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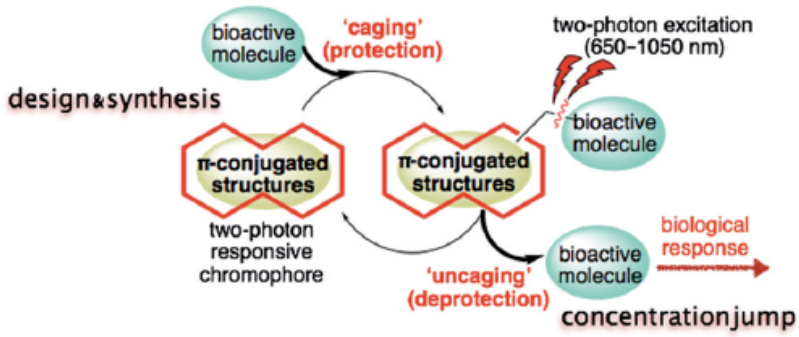


Dedicated to Professor Herbert Mayr on the occasion of his 70th birthday

Abstract Near-infrared two-photon (TP)-induced photorelease (un- caging) of bioactive molecules such as drugs has attracted considerable attention because of its ability to elucidate mechanistic aspects of bio- logical processes. This short review summarizes recent developments in the design and synthesis of TP-responsive chromophores.

Key words caging reactions, uncaging reactions, photolabile protect- ing groups, two-photon absorption, time-dependent density functional theory

1. Introduction

In general, two principle methods are used to investigate the role of biologically active compounds and their as- sociated mechanisms in biological processes: (1) imaging, and (2) caging & uncaging. Fluorescence microscopic tech- niques that use biological fluorescent stains, immunofluorescence, and fluorescent proteins can be utilized to generate real-time images of life phenomena, from which the active sites and functionalization of bioactive compounds can be clarified.1 This method is widely employed in biological and physiological studies.2

Another technique used to investigate *in vivo* activity involves concentration jumps of bioactive molecules.3 Since biological responses induced by such concentration jumps ceases within the micro- to millisecond timescale, spatio-temporal control of the release of bioactive molecules is necessary in physiological studies. Approximately 40 years ago, Schlaeger,4 Hoffmann5 and their co-workers, who were biochemists, developed a 'caging & uncaging' method for bioactive substances (Figure 1), in which the bioactive molecules are temporally inactivated (caged) by photolabile protecting groups (PPGs)6–11 and re-activated (uncaged) by flash photolysis of the caged compounds. For this reason, the meaning of 'caging & uncaging' is not derived from the shape of molecules, rather it signifies the 'masking (inactivation) & unmasking (activation)' of biologically active sub- stances such as therapeutic drugs.

To date, several types of PPGs have been used in physio-logical studies investigating the phenomena of life (Figure 2), which can be categorized into two groups based on the photorelease uncaging mechanism: (1) Those in which the release is the consequence of an intramolecular rearrangement (nitrophenyl, or cinnamyl-type PPGs), and (2) PPGs that are cleaved directly by SN1-type solvent-assisted photolysis; the latter offering inherently faster release. The orthonitrobenzyl-type PPG (o-NB)12–14 was the first PPG in the 'caging & uncaging' technique in physiological studies.

However, this PPG has the potential to thermally hydrolyze at the benzylic position to produce bioactive substances during such studies. To avoid such a possibility, Specht, Goeldner and co-workers used an *ortho*-nitrophenethyl (*o*- NPE)15–18 PPG for their physiological studies. The PPG was developed by Hasan

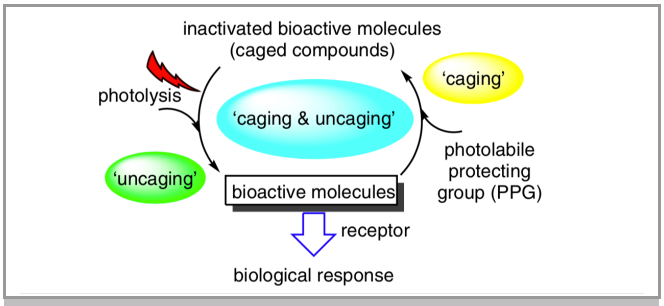


Figure 1 Concept of "caging & uncaging" of bioactive molecules

and co-workers.15 Nitro-substituted indoline derivatives19–24 have also been used for the uncaging reactions.

Givens and co-workers developed a photo-SN1-type of uncaging step using coumarin chromophores (Figure 2).25,26 Quinoline derivatives27–30 have also been used for the SN1-type photorelease of drugs. Kakiuchi and co-workers have discovered a novel chromophore employing a thiochromone for the uncaging step.31 Very recently, new visible- light-responsive PPGs with fluorescent character, i.e., BODIPY and bimane, were developed by Smith, Winter, Klán, and Singh.32 In this case, acids [X = –OC(O)R] can be released by the visible light. Furthermore, Marchán et al. developed new coumarin-derived chromophores (DEDCC) having visible-light responsiveness.32d,e Jana, Singh, and co- workers used a carbazole (CBZ) unit for the release of single and dual carboxylic acids.32f

After the pioneering studies by Schlaeger and Hoffmann in the late 1970s, the 'caging & uncaging' technique has become a general tool for physiological studies, including neurological science, cell biology, gene expression, and structural biology. In the mid-1990s, the second generation of 'caging & uncaging' technology emerged, employing a two- photon (TP) excitation process with light at ~700–750 nm. Note that living tissues are almost transparent (low absorption) to light from the near-infrared (NIR) region, i.e. be- tween 650–1050 nm. Denk,33,34 Lipp and Niggli,35 Furuta and Tsien,36 and Ellis-Davies and Kasai37 were the contributors to this revolution, which provided this next generation of 'caging & uncaging' technology based on NIR-TP irradiation. For *in vivo* studies, caged compounds in general should be water-soluble and non-toxic. In addition to the basic conditions, it is becoming essential to have large TP responses from the chromophores.

The physicist Maria Göppert-Mayer laid down the theo-retical foundation of the TP excitation process in 1931,38 in which two photons are absorbed simultaneously by the same molecule. This is illustrated for Ar–X in Figure 3. Thanks to its strong one-photon (OP) absorption band at 400 nm (3.10 eV), the molecule can be electronically excited by irradiation at 400 nm to generate the corresponding excited state [Ar–X]* (S1) (Figure 3). Of course, the excited state molecule [Ar–X]* cannot be generated using an 800 nm OP excitation process, because the molecule has a vanishing OP absorption at 800 nm (1.55 eV), that is, the absorbance is zero at 800 nm (Figure 3). However, using ultra- short pulses with high light intensity (typically via a femtosecond pulsed laser), molecules having TP absorption character can absorb simultaneously two photons, in this case at 800 nm, to generate the same molecular excited state [Ar–X]*.

