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Emergence of light-driven protometabolism upon recruitment of a photocatalytic cofactor by a self-replicator

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ABSTRACT

Establishing how life can emerge from inanimate matter is among the grand challenges of contemporary science. Chemical systems that capture life's essential characteristics - replication, metabolism and compartmentalization- offer a route to understanding this momentous process. The synthesis of life, whether based on canonical biomolecules or fully synthetic molecules, requires the functional integration of these three characteristics. Here we show how a system of fully synthetic self-replicating molecules, upon recruiting a cofactor, acquires the ability to transform thiols in its environment into disulfide precursors from which the molecules can replicate. The binding of replicator and cofactor enhances the activity of the latter in oxidizing thiols into disulfides through photoredox catalysis and thereby accelerates replication by increasing the availability of the disulfide precursors. This positive feedback marks the emergence of a light-driven protometabolism in a system that bears no resemblance to canonical biochemistry and constitutes a significant step towards the highly challenging aim of creating a new and completely synthetic form of life.

MAIN TEXT

All currently known life forms, diverse as they are, share the same set of building blocks and chemical reactions. Consequently, most research on the origins of life is focused on how these building blocks can be synthesized under prebiotically plausible conditions (1), or on how they can be combined to create a synthetic cell (2). However, there is no evidence to suggest that life in the universe, or even early life on Earth, needs to have a biochemistry similar to that with which we are familiar (3). This suggests that, in addition to the current focus on the origin of the chemical structures of known life, research into the emergence of the functional characteristics of life is of equal importance, also using systems that are chemically distinct from the canonical biomolecules (RNA, DNA, sugars and proteins). While defining life is remarkably difficult (4), the current consensus is that life requires the functional integration of three fundamental characteristics: self-replication, metabolism and compartmentalization (5). Self-replication refers to the ability of a system to produce (not necessarily exact) copies of itself. Compartmentalization refers to the ability to maintain co-localization of the components of a system. Metabolism has been defined as “the active or passive entrance of material and energy into the system which transforms them by chemical processes into its own internal constituents [...], so that the chemical reactions result in a regulated and controlled increase of the inner constituents as well as in the energy supply of the system.” (6). Metabolism, in particular, plays a fundamental role in the development from “simple” replicators to the complex entities that we typically associate with life. In the theoretical framework of replicative dynamic kinetic stability (7) (persistent state resulting from a cyclic process of formation and destruction, occurring effectively irreversibly driven by continual material and/or energy input), the emergence of metabolic reactions has been described as a necessary step to increase the complexity of pre-existing replicators (8), a selection requisite for replicators to survive in a resource-poor environment (9) and the origin of behaviors that could be interpreted as teleonomic (with a purpose) (10). It is important to note that, in this context, the main function of metabolism is not thermodynamic but kinetic – it allows for the use of more resources by the replicator, therefore leading to faster replication and a greater dynamic kinetic stability.

Efforts aimed at integrating the functional characteristics of life have predominantly focused on enclosing (mostly enzymatic) reaction networks or a (self-)replicating entity within a compartment (11-14). Although the integration of metabolism with self-replication has been

explored theoretically (15,16), its experimental implementation is lacking. A major hurdle in achieving metabolic self-replication is associated with identifying replicating systems that can play a dual role: aside from catalyzing their own formation they should also be able to catalyze other (metabolic) reactions. Few systems exist that can do so: Lehman reported RNA sets, where both self-replication and protometabolism were based on the same phosphodiester chemistry (17), and Rebek developed synthetic self-replicators capable of organocatalysis, where replication and catalysis required different solvents (18).

