein-coupled receptor (GPCR), which is involved in the release of neurotransmitters in the central nervous system. In 2016, the first human H_3R antagonist Wakix® has been approved by the European Medicine Agency for the treatment of narcolepsy. Recently, VUF14738 and VUF14862 have been developed as photoswitchable H_3R antagonists that display over 10-fold increase or decrease in binding affinity, respectively, upon photoisomerization[1]. In this study, photoswitchable antagonists VUF14738 and VUF14862 were further functionally evaluated in human H_3R-expressing HEK239T cells reporter gene and real-time guinea pig ileum contraction assays. Furthermore, following molecular docking of both ligands in the homology model of the human H_3R, the binding mode of these photoswitchable ligands at the H_3R has been characterized by site-directed mutagenesis.

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P30- Adhesive Photoswitch for Modulation of Protein Functions

Rina Mogaki, Kou Okuro, and Takuzo Aida

Department of Chemistry and Biotechnology, School of Engineering, The University of Tokyo;; email: mogaki@macro.t.u-tokyo.ac.jp

In situ modulation of biomolecular functions by light is an important issue that leads to the elucidation of biological events and the treatment of diseases with fewer side effects. An effective approach is to incorporate photoresponsive moieties to the target biomolecule. However, the applicability of this approach is limited due to the requirement of a proper functional group for covalent tethering, which is mostly incorporated by genetic mutation. We have developed "molecular glues"[1–12] that bear multiple guanidinium ion (Gu^+) pendants and therefore tightly adhere to various biomacromolecular surfaces by forming salt bridges with oxyanions (Figure 1). With an aim to develop a universal method to modulate biomolecular functions without genetic engineering, we designed an "adhesive photoswitch", a molecular glue conjugated with an inhibitor for the target enzyme via a photoresponsive spacer. The adhesive photoswitch selectively adheres to the target wild-type enzyme and can reversibly modulate the enzyme activity (Figure 1). The molecular design strategy and the application of this adhesive photoswitch will be discussed.

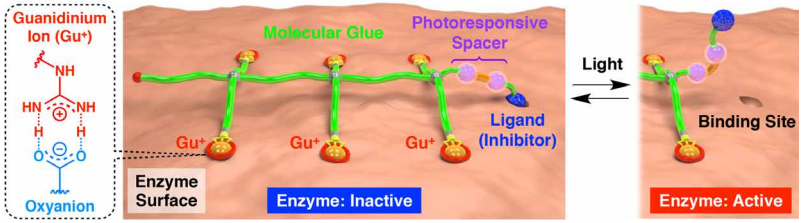

Figure 1. Modulation of protein functions with an adhesive photowitch bearing an inhibitor moiety for the target enzyme.

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P31- Photoswitchable lipids for the optical control of lipid signaling and metabolism

Johannes Morstein, Alexander J. E. Novak, Rose Hill, James A. Frank, Prashant Donthamsetti, Ehud Y. Isacoff, Diana Bautista, Dirk Trauner

Functionalized lipids are useful tools to interrogate lipid function in biological processes.[1] We have recently added photoswitchable lipids to this toolset, which allow for the control of lipid function with the spatiotemporal resolution of light. In two recent studies, we demonstrated optical control of TRPV1 channels through photoswitchable fatty acids, termed FAAzos[2] and optical control of PKC (and Munc13) through photoswitchable diacylglycerols, termed PhoDAGs.[3] Herein, we present the expansion of this approach to a series of lysophospholipids, including sphingosine and sphingosine-1-phosphate (S1P). We present efficient synthetic routes toward the respective photoswitchable analogs and demonstrate reversible control of molecular targets both in the signaling and the metabolism of these lipids.

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P32- Study of platelets activation dynamics using caged epinephrine and arachdonic acid

Alexander E. Moskalensky[a,c], Darya V. Spiryova[a] and Alexei Yu. Vorob'ev[a,b]

[a] *Novosibirsk State University, Novosibirsk, Russia;* [b] *N.N. Vorozhtsov Novosibirsk Institute of Organic Chemistry SB RAS, Novosibirsk, Russia;* [c] *Voevodsky Institute of Chemical Kinetics and Combustion SB RAS, Novosibirsk, Russia; email: a.mosk@nsu.ru*

Platelet activation is a major process in haemostasis, and also contributes to tumor growth, cancer metastasis and cardiovascular disease. We aim to measure dynamics of activation in the early stage, which can give novel information for diagnostics and therapeutics. Platelets were activated *in vitro,* and the activation was accessed by monitoring intracellular calcium. Precision of stimulation in time is very important for dynamic measurements; therefore we used "caged" agonists, which were specially developed (Figure 1).

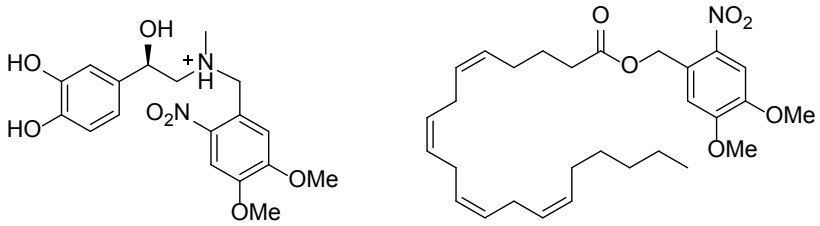

Figure 1. Compounds used for optically triggered platelet activation.

These compounds allowed us to activate platelets by a pulse of near-UV radiation (365 nm). Caged arachidonic acid showed faster decomposition and more efficient activation of platelets.

Acknowledgements
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P33- Photoswitchable allosteric ligands to decipher spatial and temporal mGluR$_1$ signaling

Silvia Panarello[a], Yevgenii Grushevskyi[b], Martin J. Lohse[b] and Amadeu Llebaria[a]

[a]*MCS, Laboratory of Medicinal Chemistry & Synthesis, Institute for Advanced Chemistry of Catalonia (IQAC-CSIC), Barcelona, Spain; emails: silvia.panarello@iqac.csic.es, amadeu.llebaria@iqac.csic.es;*
[b]*Signaling processes of Receptors Lab, Max-Delbrück-Center for Molecular Medicine, Berlin, Germany; email: Yevgenii.Grushevskyi@mdc-berlin.de, Martin.Lohse@mdc-berlin.de*

G protein-coupled receptors (GPCRs) are a large family of approximately 800 members, which include receptors for light, taste and smell, but also many receptors for transmitters and hormones. They are also the most important group of targets for drugs. The spatial and temporal aspects of GPCR activation mechanisms are a subject of huge interest[1]. The paradigm of GPCR signaling involves activation of heterotrimeric G proteins (Gαbγ). Although much progress has been made in understanding how Gα subunits interact with and regulate the activity of their downstream targets, it is not clear how activated GPCRs initiate this process by catalyzing nucleotide exchange on Gαbγ[2]. The molecular processes following the initial extracellular ligand binding to a cell membrane receptor, which induce an intracellular sequence of events, are limited by the lack of efficient tools to monitor the receptor activation kinetics. Classical GPCR molecular mechanisms models are based in conventional pharmacology assays (i.e. increases or decreases of second messenger concentrations, such as cAMP, cGMP, and inositol trisphosphate) that lack of an efficient spatiotemporal control of receptor dynamics, even though evidence for complex patterns of signaling has long been in existence[3].

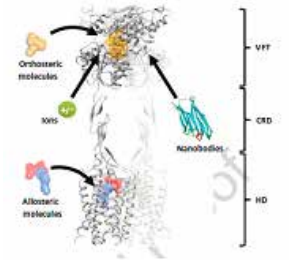

Figure 1. Complex structure of mGluRs offers different possibilities for modulation of their activity. Different types of ligands and their binding site locations are shown

Our objective is to obtain photoswitchable allosteric ligands for mGlu1, either PAMs or NAMs, to precisely control the activity of the receptor in a temporal axis. These molecular tools will enable to perform fluorescence conformational dynamic studies to understand fundamental questions regarding GPCR protein signaling after ligand binding.

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P34- Ultrafast Responsive Amphiphilic Azobenzene fo Light-controlling Cellular Membrane Potential

Giuseppe M. Paternò[a], Vito Vurro[a], Francesco Lodola[a], Mattia L. Di Francesco[b] Luca Maragliano[b], Letizia Colella[c], Cosimo D'Andrea[a,d], Chiara Bertarelli[c], Fabio Benfenati[b], Guglielmo Lanzani[a,d]

[a]*Center for Nano Science and Technology, Istituto Italiano di Tecnologia, Via Pascoli 10, 20133, Milano, Italy*
[b]*Center for Synaptic Neuroscience, Istituto Italiano di Tecnologia, Largo Rosanna Benzi 10, 16132 Genova, Italy*
[c] *Dipartimento di Chimica, Materiali e Ingegneria Chimica "Giulio Natta", Politecnico di Milano, Piazza L. da Vinci 32, 20133 Milano, Italy*
[d]*Dipartimento di Fisica, Politecnico di Milano, Piazza L.da Vinci 32, 20133 Milano, Italy*
email: giuseppe.paterno@iit.it

The precise, fast and efficient spatiotemporal control over cellular signalling via the use of external triggers has become an important topic in chemical biology[1,2] and photopharmacology[3]. In these regards, light responsive materials offer the possibility to employ light as a clean, non-invasive and spatiotemporally precise tool for controlling a variety of biological signals both in vitro and *in vivo*.
Here, we show that the affinity of an amphiphilic and sterically hindered azobenzene for membranes can be exploited to photomodulate reversibly the membrane potential in living cells using visible light. By means of state-of-the-art ultrafast spectroscopies, we demonstrate that our azobenzene molecule is photoresponsive in the ultrafast time regime (≈150 fs) despite its bulkiness, a feature that is necessary to perturbate the membrane environment. Our findings prove that simple non-covalent affinity of amphiphilic azobenzenes for membranes can be applied to modulate reversibly biological signals, and can be used to develop new membrane-specific optical triggers/probes.