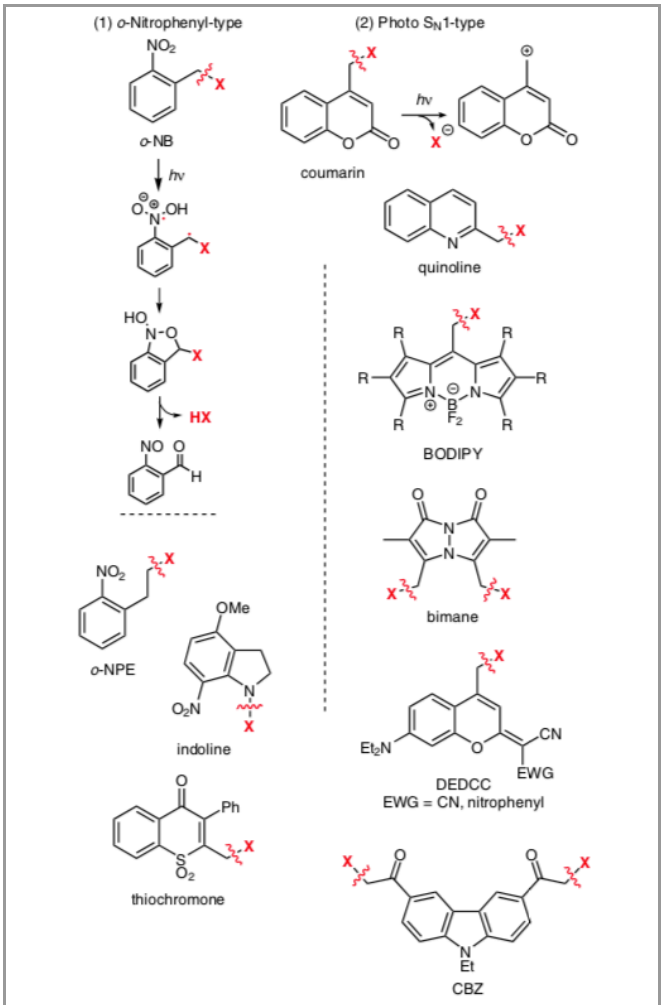


Figure 2 Selected types of photolabile protecting groups (PPGs)

Thus, in the TP excitation process, much lower energy photons can be used to bring molecules to their electronically excited state [Ar–X]* (S1). Half the energy ($h\nu' = 1/2 \times h\nu$) of the corresponding OP excitation ($E = h\nu$) can be used when the first electronic excitation band (S1) of the OP excitation process is allowed for the TP excitation process.

The efficiency of the TP absorption process is quantified by the TP cross-section (σ2), its magnitude being expressed using the Göppert-Mayer (GM) unit, 1 GM = 10–50 cm4 s photon–1 molecule–1 (Figure 4, a). The uncaging efficiency (δu) of the TP excitation process is determined by the equation $\delta u = \sigma 2 \times \varphi u$, where φu is the quantum yield of the un- caging reaction. The advantages of using the NIR-TP excitation process in place of one-photon (OP) excitation with UV-vis light include: (1) less photo-damage to living tissue, (2) higher three-dimensional (3D) spatial resolution, (3) deeper penetration in turbid tissues, and (4) lower light scattering.

With a TP excitation process, only molecules located at the focal point will be excited (Figure 4, b), since the probability (P) of excitation is proportional to the light intensity (I) squared.39 Conversely, all illuminated molecules will be excited with an OP excitation process. Thus, 3D spatial selective excitation is possible using the TP excitation technique, although a high-intensity femtosecond laser is re- quired.40,41

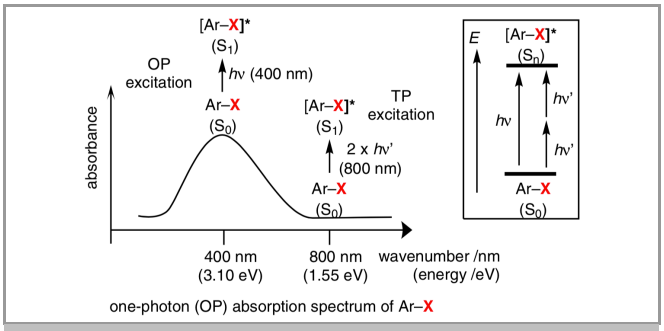


Figure 3 One-photon (OP) and two-photon (TP) excitation processes of Ar–X

In this short review, the basic strategies for designing TP-responsive chromophores for *in vivo* studies are first provided, followed by a summary of very recent developments in the TP uncaging technology using NIR light, including our contributions from the last three years, since excellent earlier reviews are also available.42–49

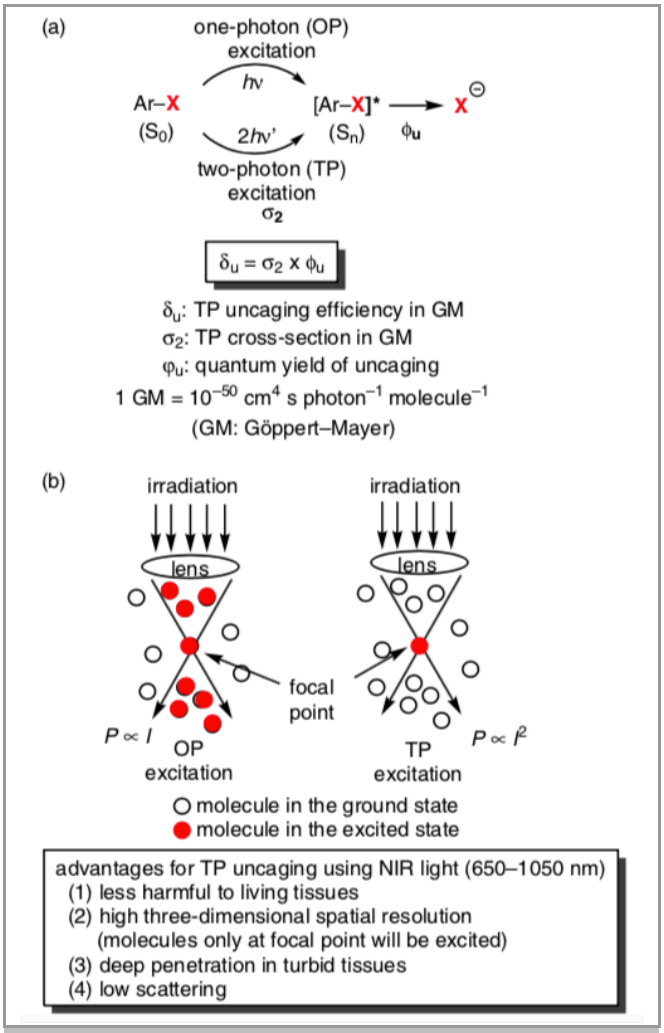


Figure 4 (a) TP uncaging reaction and (b) advantages of TP excitation versus OP excitation

In this short review, the basic strategies for designing the TP-responsive chromophores in vivo studies are first provided, followed by a summary of very recent developments in the TP uncaging technology using NIR light, including our contributions, in the last three years, since excellent earlier reviews are also available.42-49

2. Molecular Design of TP-responsive Organic Chromophores for "Caging & Uncaging"

Essentially, four strategies have been developed to de- sign chromophores with smart TP absorption character: (1) π-conjugation, and (2) dipolar, (3) quadrupolar and (4) octupolar systems (Figure 5).50 Besides, in terms of biological applications, it is useful to find out how the excitation wavelength in the NIR region of light (650–1050 nm) can be selected. In this regard, time-dependent density functional theory (TDDFT) has proved to be a powerful theoretical method for designing appropriate chromophores based on predicted TP absorption spectra.50–52

