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Photochemical Control over Oscillations in Chemical Reaction Networks

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Supporting Information

ABSTRACT: Systems chemistry aims to emulate the functional behavior observed in living systems by constructing chemical reaction networks (CRNs) with well-defined dynamic properties. Future expansion of the complexity of these systems would require external control to tune behavior and temporal organization of such CRNs. In this work, we design and implement a photolabile probe, which upon irradiation strengthens the negative feedback loop of a CRN that produces oscillations of trypsin under out-of-equilibrium conditions. By changing the timing and duration of irradiation, we can tailor the temporal response of the network.

A discipline of "systems chemistry" is developing, which aims to capture the complexity observed in natural systems within a synthetic chemical framework.[1−3] In recent years, significant progress has been made, including the design of oscillating reaction networks,[4,5] the formation of transient gels[6] and vesicles,[7] self-replicating systems,[8,9] DNA-based controllers and oscillators,[10,11] self-organizing ensembles of nanoparticles,[12] and catalytic reactions with chemo-mechanical feedback.[13] A major challenge for systems chemistry is to translate the design principles of living systems, based on feedback loops and reaction networks, into functional synthetic equivalents.

We are inspired by the regularly recurring motifs of coupled feedback loops generating much of the functional behavior in biological networks.[14,15] We recently reported an oscillating out-of-equilibrium CRN, consisting of one positive feedback loop (the autocatalytic production of enzyme trypsin (Tr) from trypsinogen (Tg)) and one negative feedback loop (Figure 1a).[5] Figure 1a gives a schematic overview of the processes that dominate during different stages of the oscillation. The negative feedback loop starts with the activation of a pro-inhibitor (Pro-I), by Tr, into an intermediate inhibitor that produces active inhibitor of Tr by the action of the enzyme aminopeptidase (Ap). The two-step negative feedback loop produces a delay in Tr inhibition, which is a key requirement for producing oscillations in flow conditions.[14] We can tailor the system-level properties of these networks (i.e., robustness and resilience of the functional output) by changing the precise structure of the small molecules.[16,17] However, incorporation of simple designs into more complex out-of-equilibrium networks will make it ever more difficult to ensure that each reaction in the network has a suitable reaction rate. Thus, we will require a method to regulate individual rates in the network, without disrupting others.

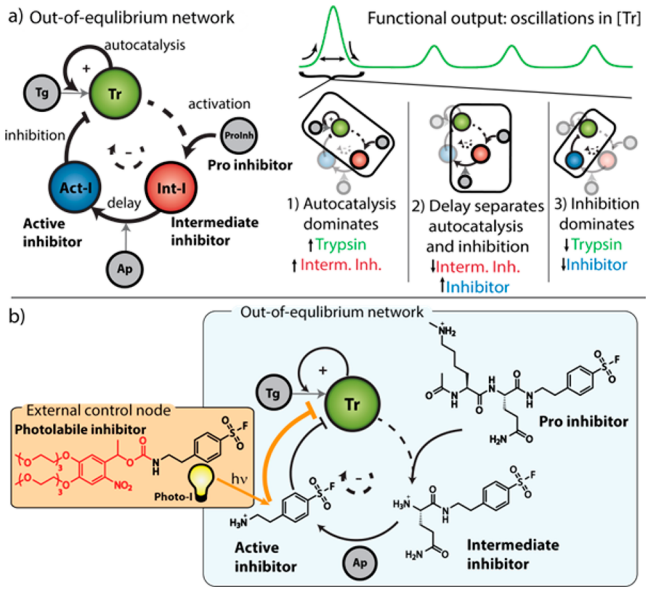

Figure 1. Introduction of a control node. (a) Schematic representation of the out-of-equilibrium system and the key processes during oscillations. (b) Details of the original network (light blue box) and the photochemical control node (orange box).

Light is an attractive external control element, as it can selectively and rapidly change reactions rates (in contrast to changes in concentration, solvent quality, or temperature). Research on the well-known BZ (Belousov−Zhabotinsky) system has shown that light can be used to alter frequency and phase of the oscillations[18] and induce formation of specific patterns.[19]

Here, we demonstrate photochemical regulation of an oscillating enzymatic reaction network. In our design, the concentration of active trypsin (Tr) is expected to fully control the output of the network. In order to change the level of active [Tr] instantaneously, external regulation is obtained via a photolabile inhibitor (Figure 1b). Upon irradiation with light at 380 nm, the active inhibitor concentration is increased, strengthening the final step in the negative feedback. We will demonstrate how light can be used to induce delay, perturb or entrain oscillations, and synchronize oscillations in separate reactors.