We set out to integrate some of the features of metabolism into a previously described self-assembly-driven self-replicator (19,20). This is an attractive system as it is currently the only self-replicator that is able to emerge spontaneously from a complex mixture and capable of exponential growth at the same time. Briefly, the replicator used in this work (**1**₆) is a hexameric macrocycle made from building block **1** (Fig. 1). When **1** is oxidized in a buffered (pH = 8.2) aqueous solution, it gives rise to a small dynamic combinatorial library of disulfide-based macrocycles of different sizes (mostly **1**₃ and **1**₄), from which **1**₆ can emerge and self-assemble into fibers. (Previous observations (19) suggest that the majority of **1**₆ is assembled into fibers, already at very low concentrations. Therefore, the terms “**1**₆” and “**1**₆ fibers” both refer to **1**₆ in the self-assembled fibers and are used interchangeably.) Once those fibers reach a critical length, they enter a templated growth/mechanical breakage cycle. In this way, **1**₆ can grow exponentially as long as the concentration of its precursors (the disulfide-based macrocycles **1**₃/**1**₄) remains high enough. However, if an additional oxidant is not used, the oxidation of **1** (occurring slowly through reaction with dissolved oxygen) can become the rate-limiting step of the replication process.

We designed a rudimentary metabolism for this system by giving **1**₆ the ability to promote the oxidation reaction of **1** → **1**₃/**1**₄, using light as an external energy source. This fulfills one of the main functions of metabolism: The use of external energy to synthesize the precursors of the self-replicating species starting from inert materials. Our strategy was inspired by cofactor recruitment encountered in present biochemistry (21-23) and involves the self-replicator binding and activating a small organic photosensitizer. This strategy was implemented using two different cofactors (**2** or **3**).

RESULTS AND DISCUSSION

Replicator-induced J-aggregation of Rose Bengal enhances its photocatalytic activity

The first photosensitizer that we selected as a cofactor was Rose Bengal (**2**), an organic dye known to photocatalyze the oxidation of thiols to disulfides via generation of ¹O₂ (24). Its hydrophobicity and two negative charges make it a good candidate for interacting with the fibers of **1**₆, that present large hydrophobic pockets (25) (Supplementary Fig. 1) and feature lysine residues that are positively charged at ca. pH 8. Spectral analysis confirmed an interaction of **2** with the macrocycles derived from **1** (Fig. 2a, Supplementary Fig. 2), but not with **1** itself, as the two thiolate groups give **1** a net negative charge (Supplementary Fig. 3). Notably, a new red-shifted band exclusively appeared when **2** and replicator **1**₆ were mixed. This sharp band, which exhibits circular dichroism (Fig. 2b), is indicative of the binding of **2** to the **1**₆ fibers in a chiral, supramolecular J-aggregate (26, 27). The band is not present when the **1**₆:**2** ratio is high (Supplementary Fig. 4) or in the presence of **1**₃/**1**₄.

The appearance of this band allowed us to design a system where only the **2**-**1**₆ complex is able to act as a photooxidation catalyst. By irradiating the system at wavelengths corresponding to the new band only, we ensured that **2** would be excited only when J-aggregated. Excited **2** then undergoes intersystem crossing, promoting ¹O₂ generation

(supported by $^1\text{O}_2$ phosphorescence (Supplementary Fig. 5), and by reaction with a $^1\text{O}_2$ sensitive probe (Supplementary Fig. 6)), which in turn mediates thiol oxidation (Fig. 3c). Indeed, upon photo irradiation, the oxidation of **1** was accelerated 2.4 fold when both **2** and the replicator **1₆** were present, compared to control experiments using only **2** or **2** and **1_{3/14}** (Fig. 3a). Samples that were not irradiated oxidized at a similar rate to the sample without **2**, confirming that the reaction proceeded via $^1\text{O}_2$ -mediated photooxidation. Thus, catalysis of the reaction **1** $\rightarrow$ **1_{3/14}** is an emergent property of the **1₆-2** complex and cannot be attributed to any of the individual species acting in isolation. The observed oxidation rates in presence of **1₆** and **1_{3/14}** were lower than the ones that could be expected by the differences in absorbance only. The IR phosphorescence of $^1\text{O}_2$ (28) shows that the quantum yield of $^1\text{O}_2$ formation by **2** is considerably reduced when **1₆** or **1_{3/14}** are present (Supplementary Fig. 5, Supplementary Table 2). This causes a decrease in oxidation rate with the formation of the **2-1₆** complex, that counters the increase in absorbance and partially explains the observed difference.

