Development of novel self-oscillating molecular robot fueled by organic acid

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Abstract— In our previous study, we first succeeded in construction of a novel-types self-oscillting molecular robot. However, the driving environment of the molecular robot was firmly restricted in strong acid conditions. This is because the molecular robots drive induced by the Belousov-Zhabotinsky (BZ) reaction, which is well known for exhibiting temporal and spatiotemporal oscillating phenomena. The overall process of the BZ reaction is the oxidation of an organic substrate, such as malonic acid (MA) or citric acid, by an oxidizing agent (bromate ion) in the presence of a strong acid and a metal catalyst. In this study, in order to drive the novel molecular robot under the physiological condition, we conducted the modification of the molecular structure of the self-oscillating polymer chain. In order to cause the self-oscillation under the biological condition, we synthesized a built-in system where the BZ substrates other than organic acid were incorporated into the molecular robot itself. As a result, the novel molecular robot drives under the biological condition. We believe that the development of the novel molecular robot lead to construction of the novel biomimetic soft robots and actuators, and may inspire novel nonlinear experimental and theoretical considerations.

INTRODUCTION

Polymer actuators with stimulus-responsive nature are receiving significant interest for application to soft actuator, micro(nano)-machines and humanoid robots. The stimuli-responsive polymer systems have a unique nature of volume change in response to external stimuli such as a temperature , pH, electric field, light, etc [1-10]. Moreover, the polymer actuators have a lot of advantages such as the light weight, the flexibility and the low noise, etc. However, in order to operate stimulus-responsive polymer system as soft actuators, external control devices and complex inter connections are needed. On the other hand, living organism drives autonomously and spontaneously under the significant mild condition. As one of characteristic behaviors in living systems, autonomous oscillation, that is, spontaneous changes with temporal periodicity (called "temporal structure") such as heartbeat, brain waves, pulsatile secreton of hormone, cell cycle, biorhythm, etc. can be exemplified. So far, the stimulus-responsive polymer systems have been extensively investigated. However, polymer systems undergoing self-oscillation under constant condition without any on-off switching of external stimuli are still insufficient.
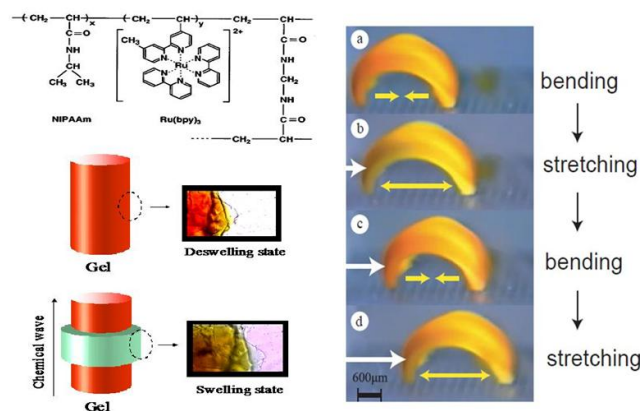

Figure 1. Self-walking gel that consists of self-oscillating molecular robot.

If such autonomous polymer systems like living organism acting under biological condition can be realized by using completely synthetic polymers, unprecedented biomimetic robots will be created.

In this study, in order to realize the autonomous polymer system by tailor-made molecular design, we investigated the biomimetic molecular robots that cause the self-oscillation under biological condition. In oreder to cause the self-oscillation under constant condition, we focused on the Belousov-Zhabotinsky (BZ) reaction, which is well known for exhibiting temporal and spatiotemporal oscillating phenomena [11-15]. The BZ reaction is often analogically compared with the TCA cycle (Krebs cycle), which is a key metabolic process taking place in the living body. The overall process of the BZ reaction is the oxidation of an organic substrate, such as malonic acid (MA) or citric acid, by an oxidizing agent (bromate ion) in the presence of a strong acid and a metal catalyst. In the course of the reaction, the catalyst undergoes spontaneous redox oscillation.

For utilizing the chemical energy of the BZ reaction as a driving source generating mechanical oscillation of polymer chains, [Ru(bipyridine)2(4-vinyl-4 '-methyl bipyridine)] $(Ru(bpy)_3)$ as the catalyst was covalently bonded to poly(Nisopropylacrylamide) (poly(NIPAAm)) or their crosslinked network (i.e., gel). The hydrophilicity of the polymer chain periodically changes synchronized with the redox changes of the $Ru(bpy)_3$ moiety. As a result,

autonomous and spontaneous the aggregation-disaggregation self-oscillations for polymer chains and swelling-deswelling self-oscillations for gels were realized under the coexistence of the three BZ substrates (MA, acid and oxidant) other than the catalyst, respectively. By utilizing the novel self-oscillating polymer gel, we first succeeded in construction of a novel-type self-walking gel robot [16] (See Figure 1). However, for the novel molecular robot, the operating condition was limited to non-physiological environment where the strong acid and the oxidant coexist. For extending application fields, more sophisticated molecular design to drive self-oscillating molecular robots under physiological condition is needed.