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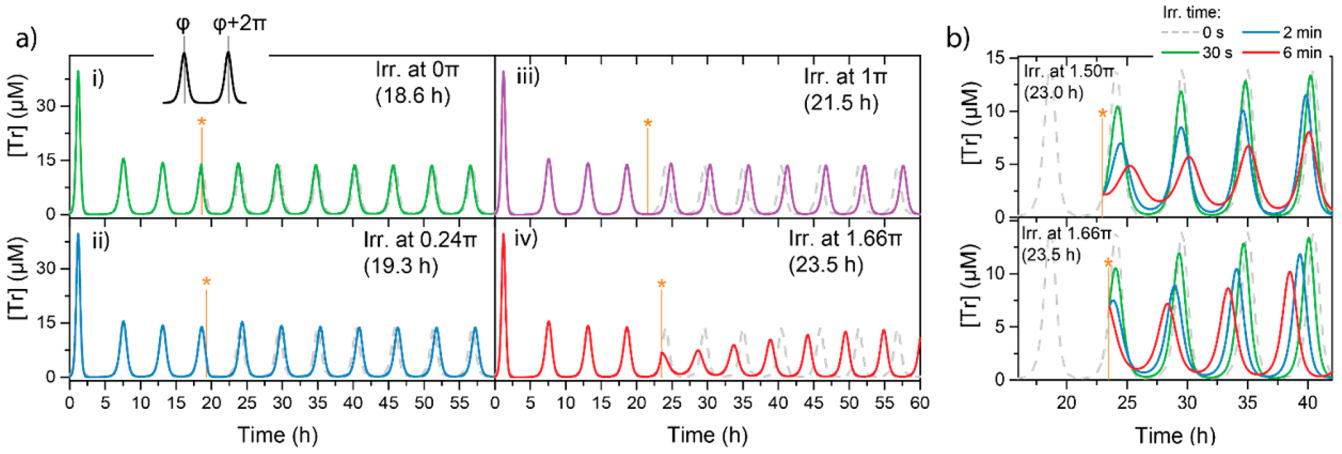

Figure 2. Modeling of a short single irradiation pulse (a) irradiation for 3 min at different phases of oscillations: 0, 0.24π, 1π, 1.66π, solid lines depict simulations with irradiation and dashed gray lines without irradiation, orange stars indicate a time point of irradiation. (b) Zoom with irradiations at 1.50π and 1.66π for different times. The conditions used for simulations: $[Tg]_0$ = 167 μM, $[Pro\text{-}I]_0$ = 1.5 mM, $[Ap]_0$ = 0.28 U/mL, $[Photo\text{-}I]_0$ = 100 μM, $[Tr]_0$ = 0.2 μM, k_f = 0.37 h^{-1}.

The photolabile inhibitor (Photo-I) consists of an inhibitor moiety coupled to a well-known methyl-6-nitroveratryloxycarbonyl (MeNVOC) photoprotection group,[20,21] further functionalized with poly ethylene glycol tails (PEG) to increase water solubility. The synthesis of Photo-I was completed in 5 steps; full experimental details and characterization can be found in the Supporting Information S2. Before introducing the Photo-I into the network, we studied the cleavage reaction as a function of UV irradiation. We first verified that Tr remains stable upon irradiation (Supporting Information S3.5). Next, we determined the apparent rate constant of the cleavage reaction, and found a cleavage rate constant of 33 h^{-1}. In comparison to the typical time scales of our oscillations (periods ~ 5 h), active inhibitor formation from the Photo-I can be considered an instantaneous process. Furthermore, the inhibition by the uncleaved photolabile inhibitor ($k_{inh}^{Photo\text{-}I}$ = 1.4 mM^{-1} h^{-1}) is roughly 40 times slower in comparison with inhibition by active inhibitor (k_{inh}^{I} = 52.7 mM^{-1} h^{-1}). The apparent inhibition rate constant of the uncleaved photoinhibitor is similar to other background inhibition reactions in the network (Supporting Information S3.4), and can therefore be treated as a background reaction. See Supporting Information S3 for full details on kinetic and spectral properties of Photo-I, including the photocleavage rate, background inhibition, background hydrolysis of the sulfonyl fluoride group, and absorbance spectra.