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P35- Metabotropic glutamate receptor mGlu4 in the amygdala differentially inhibits sensory and affective component of chronic pain.

Vanessa Pereira[a], Charleine Zussy[a], Xavier Gomez-Santacana[a,b], Xavier Rovira[a,c], Fanny Malhaire[a], Jean-Philippe Pin[a], Amadeu Llebaria[b] and Cyril Goudet[a]

[a] *IGF, CNRS, INSERM, Univ. de Montpellier, Montpellier, F-34094 Montpellier, France*
[b] *MCS, Laboratory of Medicinal Chemistry, Institute for Advanced Chemistry of Catalonia (IQAC-CSIC), Barcelona, Spain*
[c] *Molecular Photopharmacology Research Group, The Tissue Repair and Regeneration Laboratory, University of Vic - Central University of Catalonia, C. de la Laura,13, 08500 Vic, Spain*
email: vanessa.pereira@igf.cnrs.fr

Pain is an essential protective mechanism meant to prevent tissue damages in organisms. Inflammation or nerve injury can instigate the transition to chronic pain. Chronic pain is long lasting, invalidating and provokes long-term disability for patients. Therefore, chronic pain and its management represents a major public health problem. Understanding chronic pain molecular mechanisms is critical to develop innovative and efficient therapies.
Number of evidences have demonstrated a pivotal role of glutamate in nociceptive transmission. Glutamate receptors are then promising potential target for drug development. Glutamate is the most abundant excitatory neurotransmitter in the brain. Once released, glutamate acts through ionotropic and metabotropic glutamate receptors (iGluRs and mGluRs). iGluRs are ligand-gated ion channels triggering fast excitatory neurotransmission. mGluRs are G protein-coupled receptors modulating synaptic transmission.
Our work focuses on the role of mGlu4 in both sensory and affective components of pain. Using intra-amygdala delivery of optogluram, a photoswitchable PAM of mGlu4, we demonstrated that mGlu4 in the amygdala drives both sensory and affective component of inflammatory pain. In order to better understand the relationship between these two components, we used a model of neuropathic pain induced by the chronic constriction of the sciatic nerve. We first demonstrated that unilateral mGlu4 activation in the amygdala relieves neuropathic pain. Secondly, our results suggest that antidepressive effect of mGlu4 is not subsequent to antinociceptive effect.

P36- Tapered optical fibers as multifunctional interfaces with the brain

F.Pisano[a], M.Pisanello[a], L.Sileo[a], A.Rizzo[a,b], E.Maglie[a,b], A.Balena[a,b], B.Spagnolo[a], R.Peixoto[c], B.L.Sabatini[c], F.Pisanello[a], M. De Vittorio[a,b]

[a]*CBN-Istituto Italiano di Tecnologia, Via Barsanti, 73010 Arnesano, Italy;* [b]*Dip. Ingegneria dell'Innovazione, Università del Salento, 73100 Lecce, Italy;* [c] *Dept.of Neurobiology,HHMI, HMS, Boston 02115 MA, USA*
corresponding author:filippo.pisano@iit.it

Light is playing an ever increasing role in neuroscience owing to the development of optogenetic techniques and the impact of genetically encoded activity indicators[1,2]. However, harnessing the full potential of these methods requires versatile tools that can be deployed in a broad range of experimental conditions. To this end, we propose tapered optical fibers as a robust and inexpensive platform to develop a novel class of multifunctional probes for research and, potentially, therapeutic use. TFs can dynamically deliver and collect light from large brain regions or multiple, spatially confined locations along the taper (Fig. 1a,b)[3,4]. At the same time, TFs can be structured exploiting micro- and nano- fabrication techniques to restrict the interaction volumes so to fit specific experimental needs or to include extracellular recording electrodes next to light delivery points (Fig. 1c)[5–7]. In perspective, nano-fabrication on the TFs highly curved surface holds promise towards the realization of photonic structures for the detection of neurotransmitters via Surface Enhanced Raman Spettroscopy(SERS).

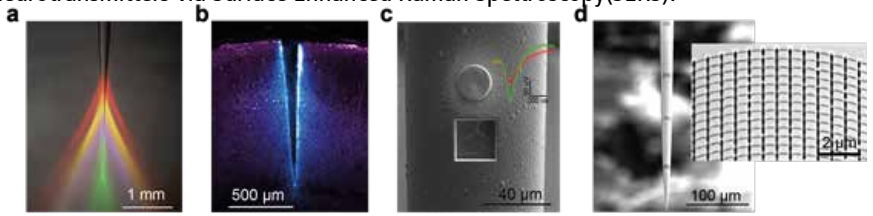

Figure 1. a) Multipoint illumination along the TF using mode division demultiplexing (false color overlay); b) light collection (cyan) from a tapered fiber inserted in a fixed brain slice imaged with 2-photon microscopy (magenta); c) extracellular electrode fabricated on the TF, next to a light delivery spot, *inset,* recordings from neural cells *in vivo*; d) multiple plasmonic structures fabricated along the taper surface coated with Au, *inset,* detail of the nanofabricated structure.

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P37- Optical control of clathrin-mediated endocytosis using Traffic Light peptides

Davia Prischich[a,b], Javier Encinar del Dedo[c], Andrés Martín-Quirós[a], Laura Nevola[d], Ernest Giralt[d], M. Isabel Geli[c], Pau Gorostiza[a,b,e]

[a] *Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain;* [b] *Centro de Investigación Biomédica en Red – Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Madrid, Spain;* [c] *Molecular Biology Institute of Barcelona (IBMB-CSIC), Barcelona, Spain;* [d] *Institute for Research in Biomedicine (IRB), Barcelona, Spain;* [e] *Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain; email: pau@icrea.cat*

Clathrin-mediated endocytosis (CME) is well known for being fundamental to a variety of physiological processes such as uptake of nutrients through the plasma membrane, signal transduction or neurotransmission. Nonetheless, the complex network of proteins involved in regulating this transient machinery makes it particularly hard to tackle only by means of genetic modification and immunological depletion. Although pharmacological tools can aid studying the dynamics of biological responses by acute inhibition or stimulation of the upstream processes, the freely diffusing nature of these molecules poses limits on the control of their activity. In this sense, photopharmacology offers a powerful tool to manipulate endogenous processes with high spatio-temporal resolution and in a non-invasive manner.

Traffic Lights (TLs) peptides were designed to be cell-permeable, photoswitchable inhibitors of the main adaptor complex in the CME machinery. Based on the structure of β-arrestin C-terminal peptide (BAP-long), they bind to the β-appendage of AP2 (β-adaptin), which mediates the binding of clathrin to the membrane or to cargo receptors. These peptides, named TL1 and TL2, proved capable of inhibiting CME in a light-regulated manner when tested in mammalian cells.[1,2]

Here we show that TL2 retain its activity in yeast, an extremely versatile eukaryotic model system. *S. cerevisiae* cells were deprived of the cell wall and the resulting spheroplasts were allowed to internalise the peptide. Subsequently, mutants expressing fluorescently tagged Sla1 - a coat-associated endocytic protein - were used to observe kinetic delays in the dynamics of vesicle formation. After having confirmed photoregulation of CME events by means of TL2, we now aim to achieve *in situ* activation of the peptide so to directly address the role of endocytosis in cellular processes such as cytokinesis or cell migration.

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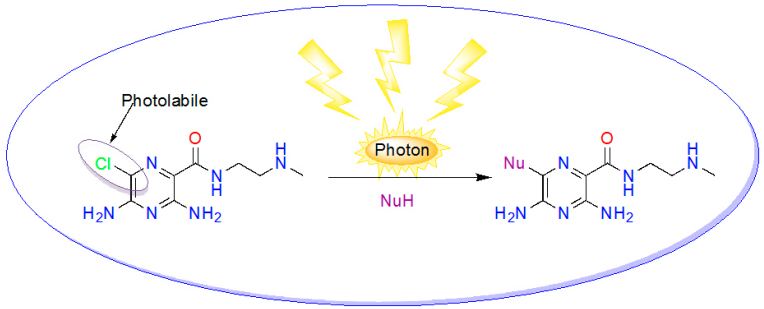
P38- Photomediated Nucleophilic Aromatic Substitution (S_NAr) Of Amiloride Derivatives

Wangchuk Rabten[1], Kjell Jorner[1], Tomáš Slanina[1], Per-Ola Norrby*[2], Henrik Ottosson*[1]

[1] *Department of Chemistry - Ångström Laboratory, Box 523, 751 20 Uppsala, Sweden.* [2]*Early Product Development, Pharmaceutical Sciences, IMED Biotech Unit, AstraZeneca, Pepparedsleden 1, SE-431 83 Mölndal, Sweden*

E-mail: Wangchuk.rabten@kemi.uu.se

Amiloride is potassium-sparing diuretic drug which is administered in combination with other diuretic drugs (e.g. hydrochlorothiazide) for the treatment of oedema and hypertension.[1] Amiloride absorbes UVA light strongly at 360 nm[1] thus, allowing to explore potential photochemical reaction. The photo-S_NAr reaction of amiloride could provide alternative methodologies to derivatize the parent molecule that could lead to potential drug candidates. The photo-S_NAr reaction has received much attention recently as an alternative to conventional transition metal cross-coupling reactions to form C-O, C-N, and C-C bonds.[2-3] It provides mild and versatile alternative methodologies to construct various chemical entities from readily available starting materials whilst retaining the fundamentals of green chemistry in addition to being cost-efficient.[4]



In this study, the amiloride derivative (3,5-diamino-6-chloro-N-(2-(methylamino)ethyl) pyrazine-2-carboxamide) was synthesized and evaluated under photochemical reactions. The photo-S_NAr reaction was carried out both at 254 nm and 365 nm in alcohols (MeOH, EtOH and *i*PrOH) and water. The clean photosolvolysis product in 90-97% conversion in 2 hours of irradiation was obtained in MeOH. Similarly, when water was used as the solvent high conversion was observed, however, the product decomposed after prolonged standing in solution. The reaction was closely evaluated computationally to understand further insight into the reaction mechanism by employing both multireference methods (e.g., CASPT2//CASSCF) and time-dependent density functional theory (TD-DFT).

