

Activated photothermal heating using croconaine dyes†

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Laser-induced photothermal heating is promoted by dyes or nanoparticles that strongly absorb light and convert it into heat. The best known near-infrared absorbing systems are gold nanorods and nanocages. The alternative strategy of using organic dyes to absorb the laser light has several inherent advantages due to the small molecular size and potential synthetic flexibility, but a major drawback is rapid photothermal bleaching. Here, we report three important findings: (a) near-infrared croconaine dyes exhibit outstanding properties for high performance photothermal heating; including an intense and narrow absorption band at around 800 nm, high chemical, photo- and thermal stability, very efficient relaxation to the ground state, and very low oxygen photosensitization ability. (b) Photothermal heating obeys the Beer–Lambert law ($1 - 10^{-A}$) and sample heating reaches an asymptotic limit when chromophore absorbance values are greater than ~ 1 . (c) Croconaine dyes form red-shifted encapsulation complexes which allows the realization of molecular recognition induced activated photothermal heating, a broadly applicable nanoscale design concept that employs chemical or supramolecular processes to switch on laser-induced hyperthermia.

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Introduction

Laser-induced photothermal heating is promoted by dyes or nanoparticles that strongly absorb light and convert it into heat.^{1–4} Applications include photothermal imaging,^{5–8} photothermal therapy,^{9–11} tissue welding,^{12,13} molecular-scale surgery,^{14,15} light-triggered drug release,^{16–20} cell membrane manipulation,^{21,22} light powered micromachines,²³ photothermal materials,^{24–26} and single molecule temperature jump experiments.^{27,28} For most applications, a significant technical advantage is gained by using near-infrared light and many studies employ wavelengths around 800 nm produced by diode lasers. The best known near-infrared absorbing systems are inorganic nanoparticles such as gold nanorods and nanocages, and efforts are ongoing to develop next generation versions.^{29–34} The alternative strategy of using organic dyes, which absorb the laser light and convert the energy into heat *via* non-radiative relaxation pathways such as molecular vibrations, has several inherent advantages due to the small molecular size and potential synthetic flexibility. While the absorption cross sections of organic dye molecules are many magnitudes lower than that of gold nanoparticles, on a mass basis gold nanoparticles and near-infrared organic dyes have been shown to exhibit roughly similar heat generation capacities.³⁵ The most

studied near-infrared organic dye is Indocyanine Green (ICG),^{3,11,36} due in large part to the fact that it is approved for use in humans. However, laser irradiation of ICG and related cyanine dyes is known to produce rapid photobleaching and loss of the photothermal effect.^{35–38} There are surprisingly few literature reports of alternative chromophores for photothermal heating.^{24,39,40} The most promising dyes appear to be porphyrin and metallo-naphthalocyanine systems,^{41–43} but these are also known to be powerful oxygen sensitizers which can be an undesired complicating feature for many hyperthermia applications. The ideal molecular dye for high performance near-infrared photothermal heating should possess an intense absorption band, very short singlet excited state lifetime with highly efficient relaxation to the ground state, negligible intersystem crossing to triplet state and thus very low oxygen photosensitization ability, high chemical and thermal stability, low molecular size, and synthetic flexibility. We report that croconaine dyes exhibit all of these desired properties. Furthermore, we use croconaine dyes to demonstrate *activated photothermal heating*, a broadly applicable nanoscale design concept that employs chemical or supramolecular processes to switch on laser-induced hyperthermia.

Results and discussion

Croconaine dyes are readily prepared from croconic acid,‡ and they are known to have very high molar absorptions at around 800 nm, very short picosecond singlet excited state lifetimes and very low fluorescence quantum yields.^{44–49} Some croconaines possess undesirable properties such as low solubility, self-aggregation, and modest chemical instability. We chose to

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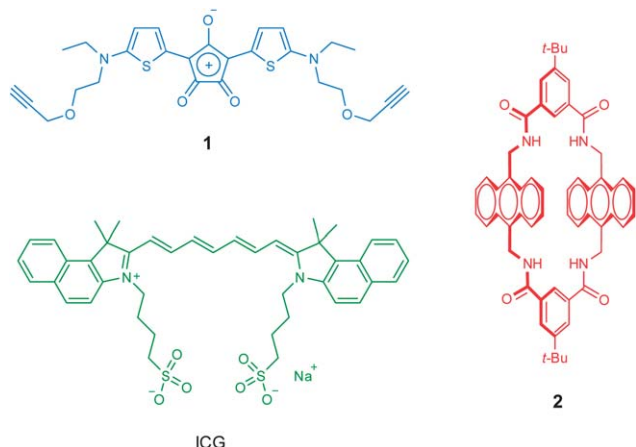


