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ARTICLE

Visible Light Initiated Release of Calcium Ions through Photochemical Electron Transfer Reactions.

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Photolysis of anthraquinone or flavin photosensitizers in the presence of calcium EDTA complexes results in decomposition of the EDTA complex, releasing free Ca^{2+}. In the case of the flavin sensitizers, it is shown that millimolar concentrations of Ca^{2+} can be released using visible light (>440 nm) and with quantum yields as high as 0.31. The utility of this system is further demonstrated by in situ photogelation of an alginate solution.

1. Introduction

Calcium ions play key roles in numerous processes having physiological, environmental, and technological significance. For example, Ca^{2+} controls several cellular functions including muscle contraction, the secretion of neurotransmitters and hormones, wound healing and even cell death.[1-2] Besides their importance in biology, calcium ions have also seen increasing utilization in the engineering of responsive biopolymers such as alginate gels. These calcium sensitive gels have applications ranging from microfluidic devices, encapsulation of cells to drug delivery.[3-8] For these reasons, there has been significant interest in developing new methods for the precise delivery of Ca^{2+} in various contexts.[1-10]

One approach to controlling the release of calcium, with several key advantages, is through the use of a photochemical light trigger. Photochemical control of a reaction allows for the generation of spatially and temporally defined concentrations of ions without direct contact to the system. These characteristics in turn enable calcium ions to be used in photo-patterning applications as well as in physiological studies.[3-8, 10] To this end there has been a considerable amount of research into the synthesis and study of "caged calcium" compounds.[2,6-9] Caged calcium compounds refer to molecules where Ca^{2+} is sequestered by a chelating group whose affinity can be lowered through a photochemical mechanism.[5] Key examples of synthesized calcium cages include nitr-5 by the Tsien group and DM-nitrophen developed by Kaplan and Ellis-Davis whose calcium binding diminishes with the addition of light.[6-7,8] More recently, Agarwal et al. have demonstrated calcium release with visible light using a nitroaromatic photoremovable protecting group.[9]

While much progress has recently been made in light triggered release of calcium there are still areas that could be further improved.[2, 11] For example, it would be desirable to continue to expand the extent these systems respond to visible wavelengths. Likewise, the ability to release Ca^{2+} with inexpensive and biocompatible reagents could increase the use of this technology by having a more attainable cost range.

The current study is based on previous success in releasing carboxylate ions through photoinduced electron transfer.[12] In these reactions light is absorbed by the redox active chromophore which then causes an excited-state oxidation or reduction. After the electron is transferred the desired event, such as removal of a protecting group or the release of the desired species, is achieved. Below are described experiments that demonstrate an extension of this concept to the release of Ca^{2+}.

Specifically, it is shown that photolysis of oxidizing photosensitizers, such as anthraquinone and flavin derivatives, can trigger the degradation of EDTA bound to calcium ions. This can rapidly generate millimolar concentrations of free Ca^{2+} and, in the case of riboflavin, can be used to photochemically generate alginate hydrogels.

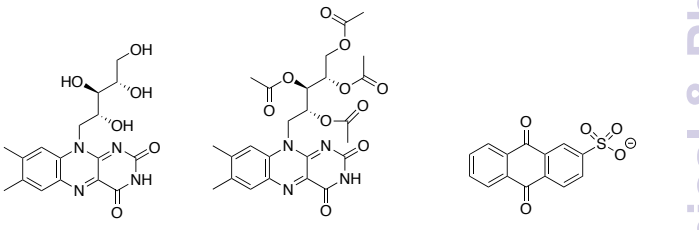

Fig. 1 Sensitizers used in this study.

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Table 1. Key photophysical properties of sensitizers.

Sensitizer	E_{red} (V)	λ_{max} (nm)	$\tau_{singlet}$ (ns)	$\tau_{triplet}$ (μs)	ε ($M^{-1}s^{-1}$)
2-AQDS	-0.620[a, b]	330[g]	-	15[h]	5200[g]
RBTA	-1.18[c,d]	445[e]	23.8[d,j]	-	8600[e]
RB	-0.507[c,d]	450[f]	2.3[i,k]	19[i]	12200[f]

[a]Potential measured vs. SCE. [b]Reference 13. [c]Potential measured vs. Fc/Fc^+. [d]Reference 14. [e]Measured in this work. [f]Reference 15. [g]Reference 16. [h]Reference 17. [i]Reference 18. [j]Measured in acetonitrile. [k]Measured in water (pH 2.2).