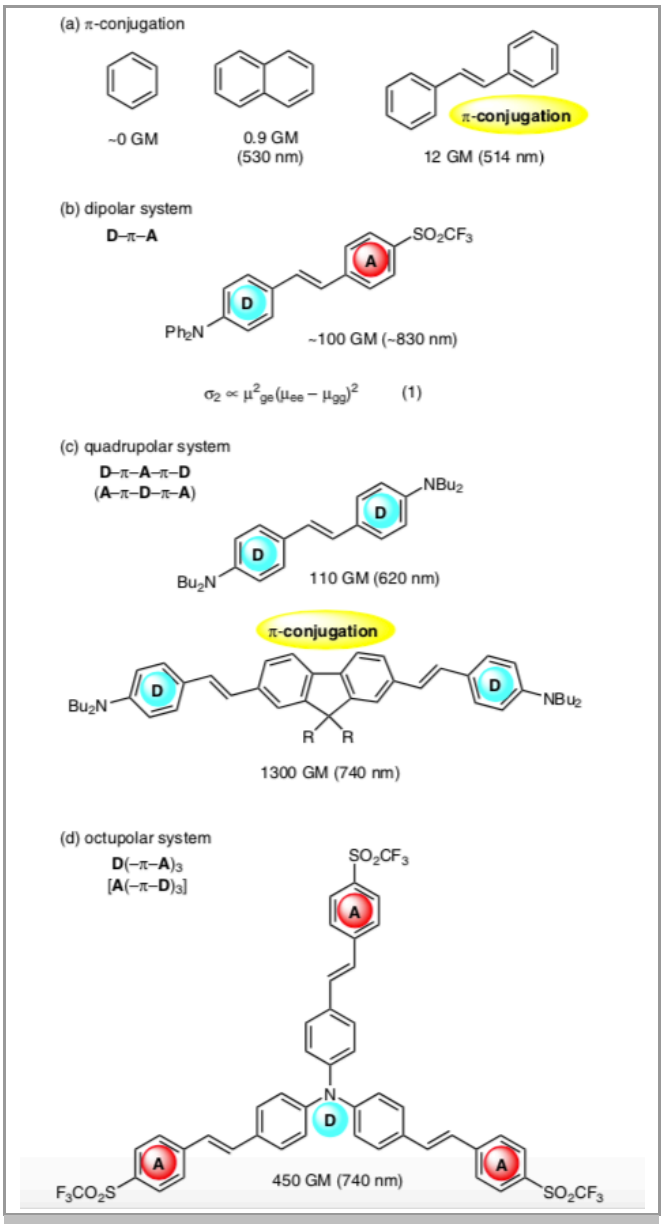


Figure 5 Strategies to induce TP absorption character into molecules

2.1. π-Conjugation

First, benzene possesses no TP-responsive character. Naphthalene shows slight TP absorption character, with 0.9 GM

at 530 nm.53 Stilbene, in which two benzene rings are connected by a double bond, demonstrates a sizable TP response, 12 GM at 514 nm (Figure 5, a).54 This basic trend clearly shows that the π-conjugation is directly correlated with TP absorption character.

2.2. A Dipolar System

The introduction of an electron-donating substituent (D) and an electron-withdrawing substituent (A), at either ends of stilbene, leads to a molecule with dipolar character (D–π–A). Figure 5 (b) highlights the dramatic increase in TP absorption cross-section, with 100 GM at ~830 nm, i.e., a wavelength of twice that of the OP absorption maximum at ~410 nm.55 The one order of magnitude increase of the TP cross-section obtained with the push–pull structure can be rationalized within a two-state model, in which the cross section (σ2) is proportional to the product of the squared transition dipole moment and the squared difference be- tween the excited-state and ground-state dipole moments (Figure 5, b, eq 1).50

2.3. A Quadrupolar System

Quadrupolar character (i.e. D–π–A–π–D or A–π–D–π–A) arises in a stilbene structure by introducing push–push or pull–pull substituent designs at either end of the core skeletal structure (Figure 5, c).56 For example, a large σ2 value of 110 GM was reported at 620 nm for a push–push structure. When the π-conjugation increases by introducing a fluorenyl core between two stilbenes moieties, σ2 undergoes a more than tenfold enhancement accompanied by significant red-shift, leading for instance to 1300 GM at 740 nm.57 A three-state model can be used to rationalize the TP response of such quadrupolar systems.

The TP absorption maxima of quadrupolar systems do not show up at twice the wavelength of the OP absorption peaks, since the selection rules for optical transitions are different for OP and TP processes in centrosymmetric structures.44,50 Often, the first TP absorption maximum shows up at a smaller wavelength than twice the first OP absorption maximum (i.e., $\lambda TP < 2 \times \lambda OP$).

2.4. An Octupolar System

Triangular structures such as 1,3,5-trisubstituted-benzene [i.e., D(–π–A)3 or A(–π–D)3] demonstrate an octupolar character via the introduction of push (D) or pull (A) substituents (Figure 5, d). Such octupolar structures also lead to sizable σ2 values, for example, of more than 1000 GM above ~740 nm for the chromophores shown Figure 5 (d).55,58

As briefly described above, both state and transition dipole moments of the molecule play crucial roles in producing molecules with high multi-photon absorption character. The absorption spectra of the TP excitation process can be predicted by TD-DFT calculations, at least qualitatively depending on available levels of theory.50 For dipolar systems, the first TP absorption maxima is expected to show up at twice the wavelength of the first OP absorption maxi- mum, both processes being symmetry allowed. Often this involves excitation to the first excited state (S1). For quadrupolar and octupolar structures, the first TP allowed electronic transition usually corresponds to a higher electronic excited state such as S2 or S3, in which the transition dipole moments may be vanishing.50,55,58

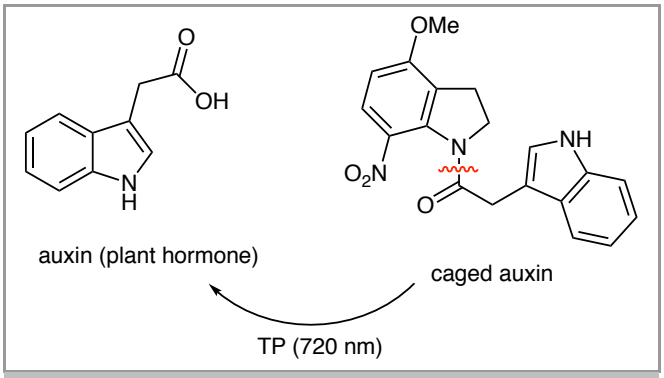
Increasing π-conjugation not only increases the TP absorption cross-section values of molecules, but also their hydrophobicity. However, caged compounds must possess suitable water solubility in physiological studies. Further- more, the TP absorption maxima should be in the NIR region of 650–1050 nm. Thus, a balance of TP absorption character and water solubility must be considered when designing TP-responsive chromophores for physiological studies.

In addition to the above-mentioned requirements for state-of-the-art caged compounds, i.e., TP responsiveness, water solubility and non-toxicity, the rate of photorelease of bioactive molecules is also a very important issue for physiological experiments. The sharp concentration jump by photolysis, e.g., less than a microsecond timescale, is es- sential for observing fast biological responses.

3. Recent Developments of TP Uncaging Reactions

3.1. 4-Methoxy-7-nitroindolinyl (MNI)-Caged Auxins

The plant hormone auxin (i.e., indole-3-acetic acid) plays a crucial role in almost every aspect of plant development. Hayashi and co-workers59 have designed and synthesized a 4-methoxy-7-nitroindolinyl (MNI)-caged auxin (Scheme 1). The caged auxin was found to be thermally stable in plants, and indole-3-acetic acid was released rapidly upon photolysis. However, the *o*-NPE-caged auxin was found to be unstable in plants. The authors determined optical control of auxin-responsive gene expression and aux- in-related physiological responses using the TP-responsive caged auxin.