Fig. 1 Chemical structures.

investigate the croconaine dye **1** with two appended thiophene units (Fig. 1) as the thiophene-croconaine chromophore is known to have excellent absorption properties and high photochemical stability, even in water.^{45,47} Croconaine **1** was prepared in straightforward fashion (ESI, Scheme S1†), and as expected, it exhibited a narrow and intense near-infrared absorption band ($\lambda_{\text{abs,max}} = 782 \text{ nm}$ (4 : 1 MeOH–H₂O), 795 nm (CHCl₃), $\epsilon = 2 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$), with weak fluorescence ($\lambda_{\text{excit,max}} = 764 \text{ nm}$, $\lambda_{\text{em,max}} = 811 \text{ nm}$, $\Phi_f = 0.061$ (CHCl₃)). Singlet oxygen trapping studies demonstrated that **1** is an extremely poor oxygen photosensitizer compared to ICG (Fig. S15 and S16†). Moreover, there was a clear difference in photostability, with no photobleaching observed for **1** compared to a large reduction in ICG absorbance under the same irradiation conditions. As a complement to its excellent photostability, croconaine dye **1** possesses high chemical stability. For example, it resists nucleophilic attack by cysteine in aqueous solvent mixtures (Fig. S17†), unlike a previously reported reactive croconaine system.⁴⁶

Comparative heat generation studies were undertaken using separate solutions of **1**, ICG, or gold nanorods (AuNRs) in 4 : 1 MeOH–H₂O. Stirred solutions were irradiated for 15 minutes with 250 mW (3.5 W cm^{-2}) coherent light from a Ti:Sapphire laser, that was tuned to the respective absorption maxima (either 780 or 808 nm), and the solution temperature was monitored using a thermocouple. Preliminary experiments examined samples at $50 \mu\text{g mL}^{-1}$, a typical concentration in many previous photothermal studies, and found that all three samples produced very similar increases in temperature. We recognised that light absorption by these samples was well above the threshold for a near-linear Beer–Lambert relationship (see below), thus we repeated the photothermal experiments at the substantially lower concentration of $2.0 \mu\text{g mL}^{-1}$. The absorption and photothermal heating data for these samples (Fig. 2a and b) highlight two notable points. First, as expected, there was a time-dependent loss in the ICG photothermal heating effect due to photobleaching of the dye (confirmed by comparing absorption spectra of the sample before and after irradiation); whereas, the croconaine dye **1** exhibited a standard