Tetraphenylporphyrin acts as a photocatalytic cofactor that can be activated with a wide range of wavelengths

A limitation of using **2** as a cofactor is that the irradiation source is restricted to a small and specific range of wavelengths. This restriction can be relaxed by using a different dye as a cofactor that can be excited with white light. Thus, we used tetraphenylporphyrin (**3**), a well-known $^1\text{O}_2$ photosensitizer (29). This nonpolar dye is poorly soluble in buffer (Fig. 2c), hampering its use as a photosensitizer in aqueous solution. However, in presence of **1₆**, **3** is solubilized, as indicated by the appearance of its Soret (425 nm) and Q bands (520, 566, and 660 nm) (Fig. 2c and Extended Data Fig. 1). Compared with its spectrum in dimethyl formamide, the Soret band of **3** was red-shifted by 9 nm and broadened (Supplementary Fig. 7). This is an indication of multiple binding sites on the **1₆** fibers, which lead to different microenvironments for the bound porphyrin molecules (30).

Binding of **3** to replicator **1₆** resulted in an increase in fluorescence from the dye (Fig. 2d and Supplementary Fig. 2) and also enhanced its production of $^1\text{O}_2$, manifested in the enhanced rate of oxidation of a probe (31) (Supplementary Fig. 6). In addition to solubilizing the dye, the hydrophobically driven binding of **3** to the replicator **1₆** may also alleviate the self-quenching of **3**, caused by its aggregation. Importantly, replicator **1₆** is better at solubilizing **3** than replicator precursors **1_{3/14}** (Fig. 2d), which is presumably due to the larger hydrophobic binding sites offered by the **1₆** fibers. An advantage of this strategy is that it can be used to bind and activate other hydrophobic porphyrins (Supplementary Fig. 8).

Upon binding to replicator **1₆** and irradiation, dye **3** induced a 2-fold enhancement in the rate of oxidation of **1** compared to the same dye in the presence of **1_{3/14}** (Fig. 3b). Thus, like **2**, also **3** acts as a photocatalytic cofactor, albeit through a different activation mechanism. Importantly, in both systems the replicator accelerates the light-driven formation of **1_{3/14}**. Given that these macrocycles are the precursors for the replicator **1₆**, this behavior corresponds to protometabolism.

Photooxidative protometabolism yields the precursors of the replicator, increasing its replication efficiency

The dynamic behavior of our systems under conditions where self-replication and oxidative protometabolism could influence each other was explored. Dynamic combinatorial libraries made from thiol building block **1** were prepared in the presence of **2** or **3**, without added

oxidant other than dissolved oxygen from air, and stirred while irradiating with yellow light (590 nm) or a halogen lamp, respectively (See Supplementary Discussion). In both experiments, as soon as self-replicator **1**₆ emerged, the rate of oxidation of thiol **1** increased (Fig. 4a and c; top half of each graph shows the concentrations of **1** in black and **1**₆ in red; bottom half of each graph shows the rates at which these species are consumed and produced, respectively), consistent with the previous experiments (Fig. 3a and b). As expected, in the controls without cofactor the oxidation rate did not change with the emergence of **1**₆, remaining relatively low and decreasing slowly due to the depletion of **1** (Fig. 4b and d). Importantly, the enhanced rate of oxidation in the presence of cofactor also induced an increase in replication rate, constituting a second positive feedback loop in the system.