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P39- An appraisal of the influence of the metabotropic glutamate 5 (mGlu5) receptor on sociability and anxiety

Arnau Ramos-Prats[a], Julia Kölldorfer[a], Elena Paolo[a], Maximilian Zeidler[a], Gabi Schmid[a] and Francesco Ferraguti[a]

[a]*Institute of Pharmacology, Medical University of Innsbruck, Innsbruck, Austria*
email: arnau.ramos@i-med.ac.at

Amongst the many neurotransmitter systems causally linked to the expression of social behavior, glutamate appears to play a pivotal role. In particular, metabotropic glutamate 5 (mGlu5) receptors have received much attention as its altered function has been reported in several mouse models of autism spectrum disorders and mental retardation. Inhibition of the activity of mGlu5 receptors by means of genetic or pharmacological manipulations improved social deficits in some of these animal models. However, in normal wild-type (WT) mice, pharmacological blockade of mGlu5 receptors yielded inconsistent results. The aim of our study was to investigate the actual contribution of decreased or absent mGlu5 receptor function in sociability and anxiety-like behavior as well as to explore the impact of mGlu5 receptor ablation on the pattern of brain activation upon social exposure. Here we show that *Grm5-/-* mice display higher social preference indexes compared to age-matched WT mice in the three-chambered social task. However, this effect was accompanied by a decreased exploratory activity during the test and increased anxiety-like behavior. Contrary to mGlu5 receptors ablation, the mGlu5 receptor negative allosteric modulator 3-((2-methyl-1,4-thiazolyl)ethynyl)pyridine (MTEP) induced anxiolytic effects without affecting social preference in WT mice. By mapping c-Fos expression in 21 different brain regions known to be involved in social interaction, we detected a specific activation of the prefrontal cortex and dorsolateral septum in *Grm5-/-* mice following social interaction. C-Fos expression correlation-based network and graph theoretical analyses further suggested dysfunctional connectivity and disruption of the functional brain network generated during social interaction in *Grm5-/-* mice. The lack of mGlu5 receptors resulted in profound rearrangements of the roles of prefrontal and hippocampal regions in the social interaction network.
In conclusion, this work reveals a complex contribution of mGlu5 receptors in sociability and anxiety and points to the importance of these receptors in regulating brain functional connectivity during social interaction.
Our current and future work will be aimed at elucidating the influence of hippocampal mGlu5 receptos in social behavior and anxiety by means of selective knockdown and photopharmacolgical modulation of this receptor.

Production of this booklet has been kindly supported by ESTEVE

P40- Development of light-responsive MRI contrast agents for imaging and theranostics

Friederike Reeßing[a,b], Marc C. A. Stuart[b], Rudi A.J.O. Dierckx[a], Ben L. Feringa[b], Wiktor Szymanski[a,b]

[a]*Department of Radiology, University, Medical Center Groningen, The Netherlands;* [b]*Stratingh Institute for Chemistry, University of Groningen, The Netherlands*
email: f.r.reessing@rug.nl

MRI is an outstanding anatomical imaging technique, due to its non-invasiveness and excellent resolution. However, the low sensitivity and limited vfunctional information obtained by MRI is a major drawback.[1-2] In order to address this problem, we aim to develop MRI contrast agents responsive to light, envisioning the use of light-emitting targeting moieties accumulating in the disease tissue. This strategy would lead to significant signal amplification and provide functional information.
Compound **1** (Figure 1) is a T1 MRI contrast agent, which - in its intact from - can be incorporated into liposomes. Photocleavage leads to the conversion of a macromolecular to a small, quickly tumbling contrast agent, causing a change in relaxivity.[3-5] The agent was synthesized *via* a Passerini multicomponent reaction (MCR), employing a precursor for the photoresponsive moiety as one of the reactants.[6] Subsequently, it was successfully incorporated into liposomes with DOPC, as confirmed by cryoTEM analysis. The selective accumulation of Gd^{3+} on liposomes was confirmed by EDX analysis. NMRD profile curves show about 40% decrease in T1 relaxation rate upon irradiation with light (λ= 400 nm), following the same kinetics as observed in the UV Vis analysis of the photocleavage. A permeation assay using calcein indicates the disintegration of liposomes upon irradiation[7] giving rise to the possibility of using the system in theranostics.[9-8]

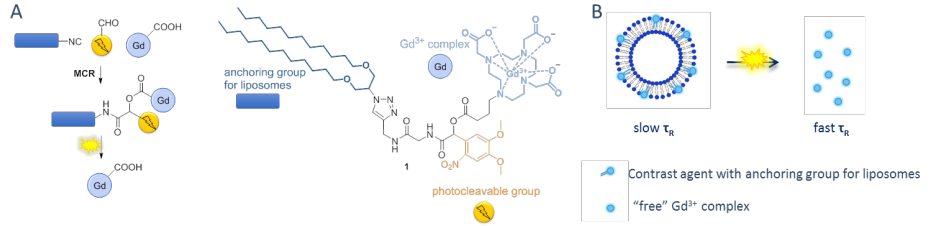

Figure 1: A) Synthetic strategy and structure of compound 1; B) Schematic representation of photoactivation.

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Acknowledgements

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P41- Structural Basis for the Optical Control of c-Jun N-terminal Kinase 3

Martin Reynders[a,b], Apirat Chaikuad[c], Katharina Bauer[d], Stefan Laufer[d], Stefan Knapp[c], Dirk Trauner[a]

[a]Current address: Department of Chemistry, Silver Center for Arts and Science, New York University, 100 Washington Square East, New York, NY 10003 (USA); [b]Department of Chemistry, Ludwig Maximilians University of Munich, Butenandtstr.5–13, 81377 Munich (Germany); [c]Institute for Molecular Life Sciences and Institute for Pharmaceutical Chemistry, Johann Wolfgang Goethe-University, 60438 Frankfurt am Main (Germany); [d]Department of Pharmaceutical / Medicinal Chemistry, Eberhard-Karls-University Tübingen, Auf der Morgenstelle 8, 72076 Tübingen (Germany); email: mr5185@nyu.edu, dirktrauner@nyu.edu

Kinase pharmacology of ATP-binding pocket inhibitors suffers from poor selectivity[1], increasingly addressed by covalent kinase inhibitors[2]. Optogenetics and photopharmacology offer an orthogonal approach to selectively target kinases. Here we present structural insight into optical control of kinases using synthetic small molecules. c-Jun N-terminal kinase 3 (JNK3), a key signaling enzyme in cellular stress response and promising target for the treatment of neurodegenerative diseases[3], is targeted by cyclic azobenzene based, photoswitchable inhibitors, thereby introducing the combination of covalent inhibition and photopharmacology. X-ray crystal structure determination of JNK3 resolving both azobenzene isomers provides insight into the underlying binding characteristics responsible for the kinase activity difference upon photoisomerization. This approach of enabling spatiotemporal control of JNK3 lays the foundation for other covalent inhibitors and kinases.

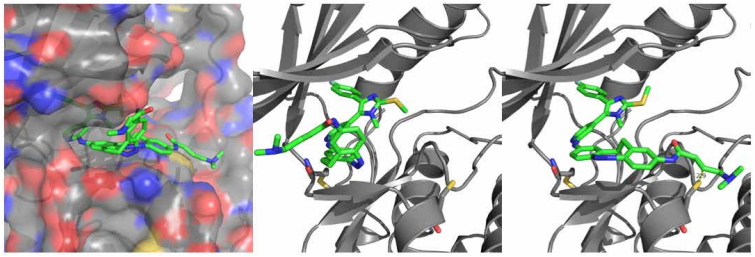

Figure 1. Crystal structure of MR-II-249 bound to JNK3.

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P42- A drug to photocontrol endogenous muscarinic acetylcholine receptors *in vivo*

Fabio Riefolo[a,b], Carlo Matera, PhD[a,b], Aida Garrido-Charles[a,b], Alexandre Gomila[a,b], Luca Agnetta[c], Enrique Claro, PhD[d], Roser Masgrau, PhD[d], Ulrike Holzgrabe, PhD[c], Montserrat Batlle, PhD[e], Michael Decker, PhD[c], Eduard Guasch, MD, PhD[e,f], and Pau Gorostiza, PhD[a,b,g,*]

[a]Institute for Bioengineering of Catalonia (IBEC), Barcelona Institute for Science and Technology (BIST); [b]Network Biomedical Research Center in Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN); [c]Pharmaceutical and Medicinal chemistry, Institute of Pharmacy and Food Chemistry, Julius Maximilian University of Würzburg, Am Hubland, D-97074 Würzburg, Germany; [d]Institut de Neurociències (INc), and, Dept. Bioquímica i Biologia Molecular, Unitat de Bioquímica de Medicina, Universitat Autònoma de Barcelona (UAB); [e]Cardiovascular Institute, Hospital Clinic; University of Barcelona (UB); IDIBAPS; [f]Network Biomedical Research Center in Cardiovascular Diseases (CIBER-CV); [g]Catalan Institution for Research and Advanced Studies (ICREA); email: pau@icrea.cat

Muscarinic acetylcholine receptors (mAChRs) are class A GPCRs involved in the control of numerous central and peripheral physiological responses. The high sequence homology of the different subtypes (M1–M5) in the transmembrane region hampers the development of subtype selective orthosteric agonists. On the other hand, the allosteric site, located in the extracellular loop, is less conserved, thus muscarinic allosteric agents are commonly endowed with a more pronounced subtype-selectivity. "Dualsteric ligands" are a new strategy for the selective modulation of mAChRs: they can bind simultaneously to both the orthosteric and the allosteric sites of such receptors, incorporating a) iperoxo, an oxotremorine-related unselective orthosteric superagonist, b) a polymethylene spacer, and c) a moiety targeting the allosteric site.[1] Inspired by this strategy, in the course of our ongoing development of photoswitchable ligands for the optical control of (neuro)biological functions,[2] we designed and synthesized a new set of light-regulated muscarinic bitopic ligands by replacing the polymethylene spacer chain with an azobenzene linker to serve as molecular photoswitch. This modification enabled the remote control of the mutual position between the two pharmacophoric moieties with light, thus potentially modulating affinity and efficacy of our compounds as a function of their photoisomerization state. One of the ligands turned out to be a potent activator of M2 receptors in the dark or under illumination with visible light (*trans* isomer), but inactive under UV illumination (*cis* isomer). We have evaluated *in vitro* photoresponses using a calcium imaging assay in genetically unmodified receptors overexpressed in mammalian cells. As stimulation of M2 mAChRs decreases heart rate (directly in atria) and force of contraction (indirectly in ventricles),[3] we have studied the applications of this compound as a photopharmacological tool to remotely control cardiac function *in vivo* in wildtype frog tadpoles and in rats.

