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EDITORIAL

President's Letter

Dear Colleagues,

Loughborough's street fair, an extravaganza where the whole town centre is transformed for four days into a giant fun-fair, first gained its charter in 1221 from King Henry III. Assuming it has been held annually, the fair has therefore been coming to Loughborough for 794 years! The arrival of the fair of course generates much excitement, but its arrival, in early November, also coincides with a change in the weather; temperatures tumble and frosty starts to the day become the norm. A stark contrast to the very agreeable weather that I and many colleagues enjoyed at the (relatively) recent IUPAC photochemistry symposium in Bordeaux. This was a very enjoyable conference, and an opportunity to catch up with many colleagues both in the general conference sessions and in the EPA reception held following the General Assembly. There was significant EPA input to the symposium, with the presentation of the Porter medal and the EPA PhD prize as well as the general assembly meeting. At this meeting we welcomed the newly-constituted Executive Committee, and bade farewell to Eric Vauthey, who stepped down from the committee after many years of service.

In composing this, my first letter as President, I thought back to my first introduction to the EPA. This was as a PhD student back in 1991, when Hans Jochen Kuhn, then Newsletter editor, sidled up to me (I presume primed by my PhD supervisor, Frank Wilkinson!) at the XVth ICP in Paris and asked me to prepare a conference report for the EPA newsletter. This duly appeared in November of that year co-authored by Dave McGarvey, now at Keele University. Thus began my association with the EPA, and over the years I have kept in touch with the activities of the EPA through the newsletter and through many friends who have served on the Executive Committee. I subsequently joined the EPA executive as newsletter editor under the presidency of David Philips, who subsequently went on to become president of the Royal Society of Chemistry in the UK. Since around this time the printed newsletter has become a regular fixture in the EPA calendar, and I believe is well received by the membership.

Also during this period the Executive have been looking at ways to better engage with the membership, and indeed to expand it. To this end we now have an active Facebook page, which we use to publicise meetings, vacant positions and other highlights; please do take a look. Simply search "European Photochemistry Association" and "like" us to be involved. If there are meetings or positions or anything else you would like publicised this way, please get in touch with Roberto; the more members who use this, the more useful it will become.

Next year (2015) sees the UNESCO International Year of Light and Light-based Technologies (IYL 2015), which obviously provides an excellent opportunity to promote photochemistry to a wider audience. Talking with colleagues in Bordeaux, the approach favoured was to provide resources to facilitate local interventions. We are therefore in the process of constructing a web page with links to video, power-point presentations and other resources which can be used as the basis for events in schools and colleges. I have been in touch with the Presidents of I-APS and APA, and they are supportive of linking from their websites and providing additional links. Thanks also are due to Axel Griesbeck and Silvia Braslavsky for providing impetus to this initiative. This can hopefully become a dynamic resource which, properly curated, will be useful in a range of future outreach activities.

So next year presents a very good opportunity for the promotion of photochemistry, and I hope that colleagues will make the most of this. At the forthcoming Executive Committee meeting in January we shall be looking at ways we can maximise impact and support colleagues in delivery. Our Facebook page seems an ideal forum to share ideas and best practice, so please "like" to stay informed and get in touch with us with anything you would like to share.

Dr. David Worrall
Loughborough University

PUBLICATIONS

…then God said, "Let light be" and there was light.
And God saw that the light was good"
(Genesis, 1, 3-4)

The international year of Light

Light, Energy, Photochemistry and Photophysics

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What is light?

Most people (not we photochemists!) take light for granted every day. They never ask themselves: what is light? In case they do, they immediately realize that it is not possible to define this omnipresent entity in clear and concise terms. The same is true with other fundamental entities, like time. St. Augustine was used to say: "What then is time? If no one asks me, I know what it is. If I wish to explain it to him who asks, I do not know".

Light is remarkable. The common answer, *light is energy*, does not allow us to make much progress in understanding what light is because even energy is difficult to define. In a famous lecture, Richard Feynman stated: "It is important to realize that in physics today, we have no knowledge what energy is" [1]. The equivalence of energy E and mass m described by the famous Einstein's equation $E = mc^2$ adds a further aura of mystery to the nature of light.

According to the Bible light was created by God: " …then God said, "Let light be" - and there was light. And God saw that the light was good" (Genesis, 1, 3-4). The first letter of John goes on saying that "God is light; in him there is no darkness at all" (1 John, 1, 5).

Current physical theories are based on the occurrence of a mysterious explosion (Big Bang) that created light, space, time, and matter.

These four fundamental entities of the Universe, and the fifth, the most important one, which is life, are interconnected to one another and cannot be reduced to or defined by something simpler. The emergence of rational thought, however, brought with it a need to question the nature of light, this extraordinary phenomenon that illuminates our world and does much more than that.

A brief history of the concept of light

Over the centuries, our view of light has changed dramatically. The first real theories about light came from the ancient Greeks: light as a ray, a straight line moving from one point to another. Pythagoras proposed that vision resulted from light rays emerging from a person's eye and striking an object. Epicurus argued the opposite: objects produce light rays, which then travel to the eye. Other Greek philosophers - for example Euclid - used ray diagrams to show how light bounces off a smooth surface or bends as it passes from one transparent medium to another.

Geometrical optics, involving mirrors, lenses and prisms, was later developed by some Arab scholars. Ibn al-Haytham (Alhacen), who lived in present-day Iraq between 965 and 1039, is considered the father of modern optics and ophthalmology. He identified the optical components of the human eye and correctly described vision as a process involving light rays bouncing from an object to a person's eye.

In 1690 the Dutch mathematician, astronomer and physicist Christiaan Huygens (1629-1695) published his *Traité de la Lumière*, which is considered to be the foundation of the *wave theory* of light. In this theory, he assumed the existence of some invisible medium (luminiferous aether) filling all empty space between objects. He further speculated that light forms when a luminous body causes a series of waves or vibrations in this aether. Those waves then advance forward until they encounter an object. If that object is an eye, the waves stimulate vision. In 1704, however, Isaac Newton argued that the geometric nature of reflection and refraction of light could only be explained if light was made of particles, referred to as *corpuscles*.

If light was a beam of particles, the questions are: what kind of particles? what are they made of? what size are they? what shape? and if light was a wave, what kind of wave? An ocean wave is not a thing, it is a property of water, something that water does. If there is no

water there is no wave. So if light was a wave, what was waving? These were the questions that physicists tried to answer. While seeking adequate answers, light ended up being described as both a particle and a wave. Yet how could it be both? This was the first of many paradoxes that began to question our common sense notion of how the Universe operates.

Newton's theory remained in force for more than 100 years. When the corpuscular theory failed to adequately explain the diffraction, interference, and polarization of light it was abandoned in favor of Huygens' wave theory. In 1847 Michael Faraday suggested light was a high-frequency electromagnetic vibration, which could propagate even in the absence of a medium such as the aether. His work inspired James C. Maxwell who discovered that self-propagating electromagnetic waves travel through space at a constant speed, which is equal to the previously measured speed of light. From this, Maxwell in 1862 concluded that light was a form of electromagnetic radiation and in 1873 he published *A Treatise on Electricity and Magnetism,* which contained a full mathematical description of the behavior of electric and magnetic fields, still known as Maxwell's equations.

In 1900 Max Planck, attempting to explain black body radiation, suggested that although light was a wave, these waves could gain or lose energy only in finite amounts related to their frequency. Planck called these "lumps" of light energy "quanta". In 1905, Albert Einstein used the idea of light quanta, later called *photons*, to explain the photoelectric effect.

The concept of a photon allowed photochemistry to emerge from its empirical stage. When it was clear that absorption of light corresponds to the capture of a photon by an atom or a molecule, Johannes Stark and Albert Einstein between 1908 and 1913 independently formulated the photoequivalence law, which is nowadays described in the first chapter of any photochemistry book.

Fundamental concepts

Experiments over the past hundred years or so have demonstrated that light has, indeed, a dual nature. In many instances, it is convenient to represent light as a "particle" phenomenon, thinking of light as discrete "packets" of energy (photons). The basic property of a photon is its energy, *E*. The other way of representing light is as an electromagnetic wave phenomenon. In this model, light is

characterized by its wavelength λ, frequency ν, and velocity c. The three quantities are related by the following equation:

$$\lambda\nu = c$$

The value of c is constant (2.998×10^8 m s^{-1} in vacuum), whereas λ (and ν) may cover a wide range of values (electromagnetic spectrum). The particle and wave nature of light are taut together by a simple relationship between the energy of a photon and the corresponding frequency of that photon:

$$E = h\nu$$

where h is the Planck constant (6.63×10^{-34} Js). These two equations allow us to convert back and forth from wavelengths (or frequencies) of photons to their energies.

The wave properties of light (λ and ν) can be measured, which makes light a quite useful *signal*. At the same time, light is *energy*. Therefore, light can be defined as a way (very fast, indeed!) of transferring signals and energy through space. Both the signal and energy properties of light are largely exploited in Nature and science. Nature has made use of light to create the early forms of life on our planet, to guide evolution, and to enable living species to recognize one another and the environment. Man has then exploited light properties for a variety of purposes and applications.

Light and matter

After some time from Big Bang our early Universe is flooded with building blocks of matter. But how did pure energy turned into matter it was a mystery until 1905 when the already mentioned Einstein's equation $E = mc^2$ showed that energy and matter are different forms of the same thing. In physics, mass–energy equivalence is the concept that the mass of an object or a system is a measure of its energy content. For instance, adding 25 kilowatt-hours (90 megajoules) of *any* form of energy to any object increases its mass by 1 microgram (and, accordingly, its inertia and weight) even though no matter has been added. During the expansion of the Universe, huge amounts of energy were converted into matter. In a nuclear explosion the opposite happens. In the frame of the US Strategic

Defense Initiative (1983), X-ray lasers powered by nuclear explosions were investigated for military purposes.
The conversion of a very small amount (not measurable!) of mass into a measurable quantity of energy takes place any time an atom or a molecule emits a photon, and the reverse occurs when a photon is absorbed

Light today
Light plays a vital role in our daily lives and is an imperative cross-cutting discipline of science in the 21st century. It has revolutionized medicine, opened up international communication via the Internet, and continues to be central to linking cultural, economic and political aspects of the global society. The importance of light reaches far beyond life on Earth. Through major scientific discoveries and technological advancements, light has helped us to see and better understand the Universe.

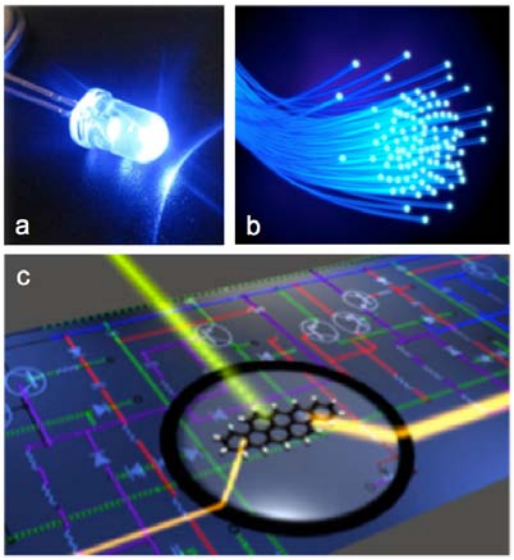

Figure 1. *Applications of photonics: (a) Light Emiting Diode (LED) that converts electricity into light with high efficiency; (b) fiber optics, a technology that uses glass (or plastic) threads (fibers) to transmit information; (c) molecular device based on light absorption and light emission for information processing.*

Lighting provides safety and security, affords access to education, improves quality of life and enhances architecture. We take lighting for granted and often notice it only by its absence. For over 1.5 billion people around the world, however, night time still means either darkness or the dim glow of a candle. Such poor-quality lighting has dramatic impact on the quality of life.

The electrification revolution has created and re-invented a multitude of industries. Ironically, the incandescent light-bulb itself seemed immune to these changes, only recently giving way to the solid state technologies. Indeed, it took a truly major development, the invention of the white light-emitting diode (LED), to displace the iconic symbol of electrification. Recognizing this extraordinary shift, the 2014 Nobel Prize in Physics has been awarded to Isamu Akasaki, Hiroshi Amano, and Shuji Nakamura for their pioneering development of gallium nitride (GaN)-based materials and devices, including the blue LED [2] (Figure 1a).

Photonics is the science and technology of generating, controlling, and detecting photons. Photonics is everywhere; in consumer electronics (barcode scanners, DVD players, remote TV control), telecommunications (internet), health (eye surgery, medical instruments), manufacturing industry (laser cutting and machining), defense and security (infrared camera, remote sensing), entertainment (holography, laser shows), etc. Fiber

optics (Figure 1b) allow us to use light to transmit large amounts of information, and to explore regions where we cannot go, such as in medical probes or endoscopes. The 21st century will depend as much on photonics as the 20th century depended on electronics. Molecular photonics (Figure 1c) is an emerging branch of photochemistry and photophysics [3].

Photochemistry and photophysics

Photochemistry and photophysics are natural phenomena as old as the world. Our life depends on photosynthesis, a natural photochemical and photophysical process. We get information about the surrounding space by photochemical and photophysical processes that occur in our eyes.

Artificial photochemical reactions have been observed as long as chemistry has been studied. Most of the earlier observations,

however, were accidental and remained unexplained. Photochemistry emerged from its empirical stage when modern physics established that light is radiated in discrete energy quanta, photons, and that light absorption corresponds to the capture of a photon by an atom or a molecule.

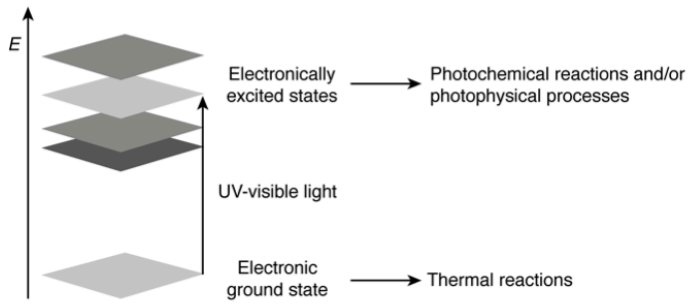

Figure 2. *Electronically excited states as novel chemical species*

After the First World War, photochemistry became a territory of physical chemists who were particularly interested in the photolysis of small molecules in the gas phase. The notion of competition among photochemical and photophysical processes for electronically excited state decay was gradually recognized. In the period between 1930 and 1950 the development of molecular orbital theory led to the interpretation of the electronic absorption spectra of organic molecules and the rationalization of trends in series of related molecules [4,5]. Some years later the main lines to interpret the absorption spectra of metal complexes became available. Since 1960 the concepts to understand the reactivity of electronically excited states emerged and correlations between structure and photochemical reactivity or photoluminescence were developed, first for organic molecules [6-8] and then for metal complexes [9]. Within a few years, the tight link between photochemistry and photophysics was established [10-14]. Furthermore, it became clear that photochemistry (a term that commonly is taken to include photophysics) is really a

distinct and separate part of chemistry because it does not concern the ground state of molecules, but concerns novel species: the electronically excited states (Figure 2). Focused photochemical experiments, improved spectroscopic techniques, and computational methods began to provide adequate characterization of electronically excited states of several classes of molecules.

Around 1990, investigations were extended to supramolecular species [15] and photochemistry and photophysics began to play a most important role in the chemistry of organic molecules [16-18] and metal complexes [18], as well as in novel scientific ventures such as creation of molecular devices and machines [19] (Figure 3), information processing at the molecular level [20] and a variety of theoretical studies and useful applications [16-18]. In recent years, a tremendous development of techniques has also permitted the investigation of photochemical and photophysical properties of molecules up to time windows as short as those allowed by the uncertainty principle and at the single-molecule level [21, 22].

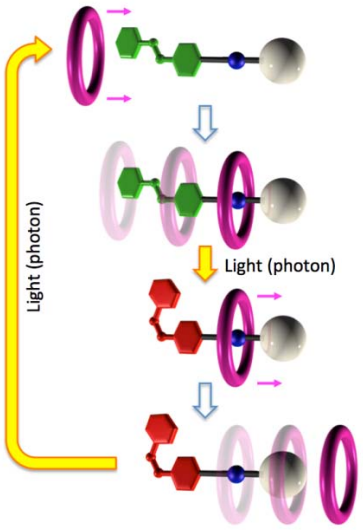

Figure 3. *Scheme of a photochemically driven molecular pump. The absorption of light causes the relative unidirectional translation of suitably designed ring and axle molecular components [19b].*

The current scientific literature shows that the frontiers of photochemistry and photophysics continue to expand with the development of new molecules, new materials, and new processes. Light is often used in chemical laboratories as a silver bullet reactant to obtain products unavailable by thermal activation [23], while sophisticated photochemical and photophysical processes are exploited for novel, unusual and unexpected applications [18-25]. We are moving toward a future in which energy and information will be the dominant features of civilization. We will be forced to exploit sunlight as our ultimate energy source, converting it into useful energy forms by photochemical and photophysical processes [26]. We will continue to miniaturize devices for information and communication technology down to the molecular level and we will use, more and more, light signals to transfer, store, and retrieve information [18-22, 24, 25] (Figure 4). There is no doubt that photochemistry and photophysics will play an increasingly important role in the development of science and technology.

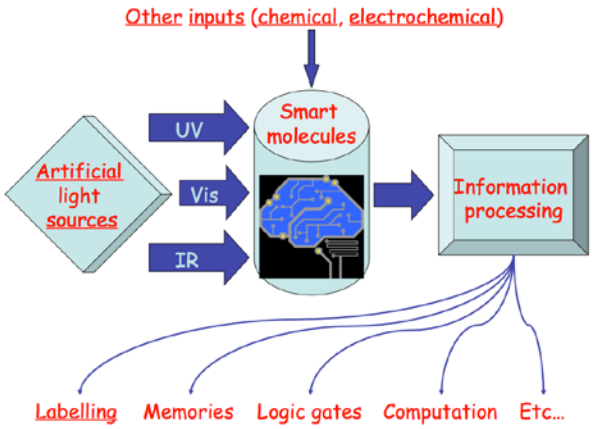

Figura 4. *Smart molecules can be used to elaborate light signals (and other inputs) for information processing.*

The role of scientists in a complex and fragile world

Which is the role of scientists in our world today? What are their duties? What does society expect from them? We are no longer in the

old days when science could be done just for fun and scientists could live in an ivory tower. Several years ago Arendt [27] observed that "Reality has the disconcerting habit of confronting us with the unexpected, for which we were not prepared". This is even truer today because the world is a system whose complexity increases further year after year. A simple example of increasing complexity related to chemistry is the following: two decades ago, a typical household owned products that altogether depended on less than 20 elements of the Periodic Table, whereas today a typical smart phone contains up to 60 different elements [28]. Complexity is a common feature of all the problems we have to solve.

Several scientists point out that the development of science increases the fragility of our world. In his book *Our last hour* Martin Rees [29] writes that there is no more than 50% probability that our civilization will survive until the end of this century because of bad or incautious use of the most recent developments of science and technology. Other scientists have warned about further development of science: "We are overcoming the boundary between enough and too much" [30] and "There is not much time to decide what we should do and what we should not do" [31]. It has also been noticed that the new technologies have the ability to change the very essence of our beings [30].

We are concerned about the increasing consumption of natural resources [32], the climate change [33], the energy crisis [26], and the degradation of the environment [34-36]. Many scientists have recently emphasized that our finite planet cannot sustain an endless expansion [26, 34-37]), and that we should take ecological constraints not as a hindrance, but a source of long-term economic security [38].

Until now, mankind has taken from spaceship Earth enormous amounts of resources [39]. We need to reverse this trend [40]. We need to create new resources. Photochemistry and photophysics can help by taking advantage of the only abundant, inexhaustible and well distributed resource on which we can rely: solar energy. Starting from seawater and the fundamental components of our atmosphere (nitrogen, oxygen and carbon dioxide), by means of sunshine, we will produce fuels, electricity, pure water, polymers, food and other things we need [41] (Figure 5). May be future generation will pay back Earth with a capital generated by using human intelligence to exploit sunlight.

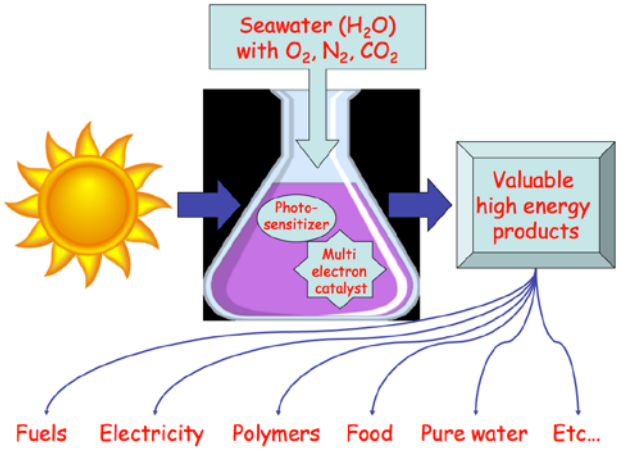

Figure 5. *With the help of photosensitizers and multi-electron catalysts, sunlight exploitation will allow us to convert abundant, low-energy substances into valuable high-energy products.*

Indeed, science can greatly benefit mankind, but science and technology alone will not take us where we need to go: a fair, open, responsible, friendly, united and peaceful society. Responsible scientists, while creating, with the greatest moral care, new science and technology, should also play an important role as authoritative, informed, and concerned citizens of the planet Earth [42].

References

1. J. Gleick, *Genius: The Life and Science of Richard Feynman*, Pantheon Books, New York, **1992**.
2. P. Von Dollen, S. Pimputkar, and J. S. Speck, "Let there be light – with gallium nitride: the 2014 Nobel Prize in Physics", *Angew. Chem. Int. Ed., 53*, 2-5, **2014**. doi: 10.1002/anie.201410693.
3. V. Balzani, A. Credi, M. Venturi: "Processing Energy and Signals by Molecular and Supramolecular Species", *Chem. Eur. J., 14*, 26-39, **2008**.

4. E. J. Bowen, *The Chemical Aspects of Light*, Clarendon Press, Oxford, **1946**.
5. H. H. Jaffé, M. Orchin, *Theory and Applications of Ultraviolet Spectroscopy*, Wiley, New York, **1962**.
6. N. J. Turro, *Molecular Photochemistry*, Benjamin, New York, **1965**.
7. J. G. Calvert, J. N. Pitts Jr., *Photochemistry*, Wiley, New York, **1966**.
8. C. A. Parker, *Photoluminescence of Solutions*, Elsevier, Amsterdam, **1968**
9. V. Balzani, V. Carassiti, *Photochemistry of Coordination Compounds*, Academic Press, London, **1970**.
10. J. P. Simons, *Photochemistry and Spectroscopy*, Wiley-Interscience, London, **1971**.
11. G.B. Porter, V. Balzani, L. Moggi, "Primary processes and energy transfer: consistent terms and definitions", *Adv . Photochem.*, *9*, 147–196, **1974**.
12. J. A. Barltrop, J. D. Coyle, *Principles of Photochemistry*, Wiley, Chichester, **1975**.
13. P. Suppan, *Chemistry and Light*, The Royal Society of Chemistry, Cambridge, **1994**.
14. C. E. Wayne, R. P. Wayne, *Photochemistry*, Oxford University Press, Oxford, **1996**.
15. V. Balzani, F. Scandola, *Supramolecular Photochemistry*, Horwood, New York, **1991**.
16. P. Klán, J. Wirz, J., *Photochemistry of Organic Compounds: From Concepts to Practice*, Wiley, Chichester, **2009**.
17. N. J. Turro, V. Ramamurthy, J. C. Scaiano, *Modern Molecular Photochemistry of Organic Molecules*, University Science Books, Sausalito, **2010**.
18. V. Balzani, P. Ceroni, A. Juris: *Photochemistry and Photophysics: Concepts, Research, Applications,* Wiley-VCH, **2014**.
19. (a) V. Balzani, A. Credi, M. Venturi, *Molecular Devices and Machines: Concepts and Perspectives for the Nanoworld*, 2nd ed., Wiley-VCH, Weinheim, **2008**; (b) G. Ragazzon, M. Baroncini, S. Silvi, M. Venturi, A. Credi, *Nat. Nanotech.*, in press, DOI: 10.1038/nnano.2014.260
20. A. P. De Silva, *Molecular Logic-Based Computation*, RSC Publishing, Cambridge, **2013**.
21. See, for example: T. Fukaminato, T. Doi, N. Tamaoki, K. Okuno, Y. Ishibashi, H. Miyasaka, M. Irie, "Single-molecule fluorescence photoswitching of a diarylethene-perylenebisimide dyad: non-

destructive fluorescence readout", *J. Am. Chem. Soc., 133*, 4984–90, **2011**.
22. L. Möckl, D. C. Lamb, C. Bräuchle, *Super-resolved Fluorescence Microscopy: Nobel Prize in Chemistry 2014 for Eric Betzig, Stefan Hell, and William E. Moerner*, *Angew. Chem. Int. Ed.*, **2014**, doi: 10.1002/anie.201410265.
23. (a) C. K. Prier, D. A. Rankic, D. W. C. MacMillan, "Visible Light Photoredox Catalysis with Transition Metal Complexes: Applications in Organic Synthesis", *Chem. Rev. 113*, 5322−5363, **2013**; (b) D. M. Schultz, T. P. Yoon, "Solar Synthesis: Prospects in Visible Light Photocatalysis", *Science 343*, **2014**. doi: 10.1126/science.1239176.
24. B. Feringa, W.R. Browne, (eds), *Molecular Switches*, 2nd ed., Wiley-VCH, Weinheim, **2011**.
25. J. Zhang, Q. Zou, H. Tian, "Photochromic materials: more than meets the eye", *Adv. Mat.*, *25*, 378-379, **2013**.
26. N. Armaroli, V. Balzani, *Energy for a Sustainable World*, Wiley-VCH, **2011**.
27. H. Arendt, *Crises of the Republic*, Houghton Mifflin Harcourt, Boston, **1972.**
28. R. G. Eggert, "Minerals go critical", *Nature Chemistry*, *3*, 688, **2011**.
29. M. J. Rees, *Our Final Hour*, Basic Books, New York, **2003**.
30. B. McKibben, *Enough*: *Staying Human in an Engineered Age*, Henry Holt, New York, **2003**.
31. S. Greenfield, *Tomorrow's People*, Pinguin Books, **2003**.
32. U. Bardi, *Extracted: How the Quest for Mineral Wealth is Plundering the Planet*, Chelsea Green, **2014**.
33. Climate Change **2013**: The Physical Science Basis, http://www.ipcc.ch/report/ar5/wg1/; Climate Change **2014**: Impacts, Adaptation, and Vulnerability *http://www.ipcc.ch/report/ar5/wg2/*
34. E. O. Wilson, *The Creation: An appeal to save life on Earth*, Norton, New York, **2006**.
35. L. R. Brown, *World on the Edge: How to Prevent Environmental and Economic Collapse,* Norton, New York, **2011**.
36. P. R. Ehrlich, A. H. Ehrlich, "Can a collapse of global civilization be avoided?" *Proc. R. Soc. B*, 280, 20122845, **2013**
37. B. McKibben,. *EAARTH: Making a Life on a Tough New Planet,* Henry Holt, New York, **2011**.
38. T. Prince, *The Logic of Sufficiency*, MIT Press, Cambridge (MA), **2005**.