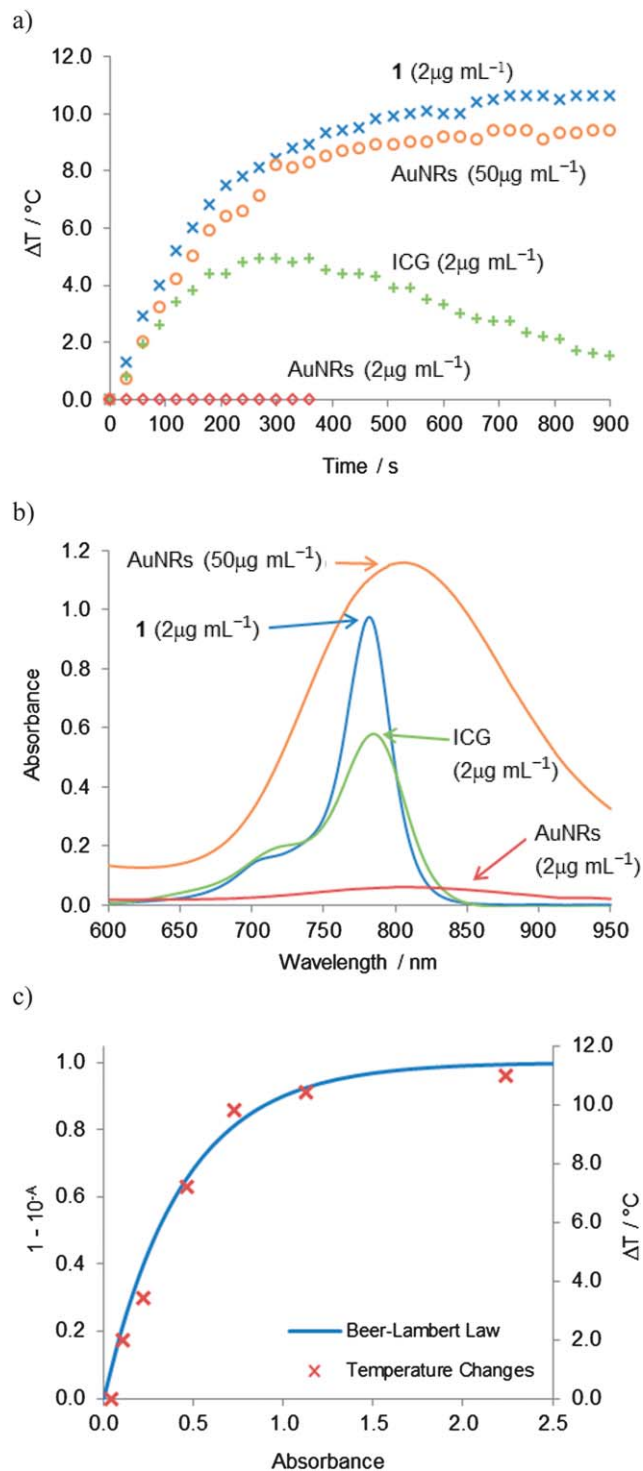


Fig. 2 (a) Temperature change profiles observed during laser irradiation (250 mW, 3.5 W cm^{-2}) for solutions of croconaine dye **1** (780 nm), ICG (780 nm) and AuNRs (808 nm) in 4 : 1 MeOH–H₂O; (b) corresponding absorbance spectra; and (c) correlation between the Beer–Lambert law ($1 - 10^{-A}$) and the temperature changes (ΔT) observed upon laser irradiation of different solutions of croconaine dye **1** under the same conditions as in (a).

heating curve and no measurable change in absorbance after laser irradiation (Fig. S18 and S19†). Second, the temperature changes in Fig. 2a correlate reasonably well with the maximum