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P43- Organ-pharmacological investigations of photochromic histamine H_1 receptor ligands

K. Rustler[a], S. Pockes[b], S. Elz[b], and B. König[a]

[a] *Institute of Organic Chemistry, University of Regensburg, 93053 Regensburg, Germany; email: karin.rustler@ur.de*
[b] *Institute of Pharmacy, University of Regensburg, 93053 Regensburg, Germany;*

The histamine H_1 receptor belongs to the family of G-protein coupled receptors (GPCRs)[1] and is mostly known for the regulation of allergic and inflammatory effects in the human body.[2] Although many antagonists to treat allergic diseases are developed and structurally optimized, they are still not free of side effects, e.g. sedation.[3] Thus, the use of light as external noninvasive stimulus to trigger pharmacological activity as used in photopharmacology is desirable.[4,5] Inspired by VUF6884 (7-Chloro-11-(4-methylpiperazin-1-yl)dibenzo[*b,f*][1,4]oxazepine)[6] and the work of *Strasser*[7,8] and *Gobleder*[9] reporting a dual H_1/H_4 receptor ligand, initially five photochromic azobenzene-based derivatives were designed, synthesized, photochemically characterized, and organ-pharmacologically tested. For this purpose, the standardized pharmacological histamine H_1 receptor assay[10] on the isolated guinea pig ileum was adapted for continuous tissue irradiation. Four of five tested photochromic oxazepine derivatives showed a decreased antagonistic activity at the gpH_1R. Yet one hit with retained antagonistic activity compared to its non-photochromic lead was identified. For this compound, the pharmacological results suggest a fourfold difference in antagonistic activity of its *trans*- and *cis*-isomeric state. As further improvement, two bathochromically shifted derivatives were synthesized, avoiding the use of UV-light and improving solubility.

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P44- Synthesis and Characterization of Photoswitchable Kinase Inhibitors

Miriam Schehr[a], Julia Ewert[a], Linda Heintze[b], Chiara Ianes[c], Jörn Weisner[d], Matthias Müller[d], Christian Pichlo[e], Uwe Knippschild[c], Daniel Rauh[d], Ulrich Baumann[e], Christian Peifer[b], Rainer Herges[a]

[a] *Otto Diels-Institute of Organic Chemistry, Christian Albrechts University Kiel, Otto Hahn Platz 4, 24118 Kiel, Germany*
mschehr@oc.uni-kiel.de , jewert@oc.uni-kiel.de and rherges@oc.uni-kiel.de
[b] *Institute of Pharmacy, Christian Albrechts University Kiel, Gutenbergstraße 76, 24118 Kiel*
cpeifer@pharmazie.uni-kiel.de

In recent years protein kinases have become one of the most employed group of drug targets. Today, there are more than forty different kinase inhibitors on the market used mostly against a variety of different cancer types. However, a fair number of kinase inhibitors exhibit poor kinome selectivity. Our goal is to design, synthesize and characterize a reversible photoswitchable kinase inhibitor, which can be spatially and temporally controlled by light to minimize side effects or the development of resistances. In addition, such a photoswitchable inhibitor could be of great interest to investigate the mechanism of inhibition on a molecular level. Therefore, we used 4,5-diarylthiazol and -imidazole core structures which already have been proven as hits of Casein Kinase 1 and p38 MAPK. Four azobenzene-containing and one diazocine-functionalized inhibitors could be successfully synthesized and characterized. Unfortunately only minor differences in activity between the *cis*- and *trans*-isomers could be observed, but nevertheless, important conclusions based on ligand-protein crystal structures could be achieved for the improvement of the design of future photoswitchable inhibitors.

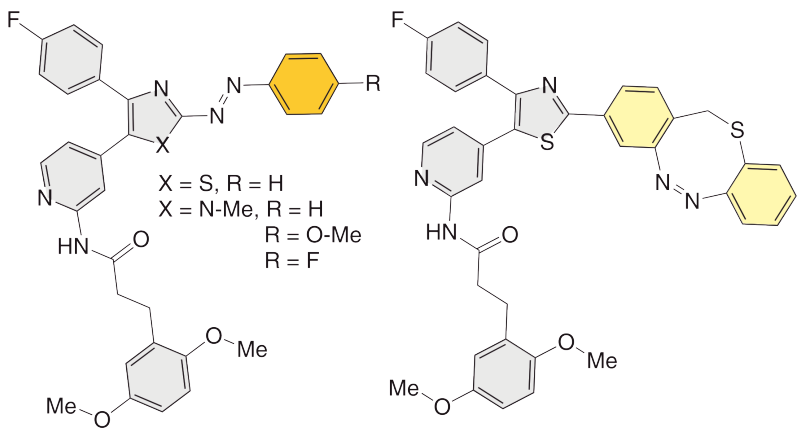

Figure 1. Sucessfully synthesized and fully characterized photoswitchable kinase inhibitors.

Production of this booklet has been kindly supported by ESTEVE

P45- A Diazocine-functionalized Derivative of the VEGFR2 Tyrosine Kinase Inhibitor Axitinib

Dorian Schmidt[1], Julia Ewert[2], Linda Heintze[1], Theo Rodat[1], Miriam Schehr[2], Rainer Herges[2], Christian Peifer[1,*]

[1]*Institute of Pharmacy, University of Kiel; Gutenbergstr. 76, 24118 Kiel, Germany;* [2]*Institute of Organic Chemistry, University of Kiel, Otto-Hahn-Platz 4, 24098 Kiel, Germany; email: dschmidt@pharmazie.uni-kiel.de*

Protein kinases are enzymes that mediate signal transduction in intracellular signal pathways and regulate cell growth and differentiation. Overactivated kinases, however, can lead to uncontrolled cell proliferation and play a crucial role in tumor progression and inflammatory diseases. Therefore, kinases are important drug targets and the development of small molecule kinase inhibitors has become a major field in pharmaceutical research.[1]
Our goal is to develop photoswitchable kinase inhibitors that can be spatially and temporally controlled by light. In this context, we became interested in the VEGFR2 kinase inhibitor axitinib that is licensed for second-line therapy of renal cell carcinoma (RCC) since 2012.[2]

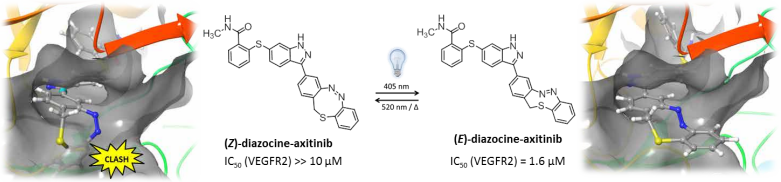

Figure 1. (Left): Superposition of (Z)-diazocine-axitinib with the ATP binding pocket of VEGFR2 (pdb code 4AG8).[3]. In the Z-configuration the diazocine moiety clashes with the protein surface. No binding mode can be found. (Middle): E/Z-isomerization of diazocine-axitinib. (Right): Calculated binding mode of (E)-diazocine-axitinib with VEGR2.

In the present study, we have functionalized the pharmacophore of axitinib with a photoswitchable sulfur-diazocine moiety.[4] In the thermodynamically stable *Z*configuration this axitinib derivative does not show activity in an *in vitro* VEGFR2 kinase assay. Upon irradiation with light (405 nm) the metastable *E*configuration is formed (PSS_{405nm} ~47 % *E*-isomer) resulting in an IC_{50} value of 1.6 µM. The *E*-isomer can be switched back to the bio-inactive *Z*-isomer either by irradiation with visible light (500-650 nm) or thermally [$t_{1/2}$ = 6.6 h, (37 °C)].

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P46- Towards photocontrol of the cell entry of cell-penetrating peptides

Tim Schober[a], Oleg Babii[b], Sergii Afonin[b], Igor V. Komarov[c], Anne S. Ulrich[a,b]

[a]*Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry (IOC);* [b]*KIT, Institute of Biological Interfaces 2 (IBG2);* [c]*Taras Shevchenko National University of Kyiv, Institute of High Technologies; email: tim.schober@kit.edu*

Cell-penetrating peptides are a powerful tool to transport otherwise impermeable cargos into cells.[1] Herein we combine this delivery concept to in-cell transport with photopharmacology, by enabling photocontrol of peptide cell-penetrating ability. We designed several cyclic cell-penetrating peptides, each of having a diarylethene-based photoswitch (DAE)[2] in the backbone. The DAE building blocks were synthesized as Fmoc-amino acids and were directly employed in standard solid phase peptide synthesis.[3] The peptides differed in the macrocycle ring size (10 – 14mers) and in amino acid composition, hence allowing a structure-activity relationship study.
Pure photoisomers were independently evaluated for their uptake efficiency in HeLa cells. Mean fluorescence intensity and flow cytometry were used to quantify the differences. Cytotoxicity was assessed through MTT-assays. For some amphipathic 10-mer peptides, we observed well-pronounced differences in uptake between the two photoforms. The ring-open forms in all cases showed higher uptake than the ring-closed counterparts. For arginine-only analogues, however, the effect was much less prominent if at all present. We explain the differences by the changes in rigidity/flexibility and in backbone exposure upon photoswitching, which differentially affect both, the entry-enabling preorganized charge display and peptide CPP-activity deteriorating intramolecular aggregation.