39. F. Krausmann, K.-H. Erb, S. Gingrich, H. Haberl, A. Bondeau, V. Gaube, C. Lauk, C. Plutzar, T. D. Searchinger, "Global human appropriation of net primary production doubled in the 20th century". *Proc. Natl. Acad. Sci.*, *110*, 10324–10329, **2013**.
40. L. Roberts, R. Stone, A. Sugden, A., "The rise of restoration ecology", *Science*, *325*, 555, **2009**.
41. H. B. Gray, "Powering the planet with solar fuel". *Nat. Chem.*, *1*, 7, **2009** .
42. V. Balzani, "The role of science and scientists in a complex and fragile world", *Toxicological & Environmental Chemistry*, in press

Enabling novel photoredox reactivity via photocatalyst selection

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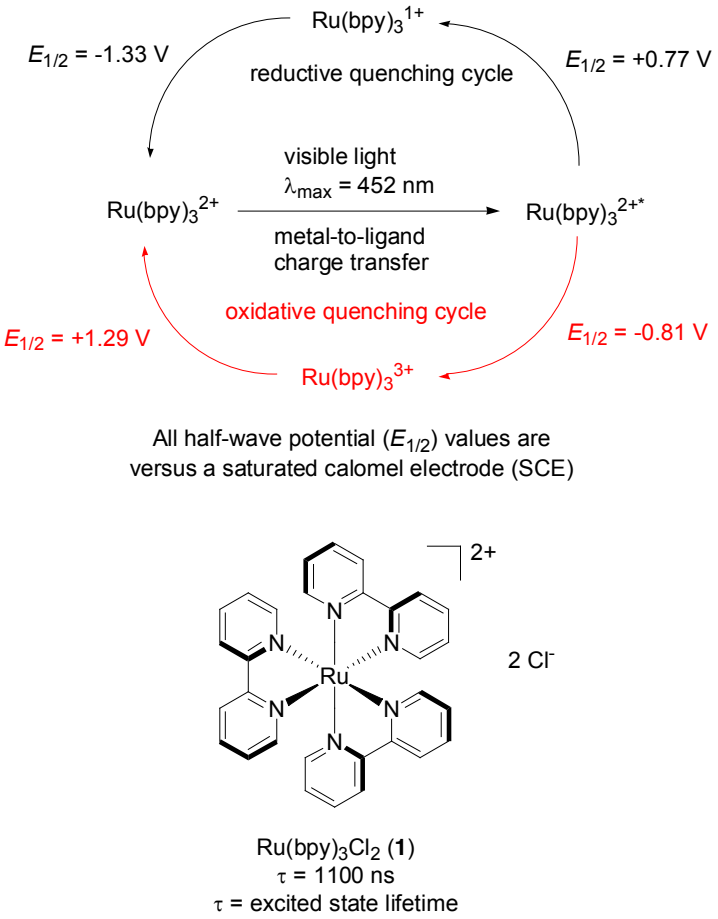
1. Introduction

Visible-light-active metal complexes have been extensively studied in numerous contexts since the first reported synthesis of tris(bipyridine)ruthenium(II chloride ($Ru(bpy)_3Cl_2$) in 1936.[1] The optical and redox properties[2] of $Ru(bpy)_3Cl_2$ and related complexes have allowed for their use in the development of such fields as photochemistry, electrochemistry, and photocatalyst.[3] For decade, with few exceptions, studies into the visible-light-induced redox properties of these catalysts were conducted by inorganic and physical chemists for applications that included water splitting,[4] photovoltaic cells,[5] and energy storage.[6] Complexes of this type have attracted the attention of synthetic chemists due in large part to the extended excited state lifetime of $Ru(bpy)_3Cl_2$ and related complexes. This property confers the ability to undergo electron- or energy-transfer processes, allowing reactivity from the visible-light-induced excited state.

The same properties that render $Ru(bpy)_3Cl_2$ and related complexes valuable in materials chemistry make them also desiderable in organic synthesis.



Scheme 1. Photocatalytic cycles of $Ru(bpy)_3^{2+}$. (Ref. 2,3a,8)

$Ru(bpy)_3Cl_2$ absorbs at 452 nm with high molar extinction coefficient (ε = 14,600 $M^{-1}cm^{-1}$),[3a] which has been assigned to a metal-ligand charge transfer (MLCT) The efficiency of the intersystem crossing is such that the quantum yield (Φ) is effectively unity for $*Ru(bpy)_3^{2+}$ $^3MLCT_1$ state,[7] which has a long lifetime (τ) of 1100 ns. As a result, fluorescence and internal conversion from $^1MLCT_1$ of $*Ru(bpy)_3^{2+}$

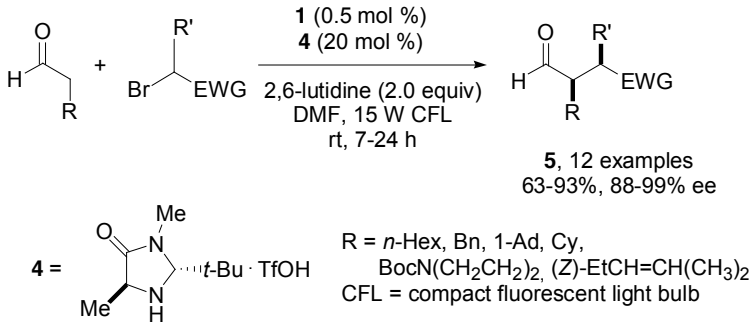
are minor deactivation pathways. The efficient energy transfer of visible light and the long excited-state lifetime allow for efficient bimolecular quenching of the excited state and provide access to single-electron redox chemistry.

The detailed photochemical processes relating to the excited-state species have been thoroughly investigated, with a synopsis designed for the organic chemist available.[8] In basic terms, upon absorption of visible light, MLCT generates an excited-state species that is "bipolar" in nature. This species can undergo either a single-electron reduction [reductive quenching; e.g., $Ru(bpy)_3^{2+*} \rightarrow Ru(bpy)_3^{+}$] or a single-electron oxidation [oxidative quenching; e.g., $Ru(bpy)_3^{2+*} \rightarrow Ru(bpy)_3^{3+}$] (Scheme 1).[2,3a,8] It is also important to note that the species resulting from either oxidative or reductive quenching [$Ru(bpy)_3^{3+}$ or $Ru(bpy)_3^{+}$] are themselves strong oxidants and reductants, respectively; thus, the possibility of single-electron transfer (SET) from multiple species must be considered.

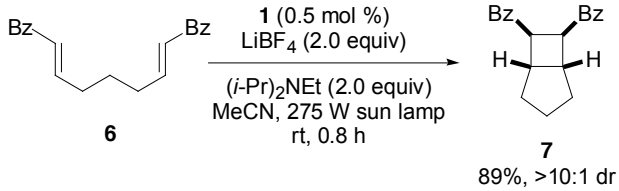
Pioneering research prior to 2008 by the groups of Deronzier, [9] Oda and Okada,[10] Kellogg,[11] and Fukuzumi[12] identified some of the key reactivities of $Ru(bpy)_3Cl_2$ that could be applied to organic synthesis. They demonstrated the ability of $Ru(bpy)_3Cl_2$ to successfully reduce a variety of C-X bonds, N-O bonds, diazonium salts, and nitroarenes. However, these were often limited to isolated and sporadic examples, with the wider utility of $Ru(bpy)_3Cl_2$ and related complexes predominantly overlooked by the broader synthetic chemistry community.

Two publications in 2008 initiated continued and directed interest in photoredox catalysis, primarily through novel applications of known modes of reactivity of $Ru(bpy)_3Cl_2$. Nicewicz and MacMillan reported an efficient merger of photoredox and organocatalysis to overcome the barriers associated with traditional two-electron strategies for the asymmetric alkylation of aldehydes. They elegantly harnessed the ability of $Ru(bpy)_3Cl_2$ to reduce C-Br bonds (such as in **3**) to create an electron-deficient radical that may add to a chiral enamine. This transformation proceeded in typically excellent yield and ee for a range of alkyl aldehydes and activated alkyl bromides (Scheme 2, Part (a)).[13]

(a) MacMillan's photoredox-catalyzed asymmetric alkylation of aldehydes



(b) Yoon's photoredox-catalyzed [2 + 2] enone cycloaddition



12 other examples (275 W floodlight at distance of 20 cm, 0.3-22 h): 54-98%, 4:1 to >10:1 dr

Scheme 2. Recent reports on photoredox catalysis that have reinvigorated interest in the field. (Ref. 13,14)

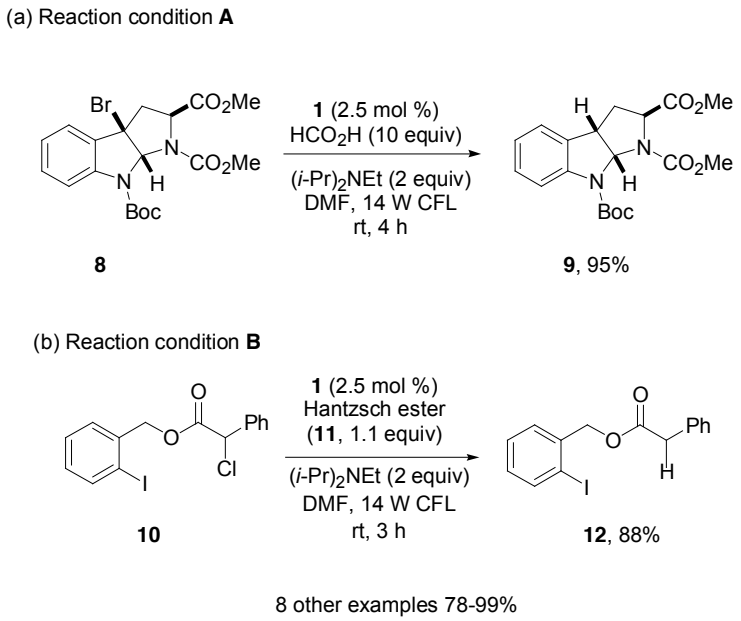
Independently, Yoon' group demonstrated the ability to perform [2 + 2] cycloadditions, traditionally the realm of high-energy UV light, with a photoredox catalyst harnessing visible light. In this methodology, a radical anion is generated from reduction of an activate enone by $Ru(bpy)_3^+$, leading to intramolecular cyclization and ultimately the cycloaddition products such as **7** (Scheme 2, Part (b)).[14] Following the preceding disclosures by MacMillan's and Yoon's groups, the synthetic community began to appreciate the wider applicability of $Ru(bpy)_3Cl_2$ and related complexes, leading to an exponential increase in the quantity and diversity of related reports.

This renewed focus has transformed photoredox catalysis from a series of independent publications into a definable field of research. Recent comprehensive reviews[15] and numerous perspective articles[16] on this topic have already appeared and can serve as excellent resource texts. This review will provide a personal account, with other relevant examples, of a portion of the development of photoredox catalysis in the Stephenson group aver the last five years. The aim this narrative approach, organized according to the mode of quenching of the photocatalyst excited state, is to impart the reader with a greater understanding of the journey that led to our current position within the research area. Specifically, how the choice of photocatalyst, and how it is employed within the catalytic cycle, drove the discovery and optimization of a wide range of photoredox-mediated processes.

2. Reductive quenching

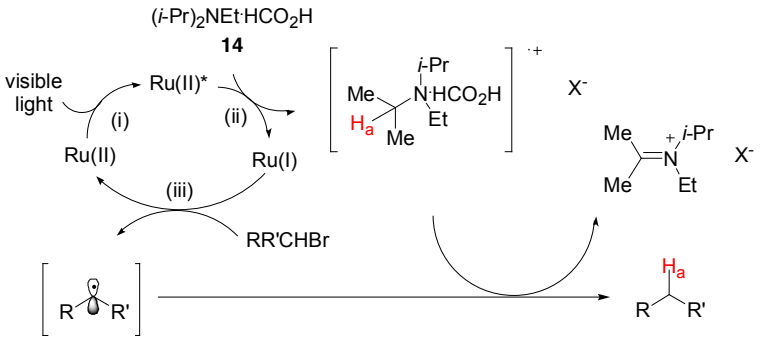
2.1. Reductive dehalogenation

Our endeavors in the field of photoredox catalysis were initiated during investigations aimed at the functionalization of bromopyrroloindolines such as **8**, and their subsequent use in complex-molecule synthesis. We were drawn to the possibility of a radical dehalogenation mediated by $Ru(bpy)_3Cl_2$, initially focusing on the reduction of activated C-Br bonds and reporting a generalized protocol to accompany pioneering initial studies by Fukuzumi and Tanaka,[12,17] Kellogg,[11] and Kern and Sauvage.[18] This method allows the tin-free reductive dehalogenation of a range of activated alkyl chlorides and bromides, and proceeds with typical yields of 70-99%.[19] Importantly, this approach displays excellent chemoselectivity, with aryl and alkenyl bromides and iodides being tolerated without competing reduction. Two complementary sets of reaction conditions (Scheme 3, Part (a) and (b)) were developed for this transformation, with the second set, Part (b), being particularly effective for substrates (such as **10**) that are prone to undergoing competing displacement of the activated halogen with formate.



Scheme 3. Reductive dehalogenation of activated carbon-halogen bonds. (Ref. 19)

We propose a catalytic cycle initiated by SET from ammonium formate complex **14** to the excited Ru(II)*. The resulting Ru(I) complex selectively reduces the carbon-halogen bond of the substrate and is oxidized back to the initial Ru(II photoactive ground state. Deuterium labeling studies showed that the hydrogen atom abstracted by alkyl radical **13** is primarily from one of the methine carbons of **15**, the radical cation of $(i\text{-Pr})_2NEt$ (Scheme 4).[19]



R = alkyl, aryl; R' = alkyl, EWG
(i) Light activation of photoactive complex; (ii) single-electron transfer from **14** and reduction of Ru(II)*; (iii) reduction of C-Br bond and re-oidation of catalyst to the photoactive ground state

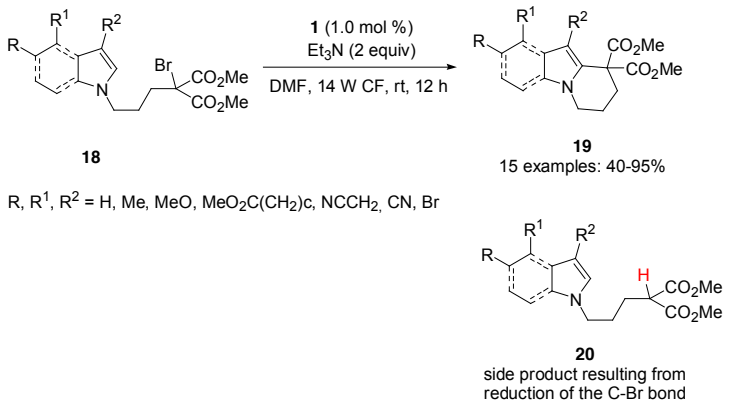
Scheme 4. Proposed catalytic cycle for the photoredox-catalyzed reductive dehalogenation. (Ref. 19)

We had initially chosen $Ru(bpy)_3Cl_2$ ($E_{1/2}^{II/I}$ = -1.33 V vs SCE) as the photocatalyst due to its commercial availability and previously demonstrated versatility. Our somewhat limited experience in the field rendered us initially slow to fully appreciate the number of well described transition-metal photocatalysts, which are, to this date, still mainly used in inorganic chemistry and material science. Recently, our research group and others have shown how a considered selection of photocatalysts has allowed the rapid expansion of methods that operate via the reductive and oxidative quenching cycles (vide infra).

2.2. Intramolecular radical additions

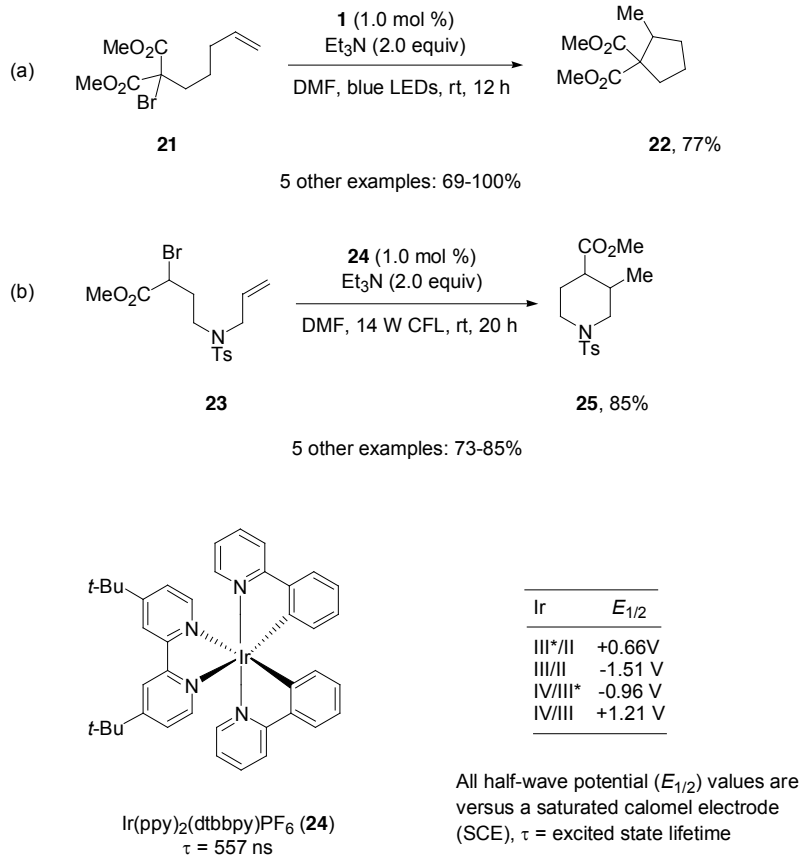
We followed our initial report on the dehalogenation reaction with the disclosure that the radical formed from the carbon-halogen bond reduction could efficiently participate in carbon-carbon bond-forming processes.[20] By employing slightly modified conditions to those of the reductive dehalogenation, a range of bromomalonates (such as **18**) were efficiently reduced and intramolecularly coupled to either indoles or pyrroles in good yields (typically >60%) (eq. 1).[20]

This approach provides an alternative to previous oxidative free-radical cyclizations[21] such as the stoichiometric $Mn(OAc)_3$-mediated oxidative cyclization reported by Kerr and co-workers.[22] Formation of the reductive dehalogenation product, e.g. **20**, from premature hydrogen-atom abstraction by the alkyl radical was minimized by use of Et_3N as the reductive quencher. Other amine quenchers such as DABCO®, Me_3N,and $(HOCH_2CH_2)_3N$ were efficient in generating the alkyl radical, but resulted primarily in the formation of reduced starting material **20**. Furthermore, while Ph_3N was completely selective for the desired cyclization product, **19**, it resulted in consistently low conversions (60%), even after prolonged reaction times (>48 h). This is presumably due to triphenylamine's increased oxidation potential, which limits access to the $Ru(bpy)_3^+$ reductant, when compared to trialkylamines.



eq 1 (Ref. 20)

We successfully applied the reaction conditions developed for the intramolecular radical coupling of electron-rich heterocycles to the analogous intramolecular addition to alkynes and alkenes.[23]



Scheme 5. Expanding the substrate scope of the intramolecular radical cyclization by the judicious choice of photocatalyst. (Ref. 23)

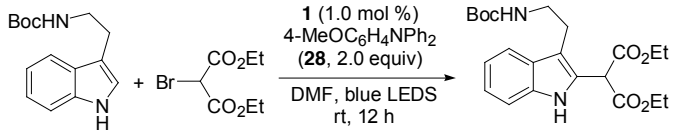
A range of 5- or 6-exo cyclization (Scheme 5, Part (a)) were realized in good-to-excellent yields (9-100%) through initial reduction of the activated C-Br bond. Consistent with MacMillan's report,[13] we found that the use of inexpensive, commercially available blue LEDs (1 W, λ_{max} = 435 nm) greatly accelerated the reaction when $Ru(bpy)_3Cl_2$ was employed as the photocatalyst.[23b] Although this method features milder initiation and greater functional group tolerance than typical

radical processes, attempts to further improve the utility of the reaction by expanding the substrate scope to less activated bromides, such as α-bromo esters, typically led only to recovery of starting material. Reasoning that a more strongly reducing photocatalyst was required, we explored $Ir(ppy)_2(dtbbpy)$-PF_6 (**24**) ($E_{1/2}^{III/II}$ = -1.51 V vs SCE,[24] compared to $E_{1/2}^{II/I}$ = -1.33 V for $Ru(bpy)_3Cl_2$), and were able to efficiently cyclize α-bromo esters (such as **23**, Scheme 5, Part (b)) and dibrominated cyclopropane susbstrates.[23] This was our first demonstration that the judicious choice of photocatalyst can allow for altered reactivity and a broader substrate scope.

2.3. Intermolecular radical addition

Our attempts to apply this method to intermolecular couplings were consistently hampered by competitive hydrogen-atom abstraction from the trialkylamine by the malonyl radical, forming the reduced malonate. This pathway also leads to further reactive components derived from the amine, such as iminium ions and enamines, that, while detrimental to the desired intermolecular coupling, could be effectively utilized as discrete intermediates in other photochemical transformations (see Section 2.4). We were able to overcome this challenge by using *N*,*N*-diphenyl-4-methoxyaniline (**28**) as a reductive quencher that cannot function as an efficient H-atom donor, showcasing the ability to drive selective reactivity by independently varying the photocatalyst or the quencher. Under the optimized conditions, a range of electron-rich heterocycles, such as Boc-protected tryptamine **26**, were efficiently coupled to diethyl bromomalonate in good-to-excellent yields (typically 60-90%) (eq 2).[25] However, significant challenges still remained as this methodology could not be applied to less activated C-Br bonds, such as methyl 2-bromo-2-phenylacetate, where typically only starting material was recovered. In this case, we postulated that charge recombination between the triarylamine radical cation and the Ru(I) outcompetes C-Br bond reduction. Employing a more electron-rich triarylamine [$(4\text{-}MeOC_6H_4)_2NPh$] successfully reactivated the reduction cycle. However, addition to the electron-rich amine, resulting in **30** rather than the desired indole, dominated-highlighting the need for further development of efficient photocatalyst quenchers. Ultimately, the reduction of unactivated carbon-halogen

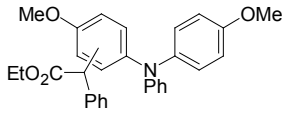
bonds was accomplished by switching the photocatalyst to *fac*-Ir(ppy)$_3$,[3e, 26] a strong excited-state reductant ($E_{1/2}^{IV/III*}$ = -1.73 V vs SCE), thus eliminating the requirement for an amine quencher (see Section 3.5).



26 **27**, 2.0 equiv **29**, 73%

14 other examples of C-H functionalization of indoles and pyrroles: 40-92%

When (4-MeOC$_6$H$_4$)$_2$NPh was used instead pf 4-MeoC$_6$H$_4$NPh$_2$, **30** predominated, not the coupled indole

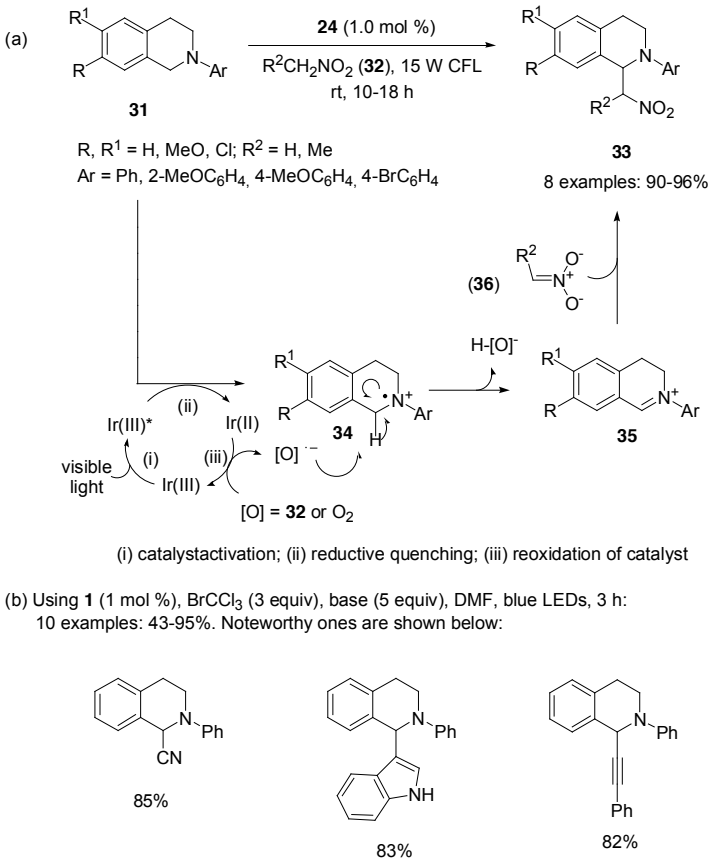


30

eq 2 (Ref. 25)

2.4. Nucleophilic addition to iminium ions derived from tetrahydroisoquinolines

Seeking to exploit the synthetic potential of iminium ions analogous to **17**, which was postulated as a detrimental side product in the debromination methodologies described above, we investigated the competency of such iminium ions as reactive species in the oxadative aza-Henry reaction with nitroalkanes (Scheme 6, Part (a)).[27]



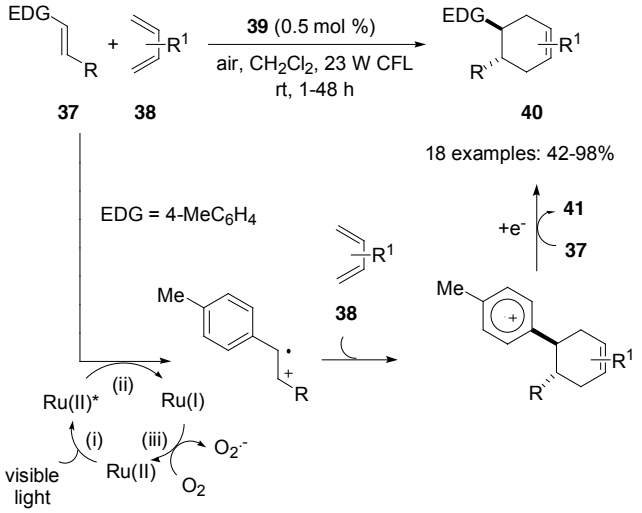
Scheme 6. Photoredox catalysis in the aa-Henryreaction and its subsequent expansion to include the addition of other nucleophiles to iminium ions. (Ref. 26,29a)

In this reaction manifold, the excited state of the photocatalyst, **1** or **24**, undergoes reductive quenching with a tetrahydroisoquinoline to generate the radical cation **34** and the reduced catalyst. Photocatalyst turnover is mediated by either adventitious oxygen and/or nitromethane to provide the ground state catalyst and a radical anion, $[O]^{-\cdot}$, that may abstract a H-atom from the amine radical cation, **34**, to form the desired iminium ion, **35**. Addition of the nitromethane-derived nitronate **36** to this iminium ion forms the observed nitroalkylated product in 90-96% yields, demonstrating that this

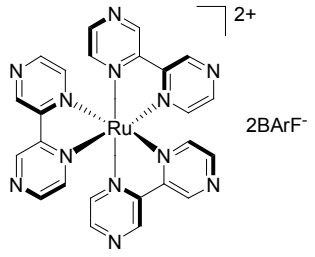
method is comparable in efficiency to the analogous Cu-mediated process by Li.[28] The general utility of this photoredox approach was subsequently demonstrated by our research group[29] and others[30] by expanding it to include the efficient addition of various nucleophiles such as cyanide, indole, and alkynes (Scheme 6, Part (b)).

2.5. Radical cation Diels-Alder reactions

Yoon's research group has also studied the reactivity of tetrahydroisoquinolines under photoredox conditions, albeit in a formal reversal of polarity whereby an α-amino radical adds efficiently to a range of α,β-unsaturated ketones.[31] This report provides two notable conclusions: (i) the reaction is greatly enhanced by Brønsted acids, and (ii) the primary mechanism is through radical propagation rather than photocatalyst turnover. The same group has also made significant contributions to the field by developing a range of visible-light-mediated cycloadditions,[32] perhaps the most striking example being the radical cation Diels-Alder cycloaddition.[33] In this methodology, $Ru(bpz)_3^{2+}$ ($E_{1/2}^{II*/I}$ = +1.45 V vs SCE)[34] was employed since, unlike $Ru(bpy)_3Cl_2$ ($E_{1/2}^{II*/I}$ = +0.77 V vs SCE), it can directly oxidize **37** (+1.1 V) to the radical cation, **41**, from it excited state (Ru^{II*}). This important realization, coupled with the correct choice of counterion ($BArF^-$ vs PF_6^-) to allow solvation in less polar solvents, permitted a range of [4 + 2] cycloaddition to occur with low catalyst loadings (typically 0.5 mol %) and in good yields (typically 60-98%) (Scheme 7).[33] The mechanism is postulated to begin with promotion of $Ru(bpz)_3^{2+}$ (λ_{max} = 440 nm) to its excited state, which is capable of oxidizing **37** to its radical cation **41**.