2. Materials and Methods

2.1 Materials

Riboflavin, anthraquinone-2-sulfonic acid sodium salt, and Sodium alginate from brown algae were purchased from Sigma Aldrich which were then used without further purification. Synthesis of 2',3',4',5'-Tetraacetylriboflavin was based on a previous procedure.[19] ^{1}H NMR signals matched previous reports.

2.2 Photolysis of Samples

Samples were photolyzed at 350 nm using a Rayonet Photoreactor from the Southern New England Ultraviolet Company. Polychromatic wavelength photolysis at other wavelengths were done on a 150 W Hg-Xe medium pressure lamp, using cut off filters as indicated in Table 2. The solutions were 1 mL in volume with a concentration of Ca-EDTA that ranged from 2.5 mM to 25 mM in deionized water (pH 6.5-7.3). Anthraquinone sensitizers were added from 2.5 to 5 mM in the photolysis solutions. Riboflavin concentrations were kept below 50 μM to avoid self-association. Visible-light alginate gels were prepared by photolysis with a 1 W, 447 nm CW diode laser.

2.3 Calcium Assay

A modified spectrophotometric dye method using o-Cresolphthalein Complexone (CPC) was used in our study to determine the amounts of calcium released.[20] The working dye solution was made by adding 1 mL concentrated HCl to 40 mg of CPC. Then the dissolved CPC is transferred into a 1 L volumetric flask by washing with deionized water. To the CPC 100 mL of dimethyl sulfoxide and 2.5 g of 8-hydroxyquinoline were added. The solution was mixed until dissolved and then diluted to volume with deionized water. The DEA buffer was made by adding 40 mL of diethylamine into a 1 L volumetric flasks. Then 0.5 g of potassium cyanide is added and dissolved with deionized water until the 1 L volumetric flask mark.

The assay involved adding 1 mL of CPC working solution, then 1 mL of diethylamine buffer solution (DEA), and 1 mL of water to make calcium testing solution. Once the solution was mixed by inversion a UV-Vis at 575 nm was taken to serve as a blank. To the calcium testing solution a 20 μL amount of test sample was added, mixed by inversion and then UV-Vis measured at 575 nm to serve as experimental. The difference in absorbance at 575 nm was then compared to a calibration curve to determine molar concentration.

2.4 Quantum Yield

Quantum yields were determined as follows. Solutions containing 25 mM Ca-EDTA and 9.0 ± 0.5 uM Riboflavin were irradiated using the output from a 1000 W Hg-Xe lamp that was passed through a monochromator set to 366 ± 10 nm. Calcium concentrations at various times were determined using the CPC assay at the intensity (I_0) above. Lamp output was calibrated using ferrioxalate actinometry as described by Hatchard and Parker.[21] In order to ensure that R_{ex} did not vary significantly throughout the sample, photolysis was carried out under optically thin conditions wherein the Abs_{366} was held at 0.1. The apparent quantum yield was determined using equations 1 and 2.

$$\phi = \frac{R_{Ca^{2+}}}{R_{EX}} = \frac{(Rate\,calcium\,release)}{(Rate\,of\,excitation)} \qquad (1)$$

$$R_{EX} = I_0(1 - 10^{-A}) \qquad (2)$$

2.5 Instrumentation

All fluorescence experiments were performed using a Hitachi F-4500 fluorescence spectrophotometer. All UV-Vis spectra were taken on a Shimadzu UV-1800.

2.6 Laser Flash Photolysis

Laser flash photolysis experiments were performed using a Nd:YAG laser pump beam source with a Continuum Surelite II-10 capable of 266, 532, or 355 nm pulses between 5-7 ns. A LeCroy 350MHz digital oscilloscope was used to observe all waveforms. The samples were prepared so that their relative absorbance was between 0.75 and 1.5 at the excitation wavelength, 355 nm. The probe beam was a 350 W Xenon arc lamp. The samples were loaded into a 1 cm quartz cuvette and were purged with pure N_2 for 10 minutes in the solution and an additional 5 minutes in the headspace. Samples were flowed continuously thorough out the experiment to avoid build-up of photoproducts.