We performed additional control experiments to confirm that the increase in oxidation and replication rates requires both photo irradiation and presence of replicator. Upon switching off photoirradiation mid-way during the experiment in Fig. 4a, both oxidation and replication rates dropped markedly, reaching values similar to those observed for control samples without cofactor (Fig. 4b) or those that were not irradiated (Extended Data Figures 2 and 3). Note that, by preventing the emergence of the replicator (by not stirring the libraries; replicator growth depends strongly on shear stress) (20), cofactor activation cannot take place, and the oxidation rate remains low throughout the experiment (Supplementary Fig. 9). Kinetic studies of libraries kept in the dark (Supplementary Figures 10 and 11), and TEM analysis (Supplementary Fig. 12) confirmed that the dyes have no significant effect on the behavior of the systems except for photooxidation. Finally, mixing the dyes studied here with a different self-replicator (32) led to different degrees of activations (Supplementary Fig. 13). This opens the door for future experiments, where replicators could compete and be selected based on the efficiency of their protometabolism.

CONCLUSIONS

In conclusion, we show how, upon photoirradiation, dyes can act as cofactors in a system of synthetic self-replicators to achieve photocatalytic conversion of molecules, present in the surroundings of the replicator, into precursors from which the replicators can grow, using light as an energy source. This positive feedback of a replicating system on the production of its own precursors can be regarded as protometabolism, but still falls short of complete metabolism since the external energy is not stored nor used to perform an endergonic reaction. Yet, we feel that the emergence of such protometabolism constitutes an important milestone in the de-novo synthesis of life. It opens the way for protometabolic activity to be further optimized and extended through selection by Darwinian evolution, since protometabolism is directly coupled to replication. While the photocatalytic activity in the present system was designed, we recently reported that, for the same system of self-replicators, catalytic activity may also emerge by chance, in the absence of cofactors and even exhibit promiscuity (33).

Photocatalytic feedback loops of the kind developed here are also attractive components for systems chemistry, as the rates of the photochemical processes can be tuned independent of other chemical processes in the system by manipulating light intensity. The ability to orthogonally manipulate the rates of different reaction pathways of a complex chemical system is essential for homing in on the right part of parameter space that harbors specific emergent phenomena (such as, for example, reaction waves or oscillations).

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AUTHOR CONTRIBUTIONS

S.O., G.M.S. and K.L. conceived the experiments; G.M.S. performed the experiments related to Rose Bengal; K.L. performed the experiments related to porphyrins; W.R.B. performed the experiments related to IR luminescence of singlet oxygen; G.M.S, K.L. and S.O. wrote the manuscript.

COMPETING INTERESTS STATEMENT

Authors declare no competing interests.

FIGURES

Fig. 1. Mechanism of light-driven protometabolic self-replication. (a) Structures of building block **1**, Rose Bengal (**2**), and tetraphenylporphyrin (**3**). (b) The reaction network combining self-replication, cofactor recruitment, and 1O_2 -mediated photo-oxidation. The oxidation of **1** leads to a dynamic combinatorial library of macrocyclic disulfides, which provides the precursors for the replicator 1_6 . The autocatalysis of 1_6 (red arrow) then takes place through a growth-breakage mechanism, featuring self-assembly onto fiber ends and mechanical fragmentation of fibers. The binding of the 1_6 fibers to a photosensitizer (**2** or **3**; present from the start of the experiment) leads to emergent photocatalytic 1O_2 production upon photo-irradiation with 590 nm light (in the case of **2**) or with white light from a halogen lamp (in the case of **3**). In both cases, the emergent production of 1O_2 accelerates the oxidation process and leads to a second positive feedback loop (represented by a second red arrow).

Fig. 2. Optical properties of 2 and 3 upon binding to macrocycles derived from 1. UV-Vis absorption spectra (a) and circular dichroism spectra (b) of **2** (4.0 μM) in buffer (black), in the presence of **1**₃/**1**₄ (80 μM in **1**; blue), and in the presence of **1**₆ (80 μM in **1**; red). The self-assembled **1**₆ fibers bind the molecules of **2** and organize them in a J-aggregate, causing the appearance of a new band (585 nm) in both its absorption and CD spectra. The macrocycles **1**₃/**1**₄ bind **2** as well, but do not cause the appearance of this new band. The shaded area represents the range of wavelengths used for irradiation in the next experiments. UV-Vis absorption spectra (c) and fluorescence spectra (excitation at 420 nm) (d) of **3** (10 μM) in buffer (black), in the presence of **1**₃/**1**₄ (1.0 mM in **1**; blue) and in the presence of **1**₆ (1.0 mM in **1**; red). In this case, the fibers of **1**₆ solubilize the hydrophobic **3** and alleviate its self-quenching, which increases its absorbance and fluorescence emission. Smaller macrocycles **1**₃/**1**₄ also cause a similar effect, but to a much smaller extent. The effect of **1**₆ and **1**₃/**1**₄ on the spectrum of **3** was also observed in conditions where scattering was minimized (Extended Data Fig. 1).