(i) catalyst activation; (ii) reductive quenching; (iii) reoxidation of catalyst



Ru(bpz$_3$(BArF)$_2$ (**39**)
τ = 740 ns

Ru	$E_{1/2}$
II*/I	+1.45 V
II/I	-0.80 V
III/II*	-0.26 V
III/II	+1.86V

All half-wave potentia ($E_{1/2}$) values are versus a saturated calomel electrode (SCE). τ = excited state lifetime

bpz = bipyrazyl; EDG = electrom-donating group; BArF = tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (a noncoordinating anion)

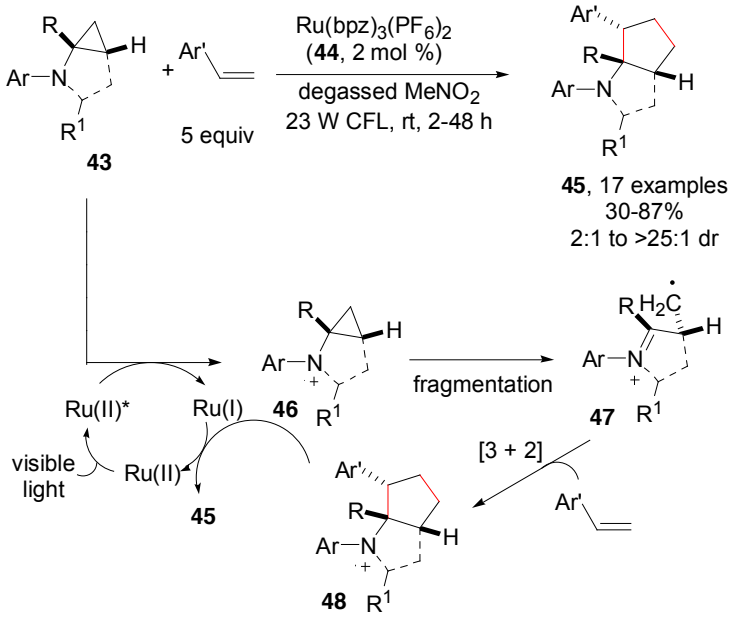
Scheme 7. Yoon's radical cation Diels-Alder reaction. (Ref. 33)

This species can then undergo intermolecular [4 + 2] cycloaddition, followed by abstraction of an electron from **37** in a chain propagation sequence. Finally, $Ru(bpz)_3^+$ is returned to the photoactive ground state, $Ru(bpz)_3^{2+}$, by oxidation with molecular oxygen. This elegant methodology displays both reversed intrinsic dienophile electronics, as well as overall regiochemical preference when compared to the traditional Diels-Alder reaction, making it highly complementary to

previously reported methods. Equally impressive is the demonstration of how the judicious selection of photocatalyst and subsequent tuning of physical properties make such a valuable method possible.

2.6. Formal [3 + 2] cycloaddition of aminocyclopropanes

Another methodology that successfully utilizes the strongly oxidizing $Ru(bpz)_3^{2+}$ excited state was recently reported by Zheng and co-workers for the intramolecular [3 + 2] cycloaddition of cyclopropylamines with alkenes.[35] In agreement with the pathway of the tetrahydroisoquinoline methodology, the amine served in the present system as the reactive species rather than the sacrificial quencher. The excited state photocatalyst initiates a cyclopropane ring opening by amine N-oxidation, generating a β-carbon radical iminium ion, **47**, that is competent in a formal [3 + 2] cycloaddition with a range of predominantly styrenyl alkenes (Scheme 8).[35] The authors postulate that the product following cycloaddition, **48**, is reduced by Ru(I), returning the photocatalyst to the parent oxidation state and furnishing a range of cyclopentanes and fused bicyclic systems in good yields (typically >70%). The reaction scope with respect to the amine is limited to either secondary or tertiary amines bearing at last one aryl substituent. This method further showcases the ability to tune the reaction conditions by the choice of photocatalyst. The yield correlated well with the excited state oxidation potential of the photocatalyst, with the weaker oxidants $Ru(bpy)_3Cl_2$ (**1**) ($E_{½}^{II*/I}$ = +0.77 V vs SCE) and $Ir(ppy)_2(dtbbpy)PF_6$ (**24**) ($E_{½}^{III*/II}$ = +0.66 V vs SCE) performing less efficiently than $Ru(bpz)_3^{2+}$ ($E_{½}^{II*/I}$ = +1.45 V vs SCE).

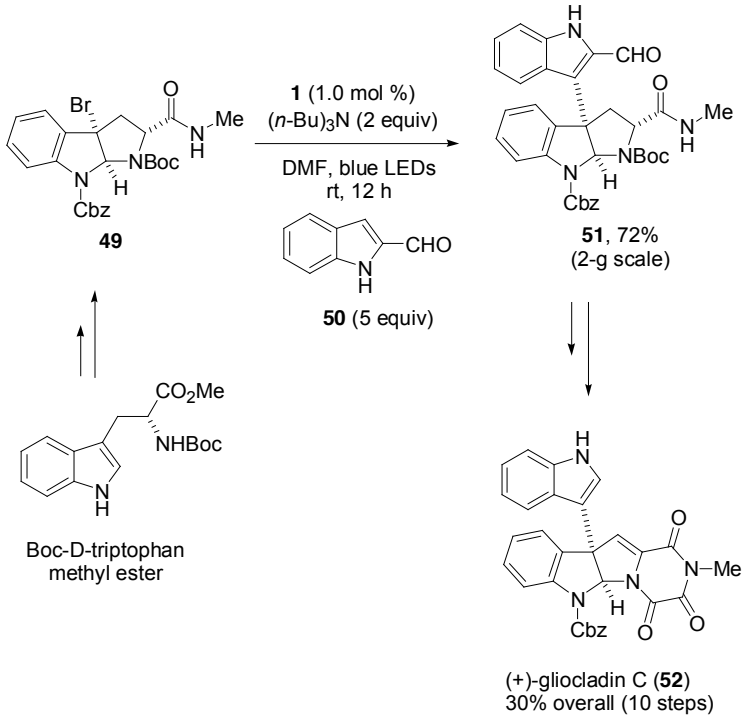


Scheme 8. Formal [3 +2] cycloaddition of aminocyclopropanes. (Ref. 35)

2.7. Application of reductive quenching in total synthesis

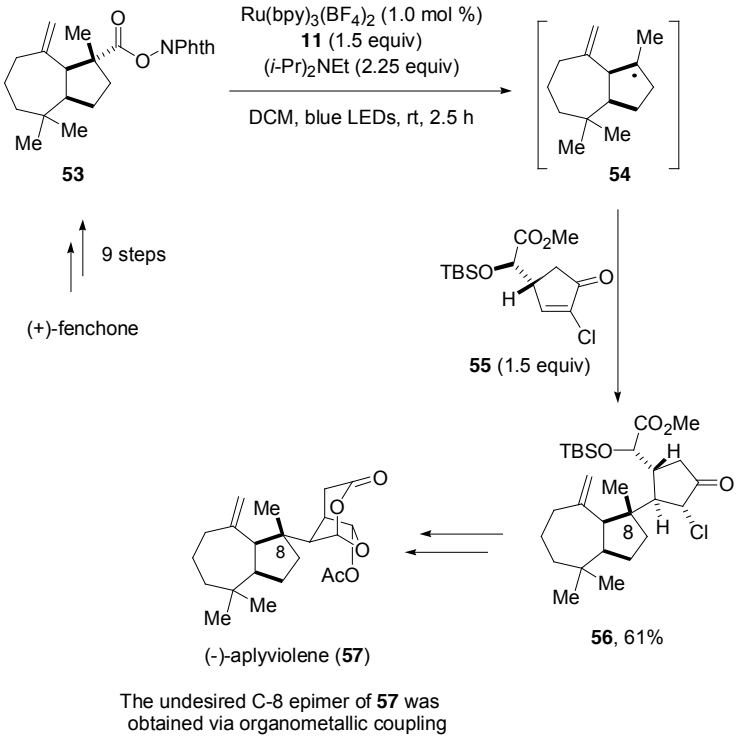
The reductive quenching cycle returned to its origins in complex-molecule synthesis when we successfully applied it to the synthesis of (+)-gliocladin C.[36] The key carbon-carbon bond was forged between the C-3 position of indole **50** and the elaborated bromopyrroloindoline radical generated by reductive dehalogenation of **49**. $Ru(bpy)_3Cl_2$ (**1**) was identified as the optimal photocatalyst in combination with $(n\text{-}Bu)_3N$ as the reductive quencher. Competing hydrogen-atom abstraction by the tertiary radical was minimized by the use of 5 equivalents of the readily available indole **50**, allowing the reaction to successfully operate on a multigram scale in good yield (72%) with only 1 mol % of the photocatalyst (Scheme 9).[36] The efficiency of this reaction allowed the total synthesis of (+)-gliocladin C in 10 steps from commercially available Boc-D-tryptophan methyl

ester in 30% overall yield, and highlights the viability of photoredox catalysis for facilitating complex molecular synthesis.



Scheme 9. Photocatalytic radical reductive coupling as a key reaction step en route to (+)-gliocladin C. (Ref. 36)

A similar strategy was elegantly employed by Schnermann and Overman in their concise, second-generation formal synthesis of (-)-aplyviolene.[37]



Scheme 10. Photocatalytic radical reductive coupling as a key reaction step in a formal total synthesis of (-)-aplyviolene. (Ref. 37)

The key transformation, relying on the seminal work of Okada and Oda,[10] accomplishes the coupling of tertiary radical **54** - generated by decarboxylative reduction of **53** - to α-chlorocyclopentenone **55** to furnish adjacent quaternary and tertiary centers with high stereoselectivity. Optimization of this challenging transformation led to conditions reported by Gagné and co-workers for the reduction of glycosyl halides under anhydrous conditions.[38] Accordingly, 1 mol % of $Ru(bpy)_3(BF_4)_2$ with $(i\text{-}Pr)_2NEt$ (2.25 equiv) and Hantzsch ester **11** (1.5 equiv) in DCM provided **56** in 61% yield and, importantly, the opposite stereoselectivity to that obtained by an analogous organometallic coupling reaction (Scheme 10).[37] This method was later expanded to a general process for the synthesis of quaternary

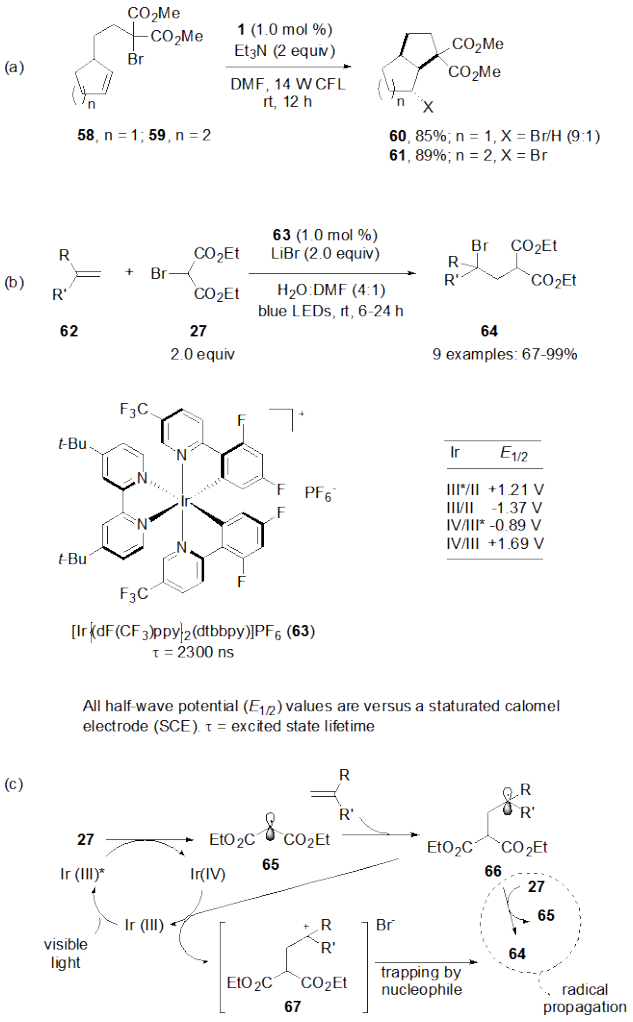
carbons from tertiary alcohols, with a range of electron-deficient alkenes employed as the coupling partners.[39]

3. Oxidative quenching

3.1. Atom-transfer radical addition (ATRA)

Our ability to efficiently utilize the oxidative quenching cycle was initially slower to develop than the corresponding reductive quenching pathway. During our investigation of reductive radical cyclizations (Section 2.2), we discovered that replacing terminal alkenes and alkynes with tethered cyclopentene **58** or cyclohexene **59** provided atom-transfer products (Scheme 11, Part (a)).[40] We found that, by removal of Et_3N, which acts as both a reductive quencher and H-atom donor, the exclusive atom-transfer product could be obtained.[41] Further optimization of this protocol, including the use of $[Ir\{dF(CF_3)\text{-}ppy\}_2(dtbbpy)]PF_6$ (**63**) as the photocalyst,[24b] greatly increased the reaction efficiency (Scheme 11, Part (b)).[41] It is postulated that **63** is optimal due to its extended excited-state lifetime (τ = 2300 ns) compared to $Ru(bpy)_3Cl_2$ (τ = 1100 ns), given their similar excited-state reduction potential: $E_{1/2}^{IV/III*}$ = -0.89 V vs SCE compare to $E_{1/2}^{III/II*}$ = -0.81 V for $Ru(bpy)_3Cl_2$.

This optimized process proceeds exclusively via oxidative quenching, whereby the excited state of the catalyst directly reduces the carbon-halogen bond of the bromomalonate substrate, **27**, to produce the desired radical. Interestingly, mechanistic studies have indicated that the process may proceed further - via propagation in a radical polar crossover and/or via catalyst turnover - to generate the same product, **64** (Scheme 11, Part (c)).[41]

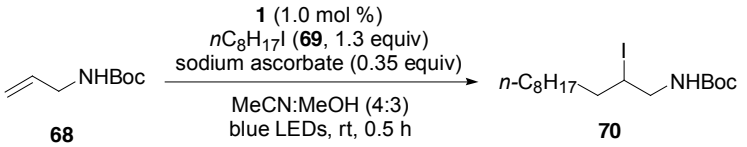


Scheme 11. Atom-transfer radical addition (ATRA): (a) Discovery, (b) Optimization, and (c) Possible mechanism. (Ref. 40. 41)

This mode of reactivity eliminates the requirement for a stoichiometric quencher, and allows the atom-transfer radical addition (ATRA) coupling of a range of halogenated compounds to

olefins under mild conditions in typically excellent yields (Scheme 11, Part (b)).[41]

Although this methodology generally performed effectively for a range of halogenated substrates, it was not efficient for the addition of perfluoroalkyl iodides (such as **69**), a system designed to achieve fluorous tagging. We, therefore, returned to the reductive quenching of $Ru(bpy)_3Cl_2$ approach, but with sodium ascorbate as an electron donor instead of a tertiary amine. This effectively prevents the premature reduction of the perfluoroalkyl radical, ad allows the efficient ATRA tagging of a range of alkenes and alkynes such as **68** (eq 3).[40]



68 **70**

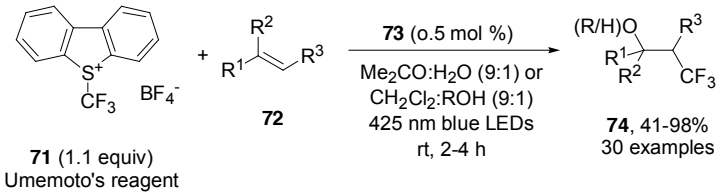
ATRA fluorous tagging of alkenes via reductive quenching of photocatalyst with sodium ascorbate

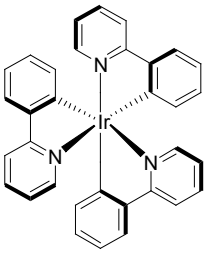
eq 3 (*Ref. 40*)

3.2. Oxytrifluoromethylation of alkenes

Yasu, Koike, and Akita recently published an elegant advancement of the ATRA methodology to oxytrifluromethylation.[42] In this protocol, Umemoto's reagent is reduced by the excited state of the photocatalyst to generate the active CF_3 radical. Following radical addition and either oxidation or chain propagation, the carbocation intermediate (analogous to **67**) is trapped by a nucleophilic additive. The use of *faci*-Ir(ppy$_3$) (**73**),[3c] the strongest excited-state reductant ($E_{½}^{IV/III*}$ = -1.73 V vs SCE) of the commonly employed photocalatalyst, in combination with Umemoto's reagent (-0.25 V vs SCE)[43] proved critical for good reactivity. A range of styrenyl alkenes were efficiently trapped in good yields (typically >75%) by a range of alcohols, carboxylic acids, or water (**eq. 4**).[42] Preliminary studies indicated moderate levels of diastereocontrol in the addition of simple alcohols to *trans*-stilbene (typically 5:1 dr), while the

application of this methodology to the synthesis of the antiestrogen drug Panomifene showcased its potential synthetic utility.





fac-Ir(ppy)$_3$ (**73**)
τ = 1900 ns

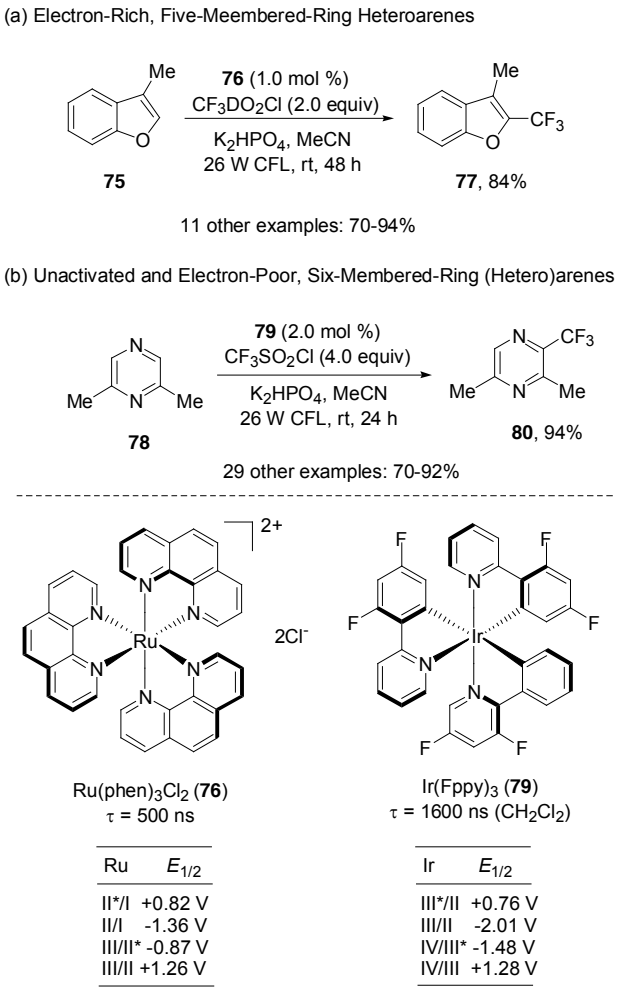
Ir	$E_{1/2}$
III*/II	+0.31 V
III/II	-2.19 V
IV/III*	-1.73 V
IV/III	+0.77 V

All halh-wave potential ($E_{1/2}$) values are versus a saturated calomel electrode (SCE). τ = excited state lifetime

eq 4 (*Ref. 42*)

3.3. Trifluoromethylation of arenes and heteroarenes

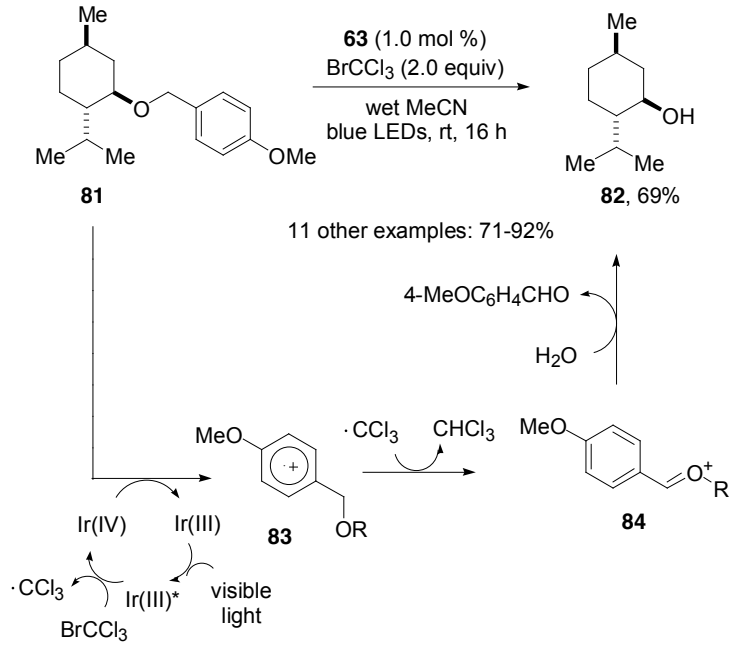
Nagib and MacMillan developed an alternate method for the generation of the CF_3 radical via photoredox catalysis, in this instance for the trifluoromethylation of arenes and heteroarenes.[44] Triflyl chloride (F_3CSO_2Cl or TfCl, -0.18 V vs SCE) represents a comparatively cost-effective and easily handled material when compared to other CF_3 sources. In this protocol, the CF_3 radical is generated by reduction of TfCl with the excited state of the photoredox catalyst and fragmentation.



Scheme 12. Trifluoromethylation of (a) electron-rich and (b) electron-poor heteroarenes. (Ref. 44)

Selective addition of this electron-deficient radical to the most electron-rich position of a range of arenes and heteroarenes provides, following rearomatization, pharmaceutically relevant building blocks in typically good yields (>30 examples with >70% yield) and

regiocontrol (Scheme 12). $Ru(phen)_3Cl_2$ ($E_{½}^{III/II*}$ = -0.87 V vs SCE)[3a,45] provided an optimal mix of reactivity and selectivity for electron-rich heteroarenes, whereas $Ir(Fppy)_3$ (Fppy = 2-(2',4'-difluorophenyl)pyridine)[46,47] was employed for more difficult substrates such as arenes and electron-poor heteroarenes (e.g. **78**). The authors propose that the higher reactivity of $Ir(Fppy)_3$ is due to increased excited-state lifetime when compared to $Ru(phen)_3$.



Scheme 13. Selective, photocatalytic oxidative radical deprotection of PMB ethers. (Ref. 48)

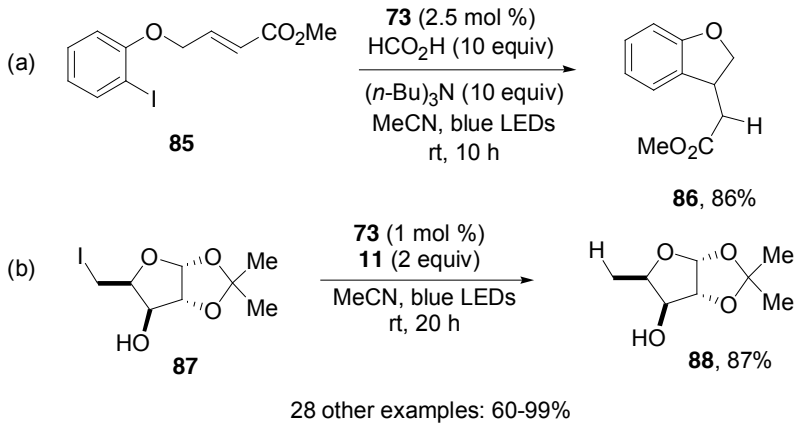
3.4. Deprotection of PMB ethers

During the course of investigations into the functional group tolerance of the ATRA methodology (Section 3.1) we discovered that PMB ethers (PMB = *para*-methoxybenzyl) were unstable to the reaction conditions, undergoing partial deprotection. Following

optimization of reactions conditions, most notably running the reactions in wet acetonitrile with bromotrichloromethane, a range of PMB ethers could be selectively deprotected (Scheme 13).[48] Functional group tolerance includes pivalate esters, substituted olefins, THP acetals, and Fmoc and Cbz groups. This mild, catalytic PMB deprotection serves as an excellent alternative to typical methods that employ DDQ, CAN, $SnCl_4$, AcOH, or Lewis acids. In analogy to the mechanism for the photoredox-catalyzed-ATRA reaction, $BrCCl_3$ oxidatively quenches Ir(III)* to generate Ir(IV) and the trichloromethyl radical ($\cdot CCl_3$). Ir(IV) then oxidizes the PMB ether to generate radical cation **83** and regenerate the ground state of the catalyst. The trichloromethyl radical ($\cdot CCl_3$) may then abstract a benzylic hydrogen from **83** to produce oxonium intermediate **84**, which is hydrolyzed by water to the free alcohol and anisaldehyde (Scheme 13).[48]

3.5. Dehalogenation of unactivated alkyl, alkenyl, and aryl iodides

The strong reductive power of *fac*-Ir(ppy)$_3$ (**73**) from the excited state ($E_{1/2}^{IV/III*}$ = -1.73 V vs SCE) returned us to our earlier studies on reductive dehalogenation and to consider the possibility of further expanding the scope to unactivated C-I bond reduction. By utilizing conditions similar to those employed in our initial entry into the area of photoredox catalysis, but harnessing the oxidative quenching cycle of **73**, a range of unactivated alkyl, alkenyl, and aryl iodides could be successfully reduced (Scheme 14).[49] Consistent with the full suite of reductive protocols developed within our group, the reaction displays excellent functional group compatibility and operational simplicity, and proceeds in typically high yields. It is important to note that the reduction potentials of many of the substrates that are effectively reduced lie outside the effective range of **73**. This observation is of merit as it indicates that reduction potentials are an effective guide to available reactivity, but are by no means the only defining factor. In this, and other instances,[50] we believe the reaction to be driven by the rapid and irreversible C-H abstraction by the radical following C-I bond reduction.



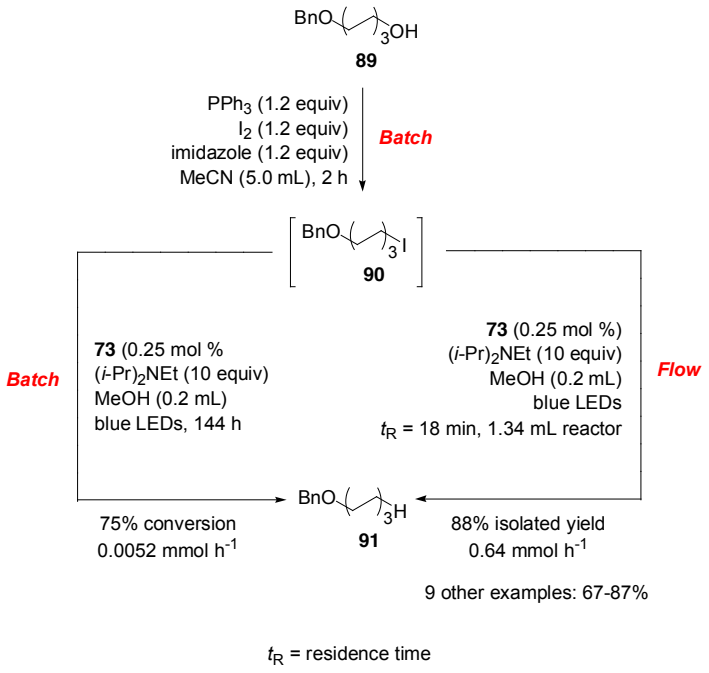
Scheme 14. Dehalogenation of unactivated C-I bonds. (Ref. 49)

3.6. Batch-to-flow deoxygenation

The efficiency of the dehalogenation described above, along with many other photoredox transformations, can be greatly improved by running the reaction in continuous flow mode. Seminal publications from our collaboration with Jamison,[51] and those from the research group of Seeberger[52] and Gagné[53], have demonstrated that conducting photoredox reactions in a flow rather than in a batch setting generally leads to shorter reaction times, improved yields, and lower catalyst loadings. This is simplistically attributed to greater light penetration, owing to the increased surface-to-volume ratio within a typical flow reactor when compared to a batch reaction.[54]

Attempts to merge our own photoredox method for the conversion of alcohols to halides[55] with the updated dehydroiodination protocol have, to this date, been largely unsuccessful. However, we have recently reported a batch-to-flow method for the efficient reduction of a range of primary and secondary alcohols.[56] This protocol proceeds by utilizing the Garegg-Samuelsson reaction[57] in batch, transforming the alcohol functionality into an iodide, which can then be reduced loadings (0.25-0.5 mol % compared to 2.5-mol % in batch) of the catalyst, *fac*-Ir(ppy)$_3$ (**73**), and provides an overall method that is competitive with deoxygenation strategies such as

those of Barton-McCombie and others that typically employ tributyltin hydride or samarium diiodide (Scheme 15).[56]

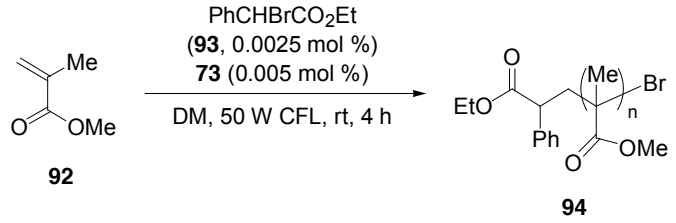


Scheme 15. Efficiency comparison of batch and batch-to-flow deoxygenations of primary and secondary alkyl alcohols. (Ref. 56)

3.7. Control of living polymerization

An excellent application of the visible-light-mediated ATRA reaction to living radical polymerization has recently been reported by Fors and Hawker.[58] In this system, atom-transfer radical polymerization (ATRP) is initiated and controlled[59] by using *fac*-Ir(ppy)$_3$ (**73**). Reduction of the alkyl bromide initiator, **93**, by the excited state of the photocatalyst, and subsequent ATRA reaction with the monomer, **92**, provide an overall cyclic process that can be turned on or off using visible light. This important feature allows excellent control over molecular weight, displaying low polydispersity while employing only between 0.005 and 0.13 mol % of **73** (eq 5).[58] When compared to other traditional copper-based ATRP processes, this

system displays excellent functional group tolerance as exemplified by the use of a free carboxylic acid monomer.