3. Results and Discussion

Several previous studies have established that flavins and other excited-state electron acceptors can photo-oxidize EDTA leading to the decomposition of the latter via a sequence of oxidation and decarboxylation events.[22] We hypothesized that photolysis of similar sensitizers in the presence of Ca-EDTA complexes would initiate similar chemistry and resulting destruction of the EDTA ligand that would, in turn, release the chelated ions. Figure 1 shows the sensitizers employed in this study along with some key photophysical parameters found in Table 1. Included in our studies are a water soluble anthraquinone derivative and two riboflavin derivatives.

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Table 2. Photolytic release of Ca^{2+}

Sensitizer	[Sensitizer] (mM)	[Ca-EDTA] (mM)	λ (nm)	Time (min)	Released Ca^{2+} (mM)
2-AQDS	2.5	2.5	350[a]	60	2.1
2-AQDS	2.5	3.5	350[a]	60	2.4
RBTA	0.02	25	350[a]	60	3.9
RBTA	0.02	25	broadband[b]	60	3.7
RBTA	0.02	25	broadband[b]	120	4.9
RB	0.035	25	> 370[c]	60	4.3
RB	0.035	25	> 418[c]	60	4.0
RB	0.035	25	> 440[c]	60	2.1

[a]Phototlyzed using a 35 W, 12-bulb Rayonet photoreactor
[b]Unfiltered light from a 150 W Hg-Xe lamp
[c]Light from 150 W Hg-Xe lamp filtered with 370, 418 and 440 nm high pass filters

Photorelease experiments were carried out using the sensitizers listed in Figure 1. These were added to aqueous solutions containing 2.5-25 mM Ca-EDTA complex (pH 6.5-7.3). The air-equilibrated solutions were exposed to light at 350 nm in a Rayonet photoreactor, or to filtered (>390 nm, >420 nm, >450 nm) light from a Hg-Xe arc lamp. The yield of free Ca^{2+} was determined spectrophotometrically by adding aliquots from the photolyzed sample to an analysis solution containing the spectrophotometric dye, o-cresolphthalein complexone.

Concentrations of Ca^{2+} were evaluated using the absorbance at 575 nm compared with a calibration curve. In several cases, the formation of Ca^{2+} was further verified using an alternative indicator, eriochrome black T or a Ca^{2+} selective electrode.

Figure 2 illustrates typical time-dependence of Ca^{2+} photorelease and Table 1 lists yields of free Ca^{2+} along with amount and identity of sensitizer, photolysis wavelengths and times. In the case of the AQ2S, the yield of free Ca^{2+} was limited to approximately 1 equivalent relative to sensitizer added.

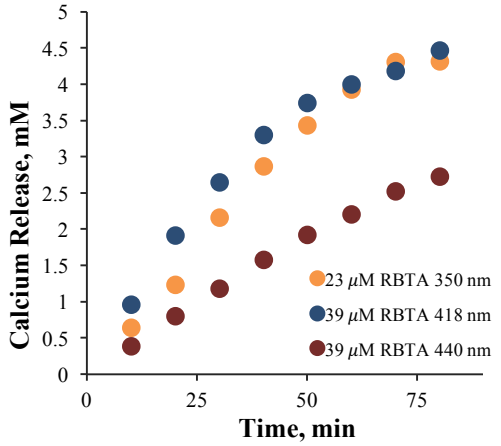

Fig. 2 Photorelease of calcium from EDTA with RBTA using various light sources. Initial [Ca-EDTA] = 25 mM in water (pH 6.5-7.3).