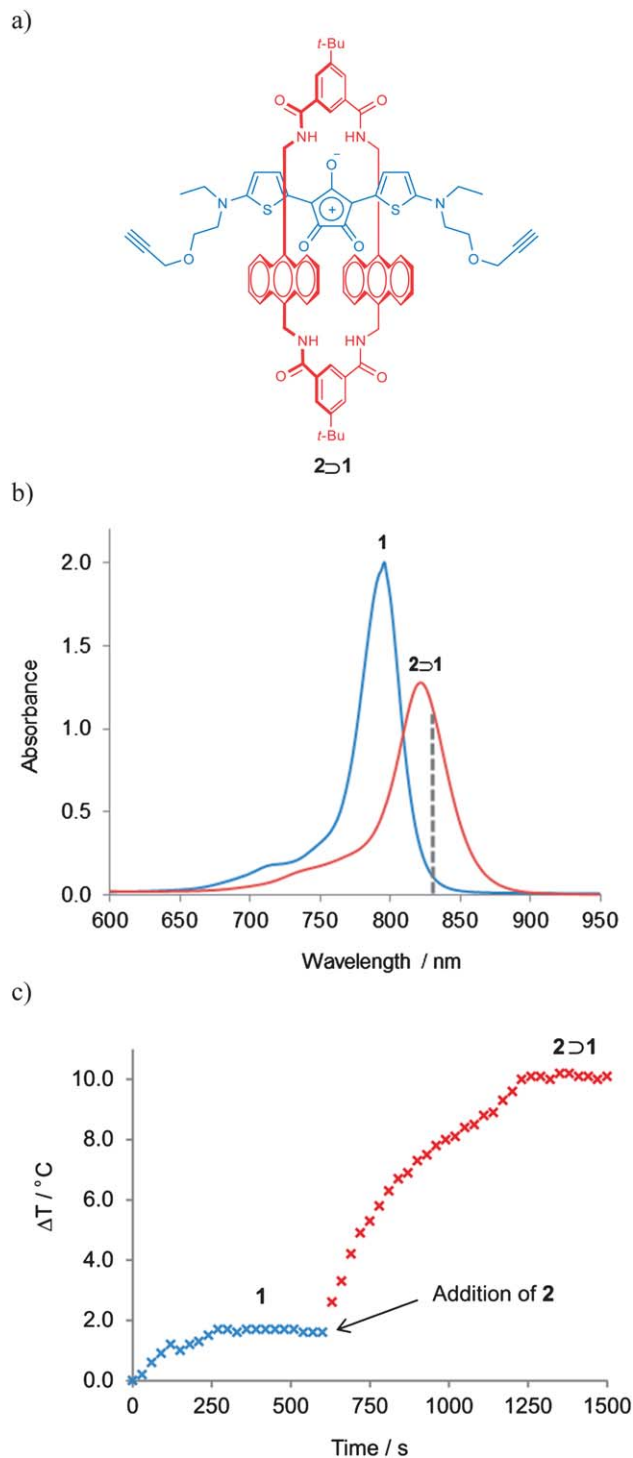


Fig. 3 (a) Inclusion complex $2\supset 1$ formed between croconaine dye **1** and macrocycle **2**; (b) absorbance spectra of croconaine dye **1** (CHCl_3 , $4.0 \mu\text{g mL}^{-1}$) and $2\supset 1$ that was formed upon addition of macrocycle **2** (50 molar equiv.); and (c) temperature changes observed for a solution of croconaine dye **1** (CHCl_3 , $4.0 \mu\text{g mL}^{-1}$) during laser irradiation (830 nm, 250 mW, 3.5 W cm^{-2}) and addition of macrocycle **2** (50 molar equiv.) at 600 s which converted **1** into $2\supset 1$.

absorbance values in Fig. 2b; for example a nanorod concentration of $50 \mu\text{g mL}^{-1}$ achieved comparable absorption and heating as dye **1** at $2.0 \mu\text{g mL}^{-1}$.

The high photothermal stability of dye **1** enabled a concentration study to fully investigate if the photothermal heating effect obeyed the Beer–Lambert law: that is, temperature change proportional to the amount of light absorbed ($1 - 10^{-A}$).^{1,50} The data in Fig. 2c confirms a near-linear relationship for dye absorbance values below $A \sim 1$, and an asymptotic temperature limit for absorbance values above $A \sim 1$. This latter feature explains why there was little photothermal heating difference when all three samples (**1**, ICG, AuNRs) were tested at high concentrations ($50 \mu\text{g mL}^{-1}$).

The correlation between the hyperthermia response and the Beer–Lambert law is a central design principle for successful development of activated photothermal heating. A corollary of this finding is that any procedure that enhances a chromophore's absorbance within the sensitive range (*i.e.*, $A < \sim 1$) will switch on laser induced hyperthermia. Croconaine dyes are especially well-suited for incorporation into activated photothermal heating strategies, since their narrow absorption bands make it easier to design nanoscale processes that greatly increase absorption levels at a specific wavelength.

To experimentally demonstrate activated photothermal heating, we developed a supramolecular process capable of modulating the heating response of croconaine **1**. The supramolecular design was based on previous observations by our group that encapsulation of structurally related squaraine dyes inside the tetralactam macrocycle **2** (Fig. 1) produces a 20–30 nm red shift in squaraine absorption.⁵¹ We were pleased to find that macrocycle **2** can efficiently encapsulate croconaine dye **1** in CHCl_3 and produce the dye inclusion complex $2\supset 1$ (Fig. 3a) with an association constant of $(3.4 \pm 0.7) \times 10^5 \text{ M}^{-1}$ (Fig. S14[†]). The structure of the inclusion complex was elucidated by standard NMR and mass spectrometric methods (Fig. S7[†]). As indicated in Fig. 3b, the croconaine encapsulation process produced the desired absorbance red shift of 28 nm with a substantial difference in absorption values for dye **1** and the inclusion complex $2\supset 1$ at 830 nm. Crucially, we were able to exploit this absorption difference to produce an activated photothermal heating process. As shown in Fig. 3c, at first, laser irradiation of croconaine **1** at 830 nm afforded a small, bulk sample temperature increase. Subsequently, this heating was greatly enhanced by the addition of macrocycle **2** and the resulting rapid assembly of $2\supset 1$.

We anticipate that a wide range of chemically and enzymatically triggered processes can be designed to control the formation of inclusion complexes that are analogous to $2\supset 1$. Many of these applications will require action in aqueous environments, and to this end we have obtained preliminary evidence that croconaine inclusion occurs very efficiently in water with a similar red shifted absorbance (Fig. S25 and S26[†]).

Conclusions

Due to their favourable photophysical properties and impressive stability, near-infrared croconaine dyes have great promise as a new class of high performance photothermal agents for a wide range of science and engineering applications. The unusually high dye stability permitted experiments

demonstrating, for the first time, that photothermal heating obeys the Beer–Lambert law and sample heating reaches an asymptotic limit when chromophore absorbance values are greater than ~ 1 . In addition, the ability of croconaine dyes to form red-shifted encapsulation complexes allowed the realization of molecular recognition induced activated photothermal heating. We foresee that activated photothermal heating will become a broadly useful technology that can be executed by various chemical and supramolecular processes.^{52–55}

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Notes and references

‡ The literature is inconsistent on the naming of dyes derived from croconic acid. Croconaines are also known as croconium, croconate and croconine dyes.

§ This prediction assumes that the dominant decay pathway for the chromophore's excited state is a heat-producing event. Alternative activation strategies can be envisioned that are based on the modulation of radiative emission pathways.

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