Fig. 3. Replicator I_6 enhances photocatalytic oxidation of I mediated by 2 and 3 . (a) Initial rates of oxidation of I ($200\ \mu\text{M}$) in the presence of 2 ($2.5\ \mu\text{M}$) and either I_6 ($80\ \mu\text{M}$) or I_3/I_4 ($200\ \mu\text{M}$). (b) Initial rates of oxidation of I ($700\ \mu\text{M}$) in the presence of 3 ($10\ \mu\text{M}$) and I_6 ($50\ \mu\text{M}$) or I_3/I_4 ($150\ \mu\text{M}$). All samples were kept at $25\ ^\circ\text{C}$ and irradiated with $590\ \text{nm}$ light (for a) or a halogen lamp (for b). Rates were measured by monitoring the concentration of I over $2\ \text{h}$ using UPLC (Methods). The data shown is the average rate of three (a) or two (b) independent samples, and the error bars correspond to their standard deviation. The oxidation rates in presence of I_6 is higher than in any of the controls, showing that the processes described in Fig. 2 lead to replicator I_6 enhancing the photooxidation of I to I_3/I_4 , which are the precursors for the replicator. (c) Jablonski diagram showing the photochemical processes involved in the formation of $^1\text{O}_2$ (excitation from the ground state (S_0) to the singlet excited state (S_1), followed by intersystem crossing to the triplet excited state (T_1) which promotes singlet oxygen production) and the subsequent oxidation of thiols to small-ring disulfides.

Fig. 4. Emergence of replicator 1_6 promotes photocatalytic production of precursors $1_3/1_4$, which promotes replication. Change in the concentrations of 1 and 1_6 in the presence (a) or absence of $1.0 \mu\text{M}$ 2 (b). Samples were stirred at 1200 rpm at 40°C , irradiated with 590 nm light for the first 6 h (orange-shaded areas), and kept in the dark for another 6 h (grey-shaded areas). Change in the concentrations of 1 and 1_6 in the presence (c) or absence of $5.0 \mu\text{M}$ 3 (d). The samples corresponding to panels c and d were stirred at 1200 rpm at 25°C and irradiated with a halogen lamp for the entire duration of the experiment. The top half of each graph represents the concentrations of 1 (black) or 1_6 (red) in units of 1 . The bottom half of each graph represents the rates of oxidation (black) and replication (red), calculated by numerical differentiation of the data in the top halves. Note the difference in abscissa (time range) between panels c and d. For both dyes, both oxidation and replication rates increase upon the emergence of the replicator 1_6 , showing that a faster oxidation benefits replication through the synthesis of $1_3/1_4$. This effect disappears upon turning off irradiation (panel a, grey area), and in controls without dye (panels b and d) or light (Extended Data Fig. 2 and 3).

Extended Data Figures

Extended Data Fig. 1: Absorption spectra of 3 recorded in reduced scattering conditions. Visible absorption spectra of **3** (10 μM) in buffer (black), and in the presence of **1**₃/**1**₄ (1.0 mM in **1**; blue) and **1**₆ (1.0 mM in **1**; red). In order to show that the increased absorbance of **3** in presence of **1**₆ is not related to scattering, this effect was minimized by recording these spectra in a different spectrophotometer (see Materials), using a wider slit (4 nm), a shorter light path (4.5 mm), and placing the cuvette immediately in front of the detector.