By contrast, when the flavin derivatives, RBTA and RB, were employed ca. 1000 fold free Ca^{2+} was released relative to the sensitizer added. This suggests that either the reduced flavin can recycle back to its original form and/or any by-products of the sensitizer retain photooxidative ability. The experiment illustrated by the UV-Vis absorption spectra in Figure 3 implies that it is largely the former. In this case riboflavin (40 μM) was photolyzed in the presence of Ca-EDTA (12.5 mM). Photolysis of the latter with unfiltered Xe-Hg lamp light for 5 minutes resulted in a diminishment of the flavin absorption band at 445 nm, consistent with the known photoreduction to the corresponding dihydroflavin derivative. When the resulting solution was allowed to stand under conditions of air equilibration for 10 minutes, the flavin absorption recovered to 98.4± 0.5% of its original value. Similar experiments with RBTA produced a 100.1 ± 0.5% recovery of the original absorbance. On this basis, we conclude that air oxidation of the dihyrdoflavin to flavin allows these sensitizers to be recycled and used in substoichiometric amounts.

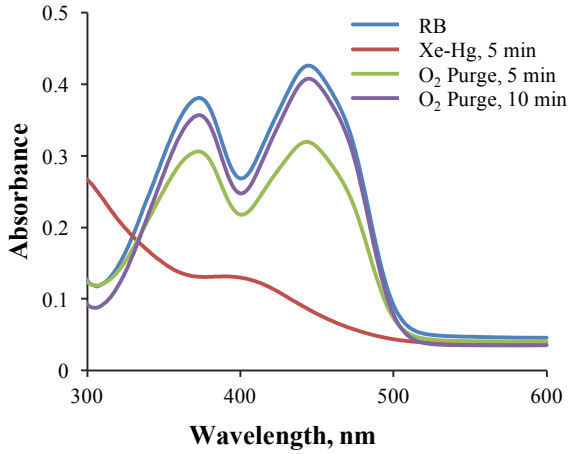

Fig. 3. Change in absorbance of sample containing [Ca-EDTA]= 12.5mM, [RB]= 40 μM. Spectra were taken before photolysis, after exposure to an unfiltered Xe-Hg lamp, and then after purging the solution with oxygen for 5 to 10 minutes.

In addition to chemical yields, the utility of a photorelease system also depends on the quantum yield of the reaction. Quantum yields were determined using ferrioxalate actinometry. For example, in the case of 20 mM Ca-EDTA with RBTA (9 μM), the quantum yield was determined to be 0.31 ± 0.03. In the current experiment the key photochemical step is a bimolecular electron transfer reaction from Ca-EDTA to excited sensitizer, and as such any observed quantum yield will be a product of quenching efficiency(ϕ_q) and the efficiency of the oxidative electron transfer reaction (ϕ_r)as described in equation 3. Thus the quantum yield determined under these conditions is a lower limit of what might be achieved under higher Ca-EDTA concentrations of if the sensitizer and the chelator were linked to one another.

$$\Phi = \phi_r \phi_q = \phi_r \frac{k_q[CaEDTA]}{k_q[CaEDTA] + k_s} \qquad (3)$$

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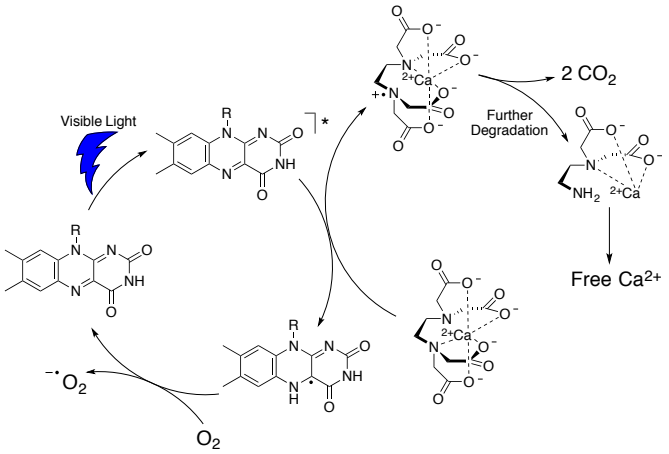

Fig. 4 Proposed mechanism for visible light release of free calcium from EDTA. After absorption of a photon, singlet Flavin abstracts an electron from EDTA which subsequently degrades resulting in the loss of its ability to chelate calcium releasing free Ca^{2+}. The reduced riboflavin can return to its original form after reaction with oxygen, thus completing the cycle and allowing the reaction to be done with substoichiometric amounts of sensitizer.