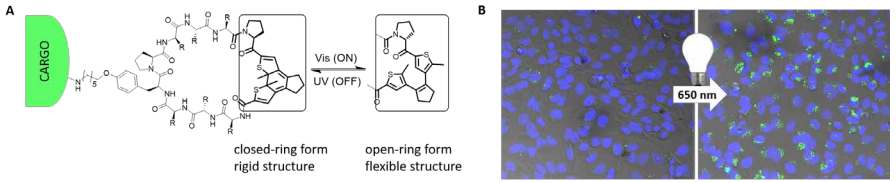

Figure 1. Overview of the photocontrol of the cell entry of cell-penetrating peptides. A) General scaffold with indicated photoswitching of the DAE-building block. B) Intracellular fluorescence (green) of different photophorms in HeLa cells after 3 h incubation at 5 µM.

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P47- Complexity from Simplicity: Rearrangement of Arenes to Bicyclo[3.1.0] hexenyl Derivatives

Tomáš Slanina,[a] Rabia Ayub,[a] Wangchuk Rabten,[a] Josene Toldo,[a] Marco Nicaso,[a] Igor Alabugin,[b] Joakim Bergman,[c] Henrik Ottosson[a]

[a] *Department of Chemistry–Ångström Laboratory, Uppsala University, Box 523, SE-751 20 Uppsala, Sweden;* [b] *Department of Chemistry & Biochemistry, Florida State University Tallahassee, FL 32306-4390, USA;* [c] *AstraZeneca R&D Mölndal, Medicinal Chemistry-KH471, Mölndal, Sweden; email: slanina.tomas@kemi.uu.se*

Aromatic molecules are abundant in biological systems. Their photochemistry often differs significantly from their ground-state reactivity and provides a simple tool for synthesis of highly complex molecules. Photochemical rearrangement of simple arenes has been first described in the 1960s.[1] The reaction forms bicyclo[3.1.0]hexenes by a single photochemical step, yet its mechanism remains controversial.[2] The bicyclo[3.1.0] hexenyl cation and benzvalene have been investigated as the main possible intermediates. While both the bicyclo[3.1.0]-hexenyl cation and benzvalene lead the same product upon nucleophilic attack by solvent, both computational and experimental results prefer the latter. The highly rigid bicyclo[3.1.0]hexene scaffold is known to be an efficient intermediate for the synthesis of natural products or biologically active compounds.[3] It contains 4-stereocenters and their strained structure and highly oriented directionality of *endo* and *exo* bonds make them interesting aliphatic scaffolds for the constructions of novel biologically active nucleotides and therapeutics.[4] The photoinduced rearrangement of simple arenes to bicyclo[3.1.0]hexene represents a powerful strategy for generation of complexity from simplicity in just one single photochemical step.

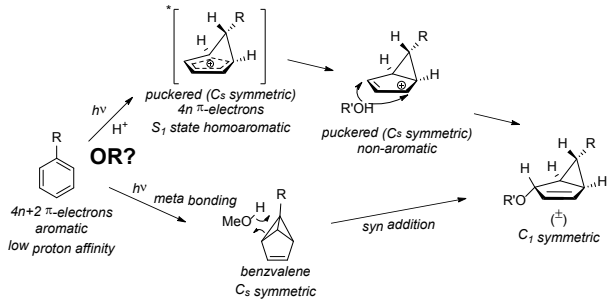

Figure 1. Photochemical rearrangement of arenes in presence of nucleophiles.

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P48-Optimization of photoresponsive Vorinostat-based HDAC inhibitor

Laura Slappendel[a,b], Mark W. H. Hoorens[a,b], Piermichele Kobauri[b], Ben L. Feringa[b], Wiktor Szymanski[a,b]

[a]*Department of Radiology,University of Groningen University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands.*
[b]*Centre for Systems Chemistry, Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands.*
Email: l.slappendel@student.rug.nl

Histone Deacetylases (HDACs) are overexpressed in many types of cancer, including gastric, prostate and colon cancer[1] and therefore HDAC inhibitors have been developed, including the FDA-approved Vorinostat[2]. Yet, current HDAC inhibitors have drawbacks, such as low tumor specificity, which result in a misbalance of eu- and heterochromatin causing apoptosis in healthy cells[3,4]. Using the photopharmacological approach[5], an HDAC inhibitor containing a photo-switchable azobenzene will provide spatial control, by which the HDAC inhibitor can be locally activated with light in cancer tissue, leaving healthy tissue unharmed[6]. As shown by Szymanski *et al*., 2015, a photo-switchable HDAC inhibitor based on Vorinostat was developed with a 39-fold difference in activity between the photo isomers on isolated enzymes. Here we show optimization towards increasing the difference in potency between *cis* and *trans* forms and improving the solubility of the compound.

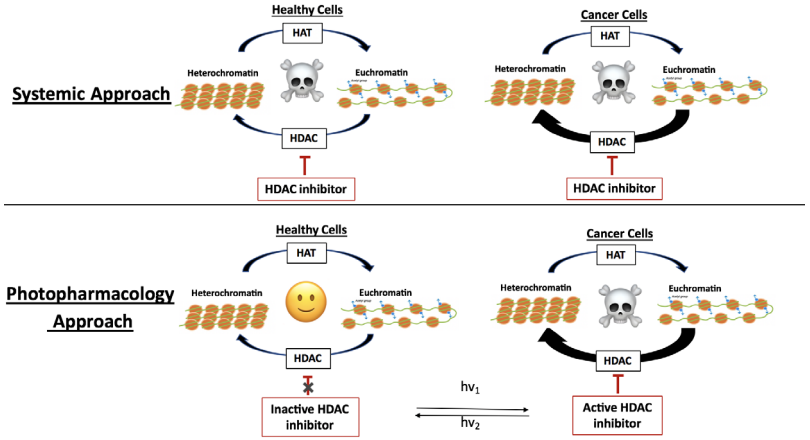

Figure 1: Systemic HDAC inhibition results in cell death in both healthy as cancer cells, yet cancer cells are more sensitive through increased HDAC expression. The photopharmacology approach allows for local activation of the HDAC inhibitors in cancer cells, leaving the healthy cells unharmed.

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P49- Design and chemical synthesis of photo-regulated melatonin receptor ligands in type 2 diabetes and neurodegenerative diseases

Gloria Somalo[a,b,c,d], Carme Serra[d], Lourdes Muñoz[d], Amadeu Llebaria[d] and Ralf Jockers[a,b,c]

[a]Inserm, U1016, Institut Cochin, Paris, France; [b]CNRS UMR 8104, Paris, France; [c]Université Paris Descartes, Paris, France; [d]MCS, Laboratory of Medicinal Chemistry, Institute for Advanced Chemistry of Catalonia (IQAC-CSIC), Barcelona, Spain
email: gloria.somalo@iqac.csic.es

G Protein-Coupled Receptors (GPCRs) are considered excellent therapeutic targets with great potential in several disorders. Among existing GPCRs, this project is focused on melatonin receptors and their cell-penetrating ligand: melatonin. Emerging evidence shows that signaling can be prolonged or initiated not only in the plasma membrane but also in intracellular compartments.[1] Deregulation of melatonin system is associated with metabolic, autoimmune and neurodegenerative diseases, even though its specific molecular mechanism still remains elusive. Therefore, the interplay between cell surface and intracellular melatonin receptors needs to be characterized by developing innovative pharmacological tools based on photo-regulated melatonin ligands.
Thus, different melatonin derivatives have been designed, among which melatonin-caged derivatives and azobenzenes can be found. None of them should be active at the level of receptor, but they can be activated by using specific light conditions, that are dependent on the chemical properties of the corresponding ligand. For caged-type derivatives, light triggers a photolytic reaction that separates the caging moiety from active melatonin, which will be released at a specific intracellular location. For azobenzene-type derivatives, light will switch reversibly the structure of the ligand derivative, in such a way that the new conformation should be recognized by the receptor. In any case, both strategies aim to achieve a spatiotemporal control of melatonin activity, in order to improve our knowledge on its specific molecular mechanism. Here the synthesis of several different compounds based on melatonin-caged methodology will be presented, including several photo-activable compounds with high responsiness to light. All of them constitute the first generation of compounds for testing, that will provide new insights about intracellular GPCRs and their promising therapeutic efficacy.

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P50- Optical allosteric modulation of NMDA receptors

Meilin Tian[a], Laetitia Mony[a,b], Zakaria Mostefai[a], and Pierre Paoletti[a,c]

[a]Institut de Biologie de l'ENS (IBENS), CNRS, INSERM, Ecole Normale Supérieure, Université PSL, 46 rue d'Ulm, 75005 Paris, France ; blaetitia.mony@ens.fr; cpierre.paoletti@ens.fr

NMDA receptors (NMDARs) are glutamate-gated ion channels widespread in the central nervous system. These receptors play fundamental roles in synaptic transmission and plasticity. NMDARs are also targets of therapeutic interest since their dysfunction is linked to a variety of neuronal disorders such as schizophrenia, depression and Alzheimer's disease[1]. NMDARs are tetramers usually composed of two GluN1 and two GluN2 subunits encoded by four different genes (GluN2A-D), resulting in a large number of receptor subtypes having distinct anatomical, biophysical, pharmacological and signalling properties. Understanding the functional role of these individual subtypes in the brain is of great importance to develop new strategies to counteract the deleterious effects of NMDAR dysregulation. However, tools allowing targeting of specific NMDAR populations in a given neuronal circuit are currently lacking.
Optogenetic pharmacology allows overcoming the limitations of spatio-temporal resolution and molecular specificity of standard pharmacology[2]. Using this approach, we aimed at selectively and reversibly enhancing the activity of GluN2B-containing NMDARs by targeting a positive allosteric site, the polyamine site[3]. Compared to previous studies targeting orthosteric (competitive) sites, targeting allosteric sites has the advantage of respecting the natural temporal pattern of receptor activation, which allows manipulation of receptors in a more physiological manner. We designed a cysteine-reactive, photoswitchable spermine derivative (MASp) containing the azobenzene photoswitch, which can alternate between a *trans* and *cis* configuration depending on the illumination conditions. By screening for cysteine substitution positions near the putative spermine binding site[3] to covalently attach MASp, we found two positions yielding strong photo-dependent potentiation of GluN2B-NMDAR activity. In one of them MASp acted as a *trans* potentiator, while in the other one it acted as a *cis* potentiator. Our work establishes a promising approach for further understanding the physiological role of GluN2B-NMDARs in brain function and pathology. More generally, it establishes photoswitchable tethered allosteric ligands (PTALs) as promising tools to control proteins with light.