Extended Data Fig. 2: Non-irradiated controls for the emergence of **1₆ in presence of **2** and **3**.**

Evolution over time of the concentrations of **1** and **1**₆ in non-irradiated libraries made from **1** and **2** (a) or **1** and **3** (b). The top halves of the graphs show the concentration of **1** (black squares) and **1**₆ (red squares, in units of **1**). The bottom halves of the graphs represent the oxidation (black circles) and replication (red circles) rates in the system, calculated by numerical differentiation of the curves in the top halves. Both libraries were prepared exactly as their equivalents in Fig. 4a and 4b, but they were kept in the dark.

Extended Data Fig. 3: Non-irradiated controls for the emergence of **1₆ in absence of photosensitizers.** Evolution over time of the concentrations of **1** and **1**₆ in non-irradiated libraries prepared from **1**, with no dyes added, in the same conditions as in Fig. 4A (left panel) or Fig. 4B (right panel). The top halves of the graphs show the concentration of **1** (black squares) and **1**₆ (red squares, in units of **1**). The bottom halves of the graphs represent the oxidation (black circles) and replication (red circles) rates in the system, calculated by numerical differentiation of the curves in the top halves.

METHODS

Materials

All reagents, solvents and buffer salts were purchased from commercial sources and used without further purification. Building block **1** was obtained from Cambridge Peptides Ltd (Birmingham, UK). Dyes **2** and **3** were obtained from Sigma-Aldrich (purities 95% and 99%, respectively). The temperature of the samples was kept constant by using a Thermo Fisher compact dry bath with a custom milled aluminium block to accommodate HPLC vials (with a dimension of 12 x 32 mm). The irradiation at 590 nm was performed with 5 mm LEDs purchased from Kingbright (model L53-LYD) through a local distributor (OKAPHONE, The Netherlands), connected to a 5V power source using a homemade setup. The light intensity of the LEDs is 2 mcd with a viewing angle of 60°, their emission wavelengths are 590 ± 12.5 nm, and their power dissipation is 105 mW, according to the supplier. Halogen lamps (5 W) whose emission encompass the entire visible region (wavelengths higher than 350 nm) were used to irradiate systems containing **3**. UPLC analysis was performed on a Waters Acquity UPLC-H class system equipped with a PDA detector. All analyses were performed using a reversed-phase UPLC column (Aeris Peptide 1.7 μm XB-C18 x 2.10 mm, Phenomenex). The column temperature was kept at 35 °C, and the sample plate was kept at 25 °C, unless otherwise specified. UV absorbance was monitored at 254 nm. For each injection, 10 μL of sample was injected. UPLC-MS analysis were performed on a Waters Acquity UPLC H-class system coupled to a Waters Xevo-G2 TOF, operated in positive electrospray ionization mode. Capillary, sampling cone and extraction cone were kept at 2.5 kV, 20 V and 4 V respectively. Source and desolvation temperatures were set as 150 °C and 500 °C. Nitrogen was used as cone and desolvation gas (5 L/h and 800 L/h respectively). The UV-Vis spectra shown in Extended Data Figure 1 were recorded using a Specord 210 Plus spectrophotometer, and all

the others were recorded using a Jasco V-630 UV spectrophotometer. CD spectra were recorded using a Jasco J-815 CD spectrometer, keeping the high tension values around 300 V for all of the measurements. Fluorescence spectra were recorded using a JASCO FP6200 fluorimeter. Solutions for UV, CD and fluorescence were prepared and measured in polystyrene cuvettes, from Brand GMBH (Werrheim, Germany), using the corresponding buffer as a blank. The use of Nile Red as a fluorescence probe for the hydrophobicity of self-assembled structures has been described in (34).

UPLC analysis

All samples were eluted using a gradient of UPLC-grade water and acetonitrile, both containing 0.1 % trifluoroacetic acid (TFA). A constant flow rate of 0.3 mL/h was kept. The gradients that were used are detailed in Supplementary Table 1.