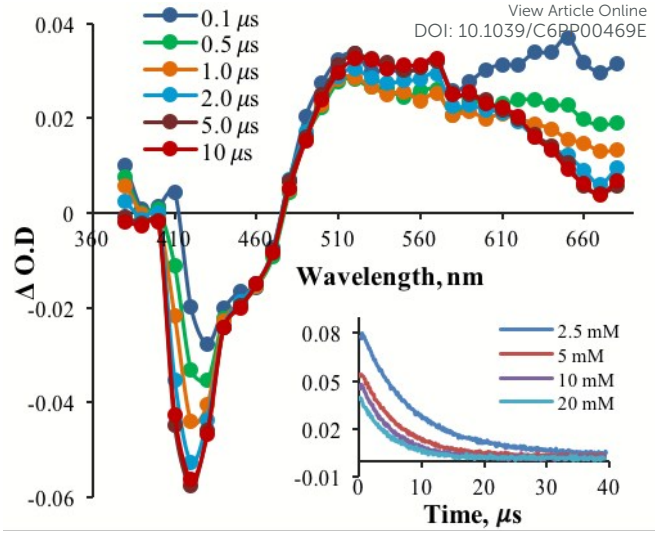

Fig. 5 Transient absorption spectrum of RB (123 μM) with 2.5 mM Ca-EDTA in phosphate buffered water (pH = 7.4). Waveforms at 660 nm with increasing [Ca-EDTA] are shown in the inset.

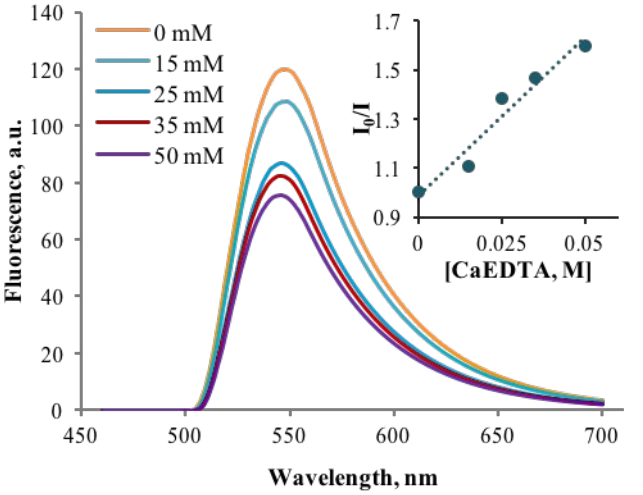

Fig. 6 Fluorescence quenching (λ_{ex} = 320 nm) of RB with varying concentrations of Ca-EDTA in water (pH = 6.8). Fluorescence quenching (inset) measured at 547 nm.

The proposed mechanism is further supported by fluorescence quenching and laser flash photolysis (LFP) experiments. As illustrated in Figure 5, pulsed laser photolysis (355 nm, 5-7 ns, 5-10 mJ) of RB with Ca-EDTA in aqueous solution produces the transient UV-Vis spectrum that includes a broad absorption near 660 nm, a sharp long-lived maximum near 510 nm and a negative bleaching signal at 420 nm. These signals have been reported previously.[23] The negative signal is caused by depletion of ground-state RB, the short-lived absorbance at ca. 660 nm is attributed to the triplet state of RB, and the long-lived 510 nm peak is the flavin radical derived from oxidation of EDTA. The result of adding Ca-EDTA is illustrated in the inset which shows the response of the signal at 660 nm to increasing concentrations of Ca-EDTA. Here, we find the Ca-EDTA decreases the initial triplet intensity, but has negligible effect on the decay rate of the triplet. On this basis we concluded the Ca-EDTA reacts with the excited singlet state of RB, but not the triplet. This conclusion is further supported by fluorescence quenching experiments shown in Figure 6, which, combined with previously reported lifetime for the excited state (RB*) given in Table 1, provided a k_q value of $5.7 \times 10^9\ M^{-1}s^{-1}$.

