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A Rhythmic Assembly System with Fireflies' Function Based on Reversible Formation of Dynamic Covalent Bonds Driven by A pH Oscillator †

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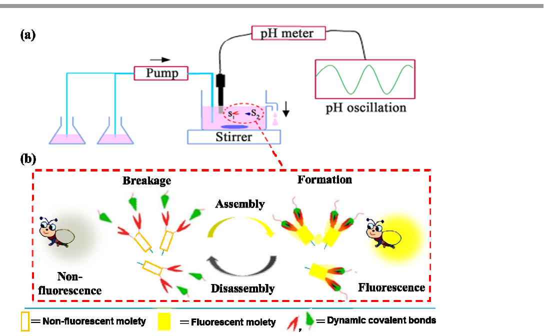
The paper proposed an approach to construct a novel kind of rhythmic assembly system with fireflies' function through combining a pH oscillator based on the hydrogen peroxide/dithionite system with pH-responsive dynamic covalent bonds composed by phenylboronic acid and alizarin red S.

Self-assembly generally refers to the process in which individual components spontaneously organize into ordered patterns or structures through various interactions. [1] According to the difference of molecular size, self-assembly can be classified into small-molecule self-assembly and polymer self-assembly. [2] With the development in the last several decades, intelligent assembly systems responsive to external stimuli, such as pH, temperature and light, have been extensively explored gradually. [3] Furthermore, as an efficient and useful tool for understanding exquisitely arranged biological processes, the study on supramolecular assembly with special functions, especially with biomimetic functions, has attracted more and more researchers' attention. [4] In general, the research on assembly systems with biomimetic functions includes three aspects: (a) providing new insights into the structure-function relationships of biomolecules at the molecular level; [5] (b) gaining self-assembly complexes similar to the structures of organisms through self-assembly process; [6] (c) gaining anticipative functions of self-assembly complexes similar to the functions of organisms through self-assembly process. [7]

However, in the current studies, the control of self-assembly systems is highly dependent on the "ON/OFF" switching of external stimuli. By learning from natural phenomena, such as heartbeat, biological rhythms, brain waves and cell cycles, Yoshida and co-workers have developed an innovative approach to create many attracting functionalities, [8] such as the autonomic intestine-like motion, worm-like motion, ciliary motion and pendulum motion, by utilizing Belousov-Zhabotinsky reaction (B-Z reaction), an oscillating chemical reaction. Our group has also reported novel kind of rhythmic self-assemble gels based on driven by a pH oscillating reaction. [9] These methods could be inferred that if an assembly process was coupled with an oscillating chemical reaction, rhythmic assembly behaviour without the "ON/OFF" switching of external stimuli could be realized. Accordingly, it also provides a new insight into the study on self-assembly with biomimetic functions.

Therefore, we herein attempt to build a firefly-inspired assembly system using an oscillating chemical reaction as a periodic stimulus. In this study, we selected a pH oscillator and pH-responsive dynamic covalent bonds as examples to construct this system (Scheme 1a). That was, we planned to find the pH-responsive dynamic covalent bonds which are fluorescent when formatting and non-fluorescent when breaking. Therefore, when the periodic pH change being provided by a pH oscillator is employed as the stimulus to trigger rhythmic reversible formation of the dynamic covalent bonds, the system would be expected to show periodically dim and fluorescent in the pH oscillator, which works in a similar fashion as observed in the body of the fireflies, as shown in Scheme 1b.



Scheme 1 The graphical schematic of the rhythmic assembly system with fireflies' function driven by a pH oscillator. (a) The experimental setup and (b) mechanism of the biomimetic fireflies' assembly system.

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To successfully fabricate the assembly system, two basic requirements must be carefully considered. One is the selection of the appropriate pH-responsive dynamic covalent bonds depicted in Scheme 1. The other is the selection of an appropriate pH oscillator that matches the pH change range of the corresponding dynamic covalent bonds. As one of the pH-responsive dynamic covalent bonds, phenylboronate ester bonds have been widely employed to fabricate pH-responsive assembly systems due to their reversibility under mild conditions. [10] Especially, because of the formation or breakage of the phenylboronate ester bond, the fluorescence intensity of the system composed of phenylboronic acid (PBA) and alizarin red S (ARS) shows a large increase while raising the pH from 4 to 7 and a dramatic drop-off in the pH range of 7-10. [11] More importantly, the reaction of PBA and ARS is so quick that approaching equilibrium within 5-10 seconds. The chemical reaction equation is shown in Fig. 1.