Simulations are required to establish the starting concentrations of Tg, Tr, and small molecules, as well as suitable flow rates and the importance of timing of irradiation. Therefore, we added the kinetic parameters of the photochemical production of inhibitor to the set of differential equations in MATLAB (see Supporting Information S4) that we previously used to simulate the dynamics of the network.[5] We simulated the response of the system to different concentrations of the Photo-I, as well as the duration and the precise timing of the irradiation. Figure 2a shows the simulated response of the network upon three min irradiation pulses at different phases (denoted here in radians from 0 to 2π) of the oscillation. We note that only upon irradiation during the rise in Tr concentration (around 1.66π; Figure 2a, iv), we see a marked damping of the amplitude followed by a slow recovery in the oscillations. In all other cases, the effect of a short pulse is quite similar and results mostly in a small delay. A closer look at irradiation around 1.66π (Figure 2b) shows that the system is rather sensitive to the precise timing of irradiation, as the sustained oscillations recover differently from perturbations at 1.50π and 1.66π.

First, we carried out a preliminary test of the photochemical switching of the inhibitor in the full network. The full network was studied experimentally in a flow reactor fed by four different syringes with Tg, Tr, Ap, combined ProInh and Photo-I. A computer-controlled circuit with three LEDs placed around the flow reactor was used to control length and timing of irradiations. The outflow of the reactor was coupled to a microfluidic chip that served as an online detection system where active [Tr] was quantified in flow by a fluorogenic assay based on benzoyl-L-arginine-7-amido-4-methylcoumarin (see Supporting Information S5 for details). As shown in Figure 3a, the network shows sustained oscillations without UV exposure, and complete inhibition of Tr during continuous irradiation of the flow reactor.

Next, we confirmed the effect observed in simulations by performing two experiments with exactly the same starting conditions and irradiated for 3 min at different phases of oscillation. Figure 3b shows an overlay of two irradiated experiments in comparison with nonirradiated oscillations. Although there are some fluctuations in the amplitude, we observed that irradiating the reactor during the initial rise in trypsin concentration, yielded a stronger perturbation, and a similar slow recovery of the amplitude as observed in the simulations shown in Figure 2b. Qualitatively, the phase dependence can be explained by considering the amount of active Tr present in the system (see also Figure 1a). When no active Tr is present, the formation of additional inhibitor will not have much impact. Similarly, when the oscillation is past its peak, the negative feedback loop has already started to outcompete the autocatalytic production of Tr, and hence the impact of additional inhibitor will be small. Therefore, the perturbation of the system will be largest during the (initial) rise in [Tr] (i.e., during the start of the autocatalytic positive feedback loop). A detailed, experimental study into the underlying mechanisms of the sensitivity of the system to the precise timing of irradiation is beyond the scope of this work.

In a next experiment, we studied the effect of irradiation on a network that operated outside the regime of sustained

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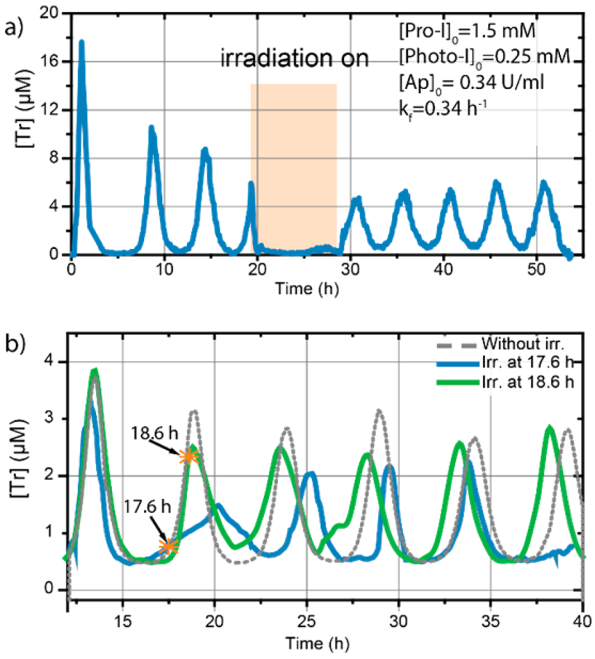

Figure 3. Dependency on time of irradiation. Three separate experiments performed with identical starting conditions, namely 1.4 mM Pro-I, 0.05 mM Photo-I, 0.34 U/mL Ap, k_f = 0.34 h^{-1}. Irradiation pulse was 3 min. The orange stars depict time of irradiation.

oscillations. External pulsing with light might "rescue" the functionality of the system. For example, it is possible to entrain a system that is in a regime of damped oscillations, by a series of irradiation pulses, to produce a forced oscillating system (Figure 4). Simulations indicate (see Supporting Information

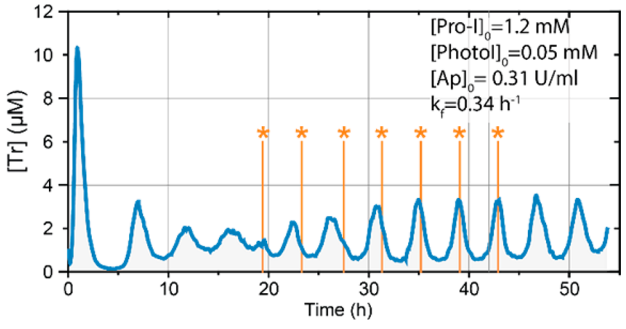

Figure 4. Demonstration of entrainment of oscillations. Experiment showing entrainment of damped oscillations by a series of 10 min irradiations.