The peaks were assigned based on controls or previous results (19). Examples of typical chromatograms for the libraries studied are shown in Supplementary Figure 15. Only the main components of the system (**1**, **1₃**, **1₄**, **1₆**) were taken into account for the calculations and graphs shown in the text.

Preparation of borate buffer

For the experiments related to **3** and their corresponding controls, sodium borate buffer was prepared by dissolving Na₂B₄O₇ in doubly distilled water to a concentration of 50 mM (200 mM in boron atoms). The pH of the buffer was adjusted to 8.2 by addition of HCl (1 M). For the experiments involving **2** and their corresponding controls, borate buffer with lower ionic strength was used. To prepare it, B₂O₃ (From Sigma-Aldrich) was dissolved in doubly distilled water and diluted to a final concentration of 25 mM (50 mM in boron atoms) after adjusting the pH to 8.2.

Preparation of 1₆

A stock solution of **1₆** was typically prepared by dissolving building block **1** in the corresponding borate buffer to a final concentration of 4.0 mM, and stirring at 1200 rpm at 40 °C for 24-48 h, while following the reaction by UPLC.

Preparation of 1₃-1₄

Stock solutions of **1₃-1₄** were prepared by mixing freshly prepared concentrated solutions of **1** and NaBO₄ and diluting to a final concentration of 4.0 mM of each of them. The oxidation level was monitored by UPLC, typically reaching 100% in less than 30 minutes.

Determination of oxidation rates using 2 and 590 nm light

Libraries were prepared by dissolving building block **1** (200 μM) and the different components of the system (**1₃-1₄**, **1₆** and/or **2**, at the concentrations indicated in the main text) in 1.0 mL of borate buffer (pH = 8.2, see above) in a 12 x 32 mm HPLC vial. A 5 mm yellow LED was fitted directly through a Teflon septum screw cap, and the temperature was kept at 25 °C using a dry bath. The cap and dry bath shielded the vials from any other irradiation source than the LED. The concentration of the different components of the library was monitored periodically for 2 h by injecting the undiluted sample onto an UPLC system. The

UPLC peak area was converted to concentration of the different components with a coefficient determined from a calibration curve ($R^2 > 0.99$). The oxidation rate of each sample was determined by linear regression of the concentration of **1** over time ($R^2 > 0.95$). The data shown is the average of three independent samples, and the error bars correspond to their standard deviation.

Determination of oxidation rates using **3 and a halogen lamp**

Aliquots of 200 μL **1₆** or **1₃-1₄** (1.0 mM in **1**) were incubated with **3** (4.0 μM) in sodium borate buffer for 2 h, followed by the addition of 300 μL of **1** (0.50 mM). The resulting solution was irradiated using a 5 W halogen lamp at a distance of 3 cm and stirred at 1200 rpm. The concentration of **1** was measured by UPLC before and after 2 h of irradiation. The sample was diluted 50 times in doubly distilled water before injection. The peak area of **1** was converted to concentration with a coefficient determined from a calibration curve ($R^2 > 0.99$). The data shown is an average of two independent samples, and the error bars correspond to their standard deviation. The control experiments were performed in the same conditions, except for the absence of **1₆**, **3**, or light.

Emergence experiments using **2 (corresponding to Figure 4 and its controls)**

Libraries were prepared by dissolving **1** (around 500 μM) with or without **2** (1.0 μM) in 1.0 mL of borate buffer (pH = 8.2, see above) in a 12 x 32 mm HPLC vial. A 5 mm yellow LED was fitted directly through a Teflon septum screw cap for the irradiated samples, and the temperature was kept at 40 °C using a dry bath. The cap and dry bath shielded the vials from any other irradiation source than the LED. Stirring was performed at 1200 rpm, using Teflon coated magnetic stirring bars (5 x 2 mm, VWR). The concentration of the different species was monitored by UPLC, injecting the undiluted sample directly. The UPLC peak area was converted to concentration of the different components using a coefficient determined from a calibration curve ($R^2 > 0.99$). The oxidation and replication rates were calculated by numerical differentiation.