M_n = number average molecular weight
M_w = weight average molecular weight

M_n = 7.8 kg mol^{-1}
M_w/M_n = 1.28

Control of living radical polymerization by ue of visible-light-mediated ATRA

eq 5

4. Conclusions and Outlook

Building upon the pioneering investigations into the use of transition-metal photoredox catalyst, our group and many others have successfully demonstrated their broader applicability in organic synthesis. While considerable research effort has been directed towards applications of previously reported transformations, many new modes of reactivity have also been outlined. This has been facilitated in part by leveraging the large number of reported transition-metal photoredox catalysts, whose origins lie outside their direct use in organic synthesis. Concurrent with driving new reaction discovery, this review has emphasized that the breadth of available photocatalysts allows for astute reaction optimization on the basis of known photophysical properties. The ability of these photocatalysts to potentially operate as either strong oxidants or reductants, combined with the relatively large range of accessible potentials, is key to their expanded use in organic synthesis. This is particularly well illustrated by our group's continued research into the reduction of carbon-halogen bonds, where both modulation of the

photocatalyst and mode of quenching have allowed the reduction of increasing more challenging substrates.
As the field continues to expand and mature, we will likely see further novel applications that harness the versatile nature of this mode of single-electron chemistry. Equally important will be endeavors aimed at addressing some of the current limitations such as the transition from one- to two-electron processes. Other areas with potential for development include the further design and synthesis of catalysts with a similar range of electronic potentials, excited-state lifetimes, and chemical stability, which do not rely on costly transition metals such as iridium and ruthenium. Another strategy to alleviate some of the cost pressures precluding wider use on scale may be analysis of catalyst recovery and re-use systems, particularly when coupled to the growing combination of photoredox and continuous processing methods. Given the frequent ambiguity over the precise mechanistic pathway for visible-light-mediated reactions, in-depth mechanistic studies are also warranted, potentially providing insights into previous processes or directing new avenues for investigation.

5. Acknowledgments

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6. References and Notes

1. Burstall, F. H. *J. Chem. Soc. (Resumed)* **1936**, 173.
2. Juris, A.; Balzani, V.; Belser, P.; von Zelewsky, A. *Helv. Chim. Acta* **1981**, *64*, 2175
3. (a) Kalyanasundaraman, K. *Coord. Chem. Rev.* **1982**, *46*, 159. (b) Kalyanasundarman, K.; Grätzel, M. *oord. Chem. Rev.* **1998**, *77*, 347. (c) Balzani, V.; Bergamini, G.; Campagna, S.; Puntoriero, F. In *Photochemistry and Photophysics of Coordination Compounds I*; Balzani, V., Campagna, S., Eds.; Topics in Current Chemistry Series, Vol. 280; Springer: Berlin Heidelberg, 2007; pp 1-36. (d) Campagna, S.; Puntoriero, F.; Nastasi, F.; Bergamini, G.; Balzani, V. In *Photochemistry and Photophysics of Coordination Compounds I*; Balzani, V., Campagna, S.,

Eds.; Topics in Current Chemistry Series, Vol. 280; Springer: Berlin Heidelberg, 2007; pp 117-214. (e) Flamigni, L.; Barbieri, A.; Sabatini, C.; Ventura, B.; Barigelletti, F. In *Photochemistry and Photophysics of Coordination Compounds II*; Balzani, V., Campagna, S., Eds.; Topics in Current Chemistry Series, Vol. 281; Springer: Berlin Heidelberg, 2007; pp 143-203.

4. (a) Meyer, T. J. *Acc. Chem. Res.* **1989**, *22*, 163. (b) Bard, A. J.; Fox, M. A. *Acc. Chem. Res.* **1995**, *28*, 141.
5. O'Regan, B.; Grätzel, M. *Nature*, **1991**, *353*, 737.
6. Gray, H. B.; Maverick, A. W. *Science* **1981**, *214*, 1201.
7. Demas, J. N.; Taylor, D. G. *Inorg. Chem.* **1979**, *18*, 3177.
8. Tucker, J. W.; Stephenson, C. R. J. *J. Org. Chem.* **2012**, *77*, 1617.
9. (a) Cano-Yelo, H.; Deronzier, A.; *J. Chem. Soc., Perkin Trans. II* **1984**, 1093, (b) Cano-Yelo, H.; Deronzier, A. *Tetrahedron Lett.* **1984**, *25*, 5517.
10. Okada, K.; Okamoto, K.; Morita, N.; Okubo, K.; Oda, M. *J. Am. Chem. Soc.* **1991**, *113*, 9401.
11. Van Bergen, T. J.; Hedstrand, D. M.; Kruizinga, W. H.; Kellogg, R. M. *J. Org. Chem.* **1979**, *44*, 4953.
12. Fukuzumi, S.; Mochizuki, S.; Tanaka, T. *J. Phys. Chem.* **1990**, *94*, 722.
13. Nicewicz, D. A.; MacMillan, D. W. C. *Science* **2008**, *322*, 77.
14. Ischay, M. A.; Anzovino, M. E.; Du, J.; Yoon, T. P. *J. Am. Chem. Soc.* **2008**, *130*, 12886.
15. (a) Xi, Y.; Yi, H.; Lei, A. *Org. Biomol. Chem.* **2013**, *11*, 2387. (b) Prier, C. K.; Rankic, D. A.; MacMillan, D. W. C. *Chem. Rev.* **2013**, *113*, 5322. (c) Narayanam, J. M. R.; Stephenson, C. R. J. *Chem. Soc. Rev.* **2011**, *40*, 102. (d) Teplý, F. *Collect. Czech. Chem. Commun.* **2011**, *76*, 859.
16. For example, see: (a) Yoon, T. P.; Ischay, M. A.; Du, J. *Nature Chem.* **2010**, *2*, 527. (b) Zeitler, K. *Angew. Chem. Int. Ed.* **2009**, *48*, 9785.
17. Hironaka, K.; Fukuzumi S.; Tanaka, T. *J. Chem. Soc., Perkin Trans. II* **1984**, 1705.
18. Kern, J. -M; Sauvage, J. -P.; *J. Chem. Soc., Chem. Commun.* **1987**, 546.
19. Narayanam, J. M. R.; Tucker, J. W.; Stephenson, C. R. J. *J. Am. Chem. Soc.* **2009**, *131*, 8756.
20. Tucker, J. W.; Narayanam, J. M. R.; Krabble, S. W.; Stephenson, C. R. J. *Org. Lett.* **2010**, *12*, 368.

21. Snider, B. B. *Chem. Rev.* **1996**, *96*, 339.
22. (a) Magolan, J.; Kerr, M. A. *Org. Lett.* **2006**, *8*, 4561. (b) Magolan, J.; Carson, C . A.; Kerr, M. A. *Org. Lett.* **2008**, *10*, 1437.
23. (a) Tucker, J. W.; Nguyen, J. D.; Narayanam; J. M. R.; Krabbe, S. W.; Stephenson, C. R. J. *Chem. Commun.* **2010**, *46*, 4985. (b) All LEDs and LED components were purchased from Creative Lighting Solutions (http://www.creativelightings.com/).
24. (a) Slinker, J. D.; Gorodetsky, A. A.; Lowry, M. S.; Wang, J.; Parker, S.; Rohl, R.; Bernhard, S.; Malliaras, G. G. *J. Am. Chem. Soc.* **2004**, *126*, 2763. (b) Lowry, M. S.; Goldsmith, J. I.; Slinker, J. D.; Rohl, R.; Pascal, R. A., Jr.; Malliaras, G. G.; Bernhard, S. *Chem. Mater.* **2005**, *17*, 5712.
25. Furst, L.; Matsuura, B. S.; Narayanam, J. M. R.; Tucker, J. W.; Stephenson, C. R. J. *Org. Lett.* **2010**, *12*, 3104.
26. Dedeian, K.; Djurovich, P. I.; Garces, F. O.; Carlson, G.; Watts, R. J. *Inorg. Chem.* **1991**, *30*, 1685.
27. Condie, A. G.; González-Gómez, J. C.; Stephenson, C. R. J. *J. Am. Chem. Soc.* **2010**, *132*, 1464.
28. Li, Z.; Li, C. -J. *J. Am. Chem. Soc.* **2005**, *127*, 3672.
29. (a) Freeman, D. B.; Furst, L.; Condie, A. G.; Stephenson, C. R. J. *Org. Lett.* **2012**, *14*, 94. (b) Bergonzini, G.; Schindler, C. S.; Wallentin, C. -J.; Jacobsen, E. N.; Stephenson, C. R. J. *Chem. Sci.* **2013**, *5*, 112.
30. Hu, J.; Wang, J.; Nguyen, T. H.; Zheng, N. *Beilstein J. Org. Chem.* **2013**, *9*, 1977 (DOI: 10.3762/bjoc.9.234).
31. Ruiz Espelt, L.; Weinsch, E. M.; Yoon, T. P. *J. Org. Chem.* **2013**, *78*, 4107.
32. Yoon, T. P. *ACS Catal.* **2013**, *3*, 895.
33. Lin, S.; Ischay, M. A.; Fry, C. G.; Yoon, T. P. *J. Am. Chem. Soc.* **2011**, *133*, 19350.
34. (a) Crutchley, R. J.; Lever, A. B. P. *J. Am. Chem. Soc.* **1980**, *102*, 7128. (b) Haga, M.; Dodsworth, E. S.; Eryavec, G.; Seymour, P.; Lever, A. B. P. *Inorg. Chem.* **1985**, *24*, 1901.
35. Maity, S.; Zhu, M.; Shinabery, R. S.; Zheng, N. *Angew. Chem., Int. Ed.* **2012**, *51*, 222.
36. Furst, L.; Nurayanam, J. M. R.; Stephenson, C. R. J. *Angew. Chem., Int. Ed.* **2011**, *50*, 9655.
37. Schnermann, M. J.; Overman, L. E. *Angew. Chem., Int. Ed.* **2012**, *51*, 9576.

38. Andrews, R. S.; Becker, J. J.; Gagné M. R. *Angew. Chem., Int. Ed.* **2010**, *49*, 7274.
39. Lackmer, G. L.; Quasdorf, K. W.; Overman, L. E. *J. Am. Chem. Soc.* **2013**, *135*, 15342.
40. Wallentin, C. -J.; Nguyen, J. D.; Finkbeiner, P.; Stephenson, C. R. J. *J. Am. Chem. Soc.* **2012**, *134*, 8875.
41. Nguyen, J. D.; Tucker, J. W.; Konieczynska, M. D.; Stephenson, C. R. J. *J. Am. Chem. Soc.* **2011**, *133*, 4160.
42. Yasu, Y.; Koike, T.; Akita, M. *Angew. Chem., Int. Ed.* **2012**, *51*, 9567.
43. Calculated from the reported value in reference 42 (-0.75 V vs CP_2Fe in MeCN).
44. Nagib, D. A.;MacMillan, D. W. C. *Nature*, **2011**, *480*, 224.
45. Skarda, V.; Cook, M. J.; Lewis, A. P.; McAuliffe, G. S. G.; Thomson, A. J.; Robbins, D. J. *J. Chem. Soc., Perkin Trans. II* **1984**, 1309.
46. Tamayo, A. B.; Alleyne, B. D.; Djurovich, P. I.; Lamansky, S.; Tsyba, I.; Ho, N. N.; Bau, R.; Thompson, M. E. *J. Am. Chem. Soc.* **2003**, *125*, 7377.
47. Excited state potentials were calculated from the reported ground state values in reference 46 by the method outlined in reference 8.
48. Tucker, J. W.; Narayanam, J. M. R.; Shah, P. S.; Stephenson, C. R. J. *Chem. Commun.* **2011**, *47*, 5040.
49. Nguyen, J. D.; D'Amato, E. M.; Narayanam, J. M. R.; Stephenson, C. R. J. *Nature Chem.* **2012**, *4*, 854.
50. (a) Kim, H.; Lee, C. *Angew. Chem., Int. Ed.* **2012**, *51*, 12303. (b) Garnier, J.; Kennedy, A. R.; Berlouis, L. E. A.; Turner, A. T.; Murphy, J. A. *Beilstein J. Org. Chem.* **2010**, *6*, 73 (DOI: 103762/bjo.6.73).
51. Tucker, J. W.; Zhang, Y.; Jamison, T. F.; Stephenson, C. R. J. *Angew. Chem., Int. Ed.* **2012**, *51*, 4144.
52. Bou-Hamdan, F. R.; Seeberger, P. H. *Chem. Sci.* **2012**, *3*, 1612.
53. Andrews, R. S.; Becker, J. J.; Gagné, M. R. *Angew. Chem., Int. Ed.* **2012**, *51*, 4140.
54. Knowles, J. P.; Elliott, L. D.; Booker-Milburn, K. I. *Beilstein J. Org. Chem.* **2012**, *8*, 2025 (DOI: 10.3762/bjoc.8.229).
55. Dai, C.; Narayanam, J. M. R.; Stephenson, C. R. J. *Nature Chem.* **2011**, *3*, 140.

56. Nguyen, J. D.; Reiss, B.; Dai, C.; Stephenson, C. R. J. *Chem. Commun.* **2013**, *49*, 4352.
57. Garegg, P. J.; Samuelsson, B. *J. Chem. Soc., Chem. Commun.* **1979**, 978.
58. Fors, B. P.; Hawker, C. J. *Angew. Chem, Int. Ed.* **2012**, *51*, 8850.
59. Leibfarth, F. A.; Mattson, K. M.; Fors, B. P.; Collins, H. A.; Hawker, C. J. *Angew.Chem., Int. Ed.* **2012**, *51*, 2.

SPECIAL REPORTS ON FLUOROGENIC PROBES

Introduction

Dear EPA members
This issue of the EPA Newsletter, December 2014, is dedicated to "Fluorogenic Probes", an emerging new area aimed at designing new dyes for monitoring and interrogating complex biological and chemical systems. New probes are currently being designed at an incredible pace to monitor specific analytes (e.g. reactive oxygen species, metals etc.) or changes in the molecular environment (e.g. viscosity, polarity, etc). By wisely exploiting either internal conversion, or intramolecular photoinduced electron transfer, among many other quenching pathways, new probes may be characterized by having their emission fully supressed. Emission is next restored following cleavage of a bond of interest or upon formation of a desired new bond in a chemical reaction that is specific to the analyte of interest. Alternatively, emission may be restored by constraining the molecular environment, resulting in the deactivation of an otherwise rapid internal conversion. The new probes thus enable monitoring their molecular environment in a non-invasive and with optimal spatio-temporal resolution.

The issue features a number of contributions summarizing recent reports on fluorogenic probes. *Jeffrey Keillor et al.* (Department of Chemistry, University of Ottawa) report on the application of fluorogenic probes bearing maleimide functional groups and relying on photoinduced electron transfer towards the intracellular labelling of specific proteins. *Christoph Fahrni* (School of Chemistry and Biochemistry, Georgia Institute of Technology) describes the rationale for preparing synthetic fluorogenic probes, relying on photoinduced electron transfer, that bind Cu(I) and may thus serve as powerful tools to interrogate and study cellular copper homeostasis. *Tetsuro Majima et al.* (The Institute of Scientific and Industrial Research (SANKEN), Osaka University) comment on a newly prepared red fluorogenic probe for intracellular $^1O_2$ mapping. With the new probe that exploits photoinduced electron transfer, $^1O_2$ generation has been successfully visualized up to the spatial resolution of a single mitochondria tubule. *Gonzalo Cosa et al.*

(Department of Chemistry, McGill University) provide an overview of fluorogenic antioxidants, lipophilic probes analogues of α-tocopherol that undergo fluorescence enhancement upon scavenging lipid peroxyl radicals within the lipid membrane of live cells. *Eduardo Peña-Cabrera et al.* (Department of Chemistry, Universidad de Guanajuato) introduces a stimulating discussion on fluorogenic probes for monitoring organic chemical reactions. The new compounds are shown to undergo dramatic emission enhancements upon undergoing chemical reactions akin to those of α,β-unsaturated carbonyl compounds. *Andrey Klimchenko and Yves Mély* (Laboratoire de Biophotonique et Pharmacologie UMR 7213 CNRS/Université de Strasbourg) discuss on environmental sensitive fluorogenic (and also chromogenic) probes and their application to cell membrane labeling. *Marina Kuimova* (Department of Chemistry, Imperial College) reports on synthetic fluorophores whose emission intensity (and fluorescence lifetime) is susceptible to the viscosity of the surrounding environment. She describes novel strategies to measure viscosity at the single cell level upon exploiting the competition between internal conversion and radiative decay of the excited state.

The EPA Newsletter Board greatly appreciates these experts' contributions to this issue and also wholeheartedly thanks Gonzalo Cosa for his assistance in expediting this endeavour encouraging the authors to contribute their work and submit it on time!

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Fluorogenic Probes to Monitor Reactive Oxygen Species in the Lipid Membranes of Live Cells.

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Formed as chemical byproducts of cell metabolism, reactive oxygen species (ROS) are connected to multiple pathologies including age-related disorders, cancer, and neurodegenerative ailments including Parkinson's and Alzheimer's diseases. New evidence is however emerging pointing to a more complex yet beneficial role ROS play in physiological processes associated with cell signaling.

This paradox has motivated our curiosity and interest in deciphering how the chemistry of ROS and the biology of ROS relate to each other. We have identified peroxyl radicals as key targets to monitor oxidative changes in the cellular environment and have pioneered the development of lipophilic fluorogenic antioxidants for the spatiotemporal imaging of lipid peroxyl radicals in the membrane of live cells.[1] Our design relies on two segment trap-reporter probes. These probes mimic the peroxyl radical-scavenging activity of α-tocopherol, the most active naturally occurring lipophilic antioxidant found in mammalian tissues. Utilizing borondipyrromethane (BODIPY) as our fluorescent reporter and coupling to it the active part of α-tocopherol (the chromanol moiety), we ensure the peroxyl radical scavenging activity of our probe is similar to that of α-tocopherol. BODIPY dyes are an ideal candidate because they are lipophilic (ensuring the probe partitions within membranes), photostable, and easily tunable to afford fluorogenicity.

In the reduced form (prior to scavenging peroxyl radicals), the chromanol (trap) moiety renders the probe non-emissive via intramolecular photoinduced electron transfer (PeT) which effectively competes with radiative decay.[2] Upon reaction with peroxyl radicals, the chromanol moiety oxidizes to chromanone, PeT is deactivated and emission is restored.

Building on our first generation probe B-TOH, our desire for probes with improved sensitivity led to the synthesis and characterization of a series of novel BODIPY dyes with versatile functionalities for improved tethering, and tunable redox properties for maximizing the PeT deactivation pathway in the off state.[4] These series of BODIPY dyes contain substituents ranging from electron donating groups (ethyl) to electron withdrawing substituents (nitrile) providing a tunable redox window of 700 mV. In addition they provide a range of handles for coupling antioxidants (or any other receptor of interest) suitable for either nucleophilic additions or electrophilic additions.

We next utilized one of the newly prepared BODIPY dyes and coupled it via a short linker to the trap chromanol segment to maximize PeT, leading to the second generation probe, H_2B-PMHC. H_2B-PMHC has enhanced sensitivity (>30 fold fluorescence enhancement upon reaction with lipid peroxyl radicals), and improved reactivity (antioxidant activity is on par with α-tocopherol) when compared to B-TOH. We exploited the high sensitivity of the new probe H_2B-PMHC towards the development of high throughput assays to monitor lipid peroxidation. By following fluorescence, real time, on parallel wells, we succeeded in monitoring multiple reaction conditions simultaneously. Combined, the new probe, the fluorescence assay, and the data analysis provide a new method to obtain in a rapid parallel format relative antioxidant activities in phospholipid membranes The significance of lipid unsaturation, peroxyl radical partitioning, and the lipophilic tail of the chromanol ring in the antioxidant activity of α-tocopherol, were put in quantitative terms with the new studies.[5] The high-throughput assay we developed with the use of H_2B-PMHC is readily extendable to other lipid mixtures and can also be applied to the evaluation of antioxidant activity of novel synthetic antioxidants.[6]

The development of mitochondria targeting fluorogenic antioxidants became our next major objective. Specifically we envisioned a probe for determining the antioxidant status of the inner mitochondrial lipid membrane; a probe that would report the onset of lipid peroxidation. The inner mitochondrial membrane is a major site of ROS production due to the formation of oxidative byproducts from the mitochondrial electron transport chain. Polyunsaturated fatty acids of the inner mitochondrial membrane are particularly vulnerable to ROS mediated oxidation. The target choice was thus a

logical step forward in our attempts to elucidate how the chemistry and biology of ROS interrelate

We thus developed Mito-BODIPY-TOH,[7] where building on the trap-reporter fluorogenic probe concept, we additionally added a third, mitochondrial-targeting segment consisting of a triphenylphosphonium cation. Mito-BODIPY-TOH readily targets the inner mitochondrial membrane and is sensitive to lipid peroxyl radicals. Our studies show that in the presence of paraquat, a neurotoxin and a known source of oxidative stress, Mito-BODIPY-TOH gives rise to an emission enhancement of 8-fold (Fig. 1). In viable untreated cells, (our controls), no emission enhancement was observed. Our studies suggest the potential of Mito-BODIPY-TOH for use in understanding the link between antioxidant load, lipid peroxidation and mitochondrial physiology.

Due to the heterogeneity of the environment of lipid membranes, understanding the dynamics of lipid peroxidation and the role ROS plays in cellular signalling has been challenging. Ensemble studies in both liposomes and cells, while providing a general overview of the processes at hand, do not show specific events. Therefore, we sought to employ single molecule spectroscopy to monitor single oxidative events in lipid membranes. We pursued the use of H_2B-PMHC (see Fig.2) in single molecule experiments given its unsurpassed sensitivity.[8] Loading H_2B-PMHC into liposomes, and observing oxidative processes at the single particle level, our studies show that lipid peroxidation takes place under standard liposome preparation conditions and underscore the prominence of lipid degradation during their formulation. Additional labelling of the liposomes with DiD as an internal standard allowed us to quantify the change in emission intensity of H_2B-PMHC in a ratiometric single particle (liposome) analysis (Fig. 2). This single molecule/particle assay provides a straightforward and sensitive way to monitor chemical reactions at the level of single liposomes, and we anticipate that it may become a valuable tool for studying oxidative events in biologically relevant systems.

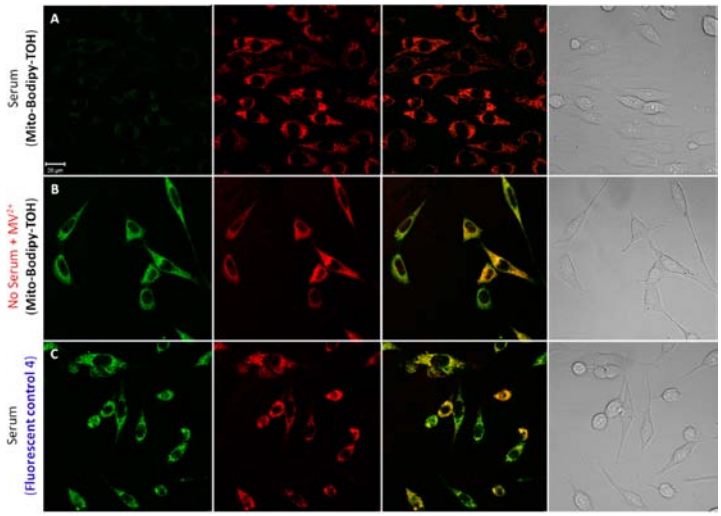

Figure 1. Detection of ROS with fluorogenic probe Mito-Bodipy-TOH in live NIH 3T3 mouse embryo fibroblast cells. The panels show (left to right) confocal fluorescence images of 1 μM Mito-Bodipy-TOH, 200 nM MitoTracker Deep Red, overlay of the first two images, and bright field image of: A) Fibroblast cells in the presence of 10 % FBS; B) Fibroblast cells treated with paraquat for 4 h prior to imaging; C) Fibroblast cells in the presence of 10 % FBS incubated with 1 μM control mitochondria-targeting fluorescent probe 4. Scale bar is 20 μm. D) Structure of mitochondria-targeting fluorogenic antioxidant (Mito-BODIPY-TOH). The three segments are colored to facilitate visualization. Adapted from reference 6.

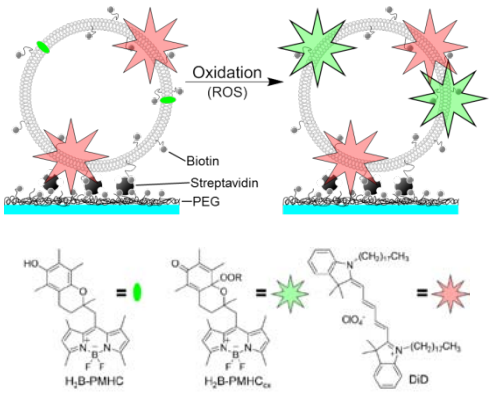

Figure 2. Experimental single molecule/particle strategy. The cartoon illustrates surface tethered liposomes embedding the fluorogenic probe H_2B-PMHC and an internal standard lipophilic cyanine dye DiD. Adapted from reference [8].

Most recently our quest for new and improved fluorogenic probes lead us to establish novel methods for the design of PeT based fluorogenic probes based on BODIPY dyes.[9] Careful tuning of the redox parameters of the BODIPY segment is integral in designing a useful and sensitive fluorogenic probe. In general, tuning of the trap segment is not desirable as it generally means disrupting the integrity and reactivity of the natural product it emulates. Therefore, the redox properties of the reporter (in our case BODIPY) must be optimized for PeT with the desired trap segment. We sought an empirical method to estimate the driving force of PeT between a trap of interest and the BODIPY dye, based on the substitution pattern of the BODIPY chromophore. We were able to formulate equations and provide the parameters towards predicting the oxidizing/reducing power of photoexcited 1,3,5,7,-tetramethyl BODIPY dyes in their singlet excited state based purely on empirical Hammett substituent constants. These equations allow for the rapid prediction of BODIPY redox potentials and thus the rapid design of PeT based sensors.[9]

In closing, we have developed several methodologies for studying the antioxidant status of both model lipid membranes and live cells. These methods include high-throughput fluorescence assays as well as single molecule spectroscopy methods. In addition we have

developed several generations of fluorogenic antioxidants and have begun developing organelle specific fluorogenic probes. Due to our interest in developing probes, we have shown the versatility of BODIPY dyes for use as PeT based fluorogenic probes, and have developed an empirical method to aid in designing exergonic PeT based sensors. We anticipate that these methods will be useful in the understanding of ROS in biological systems as well as evaluating drugs which alter the antioxidant status.

References.