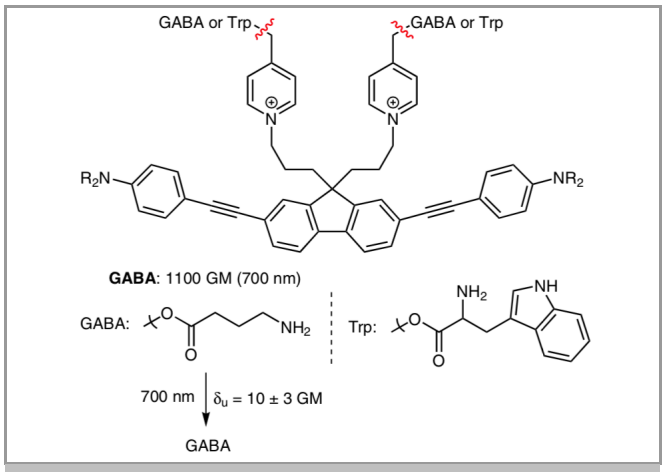


Scheme 1 TP uncaging reaction of caged auxin

3.2. Uncaging of GABA and Tryptophan Using TP-induced Electron-Transfer Reactions

Anderson and co-workers60 developed a new approach for the design of TP-responsive chromophores based on a photoinduced electron-transfer reaction (Scheme 2). They succeeded in synthesizing TP-responsive caged γ-amino butyric acid (**GABA**) and L-tryptophan (**Trp**) with an electron-donating fluorene-based chromophore and an electron-accepting pyridinium moiety. A high TP cross-section value of σ2 = 1100 GM at 700 nm was found for **GABA**. Further, a clean photochemical release of GABA was observed at 700 nm irradiation with a chemical yield of >95% and a photo chemical yield of 1% (φu = 0.01). Thus, the efficiency of the TP-induced

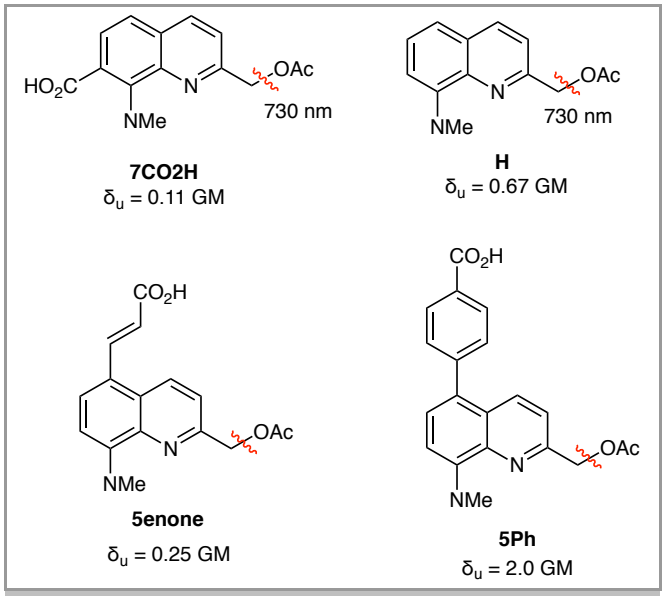
uncaging reaction of GABA was determined to be δu = 10 ± 3 GM.



Scheme 2 TP uncaging reaction of caged **GABA** and caged **Trp**

3.3. Effect of Position Isomerism in Aminoquinoline-Derived PPGs

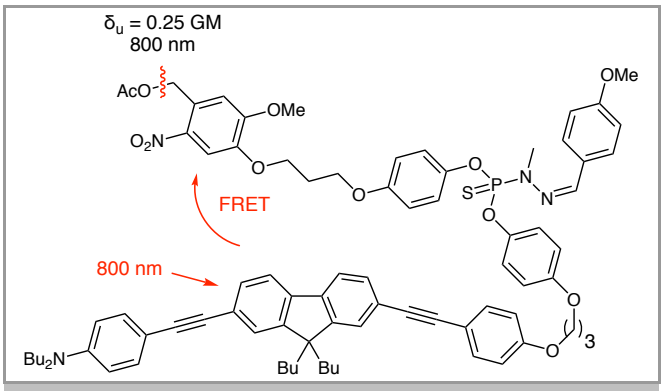
Substituent effects on the TP-induced uncaging reaction were reported by Dalko and co-workers61 for TP-responsive aminoquinoline derivatives (Scheme 3). The TP uncaging efficiency at 730 nm for the C5-carboxy isomer **5CO2H**, δu = 0.11 GM, was much smaller than the parent **H** (δu = 0.67 GM), **5Bz** (δu = 2.0 GM), and **5Acr** (δu = 0.25 GM), even though the substituent is supposed to induce a higher intramolecular charge-transfer character than the parent **H**.



Scheme 3 TP uncaging reaction of quinolone derivatives

3.4. Cooperative Dyads for TP Uncaging

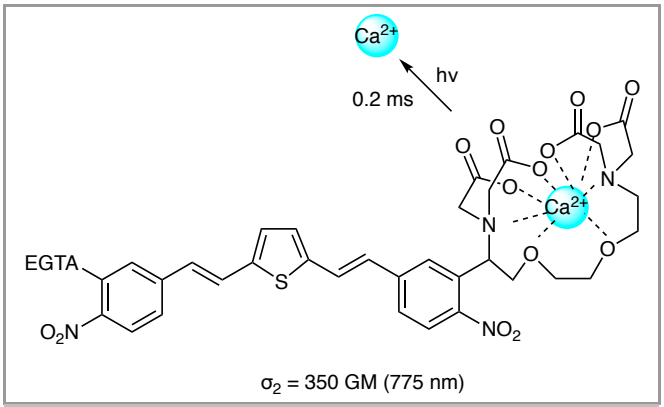
Blanchard-Desce and co-workers62 designed an intramolecular energy transfer system from the TP-responsive chromophore to the PPG unit. Donor–donor substituted (R = NR2, OR) and acceptor–acceptor substituted (R = SO2CF3) fluorene structures with quadrupolar characters were chosen for the TP-responsive chromophore (Scheme 4), and the 4-methoxy-2-nitrophenyl unit was used for uncaging. The NIR-TP-induced release of acetic acid (AcOH) was examined at 720–800 nm. The uncaging efficiency of the donor– donor substituted compound was found to provide the best TP uncaging efficiency of δu = 0.25 GM (σ2 = 310 GM) at 800 nm.



Scheme 4 TP-induced energy transfer and uncaging reaction of acetic acid. FRET: fluorescence resonance energy transfer

3.5. Caged Calcium with a Bisstyrylthiophene Backbone

Calcium ions (Ca2+) have many crucial roles in the physiology and biochemistry of organisms and cells. For example, in signal transduction pathways, Ca2+ acts as a second messenger in neurotransmitter release from neurons. Therefore, caged-calcium compounds have attracted considerable attention in the field of neuroscience. Since Ca2+ cannot be covalently bound to organic chromophores, an ethylene glycol tetraacetic acid (EGTA) unit has been used as the *in vivo* Ca2+-selective chelator. Ellis-Davies and co- workers63 were the first to develop TP uncaging of Ca2+ us- ing a nitrodibenzofuran structure. In 2016, this group developed a new EGTA-substituted chromophore with TP ab- sorption character in the NIR region; specifically a dinitro derivative of bis-styrylthiophene (Scheme 5). The bis-nitro- styryl structure provided a sizable TP cross-section of 350 GM at 775 nm. Moreover, the Ca2+ chelator was found to release Ca2+ in <0.2 ms upon flash photolysis.