The proposed mechanism for Ca^{2+} photorelease is consistent with literature precedents for EDTA electron transfer photochemistry as well as the observations described herein. The excited state sensitizer (singlet state in the case of the flavin derivatives and triplet in the case of AQ2S) abstracts one electron from the Ca-EDTA complex. The resulting aminium radical decarboxylates providing an alpha-amino radical, which we expect would be further oxidized by ground state sensitizer, providing an iminium ion. The decarboxylation of aminium radicals has been explored extensively.[24] Under the aqueous conditions the iminium ion would hydrolyze, providing ED3A and formaldehyde. The former would suffer similar oxidation hydrolysis reaction, forming mixtures of EDDA isomers and, ultimately ethylene diamine. Analysis of photolysis solutions using ^{1}H NMR spectroscopy on photolysis solutions showed a complex mixture of signals characteristic of degradation of EDTA of formate (from disproportionation of formaldehyde) as a peak at 8.4 ppm which is consistent with previous reports on the photooxidative degradation of EDTA (see SI).[22]

To further verify that the current system was capable of delivering a useful concentration of free Ca^{2+} in situ we examined its application to the photogelation of alginate. Photolysis of aqueous (pH = 9.3 in buffered water) solutions containing Ca-EDTA, riboflavin and alginate resulted the formation of a freestanding hydrogel as illustrated in Figure 7.

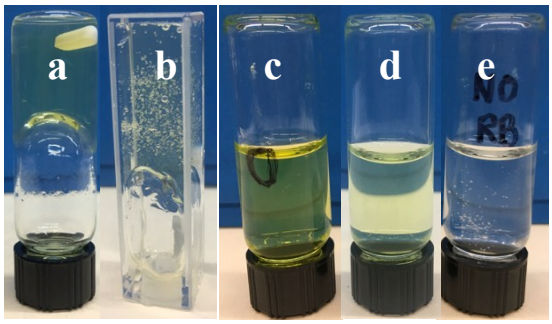

Fig. 7 Photoinduced gelation of alginate. ***a***: Photolysis at 350 nm in a Rayonet photoreactor for 2 hours. ***b***: Photolysis at 447 nm using 1 W CW diode laser for two hours. ***c***: Dark control. ***d***: Omitted Ca-EDTA. Photolysis at 350 nm in a Rayonet photoreactor for two hours. ***e***: Omitted riboflavin. Photolysis at 350 nm in a Rayonet photoreactor for 2 hours. For full experimental see SI.

Photogelation can be effected with both 350 nm broadband light, or 447 nm light from a laser diode. Control experiments wherein either light, Ca-EDTA, or riboflavin were omitted showed no gelation. Future efforts we be aimed at detailed characterization of the resulting hydrogels, and specific approaches in photopatterning and microfluidics.

Conclusions

Experiments described herein demonstrate that visible light triggered photorelease of calcium ions in up to millimolar concentrations can be accomplished using inexpensive and biocompatible reagents. Results with anthraquinone derivatives show that the release of calcium ions through photodecomposition of a chelating agent can be accomplished with exited state oxidants. Using riboflavin, it is possible to carry out release of calcium in a photocatalytic manner using substoichiometric amounts of the light absorbing component. Under steady-state illumination conditions, the overall rate of Ca^{2+} production depends on the intensity of the light source, the molar absorptivity of the sensitizer (ε) and the quantum yield for release (ϕ). The product of the latter two terms, the photochemical efficiency ($\varepsilon \times \phi$), can be used to characterize the effective release rate at a given light intensity. The value for the current system of $\varepsilon \times \phi = 2700\ M^{-1}cm^{-1}$ demonstrates that RBTA/Ca-EDTA can produce calcium ions at a rates comparable to previously characterized systems.[2] Under pulsed illumination conditions, the relevant consideration would be the rate-limiting step in the formation of free Ca^{2+} following light absorption. Additional mechanistic studies will be aimed at determining that rate constant. Finally, it has been shown that this photochemically generated free calcium can be used to trigger gelation in alginate solutions. Future studies will (a) explore extension of this method to other chelating agents and metals (b) examine additional applications of the current system, and (c) develop linked sensitizer-chelator systems that can be used in biological systems.

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Notes and references

1 (a)Berridge, M. J., The AM and RM of calcium signalling. *Nature,* **1997**, *386*, 759. (b)Berridge, M. J.; Lipp, P.; Bootman, M. D., The versatility and unversality of calcium signalling. *Nat. Rev. Mol. Cell Biol.*, **2000**, *1*, 11.