1. Oleynik, P.; Ishihara, Y.; Cosa, G. *J. Am. Chem. Soc.* **129**, 1842-1843 (2007).
2. Krumova, K.; Oleynik, P.; Karam, P.; Cosa, G. *J. Org. Chem.* **74**, 3641-3651 (2009).
3. Khatchadourian, A.; Krumova, K.; Boridy, S.; Ngo, A. T.; Maysinger, D.; Cosa, G. *Biochemistry* **48**, 5658-5668 (2009).
4. Krumova, K.; Cosa, G. *J. Am. Chem. Soc.* **132**, 17560-17569 (2010).
5. Krumova, K.; Friedland, S.; Cosa, G. *J. Am. Chem. Soc.* **134**, 10102-10113 (2012).
6. Li, B.; Harjani, J. R.; Cormier, N. S.; Madarati, H.; Atkinson, J.; Cosa, G.; Pratt, D. A. *J. Am. Chem. Soc.* **135**, 1394-1405 (2013).
7. Krumova, K.; Greene, L. E.; Cosa, G. *J. Am. Chem. Soc. 135*, 17135-17143 (2013).
8. Godin, R.; Liu, H.-W.; Cosa, G. *Chem. Sci.* **5**, 2525 (2014).
9. Lincoln, R.; Greene, L. E.; Krumova, K.; Ding, Z.; Cosa, G. *J. Phys. Chem. A* **118**, 10622-10630 (2014).

Fluorogenic and chromogenic probes for lipids and proteins in biomembranes

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Imaging biological systems at high signal to noise ratio is facilitated with the use of fluorogenic probes that turn-on their fluorescence exclusively when bound to the target.[1-3] On the other hand, chromogenic probes, which change their emission colour, provide a robust ratiometric signal for biomolecular interactions.[1,3,4] Fluorogenic and chromogenic responses are triggered by different stimuli, such as chemical reactions or non-covalent interactions. The present report will focus on environment-sensitive probes. Different photochemical mechanisms can be behind the fluorogenic and chromogenic response of a dye to its environment. The most common mechanism is charge transfer for polarity-sensitive (solvatochromic) dyes.[5,6] One of the oldest examples is the push-pull dye Prodan (Fig. 1),[7] which increases its dipole moment in the excited state. Therefore, an increase in the solvent polarity shifts its emission band to the red (chromogenic response). In addition, these molecules systematically exhibit low fluorescence quantum yield in aqueous media, due to electron transfer and twisted charge transfer processes,[6] which is the basis for their fluorogenic response to the environment. We recently developed a fluorene analogue of Prodan, presenting enhanced push-pull properties and, as a consequence, much stronger fluorogenic and chromogenic response to the environment.[8] Another important representative is Nile Red, which is a rare example of red emitting dye showing strong fluorogenic and chromogenic response to the environment.[9] Internal conversion due to molecular rotation (molecular rotors, such as CCVJ, Fig. 1) is a second common mechanism, where an increase in the environment viscosity decreases the non-radiative deactivation through rotation and thus turns on the fluorescence.[10] Finally, chromogenic probes can operate by excited state intramolecular proton transfer (ESIPT), which can be drastically modulated by the environment polarity and hydration.[11] Probably the best example is the 3-hydroxyflavone

family (Fig. 1)[11,12] that presents two emission bands. One band corresponds to the normal form, while the second, red shifted one, is the product of ESIPT. The ratio of the normal to the tautomer form increases with solvent polarity and hydration.[13] Moreover, 4'-dialkylamino-3-hydroxyflavone and its analogues exhibit clear fluorogenic character as their quantum yield strongly increases when they are transferred from aqueous to organic media.

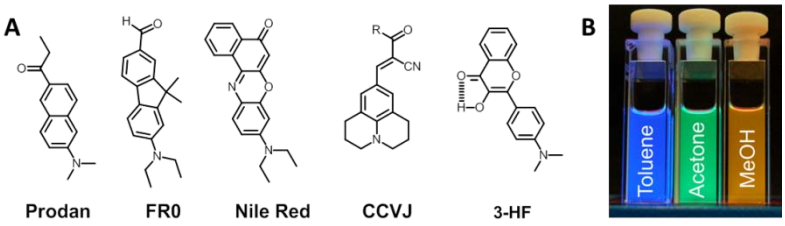

Figure 1. (A) Chemical structures of fluorogenic and chromogenic environment-sensitive dyes operating by charge transfer (Prodan, FR0 and Nile Red), internal rotation (CCVJ) and ESIPT (3-hydroxyflavone derivative, 3HF). (B) Photo of FR0 in different solvents under UV light.[8]

Biological applications of fluorogenic and chromogenic probes grow rapidly in the last years.[1,3] Here, we will present our recent works related to cell membranes. Development of fluorogenic probes for lipid membranes is important due to the limited choice of existing tools, especially for those binding specifically the outer cellular leaflet without fast internalization. Of special interest in this respect are F2N12S and NR12S (Fig. 2), bearing 3-hydroxyflavone and Nile Red dye, respectively.[14,15] Both probes are very poorly fluorescent in water, but exhibit a strong fluorescence increase (>100-fold) on binding to lipid and cell membranes (Fig. 2), so that no washing step is required to study their response on the membranes. The ratio of the two emission bands of F2N12S and the emission band position of NR12S vary as a function of the lipid order.[12,14,15] Therefore, F2N12S and NR12S enable the detection of the loss of transmembrane asymmetry during apoptosis.[14,16] They can also visualize separated phase domains in giant vesicles, monitor cholesterol content in cell membranes[12,15] and evidence a decrease in the lipid order during endosomal maturation.[17]

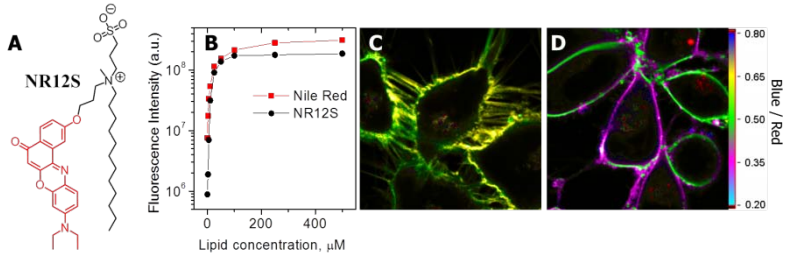

Figure 2. Probe NR12S (A) and its turn-on response to lipid membranes (B). (C, D) Ratiometric fluorescence imaging of intact astrocytoma cells (C) and after 2h of cholesterol extraction (D).[15]

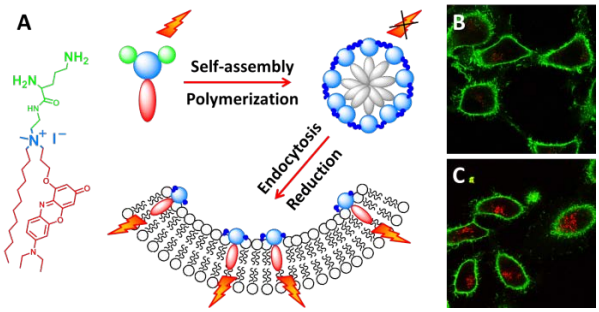

Figure 3. (A) Disassembly-driven turn on concept with a fluorescent surfactant. (B,C) Confocal fluorescence images of HeLa cells incubated for 10 min (B) and 1h (C) with the polymerized micelles. The red dots correspond to the turn-on response.[18]

Noteworthy, the mechanism of the fluorogenic response of F2N12S and NR12S is not only related to the environment-sensitivity of the dyes, but also relies on their pre-organization in micellar structures, where the dyes are efficiently self-quenched.[15] Recently, we stabilized these self-quenched nanostructures by using bio-cleavable disulphide bonds (Fig. 3).[18] The obtained polymerized micelles were non-fluorescent in buffers. After entering the cells, a reductive stimulus cleaved the disulphide bonds, inducing micellar disassembly and fluorescence turn-on of the liberated dyes.

The second important application of fluorogenic probes is sensing interaction of biomolecules with lipid membranes. In our earlier work, a 3-hydroxyflavone-based label was grafted at the N-terminus of membrane binding peptide melittin.[19] The labelled peptide showed

very poor fluorescence intensity in water. In lipid membranes, the intensity increased many-fold and its characteristic two-colour emission suggested the insertion of the probe into the lipid bilayer. Later on, we synthesized an amino acid based on the same fluorophore and incorporated it inside melittin by replacing selected amino acids.[20] This enabled us to better understand the peptide insertion and orientation in model and cell membranes. In another study, we labelled penetratin, a cell-penetrating peptide, which was shown to undergo multiple cell internalization pathways. We found that penetratin at low concentrations exhibited a clear cytosolic localization, as identified from the emission colour of the probe.[21] This confirmed the capacity of penetratin to rapidly enter the cytosol by directly crossing the cell plasma membrane.

Finally, fluorogenic probes could also be used to describe the specific interaction of a membrane receptor with its ligand. Here, the fluorogenic response is highly desirable, in order to provide direct detection of the receptor target without background from the free non-interacted ligand species. As a model system, we selected the carbetocin ligand that specifically binds the oxytocin receptor. Carbetocin was coupled to the fluorogenic dye Nile Red through a PEG(8) spacer (Fig. 4). The fluorescent ligand showed a turn-on fluorescence response to oxytocin receptor in living cells, as a result of its transfer from buffer to apolar receptor-lipid environment. We found that the PEG(8) spacer was critical to prevent non-specific interactions with lipid membranes and serum. Finally, the same ligand coupled to the non-fluorogenic dye Lissamine Rhodamine B showed much stronger background especially at high ligand concentrations, stressing again the key advantage of using fluorogenic probes.

Currently, the major limitation of the fluorogenic and chromogenic dyes sensitive to the environment is their limited brightness and photostability. Moreover, this class of dyes is poorly represented in the far red/near infrared (NIR) regions, which are particularly attractive for bioimaging. In this respect development of bright photostable fluorogenic dyes operating in far-red/NIR regions remains a challenging task for chemists and photochemists.

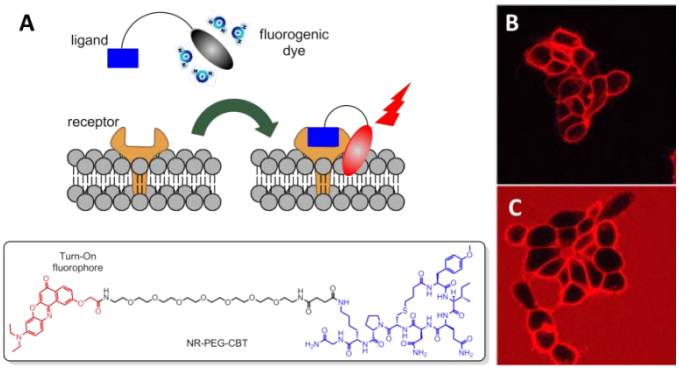

Figure 4. (A) Concept of turn-on ligand receptor detection and chemical structure of the developed ligand. Images of HEK293 cells expressing oxytocin receptor in the presence of 100 nM turn-on ligand (B) or Lissamine Rhodamine B-based ligand (C).[22]

References

(1) Li, X.; Gao, X.; Shi, W.; Ma, H. *Chem. Rev.* **2014**, *114*, 590.
(2) Grimm, J. B.; Heckman, L. M.; Lavis, L. D. *Prog. Mol. Biol. Transl. Sci.* **2013**, *113*, 1.
(3) Santos-Figueroa, L. E.; Moragues, M. E.; Climent, E.; Agostini, A.; Martínez-Máñez, R.; Sancenón, F. *Chem. Soc. Rev.* **2013**, *42*, 3489.
(4) Klymchenko, A. S.; Mely, Y. In *Prog. Mol. Biol. Transl. Sci.* 2013; Vol. 113, p 35.
(5) Reichardt, C. *Chem. Rev.* **1994**, *94*, 2319.
(6) Grabowski, Z. R.; Rotkiewicz, K.; Rettig, W. *Chem. Rev.* **2003**, *103*, 3899.
(7) Weber, G.; Farris, F. J. *Biochemistry* **1979**, *18*, 3075.
(8) Kucherak, O. A.; Didier, P.; Mèly, Y.; Klymchenko, A. S. *J. Phys. Chem. Lett.* **2010**, *1*, 616.
(9) Greenspan, P.; Fowler, S. D. *J. Lipid Res.* **1985**, *26*, 781.
(10) Haidekker, M. A.; Theodorakis, E. A. *Org. Biomol. Chem.* **2007**, *5*, 1669.
(11) Demchenko, A. P.; Tang, K. C.; Chou, P. T. *Chem. Soc. Rev.* **2013**, *42*, 1379.
(12) Demchenko, A. P.; Mély, Y.; Duportail, G.; Klymchenko, A. S. *Biophys. J.* **2009**, *96*, 3461.
(13) Klymchenko, A. S.; Demchenko, A. P. *Phys. Chem. Chem. Phys.* **2003**, *5*, 461.

(14) Shynkar, V. V.; Klymchenko, A. S.; Kunzelmann, C.; Duportail, G.; Muller, C. D.; Demchenko, A. P.; Freyssinet, J. M.; Mely, Y. *J. Am. Chem. Soc.* **2007**, *129*, 2187.
(15) Kucherak, O. A.; Oncul, S.; Darwich, Z.; Yushchenko, D. A.; Arntz, Y.; Didier, P.; Mély, Y.; Klymchenko, A. S. *J. Am. Chem. Soc.* **2010**, *132*, 4907.
(16) Darwich, Z.; Klymchenko, A. S.; Kucherak, O. A.; Richert, L.; Mély, Y. *Biochim. Biophys. Acta* **2012**, *1818*, 3048.
(17) Darwich, Z.; Klymchenko, A. S.; Dujardin, D.; Mély, Y. *RSC Adv.* **2014**, *4*, 8481.
(18) Niko, Y.; Arntz, Y.; Mely, Y.; Konishi, G.-I.; Klymchenko, A. S. *Eur. J. Chem.* **2014**, *DOI: 10.1002/chem.201405040*.
(19) Postupalenko, V. Y.; Shvadchak, V. V.; Duportail, G.; Pivovarenko, V. G.; Klymchenko, A. S.; Mély, Y. *Biochim. Biophys. Acta* **2011**, *1808*, 424.
(20) Postupalenko, V. Y.; Zamotaiev, O. M.; Shvadchak, V. V.; Strizhak, A. V.; Pivovarenko, V. G.; Klymchenko, A. S.; Mely, Y. *Bioconjug. Chem.* **2013**, *24*, 1998.
(21) Zamotaiev, O. M.; Postupalenko, V. Y.; Shvadchak, V. V.; Pivovarenko, V. G.; Klymchenko, A. S.; Mély, Y. *Org. Biomol. Chem.* **2014**, *12*, 7036.
(22) Karpenko, I. A.; Kreder, R.; Valencia, C.; Villa, P.; Mendre, C.; Mouillac, B.; Mély, Y.; Hibert, M.; Bonnet, D.; Klymchenko, A. S. *ChemBioChem* **2014**, *15*, 359.

Mapping microscopic viscosity using molecular rotors

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Diffusion-controlled processes are of paramount importance in biology, chemistry and engineering. The underlying reason for such wide-stretching influence is the fact that diffusion controlled processes often determine the rate of mass transport of species. This is seen especially within and across the boundaries of domains in heterogeneous systems and such reaction rates depend strongly on the viscosity of the microenvironment of reagents. A plethora of methods for measuring viscosity exists, however, only a few spectroscopic techniques are available that allow to probe microscopic environments and measure viscosity on the lengthscales of biological cells and individual cell organelles.

Fluorescence imaging with molecular rotors is amongst the emerging techniques for measuring diffusion and viscosity in live cells, at a single-cell level.[1-3] The term molecular rotor refers to small synthetic fluorophores in which fluorescence emission is susceptible to the

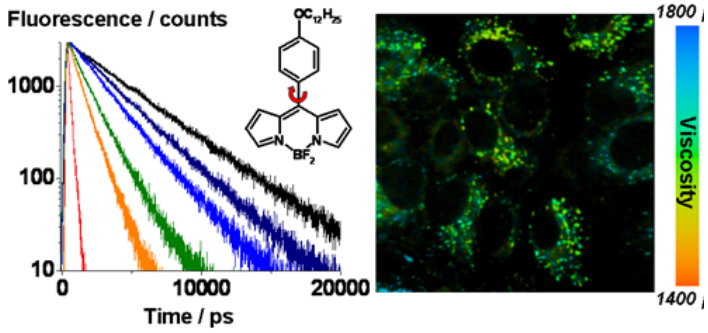

Figure 1. A series of time resolved fluorescence decays of Bodipy-C_{12} molecular rotor in methanol/glycerol calibration mixtures of viscosity between 1-950 cP. The insert shows the structure of the molecular rotor. Fluorescence Lifetime Imaging (FLIM) of Bodipy-C_{12} in live SK-OV-3 cells: a false colour scheme shows increasing viscosity (orange: low – blue: high).

viscosity of the surrounding environment. The change in fluorescence signal normally arises from competition between fluorescence (the radiative decay of the excited state) and intramolecular rotation, which changes the nature of the electronically excited state responsible for photon emission.[1,4] For molecular rotors both the quantum yield and the fluorescence lifetime depends on viscosity.[1] Whilst the fluorescence quantum yield provides a useful parameter for viscosity calibration in bulk homogeneous samples, it is rarely applicable for imaging applications. This limitation is due to the fact that in many heterogeneous samples there exist spatial variations in fluorophore concentration (and hence the value of absorbance). Since the fluorophore concentration has a major effect on the observed fluorescence intensity, it becomes impossible to decouple the changes of intensity due to viscosity variations from those that arise from the spatial variations in the concentration of the rotor. This concentration uncertainty makes the determination of the quantum yield in heterogeneous samples impossible.

In such cases, viscosity measurements based on fluorescence lifetime determination are particularly useful and provide concentration-independent microviscsoty measure.[1-2, 5-6] The lifetime response of a molecular rotor Bodipy-C_{12} can be calibrated versus viscosity in bulk methanol/glycerol mixtures and then used for viscosity imaging in cells, Figure 1.[5] Due to fast endocytosis, the Bodipy rotor is fully embedded in the intracellular domains of live cells and Fluorescence Lifetime Imaging microscopy (FLIM) can be used to record fluorecence decays in every pixel of the image. The calibration from bulk solutions can then be used to assign the viscosity value to each pixel in the intracellular environment. The viscosity of artificial lipid membranes, lipid coated microbubbles and aerosol droples was established in a similar manner using FLIM of molecualr rotors.[6]

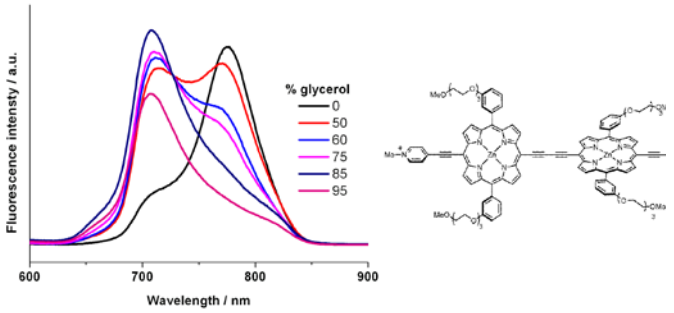

Figure 2. A structure of the conjugated porphyrin dimer used as a molecular rotor. A ratiometric calibration of this rotor in methanol glycerol mixtures of viscosity between 1-950 cP

Whereas FLIM requires a relatively complex experimental setup for fluorescence lifetime detection of molecular rotors, there is an alternative approach that allows to overcome uncertainties associated with the unknown probe concentration. This approach is based on ratiometric fluorescence measurements and its only requirement for the imaging system is the two-channel colour detection, either in a confocal or in a wide-field microscope.

In the simplest case, a ratiometric viscosity sensor is constructed to incorporate two independent chromophores. One of the chromophores is not influenced by viscosity and is used to determine the probe concentration, whilst the other acts as a molecular rotor.[7] The ratiometric fluorescence detection of such probe should allow viscosity to be measured without the bias associated with the chromophore concentration.

We have reported a new type of a ratiometric molecular rotor, which is constructed as a conjugated porphyrin dimer, Figure 2.[8] The excited state properties of this butadiyne-linked porphyrin dimer are consistent with the coexistence of two spectroscopically distinct conformations: planar and twisted, describing the relative position of the porphyrin units. Each conformer is characterised by distinct absorption and emission spectra. The planar conformer emission (a red-shifted band) dominates at low viscosities, since the relaxation pathways to this low lying state are available by rotation. However, in a high-viscosity environment the free rotation is restricted and the emission from the less stable twisted conformer can be observed.

We have also used the conjugated porphyrin dimer for the dynamic viscosity measurements in cells during Photodynamic Therapy of cancer, PDT.[8] Conjugated porphyrin dimers, such as the rotor shown in Figure 2, are very potent photosensitisers for PDT.[9]

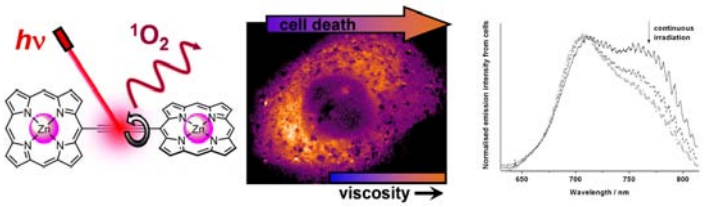

Figure 3. The red light irradiation of the conjugated porphyrin dimer leads to a significant viscosity increase in live cells, as a result of PDT. The schematic of a singlet oxygen production by the dimer is shown, along with the viscosity image of a single HeLa cell and a series of fluorescence spectra of the dimer recorded from cells during PDT.

This means that these molecules are efficiently taken up by live cells, are non-toxic to cells in the absence of irradiation, and are capable of efficiently producing cytotoxic reactive oxygen species, mainly singlet molecular oxygen.

Thus, the irradiation of a conjugated porphyrin dimer inside live cells with a high dose of light will induce cell death (apoptosis and necrosis) in response to PDT.[9] At the same time, the conjugated porphyrin dimer can act as a molecular rotor. This enabled us to monitor the change in intracellular viscosity as a result od PDT-induced cell death, Figure 3.[8]

The single point fluorescence spectral measurements in live unperturbed HeLa cells demonstrated unequivocally that viscosity in cell compartments around the hydrophobic dimer was very high, ca. 80 cP.[8] Moreover, domains of different viscosity were visualised using wide-field ratiometric imaging. The fluorescence spectra of the dimer from cells were also recorded following continuous irradiation, Figure 3, which ultimately resulted in cell death. These measurements established that the viscosity in a cell increased dramatically as a result of PDT-induced cell death,[8] and the analysis of the ratiometric fluorescence spectra in necrotic cells yielded an unprecedented high viscosity value of 360 cP.

In recent years molecular rotors have advertised their place as versatile tools for probing and imaging viscosity in biological systems, including measurements in clinical settings and fundamental studies on a single cell level. Several types of molecular rotors including malononitriles, cyanine dyes, Bodipy and porphyrin dimers have been used to measure the viscosity within individual domains of live cells. The examples of their use highlighted in this article highlight two unique advantages of molecular rotor approach: (i) the capability of quantitative *imaging* of viscosity in heterogeneous systems, with high spatial resolution and (ii) the possibility of performing measurements in *dynamically changing* systems, with fast monitoring of changing viscosity. Thus, it is perhaps not surprising that the many applications of molecular rotor technology is a fast growing and exciting field of research.

References.

1. M. K. Kuimova, *Phys. Chem. Chem. Phys.*, **14**, 12671-12686 (2012).
2. L. Wang, Y. Xiao, W. Tian and L. Deng, *J. Am. Chem. Soc.*, **135**, 2903-2906 (2013); Z. Yang, Y. He, J.-H. Lee, N. Park, M. Suh, W.-S. Chae, J. Cao, X. Peng, H. Jung, C. Kang and J. S. Kim, *J. Am. Chem. Soc.*, **135**, 9181-9185 (2013); E. Gatzogiannis, Z. Chen, L. Wei, R. Wombacher, Y.-T. Kao, G. Yefremov, V. W. Cornish and W. Min, *Chem. Commun.*, **48**, 8694-8696 (2012).
3. K. Luby-Phelps, S. Mujumdar, R. B. Mujumdar, L. A. Ernst, W. Galbraith and A. S. Waggoner, *Biophys. J.*, **65**, 236-242 (1993).
4. M. A. Haidekker and E. A. Theodorakis, *Org. Biomol. Chem.* **5**, 1669-1678 (2007).
5. M. K. Kuimova, G. Yahioglu, J. A. Levitt and K. Suhling, J. *Am. Chem. Soc.*, **130**, 6672-6673 (2008); J. A. Levitt, M. K. Kuimova, G. Yahioglu, P.-H. Chung, K. Suhling and D. Phillips, *J. Phys. Chem. C*, **113**, 11634-11642 (2009).
6. Y. Wu, M. Stefl, A. Olzynska, M. Hof, G. Yahioglu, P. Yip, D. R. Casey, O. Ces, J. Humpolickova and M. K. Kuimova, *Phys. Chem. Chem. Phys.*, **15**, 14986-14993 (2013); N. A. Hosny, G. Mohamedi, P. Rademeyer, J. Owen, Y. Wu, M.-X. Tang, R. J. Eckersley, E. Stride and M. K. Kuimova, *Proc. Natl. Acad. Sci. U. S. A.*, **110**, 9225-9230 (2013).
7. M. A. Haidekker, T. P. Brady, D. Lichlyter and E. A. Theodorakis, *J. Am. Chem. Soc.*, **128**, 398-399 (2006).

8. M. K. Kuimova, S. W. Botchway, A. W. Parker, M. Balaz, H. A. Collins, H. L. Anderson, K. Suhling and P. R. Ogilby, *Nat. Chem.*, **1**, 69-73 (2009).
9. H. A. Collins, M. Khurana, E. H. Moriyama, A. Mariampillai, E. Dahlstedt, M. Balaz, M. K. Kuimova, M. Drobizhev, V. X. D. Yang, D. Phillips, A. Rebane, B. C. Wilson and H. L. Anderson, *Nat. Photonics*, **2**, 420-424 (2008); E. Dahlstedt, H. A. Collins, M. Balaz, M. K. Kuimova, M. Khurana, B. C. Wilson, D. Phillips and H. L. Anderson, *Org. Biomol. Chem.*, **7**, 897-904 (2009).

Synthesis of *meso*-AlkenylBODIPYs and their Applications as an off-on Dosimeter.

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Among the most popular organic dyes are the so-called BODIPYs[1] (**1**) owing to the remarkable physical, chemical, and optical properties they display.[2] The typical synthesis of symmetrical BODIPYs, described by Lindsey et al., consist of the acid-catalyzed condensation of pyrrole with an aromatic aldehyde, followed by DDQ oxidation and complexation with BF_3.[3] The drawbacks of the Lindsey methodology include
laborious purifications, separate synthesis of the starting aldehyde if it is not commercially available, and incompatibility of the functional groups on the aryl ring of the aldehyde to (a) acid, (b) DDQ oxidation, and (c) strong Lewis acid exposure. In 2006, Biellmann et al. described the synthesis of 8-methylthioBODIPY (**2**) (henceforth referred to as the Biellmann BODIPY).[4]

Since then, our research group has demonstrated that **2** is a privileged building block. The MeS group endows it with a very efficient reactivity in transition-metal catalyzed cross-coupling processes such as the Liebeskind-Srogl reaction (LSR),[5] as well as S_NAr-type reactions with assorted O-, and N-centered nucleophiles.[6]
The LSR of alkenylboronic acids with Biellmann BODIPY **2** is of particular interest since it allows for the introduction of a double-bond at the *meso*-position (Fig. 1).[7]

Figure 1. Synthesis of 8-alkenylBODIPYs using the LSR and proposed reactivity thereof.

It was envisioned that, the *meso*-double bond, being attached to an electron-withdrawing moiety (i.e., the BODIPY core), would behave that of an α,β-unsaturated carbonyl compound. Thus, 8-propenylBODIPY **3** was subjected to two typical reactions of α,β-unsaturated carbonyl compound: Pd-catalyzed reduction of the double-bond and thiol addition (Fig. 2). Dramatic changes were observed in both reactions, whereas **3** is reddish in color and non-emissive, both the reduction and products and were green and highly-fluorescent. We envision very interesting applications of these derivatives by taking advantage of the unique reactivity of the *meso* double-bond.

Figure 2. Reduction and nucleophilic addition on 8-propenylBODIPY 3.

References.