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P51-Specific photopharmacological control over the function of native TRPC channels

Oleksandra Tiapko[1], Niroj Shresta[1], Sanja Curcic[1], Annarita Graziani[1], Gema Guedes de la Cruz[2], Sonja Lindinger[3], Christiane Klec[4], Wolfgang F. Graier[4], Helmut Kubista[5], Marc Freichel[6], Lutz Birnbaumer[7], Christoph Romanin[3], Toma N. Glasnov[2], and Klaus Groschner[1]

[1] Gottfried Schatz Research Center - Biophysics, Medical University of Graz, [2] Institute of Chemistry, University of Graz, [3] Institute of Biophysics, University of Linz, [4] Gottfried Schatz Research Center - Molecular Biology and Biochemistry, Medical University of Graz, [5] Institute of Pharmacology, Medical University of Vienna, [6] Pharmakologisches Institut, Universität Heidelberg, [7] Neurobiology Laboratory, National Institute of Environmental Health Sceinces, Research Triangle Park, North Carolina 27709 (USA) and Institute of Biomedical Research (BIOMED), Catholique University of Argentina; email: oleksandra.tiapko@medunigraz.at

Background: Precise and specific pharmacological control over signalling molecules in native tissues is essential to advance mechanistic understandig of organ physiopathology and to develop novel therapies. Photopharmacology achieves spatiotemporal accuracy in manipulation of a biological target typically by light-induced photoisomerization of a ligand structure. We adopted this approach to target native transient receptor potential canonical channels (TRPC) TRPC3 is one of the lipid-regulated cation channels abundantly expressed in cardiovascular tissues and brain. To obtain highly specific control over these channels, we employed photopharmocologiac stratedy to develop photoswitchable channel activators that bypass endogenous, regulatory lipid signaling.

Results: We synthetized a series of photoswitchable azobenzene derivatives based on the selective TRPC3/6 agonist GSK1702934A and identified a highly efficient photosensitive benzimidazole activator (OptoBI-1). Light-induced cycling between active (cis) and inactive (trans) conformations of OptoBI-1 allowed for repetitve activtion of recombinant TRPC3 currents and Ca^{2+} influx into HEK293 as evidence of direct optical recording of Ca^{2+} entry through the TRPC3 channel utilizing advantages of a geneticly encoded Ca^{2+} sensor (R-GECO) fused to its cytoplasmic N-terminus. Photoswitching of OptoBI-1 enabled temporally precise initiation of Ca^{2+} transients in human vascular endothelial cells (EA.hy 926) and reversible as well as repetitive suppression of firing in primary cultures of murine hippocampal neurons.

Summary/Conclusion: We report a novel benzimidazole-based, photoswitchable TRPC actuator for precise control over endogenous TRPC activity in neurons and cardiavascular cells. This approach may further be emplyed as a strategy to decipher TRPC signalling function and to advance TRPC channes as therapeutic targets.

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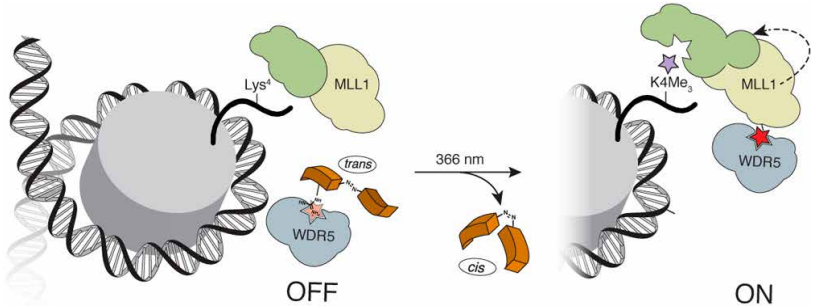
P52- OPTOEPIGENETICS: Light-controlled Modulation of Gene Expression

Lea Albert[a], Jing Xu[b], Vasundara Srinivasan[a], Yali Dou[b] and Olalla Vázquez[a*]

[a]Fachbereich Chemie, Philipps-Universität Marburg, Germany; [b]University of Michigan, USA; email: vazquezv@staff.uni-marburg.de

Epigenetics is the collection of highly dynamic processes involving multiple chromatin modifying and remodelling enzymes that control the accessibility to genes and their expression through a complex signalling network of protein-protein interactions.[1] In this context, our young group has recently described a cell-permeable photoswitchable probe capable of modulating epigenetic cellular states by disruption of an essential protein–protein interaction within the MLL1 methyltransferase core complex. Our azobenzene-containing peptides selectively block the WDR5-MLL1 interaction by binding to WDR5 with high affinity (Ki = 1.25 nM). We determined the co-crystal structure of this photoswitchable peptiomimetic with WDR5 to understand the interaction at the atomic level. Importantly, the photoswitchable *trans* and *cis* conformers of the probe display a clear difference in their inhibition of MLL1.[2]



We further demonstrate that the designed photo-controllable azo-peptidomimetics affect the transcription of the MLL1-target gene Deptor, which regulates hematopoiesis and leukemogenesis, and inhibit the growth of leukemia cells. This strategy confirms the potential of photopharmacological inhibition of methyltransferase protein–protein interactions as a novel method for external epigenetic control, providing a new toolbox for controlling epigenetic states.

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P53- Controlling Fragaceatoxin C (FraC) Pores with Light

Jana Volarić,[a] Natalie L. Mutter,[b] Wiktor Szymanski,[a] Giovanni Maglia[b] and Ben L. Feringa[a]

[a]*Stratingh Institute for Chemistry, University of Groningen, 9747 AG Groningen;* [b]*Groningen Biomolecular Sciences & Biotechnology Institute, University of Groningen, 9747 AG Groningen.*
E-mail: j.volaric@rug.nl

Fragaceatoxin C (FraC) is a potent pore-forming toxin isolated from a sea anemone. FraC has recently shown great potential for future use in DNA and protein sequence sensing.[1-3] In solution the water-soluble monomeric FraC binds to the cell membrane and undergoes a conformational change (Figure 1a). Subsequently the transmembrane protein forms a funnel-shape pore consisting of eight units with openings of 6 and 1.5 nm (Figure 1b). The goal of the project is to introduce photoswitchable molecules to the toxin in attempt to control the activity and formation of nanopores.

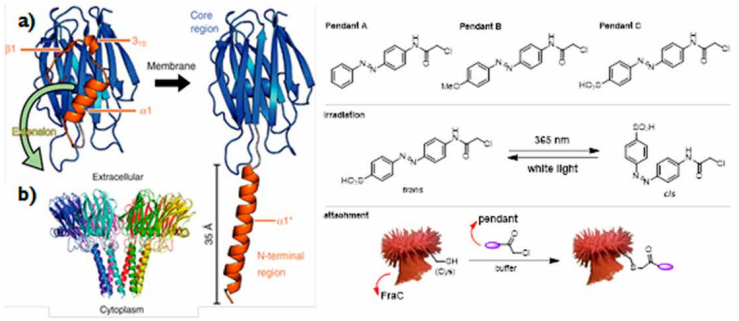

Figure 1. a) The water soluble monomeric Frac undergoes a conformational change to expose the α helix; **b)** Funnel shaped pore with eight monomers of FraC; **c)** Synthesized azobenzene photoswitches snd switching from the *trans* to the *cis* form by irradiating with 365 nm UV light or with white lgiht.; Scheme representing the attachment of the pendants to FraC.

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P54- A photoswitchable GABA receptor channel blocker

Ulrike Wirth[a], Galyna Maleeva[b], Daniel Wutz[a], Karin Rustler[a], Alba Nin-Hill[c], Mercedes Alfonso-Prieto[d,e], Elena Petukhova[f], Antoni Bautista-Barrufet[g], Alexandre Gomila-Juaneda[g], Petra Scholze[h], Franck Peiretti[i], Carme Rovira[c,j], Burkhard König[a], Pau Gorostiza[g,j,k], Piotr Bregestovski[b,f,l]

[a]*Institute of Organic Chemistry, University of Regensburg, 93053 Regensburg, Germany;* [b]*Aix-Marseille University, INSERM, INS, Institut de Neurosciences des Systèmes, 13005 Marseille, France;* [c]*Departament de Química Inorgànica i Orgànica (secció de Química Orgànica) & Institut de Química Teòrica i Computacional (IQTCUB), Universitat de Barcelona, E-08028 Barcelona, Spain;* [d]*Computational Biomedicine, Institute for Advanced Simulations IAS-5 and Institute of Neuroscience and Medicine INM-9, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany;* [e]*Cécile and Oskar Vogt Institute for Brain Research, Medical Faculty, Heinrich Heine University Düsseldorf, D-40225 Düsseldorf, Germany;* [f]*Department of Normal Physiology, Kazan State Medical University, Kazan, Russia;* [g]*Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology (BIST), Barcelona, Spain;* [h]*Department of Pathobiology of the Nervous System, Center for Brain Research, Medical University Vienna, Vienna, Austria;* [i]*Aix Marseille Université, INSERM 1263, INRA 1260, C2VN, Marseille, France;* [j]*Institució Catalana de Recerca i Estudis Avançats (ICREA), E-08020 Barcelona, Spain;* [k]*Network Biomedical Research Center in Biomaterials, Bioengineering and Nanomedicine (CIBERbbn);* [l]*Institute of Neurosciences, Kazan State Medical University, Kazan, Russia.*
Email: burkhard.koenig@ur.de

In the emerging field of photopharmacology, photochromic scaffolds e.g. azobenzenes, are used in biological investigations to trigger their activity by irradiation with light. Therefore, bioactive compounds are functionalized with molecular photoswitches to gain highly dynamic, reversible and spatiotemporal control. Ideally, the photochromic pharmacophore can be switched reversibly between high activity and low activity.[1-4]
In the presented work we report the design, synthesis and biological investigation of a photochromic GABA receptor channel blocker.