2 Ellis-Davies, G. C. R., Neurobiology with caged calcium. *Chem. Rev.*, **2008**, *108*, 1603.

3 (a) Jakkampudi, S.; Abe, M.; Komori, N.; Takagi, R.; Furukawa, K.; Katan, C.; Sawada, W.; Takahashi, N.; Kasai, H., Design and synthesis of a 4-nitrobromobenzene derivative bearing an ethylene glycol tetraacetic acid unit for a new generation of caged calcium compounds with two-photon absorption properties in the near-IR region and their application in vivo. *ACS Omega*, **2016**, *1*, 193. (b) Cui, J.; Gropeanu, R. A.; Stevens, D. R.; Rettig, J.; Del Campo, A., New photolabile BAPTA-based Ca^{2+} cages with improved photorelease. *J. Am. Chem. Soc.*, **2012**, *134*, 7733.

4 (a) Chueh, B. H.; Zheng, Y.; Torisawa, Y. S.; Hsiao, A. Y.; Ge, C.; Hsiong, S.; Huebsch, N.; Franceschi, R.; Mooney, D. J.; Takayama, S., Patterning alginate hydrogels using light-directed release of caged calcium in a microfluidic device. *Biomed. Microdevices*, **2010**, *12*, 145-51. (b) Asthana, A.; Ho Lee, K.; Kim, K. O.; Kim, D. M.; Kim, D. P., Rapid and cost-effective fabrication of selectively permable calcium-alginate microfluidic device using "modified" embedded template method. *Biomicrofluidics,* **2012**, *6*, 012821. (c) Oh, H.; Lu, A. X.; Javvaji, V.; DeVoe, D. L.; Raghavan, S. R., Smart Hydrogel-based valves inspired by the Stomata in plants. *ACS Appl. Mater. Interfaces*, **2016**, *8*, 17529.

5 Ellis-Davies, G. C., Caged compounds: photorelease technology for control of cellular chemistry and physiology. *Nat. Methods*, **2007**, *4*, 619-28.

6 Adams, S. R.; Kao, J. P. Y.; Grynkiewicz, G.; Minta, A.; Tsien, R. Y., Biologically useful chelators that release Ca^{2+} upon illumination. *J. Am. Chem. Soc.*, **1988**, *110*, 3212.

7 Adams, S. R.; LevRam, V.; Tsien, R. Y., A new caged Ca^{2+}, azid-1, is far more photosensitive than nitrobenzyl-based chelators. *Chem. Biol.*, **1997**, *4*, 867.

8 Kaplan, J. H.; Ellisdavies, G. C. R., Photoliable chelators for the rapid release of divalent-cations. *PNAS*, **1988**, *85* (17), 6571-6575.

9 Agarwal, H. K.; Janicek, R.; Chi, S.; Perry, J. W.; Niggli, E.; Ellis-Davies, G. C. R., Calcium uncaging with visible light. *J. Am. Chem. Soc.*, **2016**, *138*, 3687.

10 (a) Javvaji, V.; Baradwaj, A. G.; Payne, G. F.; Raghavan, S. R., Light-activated ionic gelation of common biopolymers. *Langmuir*, **2011**, *27*, 12591. (b) Lee, K. Y.; Mooney, D. J., Alginate, Properties and biomedical applications. *Prog. Polym. Sci.*, **2012**, *37*, 106. (c) Higham, A. K.; Bonino, C. A.; Raghavan, S. R., Khan, S. A., Photo-activated ionic gelation of alginate hydrogel: real-time rheological monitroing of the two-step crosslinking mechanism. *Soft Matter*, **2014**, *10*, 4990.

11 Bort, G.; Gallavardin, T.; Ogden, D.; Dalko, P. I., From one-photon to two-photon probes: "Caged" compounds, actuators, and photoswitches. *Angew. Chem Int. Ed.*, **2013**, *52*, 4526.

12 Borak, J. B.; Falvey, D. E., Ketocoumarin dyes as electron mediators in visible light induced carboxylate photorelease. *Photochem. Photobiol. Sci.*, **2010**, *9*, 854.

13 Bechtold, T. J., Anthraquinones as mediators for the indirect cathodic reduction of dispersed organic dyestuffs. *J. Electroanal. Chem.*, **1999**, *465*, 80.