1. Haugland, R. P. The Handbook. A Guide to Fluorescent Probes and Labeling Technology,10th ed.; Molecular Probes: Eugene, Oregon USA,

2. (a) Loudet, A.; Burgess, K. *Chem. Rev.* **107**, 4891-4932 (2007); (b) Wood, T. E.; Thompson, A. *Chem. Rev.* **107**, 1831-1861 (2007). (c) Ziessel, R.; Ulrich, G.; Harriman, A. *New J. Chem.* **31**, 496-501 (2007). (d) Ulrich, G.; Ziessel, R.; Harriman, A. *Angew. Chem., Int. Ed.* **47**, 1184-1201 (2008).

3. Li, F.; Yang, S. I.; Ciringh, Y.; Seth, J.; Martin, C. H.; Singh, D. L.; Birge, R. R.; Bocian, D. F.; Holten, D.; Lindsey, J. L. *J. Am. Chem. Soc.* **120**, 10001-10017 (1998).

4. Goud, T. V.; Tutar, A.; Biellmann, J.-F. *Tetrahedron* **62**, 5084-5091 (2006).

5. Peña-Cabrera, E.; Aguilar-Aguilar, A.; González-Domínguez, M.; Lager, E.; Zamudio-Vázquez, R.; Godoy-Vargas, J.; Villanueva, F. *Org. Lett.* **9**, 3985-3988 (2007).

6. (a) Esnal, I.; Urías-Benavides, A.; Gómez-Durán, C. F. A.; Osorio-Martínez, C. A.; Inmaculada García-Moreno, I.; Costela, A.; Bañuelos, J.; Epelde, N.; López Arbeloa, I.; Hu, R.; Tang, B. Z.; Peña-Cabrera, E. *Chem. Asian J.* **8**, 2691 – 2700 (2013). (b) Flores-Rizo, J. O.; Esnal, I.; Osorio-Martínez, C. A.; Gómez-Durán, C. F. A.; Bañuelos, J.; López Arbeloa, I.; Pannell, K. H.; Metta-Magaña, A.; Peña-Cabrera, E. *J. Org. Chem.* **78**, 5867-5877(2013).

7. Arroyo, I. J.; Hu, R.; Tang, B. Z.; López, F. I.; Peña-Cabrera, E. *Tetrahedron* **67**, 7244-7250 (2011).

Synthetic Fluorescent Probes for the Detection of Biological Copper

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As an essential trace element, copper is involved in many fundamental biological processes, including respiration, superoxide detoxification, connective tissue formation, and the mobilization and uptake of iron.[1] Due to the reducing cellular environment, the majority of copper is present in the form of Cu(I).[2] Given the rapid kinetics of copper uptake and release,[3] a significant portion of cellular copper must be present in the form of an exchangeable labile pool. Although bound to endogenous high-affinity ligands and proteins,[4] this pool is readily available for distribution by metallochaperones.[5] Likewise, synthetic fluorescent probes that can compete for Cu(I) binding may serve as powerful tools to interrogate this labile pool and thus to study cellular copper homeostasis.[6]

The design of Cu(I)-selective fluorescent probes is challenging as Cu(I) can act as an effective fluorescence quencher, usually by means of energetically low-lying metal-ligand charge transfer states. To address this problem, a sensing platform in which the Cu(I)-binding site and fluorophore moiety are electronically decoupled is best utilized. Fluorescence signalling upon Cu(I)-binding can be mediated through a photoinduced electron transfer (PET) mechanism, where the excited fluorophore acts as the electron acceptor and the electron-rich metal-ion receptor as the electron donor. Following this approach, several Cu(I)-selective fluorescent probes have been reported, all of which respond with a fluorescence enhancement upon saturation with Cu(I).[6]

In order to cross the plasma membrane and reach cellular targets, fluorescent probes must be sufficiently lipophilic, which in turn compromises their water solubility. Dynamic light scattering studies of lipophilic probes, including the BODIPY-based coppersensors CS1 and CS3,[7] revealed the formation of colloidal aggregates with hydrodynamic radii ranging between 60-100 nm.[8] Inside cells, the interaction of dye aggregates with hydrophobic proteins or lipid

bilayers may impact the cellular physiology and produce staining artifacts. The latter is particularly of concern if the fluorescence brightness and emission wavelength are different for the monomeric and aggregated forms. For example, TM-BODIPY does not self-associate and is brightly fluorescent in organic solvents, whereas upon dilution into water, the dye forms non-fluorescent aggregates.[9] Consistent with these observations, coppersensor CS3 is brightly fluorescent in the presence of lipid bilayers, and consequently, exhibits a greatly diminished response towards Cu(I).[10] Colloidal aggregation can also result in enhanced Raman scattering,[11] as routinely evident by an additional peak at higher energy in the emission spectrum of low-quantum yield dyes. Because the weak Raman signal is only enhanced in aggregates but not the monomeric form, it cannot be employed for ratiometric imaging as incorrectly assumed in the case of the low-quantum yield Cu(I)-probe RCS1.[12] In view of these pitfalls, it becomes apparent that the cellular staining patterns produced by lipophilic dyes should be interpreted with great caution.[13]

To discourage colloidal aggregation, we designed the water-soluble, Cu(I)-selective fluorescent probe **1** (CTAP-2),[8] in which a thiocrown receptor was modified with four hydroxymethyl groups and tethered to a sulfonated triarylpyrazoline fluorophore platform (Fig. 1A). The fluorescence contrast of the probe was systematically optimized through tuning of the photoinduced electron transfer driving force.[14] In contrast to all previously published Cu(I)-responsive fluorescent probes, CTAP-2 directly dissolves in water and does not require dilution from an organic stock solution. Furthermore, CTAP-2 showed a selective and reversible 65-fold fluorescence turn-on response to Cu(I) in aqueous solution with a quantum yield of 8.3% (Fig. 1B), and proved to be sufficiently sensitive for the in-gel detection of Cu(I) bound to the metallochaperone Atox1.[8] Despite the overall anionic charge at neutral pH, CTAP-2 is membrane-permeant and produced a perinuclear staining pattern in 3T3 cells, reminiscent of the subcellular copper distribution previously reported;[15] however, the Cu(I) affinity of CTAP-2 is too low to compete for Cu(I) bound to endogenous proteins, thus rendering the interpretation of these data difficult.

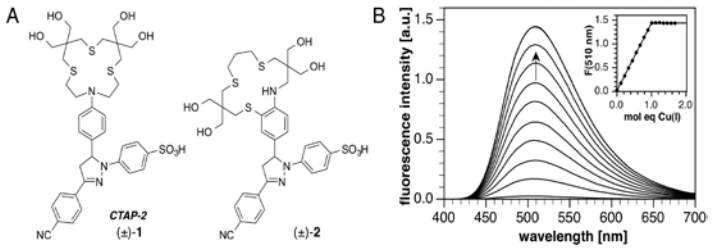

Figure 1. High-contrast fluorescence sensing of Cu(I) in aqueous solution. A) Pyrazoline-based water-soluble Cu(I)-selective fluorescent probes. B) Fluorescence emission response of CTAP-2 upon saturation with Cu(I) provided by *in situ* reduction of Cu(II) with ascorbate (pH 7.2, 10 mM MOPS, 22°C, λ_{ex} 380 nm). Inset: Fluorescence increase at 510 nm as a function of added

Electrochemical studies of the Cu(II/I) couple bound to a thiaza-crown ether receptor revealed a more favorable donor potential compared to the free ligand,[16] thus implying that Cu(I) should engage in reductive electron transfer quenching and compromise the overall fluorescence recovery of the probe. However, femtosecond time-resolved pump-probe experiments provided no evidence for the formation of a transient Cu(II) species.[16] The apparent contradiction can be reconciled by the fact that Cu(I)-promoted PET is orders of magnitudes slower compared to radiative excited state deactivation due to the reorganization barrier associated with Cu(I) oxidation.[17] Because quenching by Cu(I) cannot compete with fluorescence emission at the nanosecond time scale,
PET must occurs under kinetic control. Much to our surprise, we found that the formation of ternary complexes with solvent molecules was responsible for compromising the fluorescence recovery upon saturation with Cu(I).[16]

We reasoned that steric interactions between the ortho-hydrogen atoms of the aniline moiety and the thiaza crown ligand might encourage ternary complex formation. To alleviate these unfavorable interactions, we designed probe **2** in which the aniline ring is fully integrated into the crown ether backbone (Fig. 1A). This approach previously yielded a high-contrast Cu(I)-selective probe with a greater than 200-fold fluorescence enhancement in methanol.[18] However, probe **2** offered no significant improvement in quantum yield and

fluorescence contrast over CTAP-2.[19] Concluding from detailed photophysical studies, including responses to acidification, solvent isotope effects, quantum yields, and time-resolved fluorescence decay profiles, the fluorescence contrast of **2** is compromised by two distinct non-radiative excited state proton transfer (ESPT) deactivation pathways operating under neutral and acidic conditions, respectively, and by inadequate coordination of Cu(I) to the weakly basic arylamine nitrogen of the PET donor.[19] Nevertheless, with further alterations of the pyrazoline substituents and modification to the ligand architecture, it should be possible to inhibit the undesired ESPT pathways and thus to arrive at a water-soluble high quantum yield and high-contrast Cu(I)-responsive probe.

While much progress has been made in improving the photophysical properties of Cu(I)-selective fluorescent probes in aqueous solution, the binding affinities of currently available synthetic ligands are not well matched with the biologically relevant femto- to attomolar concentration window. For this reason, the development of new synthetic probes with greatly increased Cu(I) affinities represents a critical goal in the quest towards exploring copper homeostasis in live cells and organisms under physiologically meaningful conditions.

References

1. Kim, B.-E.; Nevitt, T.; Thiele, D. J. *Nat. Chem. Biol.* **4**, 176-185 (2008).
2. Yang, L.; McRae, R.; Henary, M. M.; Patel, R.; Lai, B.; Vogt, S.; Fahrni, C. J. *Proc. Natl. Acad. Sci. USA* **102**, 11179-11184 (2005).
3. Scheiber, I. F.; Schmidt, M. M.; Dringen, R. *Neurochem. Int.* **60**, 292-300 (2012).
4. Rae, T. D.; Schmidt, P. J.; Pufahl, R. A.; Culotta, V. C.; O'Halloran, T. V. *Science* **284**, 805-808 (1999).
5. Lutsenko, S. *Curr. Opin. Chem. Biol.* **14**, 211-217 (2010).
6. Fahrni, C. J. *Curr. Opin. Chem. Biol.* **17**, 656-662 (2013).
7. Zeng, L.; Miller, E. W.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **128**, 10-11 (2006); Dodani, S. C.; Domaille, D. W.; Nam, C. I.; Miller, E. W.; Finney, L. A.; Vogt, S.; Chang, C. J. *Proc. Natl. Acad. Sci. USA* **108**, 5980-5985 (2011).
8. Morgan, M. T.; Bagchi, P.; Fahrni, C. J. *J. Am. Chem. Soc.* **133**, 15906-15909 (2011).
9. Tleugabulova, D.; Zhang, Z.; Brennan, J. D. *J. Phys. Chem. B* **106**, 13133-13138 (2002); Bergstrom, F.; Mikhalyov, I.; Hagglof, P.; Wortmann, R.; Ny, T.; Johansson, L. B. A. *J. Am. Chem. Soc.* **124**, 196-

204 (2002); Ohsaki, Y.; Shinohara, Y.; Suzuki, M.; Fujimoto, T. *Histochem. Cell Biol.* **133**, 477-480 (2010).
10. Hong-Hermesdorf, A.; Miethke, M.; Gallaher, S. D.; Kropat, J.; Dodani, S. C.; Chan, J.; Barupala, D.; Domaille, D. W.; Shirasaki, D. I.; Loo, J. A.; Weber, P. K.; Pett-Ridge, J.; Stemmler, T. L.; Chang, C. J.; Merchant, S. S. *Nat. Chem. Biol.* **10**, 1034-1042 (2014).
11. Akins, D. L. *J. Phys. Chem.* **90**, 1530-1534 (1986); Akins, D. L.; Macklin, J. W. *J. Phys. Chem.* **93**, 5999-6007 (1989).
12. Domaille, D. W.; Zeng, L.; Chang, C. J. *J. Am. Chem. Soc.* **132**, 1194-1195 (2010).
13. Price, K. A.; Hickey, J. L.; Xiao, Z. G.; Wedd, A. G.; James, S. A.; Liddell, J. R.; Crouch, P. J.; White, A. R.; Donnelly, P. S. *Chem. Sci.* **3**, 2748-2759 (2012).
14. Cody, J.; Mandal, S.; Yang, L. C.; Fahrni, C. J. *J. Am. Chem. Soc.* **130**, 13023-13032 (2008); Fahrni, C. J.; Yang, L. C.; VanDerveer, D. G. *J. Am. Chem. Soc.* **125**, 3799-3812 (2003); Verma, M.; Chaudhry, A. F.; Morgan, M. T.; Fahrni, C. J. *Org. Biomol. Chem.* **8**, 363-370 (2010).
15. McRae, R.; Lai, B.; Fahrni, C. J. *J. Biol. Inorg. Chem.* **15**, 99-105 (2010).
16. Chaudhry, A. F.; Verma, M.; Morgan, M. T.; Henary, M. M.; Siegel, N.; Hales, J. M.; Perry, J. W.; Fahrni, C. J. *J. Am. Chem. Soc.* **132**, 737-747 (2010).
17. Rorabacher, D. B. *Chem. Rev.* **104**, 651-697 (2004).
18. Chaudhry, A. F.; Mandal, S.; Hardcastle, K. I.; Fahrni, C. J. *Chem. Sci.* **2**, 1016-1024 (2011).
19. Morgan, M. T.; Bagchi, P.; Fahrni, C. J. *Dalton Trans.* **42**, 3240-3248 (2013).

Dimaleimide Fluorogens as Protein Labelling Agents

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The fluorescent labelling of specific proteins in living cells is an important challenge in chemical biology. To this end, four general strategies have been developed. 1) A protein of interest (POI) can be genetically fused to green fluorescent protein (GFP).[1,2] 2) A POI can be genetically fused to a pendant enzyme[3,4,5] that can then be labelled using a specific fluorescent inhibitor. 3) Alternatively, modifications can be made to the POI that introduce a tag containing an enzyme recognition site.[6,7,8] A site-specific enzymatic reaction can then be used to affix a fluorescent label to the POI. 4) Finally, a POI can be genetically tagged with a minimal peptide tag that can react with small molecular probes,[9] avoiding the steric hindrance of large genetically fused proteins.

Among the small molecular agents designed for the fluorescent labelling of specific proteins, those that are fluorogenic are distinctly advantageous relative to those that are always fluorescent. Notably, the use of a fluorogenic probe to label a specific protein in a biological sample abrogates the need to wash away unreacted probe prior to imaging.[10] Some of the first fluorogenic small molecule labelling agents are the FlAsH and ReAsH organoarsenic dyes developed in the Tsien research group.[11,12] Others have advanced the field through the design of fluorogenic versions of bioorthogonal labelling agents, such as azidofluoresceins[13] and BODIPY-tetrazines.[14] Herein, we briefly review a labelling strategy we have developed that relies on the quenching properties of maleimides and their specific Fluorogenic Addition Reaction (FlARe) with a genetically encoded dicysteine peptide tag.[15]

Maleimides have long been known to quench fluorescence in their conjugated form[16] and to undergo addition reactions through their C=C double bond, showing significant chemoselectivity for thiols. Furthermore, the thioalkyl succinimide group resulting from this addition reaction does not exhibit the strong quenching effect of its parent maleimide. Several derivatives bearing a maleimide group

and blue fluorophore have thus been shown[17,18,19] to undergo a dramatic increase in fluorescence upon their reaction with thiols. Based on these properties, we conceived a simple labelling method, using fluorogens that comprise a fluorophore and *two* maleimide groups (Fig.1). We reasoned that *both* maleimide groups would have to react to restore their latent fluorescence. Further, we hoped that by requiring that the fluorogens react with two thiol groups of a dicysteine tag sequence, we would confer 'selectivity' in a method for the fluorescent labelling of proteins in complex media.

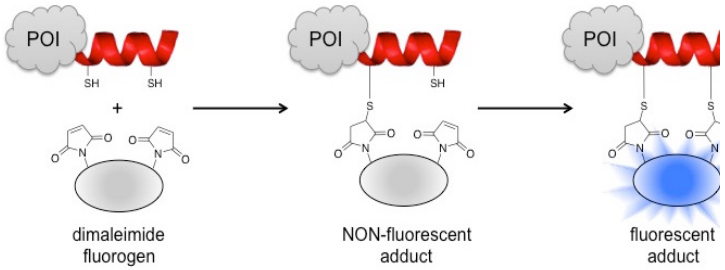

Figure 1. Fluorogenic Addition Reaction (FlARe) between dimaleimide fluorogens and a protein of interest (POI) bearing a target dicysteine tag

In our initial work[20] we synthesized a small series of compounds bearing two maleimide groups attached directly to their fluorescent cores, and demonstrated that they fluoresce very weakly until they undergo addition reactions with two equivalents of thiol. Moreover, the maleimide groups were separated by a precise distance, allowing the fluorogens to react rapidly with compounds presenting two sulfhydryl groups separated by the same distance. To that end we designed a dicysteine mutant of the helical protein Fos, and demonstrated the efficiency of its fluorogenic labelling reaction. Importantly, we also showed that our dimaleimide fluorogens would not react with two equivalents of mono-cysteine Fos mutants, validating the regioselectivity of our labelling method.

Next, we developed**Errore. Il segnalibro non è definito.** an efficient convergent synthetic route for the facile modular preparation of a library of fluorogens, in which a dimaleimide phenyl moiety was linked to a variety of fluorophores through any one of a number of

different 'spacer' segments. This route was used to prepare a series of dansyl dimaleimide fluorogens that were then used to study the quenching mechanism of the maleimide group. In this study, the potential of intersystem crossing was ruled out, since no triplet intermediate was detected by laser flash photolysis. The measurement of the Stern-Volmer rate constants suggested that quenching occurred at diffusion-controlled limits, consistent with electron transfer. Cyclic voltammetry confirmed the thermodynamic favourability of a photoinduced electron transfer (PeT); specifically, the redox potentials of the dansyl and maleimide functionalities demonstrated that electron transfer from the dansyl excited state to the pendant maleimide LUMO is exergonic. This elucidation of the PeT quenching mechanism was interesting from a fundamental point of view, but it also had two important practical consequences. Namely, it suggested that longer wavelength emission (from a lower energy excited state) may not be quenched as efficiently, and that the length and conformation of the spacer may be critical to quench efficiency.

Subsequently, we turned our attention to the design[21] of a novel minimal target peptide sequence to react with our dimaleimide fluorogens. Given the efficacy of our preliminary Fos mutant**Errore. Il segnalibro non è definito.** (*vide supra*), we took a known helical sequence from the peptide literature, inserted two Cys residues separated by two turns of the α-helix, and bracketed the minimal sequence with *N*-cap and *C*-cap sequences. CD spectroscopy showed that the new 'dC10α' sequence was strongly helical, before and after labelling. We then attempted to optimize the reactivity of the dC10α peptide by introducing basic His residues near the reactive Cys residues, but this rational design approach proved unsuccessful. Still, the dC10α sequence was shown to be much more reactive than our prototype Fos mutant. Furthermore, when we fused dC10α to the *N*-terminus of epidermal growth factor receptor (EGFR) we were able to selectively label EGFR on the surface of human cells, using a green fluorescein-based fluorogen.

In parallel work, we sought to enhance the fluorogenic nature of our labelling agents by increasing the efficiency of PeT quenching in the unreacted probe. In this quenching mechanism, electron transfer from the fluorophore to the quenching group can occur through the intervening covalent bonds or through space, the latter being the

predominant manifold within a certain distance.**Errore. Il segnalibro non è definito.**,[22] Considering the tripartite design of our fluorogens developed in our previous studies,**Errore. Il segnalibro non è definito.** we reasoned that the spacer moiety must play a critical role in quenching efficiency. We sought to confirm this by studying a series of fluorogens bearing the same coumarin fluorophore and dimaleimide quenching unit but with systematically varied spacer moieties. Our results showed that the PeT quenching efficiency was inversely correlated to the through-space distance between the fluorophore and the maleimide.[23] Following up on this conclusion, we designed and synthezied a 'spacerless' fluorogen in which a dansyl fluorophore was attached directly to a dimaleimide moiety. As expected, the fluorogen shows more than 300-fold fluorescence enhancement upon the thiol addition reaction of its maleimide groups. The discovery of such a highly efficient fluorogen opened the door to a variety of protein labelling fluorogenic reagents for different applications.

The ultimate application of fluorogens is the intracellular labelling of specific proteins; however, this poses a challenge for the FlARe method, since the intracellular concentration of the tripeptide thiol glutathione (GSH) is in the millimolar range. We therefore attempted to tune the reactivity of the maleimide groups of our probes by first designing an asymmetric dimaleimide moiety in which each maleimide bears a different substituent.[24] Kinetic characterization of the addition of two thiols demonstrated that a methoxy substituent decreases the reactivity of its maleimide with GSH, while maintaining reactivity with the dC10α tag. This selectivity may be due the dC10α tag being highly nucleophilic and relatively insensitive to the attenuated electrophilicity of the methoxymaleimide. Inspired by these results, we then developed a cyan fluorogenic labelling agent bearing two methoxymaleimide groups. This approach was shown to completely suppress the reaction with GSH but maintain reactivity with the dC10α-tagged POI. With this improvement, we were able to apply the FlARe method to intracellular labelling, demonstrating the rapid and specific labelling of a POI expressed in mammalian cells without the requirement of any washing steps (Fig. 2).**Errore. Il segnalibro non è definito.**

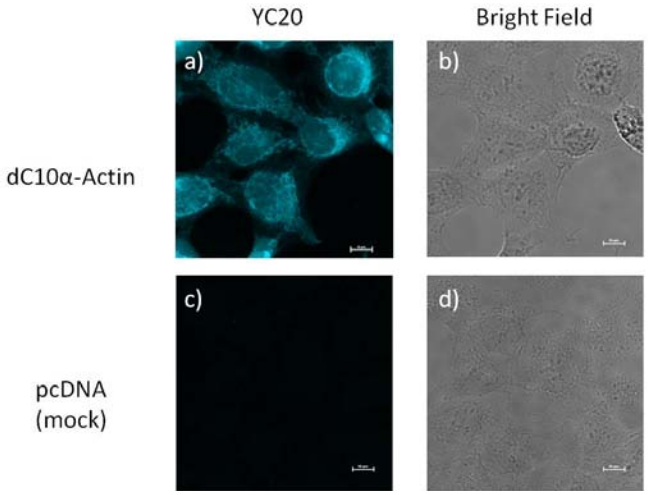

Figure 2. Fluorogenic labelling of actin protein bearing an *N*-terminal dC10α tag in HEK293 cells, with no washing.**Errore. Il segnalibro non è definito.** The absence of background labelling is shown in panel c).

References

1. Tsien, R. Y. *Annu. Rev. Biochem.* **6**7, 509-544 (1998).
2. Dean, K. M.; Palmer, A. E. *Nat. Chem. Biol.* 2014, **10**, 512-523.
3. Keppler, A.; Pick, H.; Arrivoli, C.; Vogel, H.; Johnsson, K. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 9955-9959 (2004).
4. Miller, L. W.; Cai, Y.; Sheetz, M. P.; Cornish, V. W. *Nat. Methods* **2**, 255-257 (2005).
5. Hori, Y.; Kikuchi, K. *Curr. Opin. Chem. Biol.* **17**, 644-650 (2013).
6. Oteng-Pabi, S. K.; Pardin, C.; Stoica, M.; Keillor, J. W. *Chem. Commun. (Camb.)* **50**, 6604–6606 (2014).
7. Rashidian, M.; Dozier, J. K.; Distefano, M. D. *Bioconjugate Chem.* **24**, 1277–1294 (2013).
8. Sunbul, M.; Yin, J. *Org. Biomol. Chem.* **7**, 3361–3371 (2009).
9. Chen, Y.; Tsao, K.; Keillor, J. W. *Can. J. Chem.*, in press (2014). DOI: 10.1139/cjc-2014-0405
10. Nadler, A.; Schultz, C. *Angew. Chem. Int. Ed.* **52**, 2408-2410 (2013).
11. Griffin, B. A.; Adams, S. R.; Tsien, R. Y. *Science* **281**, 269-272

(1998).
12. Adams, S. R.; Campbell, R. E.; Gross, L. A.; Martin, B. R.; Walkup, G. K.; Yao, Y.; Llopis, J.; Tsien, R. Y. *J. Am. Chem. Soc.* **124**, 6063-6076 (2002).
13. Shieh, P.; Hangauer, M. J.; Bertozzi, C. R. *J. Am. Chem. Soc.* **134**, 17428-17431 (2012).
14. Devaraj, N. K.; Hilderbrand, S.; Upadhyay, R.; Mazitschek, R.; Weissleder, R. *Angew. Chem. Int. Ed.* **49**, 2869-2872 (2010).
15. Guy, J.; Caron, K.; Dufresne, S.; Michnick, S. W.; Skene, W. G.; Keillor, J. W. *J. Am. Chem. Soc.* **129**, 11969-11977 (2007).
16. Kokotos, G.; Tzougrakic, C. *J. Heterocycl. Chem.* **23**, 87 (1986).
17. Langmuir, M. E.; Yang, J.-R.; Moussa, A. M.; Laura, R.; LeCompte, K. A. *Tetrahedron Lett.* **36**, 3989 (1995).
18. Corrie, J.E.T. *J. Chem. Soc., Perkin Trans. I* 2975 (1994).
19. Cohen, B. E.; Pralle, A.; Yao, X.; Swaminath, G.; Gandhi, C. S.; Jan, Y. N.; Kobilka, B. K.; Isacoff, E. Y.; Jan, L. Y. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 965 (2005).
20. Girouard, S.; Houle, M.-H.; Grandbois, A.; Keillor, J. W.; Michnick, S. W. *J. Am. Chem. Soc.* **127**, 559–566 (2005).
21. Guy, J.; Castonguay, R.; Campos-Reales Pineda, N. B.; Jacquier, V.; Caron, K.; Michnick, S. W.; Keillor, J. W. *Mol. Biosyst.* **6**, 976–987 (2010).
22. Le, T. P.; Rogers, J. E.; Kelly, L. A. *J. Phys. Chem. A* **104**, 6778-6785 (2000).
23. Caron, K.; Lachapelle, V.; Keillor, J. W. *Org. Biomol. Chem.* **9**, 185–197 (2011).
24. Chen, Y.; Clouthier, C. M.; Tsao, K.; Strmiskova, M.; Lachance, H.; Keillor, J. W. *Angew. Chem. Int. Ed. Engl.*, in press (2014).
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Developing a new fluorescence probe of singlet oxygen during photodynamic therapy

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Among various reactive oxygen species, singlet oxygen ($^1O_2$) has been in a particular interest due to its distinct photo-physical properties and cytotoxic effect. One representative example to exploit the oxidative power of $^1O_2$ is photodynamic therapy (PDT), a tumor treatment with photosensitizer (Sens) and photoirradiation. In this method, adequately populated Sens in tumor tissue can destroy nearby cells under photoirradiation through complex cytotoxic pathways triggered by the initial oxidative damage of $^1O_2$. In order to improve the efficiency of PDT, therefore, understanding the intracellular dynamics of $^1O_2$ is undoubtedly crucial. To this goal, time-resolved observation of $^1O_2$ phosphorescence provides us the most direct information about its generation, location, and deactivation.[1,2] Nonetheless, the intensity of $^1O_2$ phosphorescence is intrinsically too small to visualize its population with a video-rate and intracellular spatial resolution, indicating a practical limitation of using solely $^1O_2$ phosphorescence for theragnosis (cf. therapy and diagnosis simultaneously) of PDT.

A fluorescence detection using probes is a good alternative for the visualization intracellular $^1O_2$ especially in the sense of the signal-to-noise ratio, essential for high temporal and spatial resolution imaging. To design an adequate fluorescence probe for $^1O_2$ sensing, anthracene derivatives have been the most frequently exploited since the center ring of anthracene moiety can react with $^1O_2$ selectively with a reasonable reaction rate depending on the substitution groups at 9- and 10-positions.[3] In 1999, Nagano group firstly proposed the design of $^1O_2$ probe composed of fluorescein and anthracene derivatives.[4] As anthracene derivatives act as an electron donor for fluorescein in the singlet excited state, this dyad undergoes photoinduced electron transfer (PET) upon the excitation of a fluorescein part, resulting in the fluorescence quenching. In the presence of $^1O_2$, $^1O_2$ can form endoperoxide at the anthracene moiety, lowers the energy level of highest occupied molecular orbital

(HOMO), and prevents PET, resulting in the recovery of fluorescein fluorescence.[5] In light of their inspiring reports, several other sensor designs as well as a commercialized fluorescence probe of $^1O_2$, Singlet Oxygen Sensor Green (SOSG, from Molecular Probes®), has been developed.