In this report, in order to operate autonomous molecular system under the biological condition, we attempted to construct the built-in system where the BZ substrates other than organic substrate are incorporated into the polymer chain. As the first step for the purpose, acrylamide-2-methylpropane sulfonic acid (AMPS) was incorporated into the poly(NIPAAm-*co*-Ru(bpy)$_3$) chain as a pH control site (See Figure 2). Furthermore, as the second step, methacrylamidopropyltrimethylammonium chloride (MAPTAC) with a positively charged group was incorporated into the poly(NIPAAm-*co*-Ru(bpy)$_3$) as a capture site for an anionic oxidizing agent (bromate ion) (See Figure 4). The bromate ion was introduced into the MAPTAC-containing polymer chain through the ion-exchange process. For providing the pH-control site or the oxidant-supply site into the poly(NIPAAm-*co*-Ru(bpy)$_3$), the aggregation-disaggregation self-oscillation for the polymer solutions were archived under the acid-free and oxidant-free condition, respectively. As the final step for the accomplishment of this purpose, we synthesized the novel polymer chain which was introduced both the pH control and oxidant supply sites in the poly(NIPAAm-*co*-Ru(bpy)$_3$) at the same time (See Figure 6). As a result, we first succeeded in causing the aggregation-disaggregation self-oscillation under the biological condition in which only one BZ substrate (malonic acid) existed.

I. EXPERIMENTAL SECTION

A. Polymerization

A-1 Synthesis of poly(NIPAAm-co-Ru(bpy)₃-co-AMPS).

Using NIPAAm (2.0 g), AMPS (7.0 g), Ru(bpy)$_3$ monomer (1.0 g) and 2,2'-azobisisobutyronitrile (AIBN) (0.13 g) as an initiator, poly(NIPAAm-*co*-Ru(bpy)$_3$-*co*-AMPS) (Figure 2) was synthesized by radical polymerization in a mixture of methanol (32 g) and water (8 g) under a total monomer concentration of 20 wt%. The polymerization was carried out at 60°C for 24 h *in vacuo*. The resulting reaction mixture was dialyzed against water for 4 days followed by methanol for 3 days, and then freeze-dried.

A-2 Synthesis of poly(NIPAAm-co-Ru(bpy)₃-co-MAPTAC).

Using NIPAAm (2.5 g), MAPTAC (6.5 g), Ru(bpy)$_3$ monomer (1.0 g), and AIBN (0.35 g) as an initiator, poly(NIPAAm-*co*-Ru(bpy)$_3$-*co*-MAPTAC) (Figure 4) was synthesized by radical polymerization in methanol (40 g). The polymerization was carried out at 60°C for 24 h in vacuo. The resulting reaction mixture was dialyzed against methanol for 3 days and then water for 4 days. For exchanging the counter ion, the polymer was dissolved in NaBrO$_3$ aqueous solution (1 M) and dialyzed against pure water for 30 days with repeating exchanging the water to remove excess Na$^+$ and BrO$_3^-$ ions, and then freeze-dried.

A-3 Synthesis of poly(NIPAAm-co-Ru(bpy)₃-co-AMPS-co-MAPTAC).

The poly(NIPAAm-*co*-Ru(bpy)$_3$-*co*-AMPS-*co*-MAPTAC) (Figure 6) was synthesized by radical polymerization in a methanol (31.80g) and water (31.80g) under a total monomer concentration of 20 wt%, using NIPAAm (1.20g), Ru(bpy)$_3$ monomer (1.28 g), AMPS (13.05g) and MAPTAC(0.47g) and 2,2'-azobis(2-methylbutyronitrile)isobutyronitrile (V-59) (0.41 g) as an initiator The polymerization was carried out at 80 °C for 24 h in vacuo. The resulting reaction mixture was dialyzed against methanol for 3 days and then water for 4 days. For exchanging the counter ion, the polymer was dissolved in NaBrO$_3$ (1M) and NaBr (0.5M) aqueous solution and dialyzed against pure water for 15 days with repeating exchanging the water to remove excess Na$^+$, Br$^-$ and BrO$_3^-$ ions. Moreover, the counter ion in the AMPS site was exchanged Na$^+$ for H$^+$ using ion-exchange resin, and then freeze-dried.

B. LCST measurements

The lower critical solution temperature (LCST) of the self-oscillating polymer solution was measured under the reduced and oxidized states by utilizing Ce(SO$_4$)$_2$ as an oxidizing agent and Ce$_2$(SO$_4$)$_3$ as a reducing agent, respectively. The polymer solutions were prepared by dissolving the polymer chain in an aqueous solution, respectively. LCST measurements were carried out with a spectrophotometer (Shimazu, Model UV-2500) equipped with a thermostatic controller and magnetic stirrers. The transmittance (%) of the polymer solution at 570 nm was then recorded by raising the temperature at a rate of 0.5 °C /min.