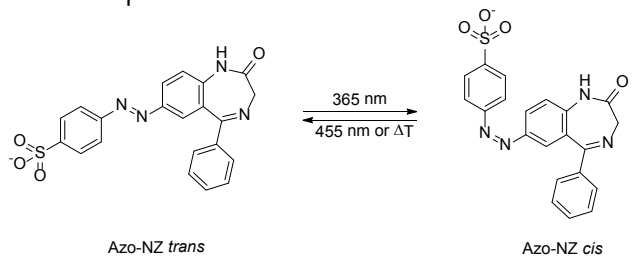

Figure 1. Light-induced isomerization of Azo-NZ.

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P55- Synthesis and Photophysical Characterization of Azoheteroarenes

Yongjin Xu[a], Chunxia Gao[a], Joakim Andréasson[b], Morten Grøtli[a]

[a] *Department of Chemistry and Molecular Biology, University of Gothenburg, SE-412 96, Gothenburg, Sweden;* [b] *Department of Chemistry and Chemical Engineering, Physical Chemistry, Chalmers University of Technology, SE-412 96, Gothenburg, Sweden;*
email: yongjin.xu@chem.gu.se

Protein kinases are involved in the regulation of almost all cellular processes. Consequently, there is a huge interest in developing kinase inhibitors to study kinase signalling and to develop small-molecule kinase drugs. Novel approaches allowing spatiotemporal control of the activity of a kinase would be key additions to the signal transduction toolbox, and can be achieved through the use of photoswitchable molecules.[1] Azobenzene compounds are known as versatile examples for photoswitchable systems because of their isomeric *cis*- and *trans*-configurations. However, aromatic nitrogen heterocycles are among the most significant structural components of kinase inhibitors. Therefore, in order to integrate the azobenzene-based molecular photoswitches into the pharmacophore of kinase inhibitors, the development of heterocyclic azobenzenes derivatives are fundamental. Herein, as the first stage towards photoswitchable kinase inhibitors, we report on the synthesis and the photophysical characterization of a series of azoheteroarenes, with Buchwald-Hartwig coupling or nucleophilic substitution, followed by O_2 oxidation as the key synthetic steps in the preparation of these compounds.[2]

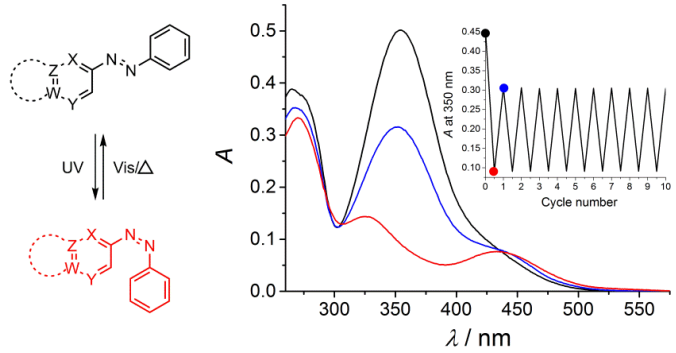

Figure 1. Photoswitchable azoheteroarenes

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P56- Two-photon activation of freely diffusible allosteric photoswitches

Silvia Pittolo[1], Hyojung Lee[1], Miquel Bosch[1], Gertrudis Perea[2], Xavier Gómez-Santacana[3], Anna Lladó[4], Sébastien Tosi[4], Julien Colombelli[4], Kira Poskanzer[5], Amadeu Llebaria[3], Pau Gorostiza[1,6,7]

[1] Institute for Bioengineering of Catalonia (IBEC), Barcelona
[2] CAJAL Institute, Madrid
[3] IQAC-CSIC & IBEC, Barcelona
[4] Advanced Digital Microscopy (ADM), IRB, Barcelona
[5] University of California, San Francisco
[6] ICREA
[7] CIBER-BBN

Photosensitive molecules can control the activity of neurons with light patterns. These compounds allow mimicking the spatiotemporal complexity of cell-to-cell communication in the brain with unprecedented precision when compared to classical pharmacological agents. An example is pulsed illumination with near-infrared (NIR) lasers of caged neurotransmitters. NIR lasers can penetrate deep in scattering tissues, such as brain or brain slices, to define a micrometric volume where the neurotransmitters are released. For instance, NIR light pulses allow uncaging of glutamate in a volume the size of a dendritic spine.

Recently, we developed alloswitch-1, an azobenzene-based photoswitch that allows to turn on/off the metabotropic glutamate receptor 5 (mGlu5) through *cis/trans* isomerization using violet/green light (Pittolo *et al.* 2014). The potency and subtype selectivity of alloswitch-1 is that of allosteric ligands. Here we aimed at photoswitching alloswitch-1 to its *cis* isoform using two-photon excitation (2PE). 2PE of azobenzene-based molecules was previously demonstrated in covalently tethered photoswitches (Izquierdo-Serra et al. 2014), but it was not obvious in the case of the freely-diffusible alloswitch-1. Since 2PE of alloswitch-1 to the *cis* isoform would constrain the optical activation of mGlu5 to a small volume, diffusion of the *trans* isoforms from outside the 2PE volume could replace the outgoing *cis* isoforms and block the signaling downstream of mGlu5.

Here, we demonstrate 2PE of alloswitch-1 and some derivatives in cell cultures, and of alloswitch-1 in rodent brain slices. This is the first evidence that optical control of freely diffusible allosteric modulators of neuronal receptors is possible with pulsed NIR light. The 2PE of freely-diffusible alloswitch-1 has an axial resolution of at least 10 µm and offers great opportunities to study neuronal circuits in intact tissues and *in vivo* with unprecedented pharmacological selectivity, tissue depth and spatial resolution.

AUTHOR INDEX

AUTHOR INDEX

PARTICIPANTS LIST

* received until October 22

PARTICIPANTS LIST

Luca Agnetta
Julius-Maximilians University, Germany
luca.agnetta@uni-wuerzburg.de

Marc Alemany
University of Vic - Central University of Catalonia, Spain

Ramon Alibés Arqués
Autonomous University of Barcelona, Spain
ramon.alibes@uab.cat

Josep Arimany
Vic Council, Spain

Valentina Arkhipova
University Medical Center Groningen (UMCG), The Netherlands
valentina.arkhipova@rug.nl

Julia Ast
University of Birmingham, United Kingdom
j.ast@bham.ac.uk

Aida Barcons
University of Vic - Central University of Catalonia, Spain

Francesco Bavo
University of Milan, Italy
francesco.bavo@unimi.it

Kristian Berg
Oslo University Hospital, Norway
Kristian.Berg@rr-research.no

Alice Berizzi
IGF, CNRS, INSERM, Univ. Montpellier, France
alice.berizzi@igf.cnrs.fr

Leo Bonato
De Gruyter Publishing, Germany
leo.bonato@degruyter.com

Roser Borras Tuduri
Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Spain
rbtqbi@cid.csic.es

Gisela Cabré Segura
Autonomous University of Barcelona, Spain
gisela.cabre.segura@gmail.com

Núria Camarero
Institute for Bioengineering of Catalonia (IBEC), Spain
ncamarero@ibecbarcelona.eu

Meritxell Canals
Nottingham University, United Kingdom
mcanalsbuj@gmail.com

Rossella Castagna
Institute for Bioengineering of Catalonia (IBEC), Spain
rcastagna@ibecbarcelona.eu

Juanlo Catena
Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Spain
jcrqbi@cid.csic.es

Francisco Ciruela
UB-IDIBELL, Spain
fciruela@ub.edu

Vincent Daria
Eccles Institute of Neuroscience, Australia
vincent.daria@anu.edu.au

Michael Decker
University of Würzburg, Germany
michael.decker@uni-wuerzburg.de

Craig Donoghue
IRB Barcelona, Spain
craig.donoghue@irbbarcelona.org

Georgina Ducet
University of Vic - Central University of Catalonia, Spain

Kate Dunning
University of Strasbourg, France
kdunning@unistra.fr

Anna Duran Corbera
Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Spain
adcqbi@cid.csic.es

Julia Ewert
Otto-Diels-Institute, Germany
jewert@oc.uni-kiel.de

Ben Feringa
University of Groningen, The Netherlands
b.l.feringa@rug.nl

Víctor Fernández-Dueñas
University of Barcelona, Spain
vfernandez@ub.edu

Joan Font-Ingles
IGF, CNRS, INSERM, Univ. Montpellier, France
joan.font-ingles@igf.cnrs.fr

Matthew Fuchter
Imperial College London, United Kingdom
m.fuchter@imperial.ac.uk

Manuel Gailer
Ludwig-Maximilians University, Germany
maaich@cup.uni-muenchen.de

Aida Garrido Charles
Institute for Bioengineering of Catalonia (IBEC), Spain
agarrido@ibecbarcelona.eu

Chloé Geoffroy
Institute of Biology of ENS (IBENS), France
chloe.geoffroy@ens.fr

Ouldouz Ghashghaei
University of Barcelona, Spain
ulduz.ghashghaie@gmail.com

Jesus Giraldo
UAB-Inc, Spain
Jesus.Giraldo@uab.es

Laurent Givalois
University of Montpellier, France
laurent.givalois@umontpellier.fr

Edith C. Glazer
University of Kentucky, USA
ec.glazer@uky.edu

Xavier Gómez-Santacana
IGF, CNRS, INSERM, Univ. Montpellier, France
xavi.gomez-santacana@igf.cnrs.fr