Emergence experiments using **3 (corresponding to Figure 4 and its controls)**

Libraries were prepared by dissolving **1** (1.0 mM) with or without **3** (20 μM) in 1.0 mL of borate buffer (pH 8.2, see above) in a 12 x 32 mm HPLC vial. The samples were kept at room temperature and irradiated with a 5 W halogen lamp from a distance of 3 cm and stirred at 1200 rpm, using Teflon coated magnetic stirring bars (5 x 2 mm, VWR). The samples were monitored periodically by UPLC analysis of an aliquot that was diluted 50 times with doubly distilled water. To correct for the error caused by dilution, the concentration of the different library members was calculated based on the ratio between each peak and the total peak area of the disulfide peaks (6-12 minutes) in the chromatogram.

Determination of the $^1\text{O}_2$ generation quantum yield of **2**

Solutions of **2** (4.0 μM) and **1**, **1₃/1₄**, or **1₆** (80 μM in **1**), prepared using D_2O as a solvent, were excited at 532 nm diode (45 mW), and $^1\text{O}_2$ phosphorescence collected by a fibre optic coupled to a Shamrock 163 spectrograph (ANDOR) and dispersed onto a iDUS-InGaAs-512 diode array detector.

Negative staining transmission electron microscopy

A small drop (5 μ L) of sample was deposited on a 400 mesh copper grid covered with a thin carbon film (Van Loenen instruments, The Netherlands). After 30 s, the droplet was blotted on filter paper. The sample was then stained twice (5 μ L each time) with a solution of 2% uranyl acetate deposited on the grid and blotted on the filter paper after 30 s each time. The grids were observed in a Philips CM12 cryo-electron microscope operating at 120 kV. Images were recorded on a slow scan CCD camera.

Supplementary Discussion

The libraries including **2** and their controls were set up using a less concentrated buffer (see above). This typically increases the time required for the emergence of **1₆**, so the temperature of these libraries was increased in the emergence experiments (as the ones shown in Fig. 4a and b) to compensate for this effect and reduce experiment times.

The emergence of **1₆** (as in Fig. 4) required longer experiments than the determination of initial oxidation rates (as in Fig. 3). In order to slow down photooxidation, and still have part of the library in the form of **1** by the time when **1₆** emerged, in these experiments the concentration of dye was reduced. Other differences in experimental conditions between the experiments containing **2** and **3** are due to their different behaviors and solubilities, which led to their separate optimization.

For both dyes, the temperature of the libraries was monitored in both irradiated and non-irradiated samples, not observing differences higher than 2 °C between them in any instance.

The time required for the emergence of **1₆** varied in different repeats of the experiment. We attribute this difference to the stochastic nature of the nucleation of fibers. The emergence of **1₆** was always followed by higher oxidation and replication rates in the irradiated libraries, compared to unirradiated controls (Supplementary Fig. 14).

The photooxidation reactions described here converted the thiols to mostly (>90%) disulfides, as observed in the chromatograms (Supplementary Fig. 15), and by studying the evolution of the total concentration of thiols and disulfides over time (Supplementary Fig. 16). Only small amounts of overoxidized **1₂** were observed as a side-product (Supplementary Fig. 17). The photobleaching of the cofactors cannot be completely discarded, since their low concentration made them hard to quantify by UPLC and their UV spectra changed with the concentration of other species. However, since catalytic activity was still observed after long irradiation times, the extent of photobleaching was limited.

REFERENCES (METHODS SECTION)

34. M. C. A. Stuart, J. C. van de Pas, J. B. F. N. Engberts. The use of Nile Red to monitor the aggregation behavior in ternary surfactant-water-organic solvent systems. *J. Phys. Org. Chem.* **18**, 929-934. (2005)

DATA AVAILABILITY STATEMENT

The UPLC data generated and analysed in this article is included in its supplementary information files, in the form of integrated peak areas and exported traces of representative chromatograms. All other chromatograms are stored locally on their native format and

available upon request. All other data generated or analysed during this study are included in this published article (and its supplementary information files).