S4.2) that irradiation during the rise in Inh oscillations, which are shifted by 0.5π phase from Tr oscillations, is most suitable if entrainment into sustained oscillations are desired. For the damped oscillations shown in Figure 4, there is insufficient active inhibitor to bring [Tr] back to baseline levels, leading to a steady state [Tr] of ~1.5 μM. Upon each irradiation, the additional active inhibitor lowers the active [Tr] to baseline levels, and subsequently the autocatalytic, positive feedback loop restarts. Remarkably, even after the irradiation pulses have terminated, the system is able to oscillate for some time more, before eventually reaching a damped state.

We have demonstrated how we can use light to modulate the output of the network. As explained earlier, future work will show the integration of multiple networks. To demonstrate how light-induced inhibition of Tr can be used in a control systems engineering approach in coupled reaction networks, we ran two oscillating networks (containing Photo-I) in separate reactors and then combined the outflow of the reactors in a Y-junction prior to analyzing the functional output of the combined set (Figure 5a). In Figure 5b, we started the two

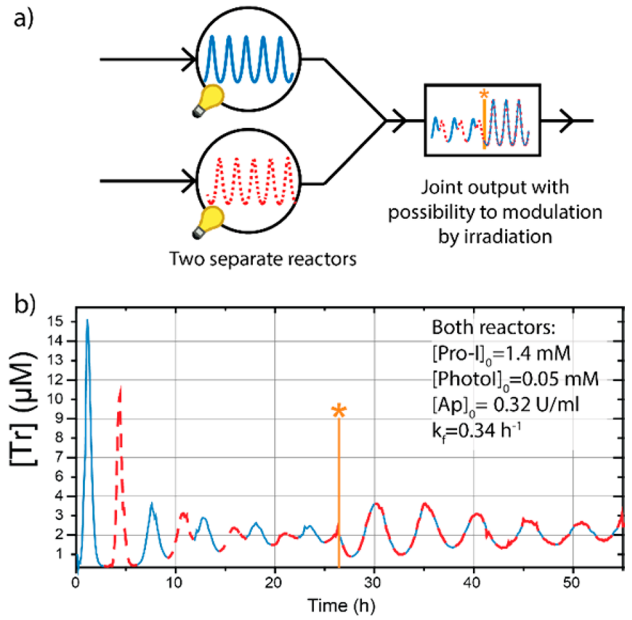

Figure 5. Phasing of two coupled oscillators. (a) Schematic representation of the experimental setup, (b) phasing of two oscillators by simultaneous irradiation of both reactor for 45 min.

oscillating reactions with a delay of 3 h, leading to a significant phase difference between the two reactors. The first part of Figure 5 shows the oscillations of both reactors combined, and one can clearly distinguish peaks from both oscillating reactors (reactor started first depicted as the solid blue line and second reactor as the dashed red line). By irradiating both reactors for 45 min, we flood both networks with active inhibitor and thus bring the system back to a state where the concentration of active Tr is minimal. Subsequently, both reactors "restart" with the positive feedback loop initiating the cycle, synchronizing the two reactors and producing a single oscillation in the combined output.

In summary, we have shown how a light-induced local perturbation of the network (i.e., we only affect concentration of a single component) can be used to gain external influence over its out-of-equilibrium function. Experimental realization of the photochemical control over the phase and amplitude of the oscillations shows strong similarity with simulations. As a first demonstration, we have shown how we can synchronize multiple oscillators, a useful feature for more integrated systems where the timing of different modules needs to be controlled. Our concept is general and we envision applications to other CRNs, whether they are based on small molecules,[4] synthetic gene networks,[22] or DNA PEN toolbox.[23] We have also observed subtle changes in the response of the network to short exposures to light at different phases of the oscillation. These results indicate that we might be able to study the dynamics of the network (robustness, resilience) using photocleavage of Photo-I as a probe.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.7b08109.

Synthesis data, kinetics, simulation info, flow experiment details (PDF)

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Notes

The authors declare no competing financial interest.

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