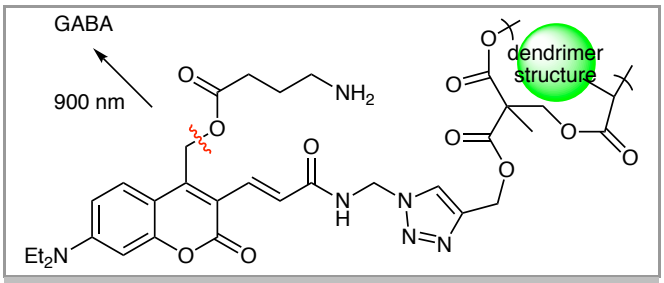


Scheme 5 Ca^{2+} uncaging reaction using a high TP-responsive chromophore

3.6. Cloaked Caged Compounds

Ellis-Davies and co-workers64 invented cloaked caged compounds (e.g., **G5-DEAC450-GABA**), in which antagonists of ionotropic γ-aminobutyric acid (GABA) were cloaked in a fifth generation of a 2,2-bis(methylol)propionic acid dendrimer

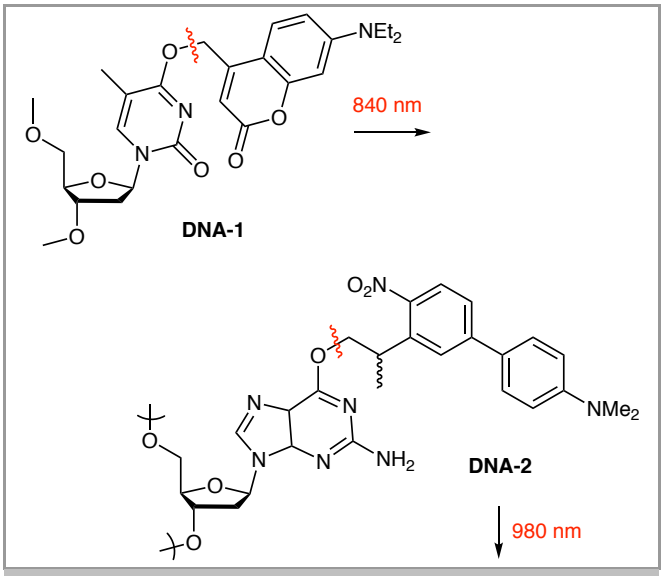
conjugated molecule (Scheme 6). Using the cloaked caged GABA compound, a physiological study of the signaling of the inhibitory neurotransmitter GABA became possible in its natural state for the first time.



Scheme 6 TP-induced (900 nm) uncaging reaction of **GABA**

3.7. Three-Dimensional Control of DNA Hybridization by Orthogonal Two-Color TP Uncaging

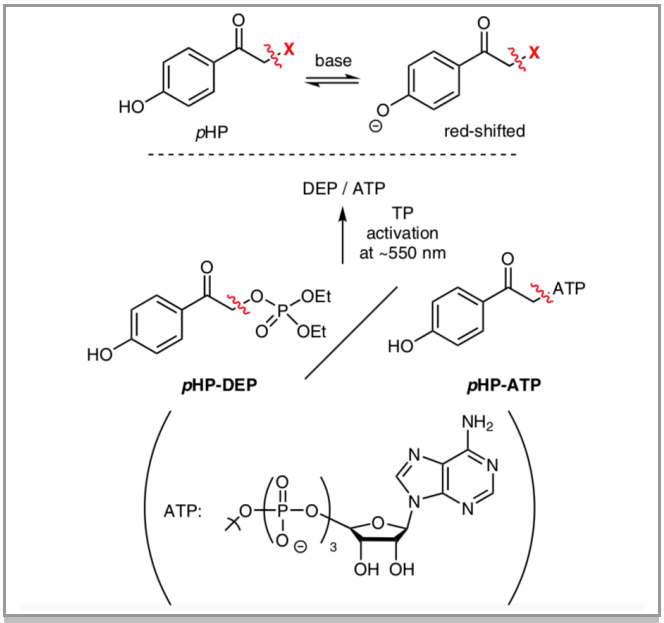
Heckel and co-workers65 reported the three-dimension- al control of DNA hybridization using TP uncaging reactions of **DNA-1** and **DNA-2** (Scheme 7). In this study, DNA strands were introduced into TP-responsive chromophores such as [7-(diethylamino)coumarin-4-yl]methyl and *p*-dialkylami- nonitrobiphenyl chromophores. These 3D uncaging reactions demonstrated that the technique is feasible for more complex scenarios. **DNA-1** can be uncaged at 840 nm, and **DNA-2** can be uncaged at 980 nm, thus, selective uncaging was found to be possible by simply changing the excitation wavelength.



Scheme 7 Wavelength-dependent release of **DNA** using TP photolysis

3.8. TP-Induced Release of Diethyl Phosphate (DEP) and ATP

The first TP activation of *p*-hydroxyphenacyl (*p*HP) PPG66a,b was reported by Houk, Givens, and Elles67 (Scheme 8). A strong TP absorption (>10 GM) was observed at ~550 nm. Monitoring the progress of the uncaging reaction of di- ethyl phosphate (DEP) and adenosine triphosphate (ATP) revealed that the TP uncaging reactions were possible using visible light in the range 500–620 nm. The authors also suggested that the TP uncaging is also possible in the range 550–720 nm under mild basic conditions, since the absorp- tion band is shifted to lower energy (3.8 eV) in both the OP and TP absorption spectra.66c



Scheme 8 TP uncaging of DEP and ATP using *p*-hydroxyphenacyl (*p*HP) PPG

4. Our Contribution to TP Uncaging Reactions

In collaboration with Professors Takayoshi Kobayashi (physicist) and Haruo Kasai (physiologist), the present authors Abe (organic chemist) and Katan (theoretical physi- cist) began investigating TP uncaging reactions in 2012 to better understand bioactive substrates in biological processes and clarify their roles. The target bioactive molecules were neurotransmitters such as glutamate (Glu), i.e., 2-ami- nopentanedioic acid.

Initially, the molecular design of TP-responsive PPGs was investigated. As noted in Section 2, the molecular size of TP-responsive chromophores should be small to enable water solubility and must also possess an appropriate TP absorption cross-section in the NIR region for utility in physiological experiments. Our target σ2 value is at least over 100 GM in the NIR region. Although the quantum yields (φu) are difficult to predict and refine, exceeding a quantum yield over 5% is achievable. The reported largest value of TP uncaging efficiency, δu = σ2 × φu, was approximately 1 GM when we began the study.

The stilbene core was selected as the platform to design a TP-responsive chromophore for physiological studies, be- cause stilbene derivatives have relatively large σ2 values de- spite their compact structures. However, stilbene derivatives are linear compounds, thus, it is possible for *cis*/*trans* isomerization to occur via their electronically excited states, which would diminish the quantum yield of the un- caging process of bioactive molecules. Therefore, we decided to use cyclic 7,8-dihydronaphthalene cores as the TP-responsive chromophores (Figure 6).68 As newcomers to this field, we initially chose the well-known *o*-nitrobenzyl (*o*- NB) PPG group on account of the strong electron-withdrawing ability of the nitro group. To

impart water solubility to the chromophore, hydrophilic substituents such as carboxylic groups and polyethylene glycol units were introduced to the core structure.