In our laboratory, we have aimed to visualize intracellular $^1O_2$ produced during PDT using fluorescence probes. As the first attempt, SOSG can be considered since it is commercially available and the most frequently used in a variety of fields. It was previously reported, however, that SOSG can induce $^1O_2$ by itself under photoirradiation, leading to a false fluorescence signal.[6,7] First of all, hence, photochemical dynamics of SOSG have been thoroughly studied using time-resolved spectroscopic measurements.[8] According to the previous reports,[6,7] SOSG is a dyad composed of fluorescein and anthracene moieties (Figure 1), and its fluorescence increases in the presence of $^1O_2$ (fluorescence quantum yield, Φ_{fl} = 0.02 to 0.37)[8] because of the same mechanism as explained above. The visible-light excitation of SOSG (λ = 500 nm) induces intramolecular PET as a major deactivation process (k_{PET} = 9.7 × 10^{11} s^{-1}), resulting in the fluorescence quenching. In addition, $^1O_2$ phosphorescence was observed upon 532-nm excitation of the fluorophore of SOSG (i.e., 5(6)-carboxy-2',7'-dichlorofluorescein) with $^1O_2$ generation quantum yield (Φ_Δ) of 0.06. This result indicates that intersystem crossing of a fluorescein moiety of SOSG, which is a minor deactivation process, induces $^1O_2$ generation via the bimolecular triplet–triplet energy transfer (k_q = 1.2 × 10^9 M^{-1} s^{-1}). Meanwhile, UV-light excitation of SOSG at 355 nm causes the two-photon ionization to give a SOSG cation (Φ_{ion} = 0.003 at 24 mJ cm^{-2}), leading to the decomposition of SOSG. To summarize, we quantitatively investigated the dynamics of

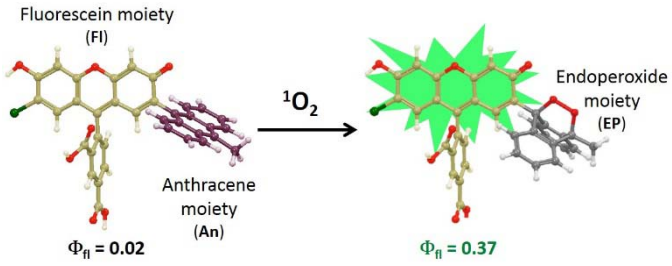

Figure 1. Expected molecular structures of SOSG (left) and its endoperoxide form (right).

SOSG in the excited state and found an exact origin for the self-oxidation: the fluorescein of SOSG in the triplet excited state.
Not only self-oxidation, SOSG also exhibits several drawbacks which make it inadequate for cell imaging: cell-impermeability (or non-specific staining) and absorption and emission in green region where significant cell autofluorescence and scattering signals are monitored together. Thus, a redder fluorescent $^1O_2$ probe is ultimately required for intracellular mapping. To this end, we recently reported a new far-red fluorescence probe for intracellular $^1O_2$ mapping.[9] This probe is a dyad of silicon containing rhodamine (Si-rhodamine) and dimethylanthracene (DMA), named Si-DMA (Figure 2a). Si-DMA takes advantages of superior photochemical properties of Si-rhodamine chromophore, such as absorption and emission in far-red region (λ_{abs} and λ_{em} = 650 and 678 nm, respectively) and the negligible self-oxidation by photoirradiation (Φ_Δ = 0.02). In the presence of $^1O_2$, similar to SOSG, fluorescence of Si-DMA increases from Φ_{fl} = 0.01 to 0.17 due to the endoperoxide formation at the DMA moiety. Si-DMA is especially suitable for imaging $^1O_2$ during PDT because of the selective mitochondrial localization due to its net charge, +1, under the physiological condition. Among three different Sens, Si-DMA could selectively detect $^1O_2$ generated by 5-aminolevulinic acid-derived protoporphyrin IX (PpIX), colocalized with Si-DMA in mitochondria (Figure 2b). Besides, mitochondria-targeted KillerRed and lysosomal porphyrins could not induce fluorescence change of Si-DMA. These selective responses of Si-DMA depending on the localization and mechanism of Sens are caused by a limited intracellular $^1O_2$ diffusion distance (at maximum,

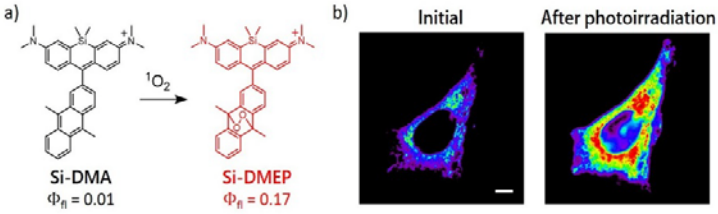

Figure 2. a) Molecular structures of Si-DMA and its endoperoxidized fluorescent form, Si-DMEP. b) Reconstructed images of Si-DMA fluorescence in a HeLa cell with PpIX. Red region after photoirradiation (λ = 640 nm at 0.6 W cm^{-2}, 60 s) indicates $^1O_2$ generation from photosensitizers. Scale bar: 10 μm.

300 nm) and negligible $^1O_2$ generation by Type-I Sens, respectively.
To conclude, using Si-DMA, $^1O_2$ generation has been successfully visualized up to the spatial resolution of a single mitochondria tubule. On the other hand, we have failed to monitor fluorescence turn-on events by $^1O_2$ generation at the single molecule level either with Si-DMA and SOSG. The problem in both cases was a bright fluorescence signal observed even in the absence of $^1O_2$. From this result, we tentatively assume that a fluorescence probe based on PET mechanism is not suitable for intracellular environment that is highly heterogeneous in terms of polarity, viscosity, and crowding effects. Those factors can significantly affect the kinetics of PET, resulting in an initially bright state. Provided more improvements in the molecular design of a fluorescence probe, nevertheless, we believe higher resolution imaging of $^1O_2$ during PDT will be realized by virtue of currently available state-of-the-art detectors and fluorescence microscope set-ups.

References.

1. Ogilby, P R. *Chem. Soc. Rev.* **39**, 3181-3209 (2010).
2. Scholz, M.; Dedic, R.; Valenta, J.; Breitenbach, T.; Hala, J. *Photochem. Photobiol. Sci.* **13**, 1203-1212 (2014).
3. Stevens, B.; Perez, S. R.; Ore, J. A. *J. Am. Chem. Soc.* **96**, 6846-6850 (1974).
4. Umezawa, N.; Tanaka, K.; Urano, Y.; Kikuchi, K.; Higuchi, T.; Nagano, T. *Angew. Chem. Int. Ed.* **38**, 2899-2901 (1999).
5. Tanaka, K.; Miura, T.; Umezawa, N.; Urano, Y.; Kikuchi, K.; Higuchi, T.; Nagano, T. *J. Am. Chem. Soc.* **123**, 2530-2536 (2001).
6. Ragas, X.; Jimenez-Banzo, A.; Sanchez-Garcia, D.; Batllori, X.; Nonell, S. *Chem. Commun.* 2920-2922 (2009).
7. Gollmer, A.; Arnbjerg, J.; Blaikie, F. H.; Pedersen, B. W.; Breitenbach, T.; Daasbjerg, K.; Glasius, M.; Ogilby, P. R. *Photochem. Photobiol.* **87**, 671-679 (2011).
8. Kim, S.; Fujitsuka, M.; Majima, T. *J. Phys. Chem. B* **117**, 13985-13992 (2013).
9. Kim, S.; Tachikawa, T.; Fujitsuka, M.; Majima, T. *J. Am. Chem. Soc.* **136**, 11707-11715 (2014).

PILLS OF HISTORY

At the origin of photochemistry. An article of Paternò written in 1875

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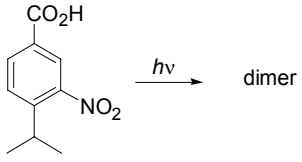
Usually, the beginning of the photochemical research in Italy is connected with the article of Ciamician on the photoreduction of quinone published in 1886.[1] This type of periodization can induce some errors, and the first is to attribute a role to Ciamician that could not be realistic. In the previous articles on this bulletin we showed that, in same period, Cannizzaro studied the photochemical behavior of santonin.[2,3] Some years ago we showed that Marussia Bakunin in 1898 studied the photochemical dimerization of some compounds she synthesized.[4]

Here I want to put another brick useful to rebuild a period in history of Italian and European research where the development of studies on the photochemical behavior of organic compounds was probably considered an opportunity that should not be missed.

We report an article of Paternò and Fileti, written when Paternò was at the University of Palermo in the 1875, eleven years before the famous work of Ciamician. Paternò reported the photochemical behavior of nitrocuminic acid (**1**) (Scheme 1). They were not able to identify the (brillant red) reaction product but they supposed, on the basis of the elemental analyses of the product, that it was a dimer of the starting material.



1

Scheme 1

In our knowledge the reaction described here has not be carried out by other scientists and then the product has not be identified.

"Azione della luce sull'acido nitrocuminico[5]

di E. Paternò e M. Fileti

Notizia preliminare comunicata al XII Congresso degli Scienziati

L'acido mononitrocuminico è uni di quei corpi che si alterano profondamente sotto l'influenza della luce; difatti si sa che quando esso è esposto alla luce diretta, od anche a quella diffusa, va colorandosi mano mano di rosso.
Noi abbiamo studiato questa trasformazione, ed abbiamo potuto constatare la formazione di una sostanza dotata di caratteri nettamente acidi, e di una composizione elementare molto vicina a quella dell'acido nitrocuminico dal quale trae origine.
L'acido mononitrocuminico adoperato nelle nostre ricerche, è stato preparato col metodo descritto nella nostra nota sui due acidi amidocuminici isomeri (p. 383).
Sciogliendo quest'acido nella benzina bollente, mettendo la soluzione in un pallone esposto alla luce diretta connesso con refrigerante a ricadere, riscaldando continuamente per mantenere il liquido in ebollizione, si va depositando mano mano la sostanza rossa fioccosa. Noi adoperiamo in ciascuna operazione 5 gr. di nitroacido sciolto in mezzo litro circa di benzina (bollente da 80° a 100°), e facciamo funzionare l'apparecchio per l'intero giorno.
Inoltre usiamo la precauzione di separare ogni sera per filtrazione la sostanza rossa formatasi durante il giorno, dal liquido ancora caldo, raccogliendola sempre sullo stesso filtro. La quantità di sostanza rossa che ogni giorno va formandosi è sempre più piccola, e l'operazione deve essere sospesa quando il liquido resta perfettamente trasparente per l'ebollizione alla luce diretta. Inoltre si bisogna osservare che la produzione della sostanza stessa si fa molto lentamente; di fatti per trasformare completamente 5 gr. dell'acido nitrocuminico, sono necessarie da 70 ad 80 ore in media, e sembra che quanto più spesso si separi dal seno del liquido il prodotto formatosi, tanto più diminuisca il tempo necessario alla totale trasformazione. Da 5 gr. di nitroacido noi abbiamo ottenuto 4 gr. circa di prodotto grezzo, e per

lo svaporamento della soluzione benzinica un residuo vischioso bruno.
La sostanza così ottenuta è di un bel colorito rosso, fioccosa, leggerissima ed amorfa; non si fonde quando riscaldata sino a 260°, ma a questa temperatura comincia a decomporsi. Si scioglie negli alcali caustici, e sposta l'acido carbonico dai carbonati alcalini: dalla soluzione ammoniacale, che è di un rosso bellissimo ed intenso ed ha un forte potere colorante, è riprecipitata per mezzo dell'acido cloridrico, ed allora diventa polverosa e di cattivissima apparenza per il disseccamento all'aria; in questo stato però, dopo cioè essere stata sciolta nell'ammoniaca e riprecipitata dall'HCl, acquista la proprietà di colorare fortemente in un bel rosso l'acqua colla quale si a bollire. Noi non abbiamo potuto trovare un mezzo sicuro di purificazione di quest'acido, e ci siamo dovuti contentare di scioglierlo nell'ammoniaca e riprecipitarlo con l'acido cloridrico.
La sostanza rossa in esame contiene dell'azoto, che abbiamo constatato coll'analisi qualitativa; in quanto poi all'analisi quantitativa, fatta bruciando la sostanza tanto con l'ossido di rame che col cromato di piombo, non abbiamo avuto cifre molto concordanti, probabilmente per la poca purezza del prodotto e per la difficoltà che presenta nel bruciare. Sembra però che la composizione elementare del nostro acido, sia molto prossima a quella dell'acido nitrocuminico; e non crediamo improbabile che la sua molecola risulti dalla unione di due molecole del nitroacido, forse in modo simile che negli azoossicomposti, dei quali però contiene più ossigeno.
Onde fare qualche po' di luce sulla costituzione della sostanza in esame, ne abbiamo tentato la riduzione e la ossidazione.
La riduzione è stata operata sciogliendo la sostanza nell'ammoniaca, saturando la soluzione con idrogeno solforato e riscaldandola per due ore in tubi chiusi alla temperatura di 100°. Il liquido, che avea già perduto il suo intenso colore rosso ed era di un rosso vinaceo, fu addizionato di acido acetico, il precipitato fu separato dallo zolfo che conteneva per dissoluzione nell'ammoniaca e riprecipitazione con acido acetico, e finalmente l'acido così ottenuto fu lavato con acqua ed asciugato. In questo stato di presenta come una polvere giallo brunastra di brutto aspetto, che si decompone pel riscaldamento, e che non fu analizzata non avendo trovato un mezzo sicuro di purificazione.
L'ossidazione dell'acido rosso è stata fatta con bicromato potassico ed acido solforico; si sviluppa acido carbonico, e si forma una polvere

gialla, che appena tolta dal miscuglio ossidante va colorandosi in bruno; per questa ragione siamo stati costretti ad operare la filtrazione ed il lavaggio in una atmosfera di acido carbonico, asciugandola poi nel vuoto. Quando essa è asciutta conserva il suo colorito giallo sporco, e non si altera in contatto dell'aria.
Queste esperienze saranno regolarmente continuate."

References

1. Ciamician, G. "Sopra una trasformazione del chinone in idrochinone". *Rend. R. Acc. Lincei* 1886, 2, 22.
2. D'Auria, M. At the origin of photochemistry. The photochemical behaviour of santonin. Some documents in this field. *EPA Newsletter*, 2013, 85, 79-86.
3. D'Auria, M. At the origin of photochemistry. The photochemical behavior of santonin. Some considerations on the published documents. *EPA Newsletter* 2014, 86, 76-80
4. D'Auria, M. Maria Bakunin e i primi studi in fotochimica in Italia, *Rendiconti dell'Accademia Nazionale delle Scienze detta dei XL*, Memorie di Scienze Fisiche e Naturali Serie V, 2009, 33, Parte 2, Tomo 2, 161.
5. E. Paternò, M. Fileti, Azione della luce sull'acido nitrocuminico, *Gazz. Chim. Ital.* **1875**, *5*, 385-387.

ABSTRACTS OF THESIS IN PHOTOCHEMISTRY

From Paternò-Büchi's Reactions to First Generation Molecular Motors: a Combined Application of Experimental and Computational Methodologies in the Field of Organic Photochemistry

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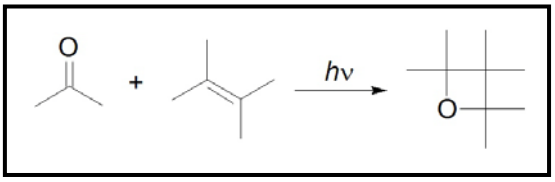
Ph.D. Thesis (in English), 2013; Research Adviser: Maurizio D'Auria

The Paternò-Büchi's reaction is one of the most ancient reactions in organic photochemistry; it is a photocycloaddition reaction of a n,π* carbonyl compound, in its singlet or triplet state, to an alkene in its ground state giving rise to the corresponding oxetane (Scheme 1).[1]



Scheme 1. The Paternò-Büchi's reaction: general scheme.

The electrophylic oxygen atom of the excited carbonylic compound could attack the electron-reach alkene to generate the 1,4 biradicalic intermediate which, in turn, would form the C,C bond to obtain an oxetane. The 1,4 biradicals exist for a time long enough to allow bond rotations before the ring closure.
When the HSOMO-LUMO interaction prevails, in which the half occupied π* carbonyl orbital interacts with the unoccupied π* molecular orbital of an electron- deficient alkene, a C,O-type biradical is formed.
Whereas, when the LSOMO-HOMO interaction prevails, in which the half-occupied n carbonyl orbital interacts with the occupied π

molecular orbital of an electron-reach alkene, a C,C-type biradical is formed.[2]

In most cases, UV radiation is used which normally leads to exited state carbonylic compounds which attack the alkene double bond usually in their triplet excited state to produce oxetanes. When asymmetric double bonds are used as substrates, stereochemically-different products can be obtained which differ by the position of the oxygen atom in the oxetane cycle. Furthermore, different enantiomers can be obtained when asymmetric carbon atoms are generated in the oxetane ring.[3]

Most of the synthetic procedures described by using a Paternò-Büchi's reaction have been obtained by using electron-reach alkenes [3] and very few examples have been reported of Paternò-Büchi's reactions on electron-deficient alkenes.[4] The change of the electronic properties of the alkene could induce a change of the mechanism of the reaction and, consequently, different regio- and stereochemistry.[4,5]

In this thesis work, we present the results of our combined synthetic and computational studies performed on electron-deficient alkenes such as alkenyl boronates.

The Paternò-Büchi's reaction has been performed on the six substrates in Figure 1 and benzophenone was used as UV absorbing compound.

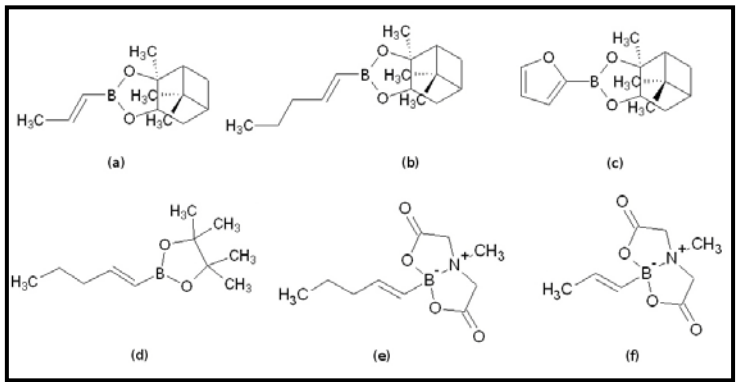


Figure 1. The six alkenylboronic esters used as substrates.

The Paternò-Büchi's reactions were performed on the pinandiol esters (a), (b) and (c), pinacol ester (d) and MIDA derivatives (e) and

(f). Such substrates reasonably differ by electron withdrawing action on the double bond and, for this reason, they are good candidates for understanding the effect of this property on the regiochemical and stereochemical aspects. Furthermore, to our knowledge, no Paternò-Büchi's reactions have been reported on alkenylboronic esters.
We were able to obtain the oxetane only in one case (Figure 2): it was isolated and characterised by using as substrate the pinacol ester (d).
For stereochemical aspects, the analysis of the NOE effects of oxetane showed a *cis* configuration of the two hydrogen atoms in the oxetane ring.
In the case of the MIDA-derivative (e), we obtained a mixture of *cis* and *trans* alcohols (see Figure 2) whilst in the case of the other MIDA-derivative (f), we obtained only the corresponding *trans* alcohol (Figure 2).

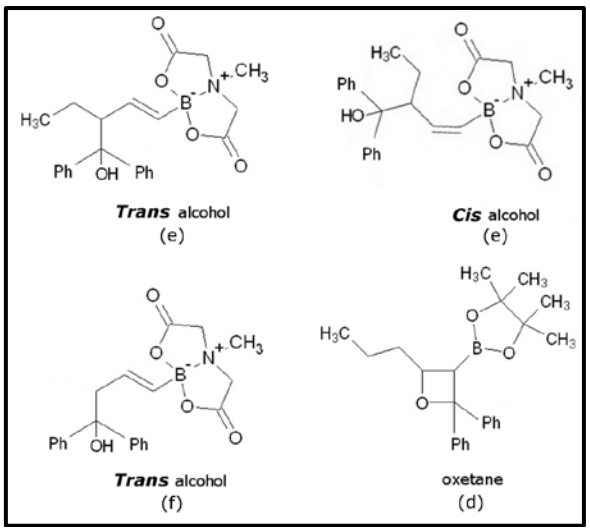


Figure 2. Reaction products obtained after irradiation with benzophenone.

With the aim to justify the observed stereochemistry of oxetane formation and to understand the obtainment of the alcoholic compounds, DFT *ab-initio* computations have been applied at the

B3LYP/6-31+G(d), M06/6-31+G(d) and CAM-B3LYP/6-31+G(d) levels of approximation.

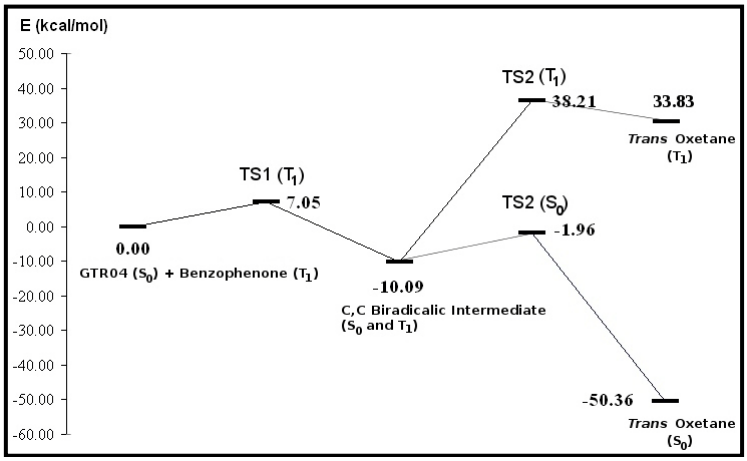


Figure 3. The computed reaction path leading to the (e) *trans* oxetane.

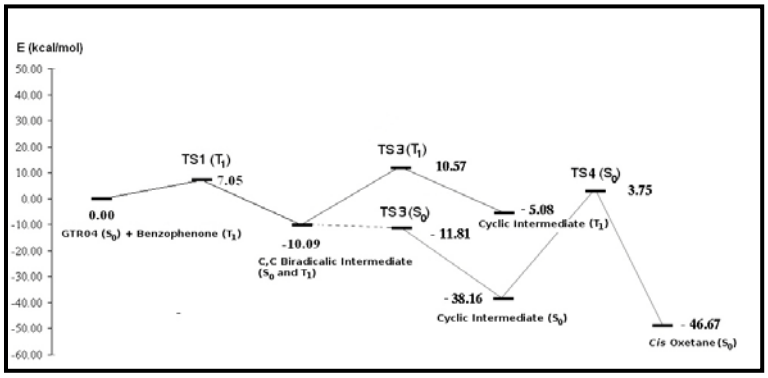


Figure 4. The computed reaction path leading to the (e) *cis* oxetane.

The computed calculations were performed to determine minima and transition structures connected to each other along the reaction path. Different reaction walks have been characterised; all of them start

with the benzophenone oxygen attack to the double bond in the T_1 excited state. The result is a C,C-type biradicalic intermediate on the T_1 PES (Potential Energy Surface) which can give rise to efficient intersystem crossing toward S_0 due to the closeness between the S_0 and T_1 PESs around the hole corresponding to the biradicalic intermediate.

According to the performed computations, only after intersystem crossing the reaction can go further leading to an oxetane. In Figures 3 and 4, we report two computed reaction paths which lead to a *trans* and a *cis* oxetane.

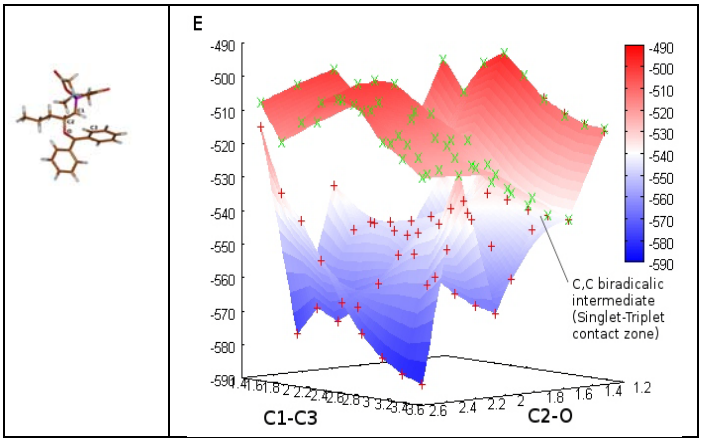


Figure 5. Computed triplet PES (the red one above) and singlet PES (the blue one). It is evident the contact zone around the triplet C,C biradicalic intermediate minimum point.

Figure 5 shows the point of probable intersystem crossing from T_1 to S_0 (at the C,C biradicalic intermediate hole on the T_1 red-coloured PES) as computed by using a bidimentional surface.

The alcoholic products have been justified as due to a competitive process whose scheme is showed in Figure 6. Our computation was in line with this description.

In conclusion, in this work we have shown the effect that the presence of an electron-withdrawing group, such as the boronate esters, induces on the possibility to have a Paternò-Büchi's reaction. We were able to obtain the oxetane only in one case. In this case, the

reaction probably occurs through the formation of a C,C-type biradicalic intermediate. We have to be cautious on this conclusion considering that the calculations have been performed on a substrate (compound e in Figure 1) that does not give the corresponding oxetane. However, the electron withdrawing properties of the MIDA ester are not so different from those of the pinacolic ester d (see Figure 1), which is able to give the corresponding oxetane. On this basis, we can extend the result of our calculations to the other alkenyl boronates.

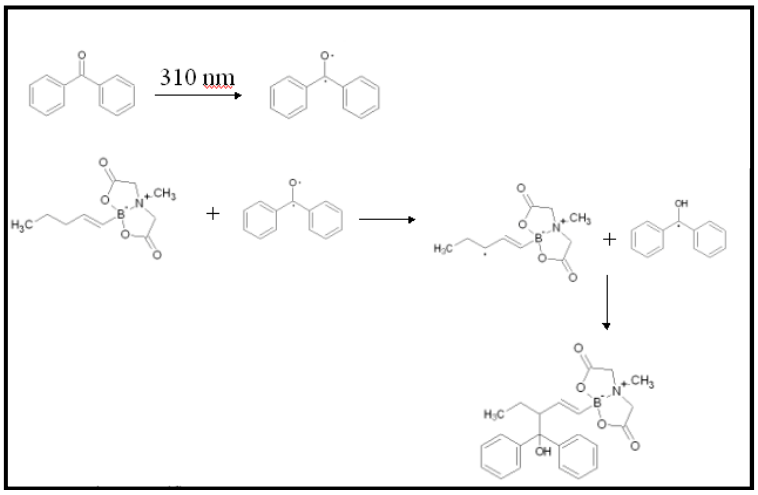
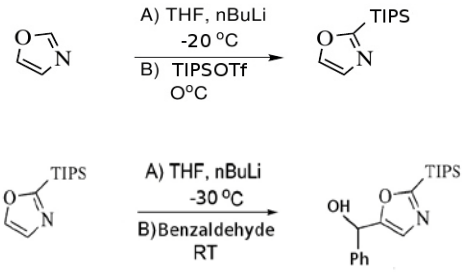


Figure 6. The hypothesised mechanism producing the (e) *trans* tertiary alcohol.

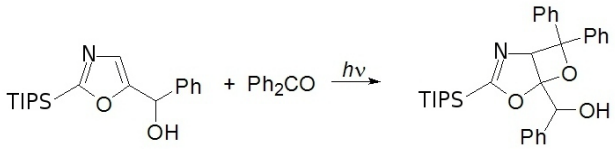
Furthermore, this is an unexpected result on the basis of the previous studies in this field where the presence of an electron withdrawing group seemed to favour the formation of a C,O-type biradicalic intermediate. We have determined several pathways allowing the formation of the expected oxetane with the observed stereochemistry. Furthermore, we have shown the hydrogen abstraction can be a competitive reaction and that, in the case of MIDA esters, the formation of the tertiary alcohol is the kinetically favoured process, in agreement with the experimental results.