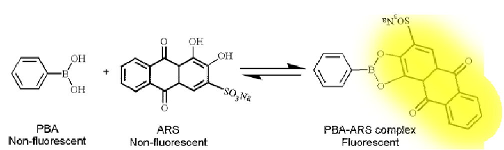

Fig. 1 The chemical structures and fluorescence properties of PBA, ARS and PBA-ARS complex

To match well with the pH response range of formation of PBA-ARS complex, a pH oscillator that allows for the breakage and formation of phenylboronate ester bond in PBA/ARS system within a given time period is required. The so-called hydrogen peroxide/dithionite (HPD) pH oscillator reported in the literature is a good choice. [12] As shown in Fig. 2a, the solution displayed continuous and cyclic pH changes with period of about 100 seconds and amplitude as large as 7 pH units ranging from 3 to 10. The detailed preparation procedure can be seen in the ESI†. In particular, the durations at high and low pH levels within an oscillatory period were sufficient to induce the formation and breakage of the phenylboronate ester bond, respectively. Such a perfect match between the dynamic covalent bond and HPD pH oscillator offered the opportunity to further study the self-assembly system with fireflies' function.

Once a stable pH oscillator appeared (Fig. 2a), the equal moles of PBA and ARS were put in the HPD pH oscillator immediately. The process was recorded by a Nikon COOLPIX S3000 digital camera (Video S1, ESI†). The fluorescence of the system at different time was shown in Fig. 2b. At first, the system, with a pH value of about 10, was non-fluorescent. At the time, the phenylboronate ester bonds had not formed (disassembly). With the decrease of the pH value as the HPD oscillating reaction progressed, the system became fluorescent gradually and gained the maximum intensity of fluorescence at around pH 7 after approximately 48 seconds. This indicated that PBA had bonded ARS (assembly). Then, the fluorescence intensity was gradually weaker in the pH value from 7 to 3 and gained the minimum at around pH 3 after approximately 55 seconds. This indicated that the phenylboronate ester bonds had broken (disassembly). Next, the fluorescence intensity become gradually stronger in the pH value from 3 to 7 and gained the maximum at around pH 7 after approximately 78 seconds. And the system was non-fluorescent once more at around pH 9 after approximately 83 seconds. Subsequently, the system experienced by the changes of non-fluorescence to fluorescence and then to non-fluorescence continuously.

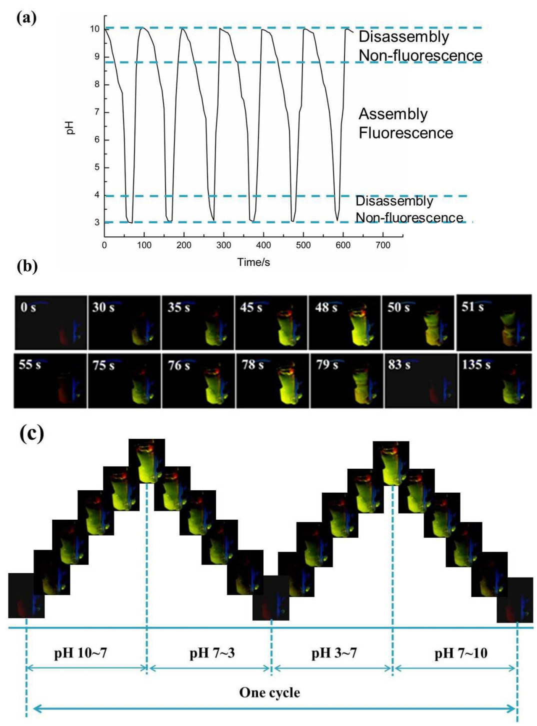

Fig. 2 The self-assembly system with fireflies' function with PBA and ARS driven by the HPD pH oscillating reaction. (a) pH-time curve of the HPD pH oscillator. (b) Snapshots of the system at different times. (c) Snapshots of the system at different pH value in one cycle.