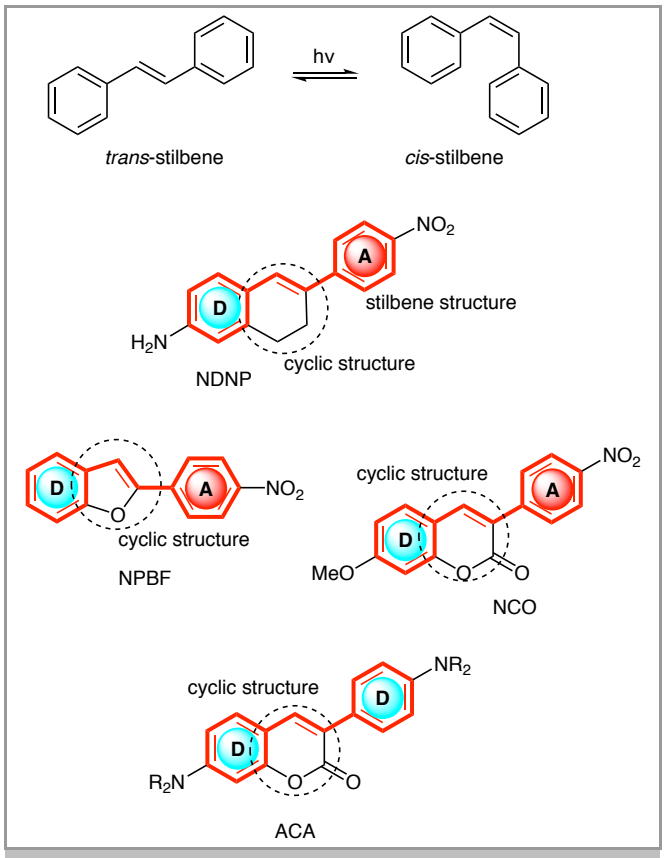
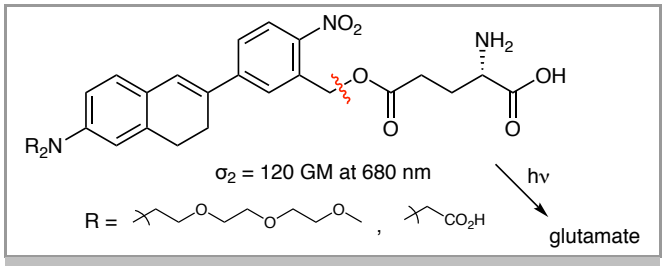


Figure 6 Our first molecular design for TP-responsive chromophores for physiological studies

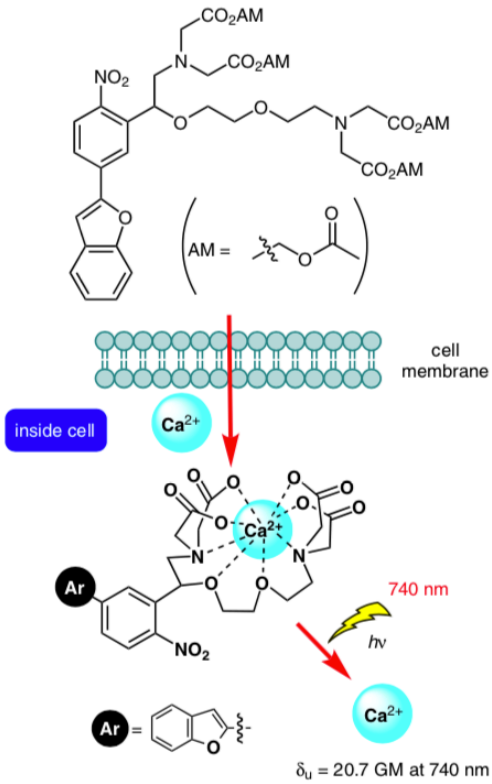


Scheme 9 TP-uncaging reaction of glutamate

The TP absorption spectrum of the newly designed TP- responsive chromophore, 6-(4-nitrophenyl)-7,8-dihydronaphthalen-2-amine, was computed at the TD-B3LYP/6-31G(d) level of theory. The TP absorption maximum was predicted to be 150 GM at ~810 nm. Therefore, the synthesis of the target molecules with a glutamate unit (Glu) began from 4-bromo β-tetralone and 3-bromobenzaldehyde (Scheme 9). Clean formation of Glu was observed using OP and TP photolysis. However, the quantum yield of the un- caging reaction was low, φu = 0.01 (1%).

Next, we designed a new TP-responsive chromophore, 2-(4-nitrophenyl)benzofuran (NPBF) (Scheme 10).69 Previously, Ellis-Davies reported that the 3-nitrodibenzofuran (NDBF) core possessed a TP uncaging efficiency (δu) of 0.6 GM with a high quantum yield of φu = 0.7.70 The computed TP absorption maximum of the NPBF chromophore was found to be 150 GM at 700 nm, while that for the NDBF structure amounted to 75 GM at 600 nm (i.e., twice smaller and blue-shifted). The TP uncaging efficiencies for the caged benzoic acid (φu = 0.09) and caged Ca2+ with EGTA structure (φu = 0.3) were determined to be 5 GM and 20.7 GM at 740 nm TP excitation, respectively. The NPBF chromophore was successfully applied *in vivo* for the caged Ca2+ ion.71

Subsequently, we investigated the donor–acceptor-substituted and donor–donor-substituted coumarin derivatives **NCO**72 and **ACA**73 as dipolar and quadrupolar systems, respectively (Scheme 11). The dipolar system **NCO** (Figure 6) was found to possess a sizable TP absorption maximum at 700 nm with 150 GM. A more than four-fold increase with a small blue-shift TP (700 GM at 650 nm) was predicted for the quadrupolar analogue **ACA**. The corresponding caged benzoic acids **NCO-BA** and **ACA-BA** were synthesized and their TP uncaging reactions were conducted with NIR irra- diation. The TP uncaging efficiency for **NCO-BA** was found to be 3.4 GM at 710 nm, and ~16 GM at 650 nm for the quadrupolar system **ACA-BA**. This shows that quadrupolar structures are promising candidates for TP uncaging.

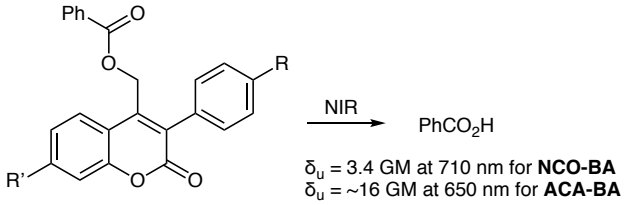


Scheme 9 TP uncaging of Ca^{2+} using NPBF chromophore

5. Summary

In this short review, recent developments of near-infrared (NIR) two-photon (TP) induced photorelease (uncaging) of bioactive molecules such as drugs have been summa- rized. The TP excitation process at 650–1050 nm is useful for physiological studies *in vivo*, since the spatiotemporal release of bioactive molecules can be achieved with high 3D control conditions in

deep areas of living tissues. The mo- lecular engineering of the next generation of chromophores suitable for the 'caging & uncaging' technique should con- tribute to unravel more precisely the mechanisms of biolog- ical activity *in vivo*.