The Paternò-Büchi's reaction has been tested also on a oxazolylcarbinole derivative to verify the role of the ring heteroatom and of the external OH group on the stereochemistry of the reaction. Such research is strictly related to previous ones performed in our laboratories on analogue furyl-based compounds. Analogues researches were performed on different oxazolyl-based compounds in which the regiochemistry was extensively studied.[6,7]
The substrate was produced in this work according to Scheme 2.



Scheme 2. The performed synthesis of studied oxazolylic compound.

The Paternò-Büchi's reaction with benzophenone as absorbing reactant was successfully performed (Scheme 3) and the stereochemical characterisation of the obtained oxetane is currently under investigation.



Scheme 3. The observed oxetane after the Paternò-Büchi's reaction.

References

1. Paternò, E. *Gazz. Chim. Ital.* **1909**, 39,341-361.

2. Kopecky, J. In *Organic Photochemistry*; VCH: New York, NY, **1992**; p 126; Turro, N. J.; Dalton, J. C.; Dawes, K.; Farrington, G.; Hautala, R.; Morton, D.; Niemczyk, M.; Schore, N. *Acc. Chem. Res.* **1972**, 5, 92e101

3. Arnold, D. R. *Adv. Photochem.* **1968**, 6, 301-423; Jones, G. *Org. Photochem.* **1981**,5, 1-122; Carless, H. A. J. In *Synthetic Organic Photochemistry*; Horspool, W. M.,Ed.; Plenum: New York, NY, **1984**; pp 425-487; Porco, J. A.; Schreiber, S. L. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Paquette, L. A., Eds.; Plenum: New York, NY, **1991**; Vol. 5, pp 151-192; Griesbeck, A. G. In *Handbook of Photochemistry and Photobiology*; Horspool, W. A., Song, P. S., Eds.; CRC: BocaRaton, USA, **1994**; pp 522-535; 550-559; Bach, T. *Synthesis* **1998**, 683-703; D'Auria, M.; Emanuele, L.; Racioppi, R.; Romaniello, G. *Curr. Org. Chem.* **2003**, 7,1443-1459; D'Auria, M. In *Targets in Heterocyclic Systems, Chemistry and Properties*; Attanasi, O. A., Spinelli, D., Eds.; Italian Society of Chemistry: Rome, Italy, **2003**; Vol. 7, pp 157-173; Griesbeck, A. G.; Bondock, S. In *Handbook of Photochemistry and Photobiology*; Horspool, W. A., Lenci, F., Eds.; CRC: BocaRaton, USA, **2004**; pp 59-1-59-19; Griesbeck, A. G.; Bondock, S. In *Handbook of Photochemistry and Photobiology*; Horspool, W. A., Lenci, F., Eds.; CRC: Boca Raton, USA, **2004**; pp 60-1-60-21; Abe, M. In *Handbook of Photochemistry and Photobiology*; Horspool, W. A., Lenci, F., Eds.; CRC: Boca Raton, USA, **2004**; pp 62-1-62-10; D'Auria, M.; Emanuele, L.; Racioppi, R. In *Advances in Photochemistry*; Neckers, D. C., Jenks, W. S., Wolff, T., Eds.; John Wiley & Sons: Hoboken, NJ, **2005**; Vol. 28, pp 81-127; D'Auria, M.; Emanuele, L.; Racioppi, R. In *Photochemistry Research Progress*; Sanchez, A., Gutierrez, S. J., Eds.; Nova Science: Hauppage, NY, **2008**; pp 373-438; D'Auria, M.; Racioppi, R. *Curr. Org. Chem.* **2009**, 13, 939-954; Abe, M. In *Handbook of Synthetic Photochemistry*; Albini, A., Fagnoni, M., Eds.; Wiley: New York, NY, **2010**; pp 217-239; Griesbeck, A. G. In *Synthetic Organic Photochemistry*; Griesbeck, A. G., Mattay, J., Eds.; Marcel Dekker: New York, NY, **2005**; pp 89-139; D'Auria, M. In *CRC Handbook of Organic Photochemistry and Photobiology*; Griesbeck, A. G., Oelgemoller, M., Ghetti, F., Eds.; CRC: Boca Raton, USA, **2012**; pp 653-681.

4. Beereboom, J. J.; von Wittenau, M. S. *J. Org. Chem.* **1965**, 30, 1231-1234; Turro, N. J.; Wriede, P.; Dalton, J. C.; Arnold, D.; Glick, A. *J. Am. Chem. Soc.* **1967**, 89, 3950-3952; Turro, N. J.; Wriede, P. A.; Dalton, J. C. *J. Am. Chem. Soc.* **1968**, 90, 3274-3275; Baltrop, J. A.;

Carless, H. A. J. *Tetrahedron Lett.* **1968**, 3901-3903; Turro, N. J.; Wriede, P. A. *J. Org. Chem.* **1969**, 34, 3562-3565; Dalton, J. C.; Wriede, P. A.; Turro, N. J. *J. Am. Chem. Soc.* **1970**, 92, 1318-1326; Turro, N. J. *Pure Appl. Chem.* **1971**, 27, 679-705; Baltrop, J. A.; Carless, H. A. J. *J. Am. Chem. Soc.* **1972**, 94, 1951-1959; Schuster, G. B.; Turro, N. J.; Steinmetzer, H. C.; Schaap, A. P.; Faler, G.; Adam, W.; Liu, J. C. *J. Am. Chem. Soc.* **1975**, 97, 7110-7118; Turro, N. J.; Farrington, G. L. *J. Am. Chem. Soc.* **1980**, 102, 6056-6063; Chung, W.-S.; Liu, Y.-D.; Wang, N.-J. *J. Chem. Soc., Perkin Trans. 2* **1995**, 581-586.

5. Mattay, J.; Gersdorf, J.; Leismann, H.; Steenken, S. *Angew. Chem., Int. Ed. Engl.* **1884**, 23, 249-250; Mattay, J. *Angew. Chem., Int. Ed. Engl.* **1987**, 26, 825-845.

6. A. G. Griesbeck, M. Fiege and J. Lex, *Chem. Commun.* **2000**, 589-590.

7. A. G. Griesbeck, S. Bondock and J. Lex*, J. Org. Chem.*, **2004**, 68, 9899-9906.

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TECHNICAL NOTES

Photography and the Spectrometer

During the early part of the 19th century when Niepce, Fox-Talbot and others were experimenting with methods to produce pictures with the aid of sunlight, a new and parallel investigation was underway in the investigation of the properties of light by using a prism, and much later, a diffraction grating, to separate light into their separate colours.

Thus both photography and optical spectroscopy have largely developed in parallel and have also mutually benefited from each other. Indeed, it is true that photography has been a key recording method in terms of spectroscopic measurements.

The early experiments of Sir Isaac Newton in the late 17th century used a glass prism and a round entrance aperture and then a rather wide slit type aperture. These experiments demonstrated a broad visible spectrum and artists often spoke of this demonstrating the primary colours. Almost one hundred and twenty five years later William Hyde Wollaston in 1802 substituted a narrow slit for the large aperture used by Newton and observed "a purer set of colours and better graduation of tint". At the same time he observed fine black lines. Within 12 years, by 1814, Joseph von Fraunhofer had developed the technique further and as a glass maker had developed Wollaston's work into an operating spectroscope using which he was able to clarify 574 black or absorption lines in the solar spectrum. These lines were later shown to be atomic absorption lines by Kirchoff and Bunsen in 1859. Meanwhile, James Gregory, the Scottish mathematician and astronomer, in 1673 demonstrated the splitting of colours from sunlight using a bird feather – this was really the first demonstration of the principle of the diffraction grating. David Rittenhouse manufactured a 'man-made' diffraction grating in 1785 using threads separated using a fine pitch screw. Fraunhofer used the same technique in 1821 using fine wire instead of threads. Thus practical and affordable diffraction gratings had been formed to act as spectroscopic elements.

In parallel, developments in the science of photography had greatly advanced and by the end of the 19th century acetate coated

photographic film was widely available along with the processing possibilities.

Combining these two instruments: the spectroscope and the photographic camera, we would now recognise this instrument as the modern spectrometer. Such combinations of spectrograph and photographic plate instruments have lead to the observation of effects such as Raman scattering, by C.V. Raman in 1928, as well as many more discoveries.

Today nearly all spectrometer systems detectors are based upon either single element or multi-element detectors that convert light into electrical charge. In particular, array detectors such as Charge Coupled Devices (CCD) and latterly similar detectors using CMOS structures have become the key elements for digital photography and scientific measurement and light recording.

Array detectors such as Charge Coupled Device (CCD) and Indium Gallium Arsenide (InGaAs) detectors have represented a revolutionary step forward in detecting light at wavelengths from ultra-violet to near infrared, particularly for spectroscopy. Their two-dimensional nature and unique combination of sensitivity, speed, low noise, ruggedness and durability in a compact and relatively economical package deliver significant advantages over single-channel detectors. CCD detector arrays are most useful when combined with an optical system to create either a conventional image or a spectral one.

A multichannel CCD can simultaneously collect spectrally dispersed information over a wide range at high speed when used in combination with an aberration-corrected imaging spectrograph. Indeed, the two-dimensional nature of the detector in such a system enables the simultaneous measurement and analysis of multiple spectra from several spatial locations or sources.

Detector choice — and a caveat

Choosing the correct detector is important to the success of a spectroscopic experiment. But while the detector's specifications can be a good place to start an evaluation, they are only one factor in a system's performance.

Equally or more important is the optical design that collects the signal and images the light from the sample into the spectrograph,

along with the choice of spectrograph, its configuration and its optimization for the particular application. After all, if you can't efficiently transmit the light to the detector, it makes no difference whether you use a $10 CMOS linear array or a $50,000 ultra-high performance scientific CCD.

In selecting an array detector for a spectroscopic application, the user must therefore evaluate detector type, size, cooling method, quantum efficiency, read- and dark-noise performance, pixel size, full-well capacity and controller dependent specifications such as analog-to-digital conversion operation. From these values, the maximum and minimum signals can be calculated, along with the signal-to-noise and dynamic range of the detector under various illumination conditions. A more rigorous evaluation also may take into consideration the charge-transfer efficiency, linearity, pixel uniformity and etaloning performance.

A variety of full-frame CCD devices are available, including front- and back-illuminated and open-electrode configurations. Deciding which are the best demands that one consider the requirements of the application. In general, selections should be made based on the wavelength range of interest, spectral coverage and resolution, and the expected optical signal level. These requirements determine the chip type, total area, pixel size and cooling method.

There are a range of CCD sensor configurations, each have advantages and disadvantages. They are summarised in table 1.

Table 1

Sensor	Abbreviation	Comments
Front Illuminated	FI	The incident photons must penetrate a poly-silicon electrode before reaching the depletion region. The transmittance of the electrode depends on its thickness, so chips from different manufacturers demonstrate different quantum efficiency responses in the 400- to 600-nm range. The electrode becomes opaque to wave- lengths below 400 nm.

Open Electrode	OE	A variant of the FI CCD is the open-electrode configuration, in which the central area of the electrode is etched to expose the under- lying photosensitive silicon. This offers an uninterrupted pathway for the incident radiation to reach the depletion region. Such detectors may exhibit quantum efficiencies of 30 percent or greater in the UV. In addition, their visible and near- IR response often is superior to front illuminated devices. Compared with back-illuminated devices, open electrode CCDs do not exhibit interference fringe effects (also called etaloning) in the near-IR
Back Illuminated	BI	Back-illuminated, or back-thinned, CCDs are full-frame image sensors in which the substrate is polished and thinned to remove most of the bulk silicon substrate. They are illuminated from the back, and the poly- silicon on the front does not influence the quantum efficiency of the detector. They are usually antireflection-coated for enhanced response in either the UV or the near-IR. Back-illuminated devices, however, are significantly more expensive than their front-illuminated and open-electrode counterparts. They often require deeper cooling to reduce noise, and they can exhibit problems in response uniformity across the chip as a result of the thinning process. Most significantly,

		etaloning can be severe when the devices are used to measure at wave-lengths longer than ~650 nm.
Back Illuminated, Deep Depleted	BI-DD	Charge Coupled Devices (CCDs) requiring enhanced performance in the Near Infrared (NIR) region of the spectrum or at higher X-ray energies can benefit from being constructed on specially selected silicon with an optimised thickness. Such a CCD, where the electron generation is within this thick silicon, is known as a Deep Depletion Device (High Resistivity).

Quantum Efficiency and cooling performances

The quantum efficiency of the detector is a reasonable indicator of its spectral response. All CCD detectors display wavelength-dependent quantum efficiencies because the absorption coefficient of silicon is wave- length-dependent. Short-wavelength photons (i.e., blue) are absorbed at much shallower depths than those with longer wavelengths (i.e., red).
In fact, the absorption coefficient is essentially zero at wavelengths longer than 1.1 μm because the photon energy is less than the silicon band gap energy and the detector becomes transparent to the light. This translates into a long-wavelength cutoff of 1.1 μm. If the application requires spectral measurement in the near-IR (for example, in the 1 to 1.7 μm range), a linear InGaAs array may be the best choice.
Combinations of imaging spectrographs and 2-Dimensional detectors can make spectral imaging systems or hyper-spectral imaging systems.

Spectral Imaging tools

The optical and spectral characteristics of a hyper-spectral imaging system are determined largely by the application requirements. However, all systems have the same basic components in common: a means to image the object, a means to provide both spectral and spatial resolution, and a means of detection.

The classic, though inefficient, method to make spectral images has been to sequentially take the same "photograph" through a series of wavelength bandpass filters and construct an image spectral cube data set — X-position vs. Y-position vs. wavelength. Traditional methods of wavelength selection have used dielectric optical bandpass filters but the use of either an acousto-optic tunable filter (AOTF) or a liquid crystal tunable filter have permitted electronic sweeping of the spectral bandpass.

In each of these cases, an image is collected one wavelength after the other and the field of view (FOV) of the imaging system is fixed. Filter based methods place a huge burden on the sample and illumination stability as well as being a time- and computation-intensive process. In the minimum experiment, it is necessary to wait until all wavelength images have been recorded, which may take from minutes to hours of measurement time depending upon samples, illumination and integration conditions. Therefore, if an object is moving or is spectrally unstable due to its chemical, physical or physiological characteristics, then the integrity of the resulting hyper-spectral data cube may be seriously in question.

True spectral imaging requires all of the wavelengths to be recorded simultaneously — that is, a wave- length-dispersive system is required. In this case, the FOV is generated sequentially and the hyper-spectral integrity of the data is maintained for each and every measurement, even if the sample dynamics are changing or if the sample is moving. An image of the FOV is collected by translating the sample across the slit aperture of the spectrograph in a method known as push broom acquisition. Thus the wavelength or spectral data are measured simultaneously and the image or FOV is generated sequentially. There is essentially no delay in acquisition, which guarantees the integrity of spectral data and ratios. The spectral image builds up in real time and can be stopped at any time. This means that spectral snapshots are possible without the need to take the full image field of view. Figure 1 shows the arrangement of the spectral hyper cubes. In principle, the data collected in either the filter or

push broom approach are complementary to each other in terms of the basic concept.

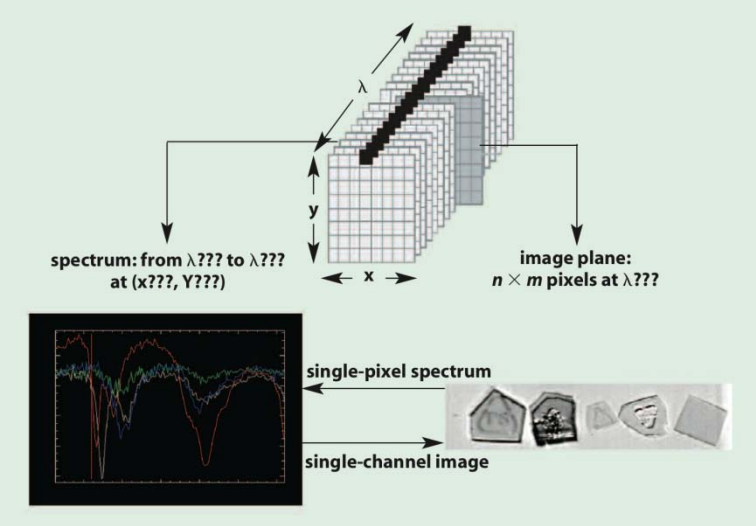

Figure 1. Hyper spectral Data Cube

Optical arrangement

The complete optical system for a hyper-spectral imaging system consists of a suitable objective lens matched to the spatial and spectral requirements of the application, the imaging spectrograph proper and a two-dimensional detector to simultaneously collect the spectral vs. spatial information.
Figure 2 illustrates the general arrangement of a typical push broom system.
The objective lens images the target onto the slit of the main element of the spectral camera: the transmission grating spectrograph. The slit, grating and detector characteristics determine the spectral resolution. The spatial resolution is determined by the pixel size of the two-dimensional camera and the objective lens as the spectrograph is designed with a magnification of 1. The transmission grating approach offers a compact, robust and

economical means to provide the required wavelength dispersion and also provides excellent spatial resolution.

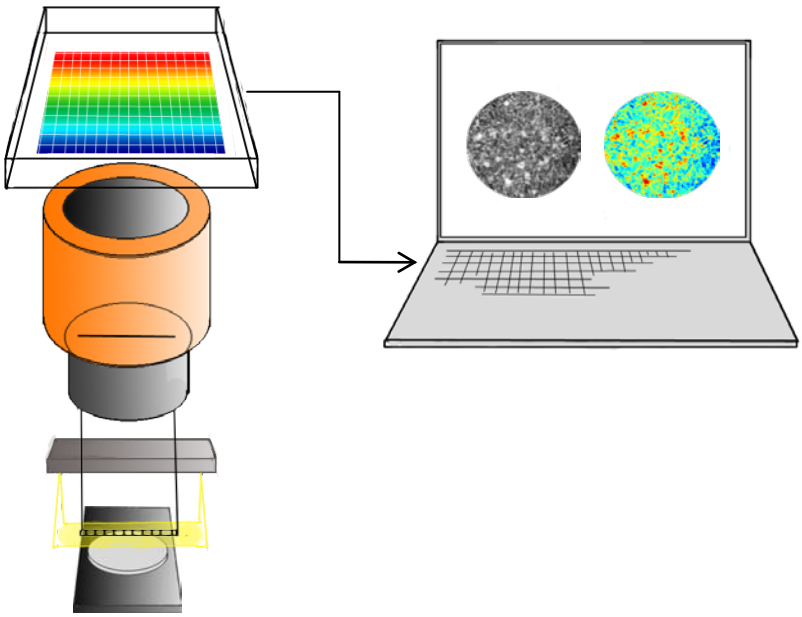

Figure 2. Typical Push Broom Spectral Imager, courtesy of SPECIM OY

In general, such spectrographs offer excellent optical and dispersive efficiency, up to 80 percent, and almost no polarization dependence. This contrasts with other spectro- graphs based on reflective gratings in either an asymmetric Czerny-Turner (CT) or Offner design. Alignment, wave front flatness and device stability are critical in such spectrographs, although designs are available that offer excellent imaging performance; though in a larger physical package than the ImSpector-type design. Because C-T and Offner designs use reflection gratings; the polarization dependences can also be strong. This may cause issues of spectrograph efficiency in, for example, fluorescence and biophysical applications, where the anisotropy of a sample is one of the key measurements to understanding cell / drug interactions.

Using spectral imaging systems, one can achieve very significant additional information about sample behaviour and dynamics and imaging systems can present close to photographic imaging quality.
Due to the fact that each individual image pixel has a complete optical spectrum associated with it, the resulting image contains a wealth of data that can be processed to provide spatial, spectral, and concentration information.
Hyper-spectral systems have been very successfully deployed in absorption, emission, luminescence, Raman applications. The latest developments encompass hyper-spectral imaging with time resolution and we have achieved technical performances that allow spatial resolution as fine as 1μm range and time resolution as good as 200ps FWHM. Thus hyper-spectral imaging has great opportunities to capture data rapidly and optically efficiently in a range of challenging experimental situations. Its really interesting to note that we continue the strong parallel development of photography and spectroscopy.

Dr. John R. Gilchrist
Gilden Photonics Ltd

PHOTOCHEMICAL AND PHOTOBIOLOGICAL SCIENCES

CONFERENCE REPORTS

Chair's Report on the 16th International Congress on Photobiology, Córdoba, Argentina September 8–12, 2014

The 2014 International Congress of Photobiology was held in the "Pavillion Argentina" located within the University Campus of the National University of Córdoba, Argentina. This conference was the 16th in a series sponsored by the International Union of Photobiology (IUPB, www.iuphotobiology.com). This was the first time that the IUPB Congress was held in the Southern Hemisphere and also the first time held south of the Rio Grande. The University of Córdoba is the oldest in Argentina (founded 401 years ago) and the City of Córdoba offered a wonderful frame (and great weather) for the Congress.

The International Organizing Committee, listed in the web page of the Congress (**www.photobiology2014.com.ar**), addressed nearly all areas of photobiology and were from many countries. All areas of the interaction of light with the biosphere were covered, such as photosynthesis, photomorphogenesis, photomovement of plants and bacteria, the interaction of UV light with ecosystems (including bacteria, phytoplankton, zooplankton, algae, plants, mammalian cells, andhumans), circadian rhythms in plants and animals, vision and light-induced damage to the retina, UV induction of skin cancer, as well as the use of light for the treatment of various illnesses and the photochemistry of xenobiotics and biological molecules. The use of light-based technologies for the study of biological processes was also the subject of various symposia.

The Congress registered 507 participants from 38 Countries. 160 participants were from Argentina, 44 from Brazil, 17 from Chile, 60 from the USA, 50 from Germany, 20 from Japan, etc. 280 of the registered participants were young fellows (graduate students and young researchers).

The **Scientific Programme (www.photobiology2014.com.ar/programme)** had 3 Plenary

Lectures: Nathan Nelson (Israel) on the "Evolution of the Photosynthetic Apparatus", Thomas Schwarz (Germany) on "Photoimmunology", Ernst Bamberg (Germany) on "Channel Rhodopsins and Optogenetics", 9 Keynote speakers highlighting the frontiers of research in various areas: Carlos Ballaré (Argentina), Rosalie Crouch (USA), Anderson Garbuglio (Brazil), Mario Guido (Brazil), Hideki Kandori (Japan), Alberto Kornblihtt (Argentina), Dimitra Markovitsi (France), Frank Vollmer (Germany), Horacio Zagarese (Argentina), and 51 Symposia (each 130 minutes with between 4 and 6 participants), organized each one by two (sometimes one) scientists who were (was) also contributors to the Symposium. There were also two marvelous special (Historical) Lectures: Winslow Briggs (USA) on his "Scientific and Life Experience", Phil Hanawalt (USA) on the "History of Research on the DNA Repair Mechanism". A Symposium on Photomovement was held in Memoriam of Masamitsu Watanabe (deceased in 2013), who had a major role in the discovery of photoreceptors implied in photomovement.

IUPB awarded three Finsen Medals with Lecture: Masamitsu Wada (Japan), Herbert Hönigsmann (Austria), Douglas Brash (USA), one Finsen Lecture: Roman Ulm (Switzerland), as well as one Edna Roe Lecture: Chikako Nishigori (Japan).

Graduate students and young researchers presented 200 posters on all areas of photobiological research. Six poster prizes in the form of book vouchers were awarded on Friday during the closing ceremony: two from Springer Verlag, two from the Royal Society of Chemistry and two from the International Union of Pure and Applied Chemistry (IUPAC).

Most symposia were organized with strong collaboration of colleagues from Latin-America. Some research areas are strong in Argentina (e.g., plant photomorphogenesis, blue-light-induction of microorganism behaviour, vision and UV damage to retina, circadian rhythms, photoecology, UV influence on the environment) and in Brazil (PDT, DNA photodamage, bioluminescence, biodiesel photoproduction), whereas some others are weak (e.g., molecular aspects of photomedicine, optogenetics, and areas of research that require complex instrumentation: e.g., ultra fast reactions). All symposia were well attended, especially by the young colleagues.

Many of the subjects treated were directly related to the problems and or peculiarities encountered in Latin America, such as the photobiology of extremophile bacteria at high altitude in the Puna

(North of Argentina and Chile, Bolivia and Perú) as well as in Antarctica, the effect of the ozone hole in the ecosystems in Argentina and Chile, the special properties of alga in Chile, the increase of UV-induced skin diseases in Brazil and others.

The participation of Argentine Scientists working abroad: Víctor Batista, Roberto Bogomolni, Gonzalo Cosa, Raquel Galián, Thomas Jovin, Diana Kirilovsky, Maria Andrea Mroginski, Ana Moore, Juan C. (Tito) Scaiano, Graciela Spivak, Cristian Strassert, María Vernet, Matias Zurbriggen, and Silvia Braslavsky was very important for the consolidation of the research ties between Argentine research groups and groups abroad. This has a special value in view of the very dramatic brain drain the Country suffered since 1966 and until 2001, which has being reverted by several actions taken in the last few years, in particular since the creation of the Minister of Science, Technology and Innovative Production, MINCyT, in 2011.

The Argentine science administration Agencies strongly supported the Congress with grants from the Argentine Research Council, CONICET, (ca. 10.000 U$S) and from MINCyT (ca. 12.000 U$S). This permitted waiving the registration fee of all Argentina graduate students and several young scientists. In addition, the programme **Raices** from MINCYT financed the travel to the Congress of several of the Argentine colleagues working abroad.

There was also important support (both financial and logistic) by the German Institutions (**Research in Germany** grouping DAAD, DFG, Fraunhofer, Humboldt Foundation) and the Max Planck Society as well as financial support from **IUBS** (International Union of Biological Societies), **IUPAC** (International Union of Pure and Applied Chemistry), **TWAS** (The World Academy of Sciences) as well as **ESP**, **ASP** and the French Society of Photobiology, who helped financing the participation of young graduate students. These grants permitted waiving the fees of Latin-American graduate students and young researchers.

Several International companies and Argentine representatives of instrumentation (see logos in Programme Booklet and Webpage) supported the Congress; major contributors were: L'Oreal: in particular for sponsoring the contributors of the Symposium on Photoprotection, BASF and Johnson&Johnson. Some of the sponsoring companies had an exhibition booth in the foyer of the Pavillion.

All abstracts of Plenary, Special, and Keynote Lectures, as well as of the contributions to the Symposia and the posters presented were published on-line and can be found at: http://www.photobiology2014.com.ar/website/wp-content/uploads/2014/09/abstractBook.pdf.

The Editors of the Journals *Photochemical and Photobiological Sciences* (*PPS*, the Journal of the European Society of Photobiology, ESP, and the EuropeanPhotochemical Association, EPA), *Photochemistry and Photobiology* (*P&P*, the Journal of the American Society of Photobiology, ASP), and *Pure and Applied Chemistry* (*PAC*, the Scientific Journal of the Union of Pure and Applied Chemistry, IUPAC) have agreed to publish, in each Journal, some of the Lectures and Symposia presented during the Congress. A letter of intention to publish is expected before November 15th. All submitted papers will undergo the normal evaluation procedure. The submission deadline for all three Journals will be March 31st, 2015 and it is planned to publish each of the papers immediately after acceptance. At the end of the publication procedures a virtual issue will collate all contributions belonging to the Congress.

A major spin-off (s) of the Congress is the creation of the Argentine Group of Molecular Photobiologists (GRAFOB in Spanish, http://grupoargentinodefotobiologia.info/drupal/). This group already held two meetings in preparation of the 16th ICP: one in 2011 in La Plata and the second in 2013 in Córdoba (same place as 16th ICP 2014), with ca. 90 participants in each. Several contacts were established between Latin American Research Groups, including some that could not participate of the Congress. The Argentine photobiology group met during the Congress and it was agreed to organize a third GRAFOB meeting in Tucumán in 2016.

The Executive Board of IUPB had a regular meeting during the Congress and also held a general assembly. The new elected Executive Board is: President: John Spudich (USA), Secretary: Evelyn Sage (France), Treasurer: Franz Trautinger (Austria), Vice-Presidents: Roberto Bassi (Italy), Carlos Ballaré (Argentina), Gary Halliday (Australia), Yoshitaka Fukada (Japan), Liason member as organizer of the 16th ICP: Silvia Braslavsky (Germany).

The participants had the oportunity of enjoying a tango show during the opening reception on Sunday evening and some Argentine folk dancing on Thursday evening, whereas they could also witnes how several students drank their mate during the lectures.

The 17th ICP will be held most probably in 2018 in the UK.

Silvia Braslavsky
Chair, 16th ICP
October 2014

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