It also can be seen in Fig. 2c. The system was fluorescent in neutral pH value and non-fluorescent with an overly basic or acidic. In addition, in each cycle of the pH oscillating reaction, the pH value changes from 10 to 3 then back to 10, meanwhile, the fluorescence intensity of the PBA-ARS system shows a large increase while the pH raising from 3 to 7 and a dramatic drop-off in the pH range of 7-10. As a result, there were two fluorescent oscillations in each period of pH oscillation. However, due to the decline of pH value slower than its growth in the HPD pH oscillator, the period of fluorescent oscillation was different in a pH oscillation cycle. Herein, the brightness in Colour Picker is used on behalf of the fluorescent intensity. The graphic curve of B of the system changing with time in the pH oscillator was shown in Fig. 3. In each period of the pH oscillator, the period of the first fluorescent oscillation was about 75 seconds and the period of the second fluorescent oscillation was about 27 seconds. Consequently, the self-assembly system with fireflies' function was fabricated while the reversible, autonomic and sustainable assembly/disassembly behaviour of the dynamic covalent bonds was clearly synchronized with changes in the pH value of the HPD pH oscillator.

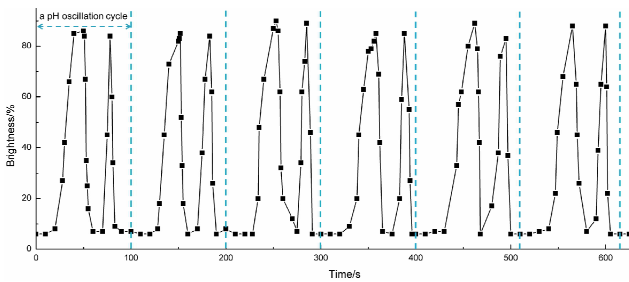

Fig. 3 The graphic curve of brightness of the system changing with time in the pH oscillator

The reason for the maximum intensity of fluorescence occurred at neutral pH is due to pH-dependent binding strength of the PBA-ARS complex. [13] And the graphical schematic is showed in Figure 4. It is known that the affinity of boronic acids with diols at low pH is small and the large increase in fluorescence while raising the pH from 3 to 7 is consistent with an increase in the binding constants in this pH range. At high pH (7-10), however, the results show a drop-off in intensity in the pH range of 7-10, especially when the pH is higher than the pKa of PBA (pKa=8.8). This indicates that the binding constants reach their maximum at around pH 7, and any further increase in pH results in a decrease of the binding affinity.

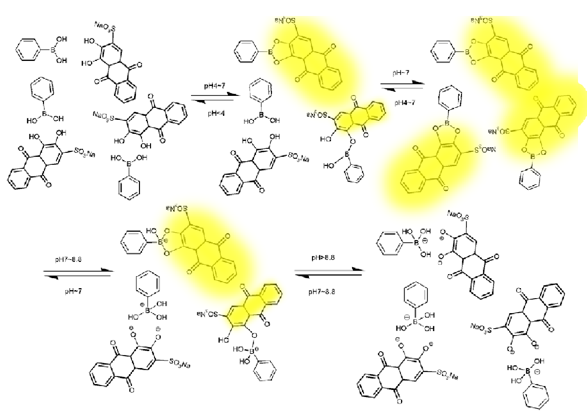

Fig. 4 The graphic mechanism of the self-assembly system with fireflies' function with PBA and ARS at different pH value.

In conclusion, a novel assembly system with fireflies' function by coupling dynamic covalent bonds with a pH oscillator has been developed. The system can exhibit reversible, autonomic and sustainable assembly/disassembly behaviour accompanying cyclic fluorescence/non-fluorescence oscillation, just like the function as observed in the body of the fireflies. Such the system is unlike conventional stimuli-responsive assembly systems controlled by the "ON/OFF" switching of external stimuli. This approach provides the opportunity to obtain smart self-oscillating materials and expands the self-assembly fields. But above all, the research provides a new insight into achieving the biomimetic materials. In addition, the system of polymer self-assembly with fireflies' function is studying.

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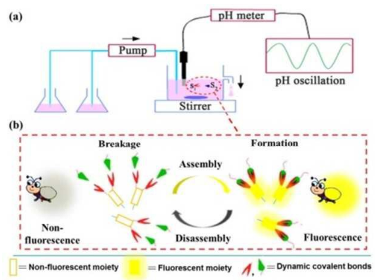

A rhythmic assembly system with fireflies' function driven by a pH oscillator was constructed through the reversible formation of dynamic covalent bonds