Scheme 11 TP uncaging reactions of benzoic acid using coumarin derivatives with dipolar and quadrupolar characters

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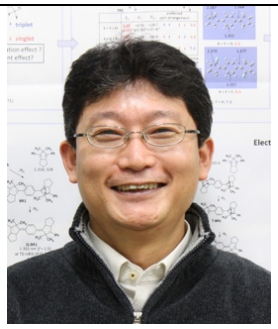
References

(1) Yuste, R. Nat. Methods, 2005, 2, 902.
(2) (a) In Fluorescence Microscopy, Cornea, A.; Conn, P. M. Eds. Elsevier, 2014. (b) Kim, H. M.; Cho, B. R. Small molecule two-photon probes for bioimaging applications. Chem. Rev. 2015, 115, 5014.
(3) In Dynamic Studies in Biology, Goeldner, M.; Givens, R. Eds., Wiley-VCH, 2005.
(4) Engels, J.; Schlager, E.-J. J. Med. Chem. 1977, 20, 907.
(5) Kaplan, H.; Forbush, B.; Hoffmann, J. F. Biochemistry, 1978, 17, 1929.
(6) Barltrop, J. A.; Schofield, P. Tetrahedron Lett. 1962, 3, 697-699.
(7) Givens, R. S.; Conrad, III, P. G.; Yousef, A. L.; Lee, J.-I., In CRC Handbook of Organic Photochemistry and Photobiology 2nd Ed., Boca Raton, CRC Press, Chap. 69.
(8) Bochet, C. G., J. C. S. Perkin Trans. 2002, 1, 125.
(9) Hoffmann, N. Chem. Rev. 2008, 108, 1052.
(10) Wang, P. Asian J. Org. Chem. 2013, 2, 452.
(11) Klán, P.; Šolomek, T.; Bochet, C. G.; Blanc, A.; Givens, R.; Rubina, M.; Popik, V.; Kostikov, A.; Wirz, J. Chem. Rev. 2013, 113, 119.
(12) Barltrop, J. A.; Plant, P. J.; Schofield, P. Chem. Comm. 1966, 822.
(13) Patchornik, A.; Amit, B.; Woodward, R. B. J. Am. Chem. Soc. 1970, 92, 6333.
(14) Il'ichev, Y. V.; Schwörer, M. A.; Wirz, J. J. Am. Chem. Soc. 2004, 126, 4581.
(15) Hasan, A.; Stengele, K.-P.; Giegrich, H.; Cornwell, P.; Isham, K. R.; Sachleben, R. A.; Pfleiderer, W.; Foote, R. S. Tetrahedron. 1997, 53, 4247.
(16) Gug, S.; Charon, S.; Specht, A.; Alarcon, K.; Ogden, D.; Zietz, B.; Leonard, J.; Haacke, S.; Bolze, F.; Nicoud, J.-F.; Goeldner, M. ChemBioChem. 2008, 9, 1303.
(17) a) Donato, L.; Mourot, A.; Davenport, C. M.; Herbivo, C.; Warther, D.; Lonard, J.; Bolze, F.; Nicoud, J.-F.; Kramer, R. H.; Goeldner, M.; Specht, A. Angew. Chem. Int. Ed. 2012, 51, 1840.
(18) Specht, A.; Bolze, F.; Donato, L.; Herbivo, C.; Charon, S.; Warther, D.; Gug, S.; Jean-FranÅois, N.; Goeldner, M. Photochem. Photobiol. Sci. 2012, 11, 578.
(19) Amit, B.; Patchornik, A. Tetrahedron Lett. 1973, 14, 2205.
(20) Amit, B.; Ben-Efraim, D. A.; Patchornik, A. J. Am. Chem. Soc.1976, 98, 843.
(21) (a) Morrison, J.; Wan, P.; Corrie, J. E. T.; Papageorgiou, G. Photochem. Photobiol. Sci. 2002, 1, 960. (b) Cohen, A. D.; Helgen, C.; Bochet, C. G.; Toscano, J. P. Org. Lett. 2005, 7, 2845. (c) Corrie, J. E. T.; Barth, A.; Papageorgiou, G. J. Labelled Compd. Radiopharm. 2001, 44, 619.
(22) Pass, S.; Amit, B.; Patchornik, A. J. Am. Chem. Soc. 1981, 103, 7674.
(23) Papageorgiou, G.; Ogden, D. C.; Barth, A.; Corrie, J. E. J. Am. Chem. Soc. 1999, 121, 6503.
(24) Matsuzaki, M.; Hayama, T.; Kasai, H.; Ellis-Davies, G. C. R. Nat. Chem. Biol. 2010, 6, 255
(25) Givens, R. S.; Matuszewski, B. J. Am. Chem. Soc. 1984, 106, 6860.
(26) Kamatham, N.; Mendes, D. C.; Da Silva, J. P.; Givens, R. S.; Ramamurthy, V. Org. Lett. 2016, 18, 5480.
(27) Fedoryak, O. D.; Dore, T. M. Org. Lett. 2002, 4, 3419.
(28) Zhu, Y.; Pavlos, C. M.; Toscano, J. P.; Dore, T. M. J. Am. Chem. Soc. 2006, 128, 4267.
(29) Davis, M. J.; Kragor, C. H.; Reddie, K. G.; Wilson, H. C.; Zhu, Y.; Dore, T. M. J. Org. Chem. 2009, 74, 1721.
(30) Li, Y. M.; Shi, J.; Cai, R.; Chen, X. Y.; Guo, Q. X.; Liu, L. Tetrahedron Lett. 2010, 51, 1609.
(31) Kitani, S.; Sugawara, K.; Tsutsumi, K.; Morimoto, T.; Kakiuchi, K. Chem. Commun. 2008, 18, 2103.
(32) (a) Goswami, P. P.; Syed, A.; Beck, C. L.; Albright, T. R.; Mahoney, K. M.; Unash, R.; Smith, E. A.; Winter, A. H. J. Am. Chem. Soc. 2015, 137, 3783-3786. (b) Palao, E.; Slanina, T.; Muchová, L.; Šolomek, T.; Vítek, L.; Klán, P. J. Am. Chem. Soc. 2016, 138, 126-133.
(33) Denk, W.; Stricker, J. H.; Webb, W. W. Science, 1990, 248, 73.
(34) Denk, W.; Proc. Natl. Acad. Sci. USA 1994, 91, 6629.
(35) Lipp, P.; Niggli, J. Physiol. 1998, 508, 801.
(36) Furuta, T.; Wang, S. S.-H.; Dantzker, J. L.; Dore, T. M.; Bybee, W. J.; Callaway, E. M.; Denk, W.; Tsien, R. Y. Proc. Natl. Acad. Sci. U.S.A. 1999, 96, 1193-1200.
(37) Matsuzaki, M.; Ellis-Davies, G. C. R.; Nemoto, T.; Miyashita, Y.; Iino, M.; Kasai, H. Nat. Neurosci. 2001, 4, 1086.
(38) Göppert-Mayer, M. Ann. Phys. 1931, 401, 273–294.
(39) Boyd, W. In Non-Linear Optics, 2nd ed., Elsevier, London, 2003.
(40) Maruo, S.; Nakamura, O.; Kawata, S. Opt. Lett. 1997, 22, 132.
(41) Kawata, S.; Sun, H.-B, Tanaka, T.; Takada, K. Nature 2001, 412, 697.
(42) Mayer, G.; Heckel, A. Angew. Chem. Int. Ed. 2006, 45, 4900-4921.
(43) Specht, A.; Bolze, F.; Omran, Z.; Nicoud, J.-F.; Goeldner, M. Hfsp J. 2009, 3, 255–264.
(44) Pawlicki, M.; Collins, H. A.; Denning, R. G.; Anderson, H. L. Angew. Chem. Int. Ed. 2009, 48, 3244-3266.
(45) Ellis-Davies, G. C. R. Two-Photon microscopy for chemical neuroscience. ACS Chem. Neurosci. 2011, 2, 185–197.
(46) Zhao, Y. Macromolecules, 2012, 45, 3647-3657.
(47) Brieke, C.; Rohrbach, F.; Gottschalk, A.; Mayer, G.; Heckel, A. Angew. Chem. Int. Ed. 2012, 51, 8446–8476.
(48) Bort, G.; Gallavardin, T.; Ogden, D.; Dalko, P. I. Angew. Chem. Int. Ed. 2013, 52, 4526–4537.
(49) Piant, S.; Bolze, F.; Specht, A. Opt. Mat. Exp. 2016, 1679-1691.
(50) Terenziani, F.; Katan, C.; Badaeva, E.; Tretiak, S.; Blanchard-Desce, M. Adv. Mater. 2008, 20, 4641-4678.
(51) Tretiak, S. ; Chernyak, V. J. Chem. Phys. 2003, 119, 8809.
(52) Xue, B.; Katan, C.; Bjorgaard, J. A.; Kobayashi, T. AIP Adv., 2015, 5, 12.
(53) Weisz, S. Z.; Zahlan, A. B.; Gilreath, J.; Jarnagin, R. C.; Silver, M. J. Chem. Phys. 1964, 41, 3491-3495.
(54) Anderson, R. J. M.; Holtom, G. R.; McClain, W. M. J. Chem. Phys. 1979, 70, 4310–4315.
(55) Katan, C.; Terenziani, F.; Le Droumaguet, C.; Mongin, O.; Werts, M. H. V., Tretiak, S.; Blanchard-Desce, M. Branching of Dipolar Chromophores: Effects on Linear and Nonlinear Optical Properties, Proc. SPIE 5935, Linear and Nonlinear Optics of Organic Materials V, 593503 (September 02, 2005); doi:10.1117/12.618464.
(56) Albota, M.; Beljonne, D.; Brédas, J. L.; Ehrlich, J. E.; Fu, J. Y.; Heikal, A. A.; Hess, S. E.; Kogej, T.; Levin, M. D.; Marder, S. R.; Maughon, D.