View Article Online
DOI: 10.1039/C6PP00469E

14 König, B. *Chemical Photocatalysis*, De Gruyter: Berlin, 2013.
15 Whitby, L. G., A new method for preparing flavin-adenine dinucleotide. *Biochem. J.*, **1953**, *54*, 437.
16 Liu, C. X.; Zachara, J. M.; Foster, N. S.; Strickland, J., Kinetics of reductive dissolution of hematite by bioreduced anthraquinone-2,6-disulfonate. *Environ. Sci. Technol.*, **2007**, *41*, 7730.
17 Leoff, I.; Treinin, A.; Linschitz, H., Photochemistry of 9,10-anthraquinone-sulfonate in solution. 1. Intermediates and mechanism. *J. Phys. Chem.*, **1983**, *87*, 2536.
18 Murov, S. L.; Carmichael, I.; Hug, G. L. *Handbook of Photochemistry*; 2nd ed.; Marcel Dekker, Inc.: New York, 1993.
19 Banekovich, C.; Ott, I.; Koch, T.; Matuszczak, B.; Gust, R., Synthesis and biological activities of novel dexibuprofen tetraacetylriboflavin conjugates. *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 683.
20 Baginski, E. S.; Marie, S. S.; Clark, W. L.; Zak, B., Direct microdetermination of serum calcium. *Clin. Chim. Acta*, **1973**, *46*, 46.
21 Hatchard, C. G.; Parker, C. A., A new sensitive chemical actinometer. II. Potassium Ferrioxalate as a standard chemical actinometer. *Proc. R. Soc. London, Ser. A*, **1956**, 235, 518.
22 (a) Fife, D. J.; Moore, W. M., The Reduction and quenching of photoexcited flavins by EDTA. *Photochem. Photobiol.,* **1979**, *29*, 43. (b) Heelis, P. F.; Parsons, B. J.; Phillips, G. O.; McKellar, J. F., The Photoreduction of flavins by amino acids and EDTA. A continuous and flash photolysis study. *Photochem. Photobiol.*, **1979**, *30*, 343. (c) Babay, P. A.; Emilio, C. A.; Ferreyra, R. E.; Gautier, E. A.; Gettar, R. T.; Litter, M. I., Kinetics and mechanisms of EDTA photocatalytic degradation with TiO_2 under different experimental conditions. *Int. J. Photoenergy*, **2001**, *3*, 193. (d) Monokhova, Y. B.; Kuballa, T.; Mildau, G.; Kratz E.; Keck-Wilhelm, A.; Tschiersch, C.; Lachenmeier, D. W., Formaldehyde in hair straightening products: Rapid ^{1}H NMR determination and risk assesment. *Int. J. Cosmet. Sci.*, **2013**, *35*, 201.
23 Bernt Melø, T.; Ionescu, M. A.; Haggquist, G. W.; Naqvi, K. R., Hydrogen abstraction by triplet flavins. I: time-resolved multi-channel absorption spectra of flash-irradiated riboflavin solutions in water. *Spectrochim. Acta, Part A*, **1999**, *55*, 2299.
24 (a)De la Fuente, J. R.; Cañete, A.; Carathanassis, N.; Bernazar, L.; Saitz, C.; Díaz-Hernández, D., Spectral and kinetic study of 3-methylquinoxalin-ones photoreduced by amino acids: *N*-Phenylglycine radical chain reactions and *N*-acetyltryptophan decarboxylation. *J. Phys. Chem. A*, **2016**, *120*, 2797. (b) Gould, I. R.; Lenhard, J. R.; Farid, S., A curve-crossing model for oxidative decarboxylation. Kinetics of anilino carboxylate fragmentations. *J. Phys. Chem. A*, **2004**, *108*, 10949. (c) Su, Z.; Mariano, P. S.; Falvey, D. F.; Yoon, U. C.; Oh, S. W., Dynamics of anilinium radical α-heterolytic fragmentation process. Electrofugal group, substituent, and medium effects on desilyation, decarboxylation, and retro-aldol cleavage pathways. *J. Am. Chem. Soc.*, **1998**, *120*, 10676.