Alex Gomila
Institute for Bioengineering of Catalonia (IBEC), Spain
agomila@ibecbarcelona.eu

Pau Gorostiza
Institute for Bioengineering of Catalonia (IBEC), Spain
pgorostiza@ibecbarcelona.eu

Cyril Goudet
IGF, CNRS, INSERM, Univ. Montpellier, France
cyril.goudet@igf.cnrs.fr

Xavier Gregori
Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Spain
xavier.gregori@iqac.csic.es

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Yevgenii Grushevskyi
Max Delbrück Center for Molecular Medicine, Germany
yevgenii.grushevskyi@mdc-berlin.de

Monica Victoria Gutiérrez Salazar
Instituto de Ciencia Molecular (ICMol), Spain
movigusa@gmail.com

Niels Hauwert
Vrije Universiteit Amsterdam, The Netherlands
N.J.Hauwert@vu.nl

Linda Heintze
Pharmaceutical Institut, University of Kiel, Germany
lheintze@pharmazie.uni-kiel.de

Jordi Hernando
Autonomous University of Barcelona, Spain
jordi.hernando@uab.cat

David Hodson
University of Birmingham, United Kingdom
d.hodson@bham.ac.uk

Anja Hoffmann-Röder
Ludwig-Maximilians University, Germany
anja.hoffmann-roeder@cup.uni-muenchen.de

Mark Hoorens
University of Groningen, The Netherlands
m.w.h.hoorens@rug.nl

Katharina Hüll
New York University, USA
kh2785@nyu.edu

Jae-Woong Jeong
Korea Advanced Institute of Science and Technology (KAIST), Republic of Korea
jjeong1@kaist.ac.kr

Laia Josa Culleré
University of Oxford, United Kingdom
laia.josacullere@chem.ox.ac.uk

Julie Kniazeff
IGF, CNRS, INSERM, Univ. Montpellier, France
julie.kniazeff@igf.cnrs.fr

Piermichele Kobauri
University of Groningen, The Netherlands
p.kobauri@rug.nl

Dusan Kolarski
University of Groningen, The Netherlands
d.kolarski@rug.nl

Burkhard Köning
University of Regensburg, Germany
burkhard.koenig@chemie.uni-regensburg.de

Daniel Lachmann
University of Regensburg, Germany
daniel.lachmann@ur.de

Ranit Lahmy
University of Regensburg, Germany
ranit.lahmy@gmail.com

Lucien Lameijer
Stratingh Institute for Chemistry, The Netherlands
l.n.lameijer@rug.nl

Rodolfo Lavilla
University of Barcelona, Spain
rlavilla@ub.edu

Hyojung Lee
Institute for Bioengineering of Catalonia (IBEC), Spain
hlee@ibecbarcelona.eu

Àngels Leiva
University of Vic - Central University of Catalonia, Spain

Rob Leurs
Vrije Universiteit Amsterdam, The Netherlands
r.leurs@vu.nl

Amadeu Llebaria
Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Spain
amadeu.llebaria@iqac.csic.es

Fernando Lobo Palacios
Instituto de Productos Naturales y Agrobiología (IPNA-CSIC), Spain
fernando.lobo@csic.es

Elena Longhi
Dept. of Pharmacy and Chemistry, LMU Munich, Germany
Elena.Longhi@lmu.de

Marc López Cano
University of Barcelona, Spain
Marclopezcano@hotmail.com

Victor Lorenz Fonfria
University of Valencia, Spain
victor.lorenz@uv.es

Fanny Malhaire
IGF, CNRS, INSERM, Univ. Montpellier, France
fanny.malhaire@igf.cnrs.fr

Nathalie Marcela
University of Vic - Central University of Catalonia, Spain

Vicente Marchán
University of Barcelona, Spain
vmarchan@ub.edu

Joan Masnou
University of Vic - Central University of Catalonia, Spain

Carlo Matera
Institute for Bioengineering of Catalonia (IBEC), Spain
cmatera@ibecbarcelona.eu

Carla Miro
University of Groningen, The Netherlands
c.miro@student.rug.nl

Tamara Mocking
Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), The Netherlands
t.a.m.mocking@vu.nl

Rina Mogaki
University of Tokyo, Japan
mogaki@macro.t.u-tokyo.ac.jp

Laetitia Mony
Institute of Biology of ENS (IBENS), France
mony@biologie.ens.fr

Johannes Morstein
New York University, USA
Johannes.Morstein@gmail.com

Alexander Moskalensky
Novosibirsk State University, Russian Federation
sunmosk@mail.ru

Santi Nonell
IQS-URL, Spain
santi.nonell@iqs.url.edu

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Vasilis Ntziachristos
Technical University of Munich, Germany
sekretariat.ibmi@helmholtz-muenchen.de

Marta Otero
University of Vic - Central University of Catalonia, Spain

Silvia Panarello
Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Spain
silvia.panarello@iqac.csic.es

Pierre Paoletti
Institute of Biology of ENS (IBENS), France
pierre.paoletti@ens.fr

Giuseppe Maria Paternò
Fondazione Istituto Italiano di Tecnologia, Italy
giuseppe.paterno@iit.it

Vanessa Pereira
IGF, CNRS, INSERM, Univ. Montpellier, France
vanessa.pereira@igf.cnrs.fr

Jean-Philippe Pin
IGF, CNRS, INSERM, Univ. Montpellier, France
jppin@igf.cnrs.fr

Ferruccio Pisanello
Istituto Italiano di Tecnologia, Italy
ferruccio.pisanello@iit.it

Filippo Pisano
Istituto Italiano di Tecnologia, Italy
filippo.pisano@iit.it

Davia Prischich
Institute for Bioengineering of Catalonia (IBEC), Spain
dprischich@ibecbarcelona.eu

Romain Quidant
Institute of Photonic Sciences (ICFO), Spain
romain.quidant@icfo.eu

Wangchuk Rabten
Uppsala University, Sweden
wangchuk.rabten@kemi.uu.se

Arnau Ramos Prats
Medical University of Innsbruck, Austria
arnau.ramos@i-med.ac.at

Friederike Reeßing
University Medical Center Groningen (UMCG), The Netherlands
f.r.reessing@rug.nl

Martin Reynders
New York University, USA
xmr128@gmail.com

Maria Ricart Ortega
Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Spain
mroqbi@cid.csic.es

Fabio Riefolo
Institute for Bioengineering of Catalonia (IBEC), Spain
friefolo@ibecbarcelona.eu

Theo Rodat
Pharmaceutical Institut, University of Kiel, Germany
trodat@pharmazie.uni-kiel.de

Diego Alejandro Rodriguez Soacha
Julius-Maximilians Universität Würzburg, Germany
diego.rodriguez@uni-wuerzburg.de

Xavier Rovira
University of Vic - Central University of Catalonia, Spain
xavier.rovira@uvic.cat

Karin Rustler
University of Regensburg, Germany
karin.rustler@ur.de

Diego Sampedro
University of La Rioja, Spain
diego.sampedro@unirioja.es

Elisabet Sarri
University of Vic - Central University of Catalonia, Spain

Miriam Schehr
Otto-Diels-Institute, Germany
mschehr@oc.uni-kiel.de

Dorian Schmidt
Institute of Pharmacy, University of Kiel, Germany
dschmidt@pharmazie.uni-kiel.de

Tim Schober
Institute of Organic Chemistry, Germany
tim.schober@kit.edu

Tindara Scolaro
Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Spain
scolaro.at@gmail.com

Carmen Serra Comas
Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Spain
carme.serra@iqac.csic.es

Nadja Simeth
University of Groningen, The Netherlands
nadja.simeth@gmail.com

Tomás Slanina
Uppsala University, Sweden
slanina.tomas@seznam.cz

Laura Slappendel
University of Groningen, The Netherlands
laura_slappendel@hotmail.com

Gloria Somalo Barranco
Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Spain
gsbqbi@cid.csic.es

Rosalba Sortino
Institute for Bioengineering of Catalonia (IBEC), Spain
rsortino@ibecbarcelona.eu

Darya Spiryova
Novosibirsk State University, Russia
gerd.raz@yandex.ru

Wiktor Szymanski
University Medical Center Groningen (UMCG), The Netherlands
w.szymanski@umcg.nl

Michael Telias
University of California Berkeley, USA
mtelias@berkeley.edu

Oliver Thorn-Seshold
Ludwig-Maximilians University, Germany
oliver.thorn-seshold@cup.lmu.de

Meilin Tian
Institute of Biology of ENS (IBENS), France
mtian@biologie.ens.fr

Oleksandra Tiapko
Medical University of Graz, Austria
oleksandra.tiapko@medunigraz.at

Dirk Trauner
New York University, USA
dirktrauner@nyu.edu

Gooitzen van Dam
University of Groningen, The Netherlands
g.m.van.dam@umcg.nl

Olalla Vázquez
Philipps-Universität Marburg, Germany
olalla.vazquez@staff.uni-marburg.de

Jordi Villà
University of Vic - Central University of Catalonia, Spain
jordi.villa@uvic.cat

Jana Volaric
University of Groningen, The Netherlands
j.volaric@rug.nl

Maikel Wijtmans
Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), The Netherlands
m.wijtmans@vu.nl

Ulrike Wirth
University of Regensburg, Germany
wirth-ulrike@web.de

Andrew Woolley
University of Toronto, Canada
awoolley@chem.utoronto.ca

Yongjin Xu
University of Gothenburg, Sweden
yongjin.xu@chem.gu.se

Charleine Zussy
University of Montpellier, France
charleine.zussy@yahoo.fr

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SCIENTIFIC COMMITTEE:

IDIBELL · university of groningen · ferrer Advanced Biotherapeutics · INc Institut de Neurociències · MDC Max Delbrück Center for Molecular Medicine in the Helmholtz Association · IQS Persona Ciencia Empresa Universitat Ramon Llull · iGF Institut de Génomique Fonctionnelle

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