M. C.; Perry, J. W.; Röckel, H.; Rumi, M.; Subramaniam, G.; Webb, W. W.; Wu, X. L.; Xu, C. Science, 1998, 281, 1653-1656.
(57) Mongin, O.; Porrs, L.; Charlot, M.; Katan, C.; Blanchard-Desce, M. Chem. Eur. J. 2007, 13, 1481 – 1498.
(58) Katan, C.; Charlot, M.; Mongin, O.; Le Droumaguet, C.; Jouikov, V.; Terenziani, F.; Badaeva, E.; Tretiak, S.; Blanchard-Desce, M. J. Phys. Chem. B 2010, 114, 3152
(59) Hayashi, K.-I.; Kusaka, N.; Yamasaki, S.; Zhao, Y.; Nozaki, H. Bioorg. Med. Chem. Lett. 2015, 25, 4464-4471.
(60) Korzycka, K. A.; Bennett, P. M.; Cueto-Diaz, E. J.; Wicks, G.; Drobizhev, M.; Blanchard-Desce, M.; Rebane, A. Anderson, H. L. Chem. Sci. 2015, 6, 2419-2426.
(61) Tran, C.; Gallavardin, T.; Petit, M.; Slimi, R.; Dhimane, H.; Blanchard-Desce, M.; Acher, F. C.; Ogden, D.; Dalko, P. I. Org. Lett. 2015, 17, 402-405.
(62) Cueto Diaz, E. J.; Picard, S.; Chevasson, V.; Daniel, J.; Hugues, V.; Mongin, O.; Genin, E.; Blanchard-Dedcs, M. Org. Lett, 2015, 17, 102-105.
(63) Agarwal, H. K.; Janicek, R.; Chi, S.-H.; Perry, J. W.; Niggli, E.; Ellis-Davies, G. C. R. J. Am. Chem. Soc. 2016, 138, 3687-3693.
(64) Richers, M. T.; Amatrudo, J. M.; Olson, J. P.; Ellis-Davies, G. C. R. Angew. Chem. Int. Ed. 2017, 56, 193-197.
(65) Fichte, M. A. H.; Weyel, X. M. M.; Junek, S.; Schäfer, F.; Herbivo, C.; Coeldner, M.; Specht, A.; Wachtveitl, J.; Heckel, A. Angew. Chem. Int. Ed. 2016, 55, 8948-8952.
(66) (a) Givens, R. S.; Park, C. H. Tetrahedron Lett. 1996, 37, 6259. (b) Park, C. H.; Givens, R. S. J. Am. Chem. Soc. 1997, 119, 2453. (c) Klicova, L.; Sebej, P.; Solomek, T.; Hellrung, B.; Slavicek, P.; Klan, P.; Heger, D.; Wirz, J. J. Phys. Chem. A 2012, 116, 2935.
(67) Houk, A.; Givens, R. S.; Elles, C. G. J. Phys. Chem. B 2016, 120, 3178.
(68) Boinapally, S.; Huang, B.; Abe, M.; Katan, C.; Noguchi, J.; Watanabe, S.; Kasai, H.; Xue, B.; Kobayashi. T. J. Org. Chem. 2014, 79, 7822-7830.
(69) Komori, N.; Jakkampudi, S.; Motoishi, R.; Abe, M.; Kamada, K.; Furukawa, K.; Katan, C.; Sawada, W.; Takahashi, N.; Kasai, H.; Xue, B.; Kobayashi, K. Chem Commun 2016, 52, 331–334.
(70) Momotake, A.; Lindegger, N,; Niggli, E.; Barsotti R. J.; Ellis-Davies, G. C. R. Nat. Methods, 2006, 3, 35-40.
(71) Jakkampudi, S.; Abe, M.; Komori, N.; Takagi, R.; Furukawa, K.; Katan, C.; Sawada, W.; Takahashi, N.; Kasai, H. ACS Omega, 2016, 1, 193-201.
(72) Chitose, Y.; Abe, M.; Furukawa, K.; Katan, C. Chem. Lett. 2016, 45, 1186–1188.
(73) Chitose, Y.; Abe, M.; Furukawa, K.; Lin, J.-Y.; Lin, T.-C.; Katan, C. Org. Lett. 2017, 19, 2622.



Manabu Abe was born in Sakai City, Osaka Prefecture, Japan. He received his Ph.D. from the Kyoto Institute of Technology (KIT) in 1995, under the guidance of Professor Akira Oku, studying the oxidative ring- opening reaction of cyclopropanone acetals and its application to organic synthesis. In 1995, he became a member of the faculty staff at Osaka University (Professor Masatomo Nojima's group). From 1997– 1998, he was an Alexander von Humboldt (AvH) Fellow with Professor Dr. Waldemar Adam at Universität Würzburg in Germany. He was also a visiting researcher at the LMU München (Professor Dr. Herbert Mayr) in 2007. He moved to Hiroshima and became a Full Professor in Organic Chemistry at the Department of Chemistry, Graduate School of Sci- ence, Hiroshima University in 2007. His research focuses on reactive intermediates chemistry, especially on diradicals, organic photochemistry, and unusual molecules.

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