C. Measurement of transmittance self-oscillations.

The self-oscillating polymer solutions were prepared by dissolving the polymer into an aqueous solution. The transmittance self-oscillations for the polymer solutions were measured under constant temperature and stirring condition. In order to detect the transmittance change which is based on the autonomous aggregation-disaggregation change, 570-nm wavelength was used because it is the isosbestic point for the reduced and oxidized states of the Ru(bpy)$_3$. The time course of the transmittance at 570 nm was monitored by a spectrophotometer.

II. RESULT AND DISCUSSION

First step for the construction of the novel molecular robot: Experimental results for the poly(NIPAAm-co-Ru(bpy)₃-co-AMPS) that cause the self-oscillation under the acid-free condition.

In the oxidized state, poly(NIPAAm-*co*-Ru(bpy)$_3$-*co*-AMPS) have no LCST for any of polymer concentrations. This is because the polymer chain in the oxidized state contains the strongly hydrophilic oxidized Ru(bpy)$_3^{3+}$ part, in addition to the hydrophilic anionic charged AMPS component. These two strongly hydrophilic components of the polymer chain prevent the detachment of water molecules even at high temperatures. As a consequence, there were no LCSTs in the oxidized state in the all polymer concentrations. Generally, this phenomenon is observed in polyelectrolyte polymer chains which consist of the NIPAAm component. This tendency becomes remarkable with the increasing content of the ion-charged component in the polymer chain. On the other hand, the LCST of the AMPS-containing polymer chain was 41 °C (1.25 wt%), 32 °C (2.0 wt%), 28 °C (2.75 wt%) and 23 °C (3.5 wt%). The LCST decreased with increasing the polymer concentration. The reduced Ru(bpy)$_3$ in the polymer chain is extremely hydrophobic, because the bipyridine ligands surrounding the Ru ion exert a greater influence on the solubility of the polymer chain as compared with the ionization effect of the Ru ion. In our previous study [17], we observed that, as the Ru(bpy)$_3$ content increased, the sharpness of the change in the transmittance with the temperature became duller in the reduced state, due to the hydrophobic interaction between the polymer chains.

Figure 3 shows the self-oscillating transmittance change for these polymer solutions at three constant temperatures (12 and 15°C). The autonomous transmittance self-oscillation was attributed to the different solubility of the polymer chain in the reduced and oxidized states. Only in the 0.5 wt% polymer solution, the self-oscillating behavior was not observed at any of these temperatures. As shown in Figure 3, the waveforms of the transmittance self-oscillation were remarkably influenced by the temperature.
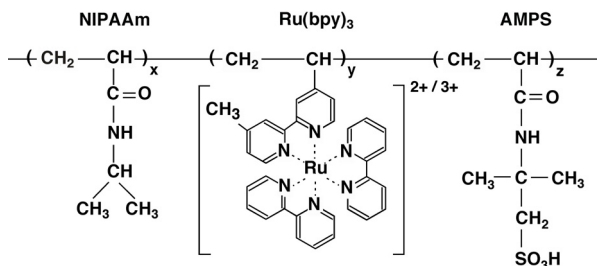

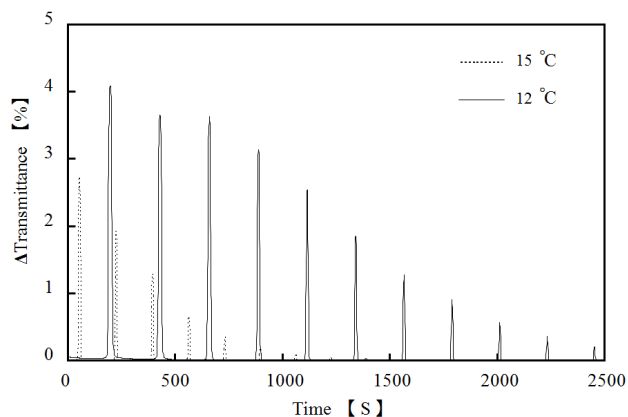Figure 2. The chemical structure of poly(NIPAAm-*co*-Ru(bpy)$_3$-*co*-AMPS).

Figure 3. Oscillating profiles of the optical transmittance for the poly(NIPAAm-*co*-Ru(bby)$_3$-*co*-AMPS) solutions at constant temperatures.

Aggregation-disaggregation self-oscillations of the AMPS-containing polymer solution caused damping, that is, the amplitude of the transmittance self-oscillation decreased with time. This phenomenon is originating in the increase in the size of the polymer aggregation in the self-oscillating behavior. Once the attenuation of the transmittance self-oscillation starts, the decreased amplitude of the self-oscillation never recovers in the self-oscillating behavior because the polymer aggregation state is thermodynamically more stable for the polymer solution.

Second step for the construction of the novel molecular robot: Experimental results for the poly(NIPAAm-co-Ru(bpy)₃-co-MAPTAC) that cause the self-oscillation under the oxidant-free condition.

In the reduced state, the LCST of the poly(NIPAAm-*co*-Ru(bpy)$_3$-*co*-MAPTAC) was 55 °C. This LCST is higher than that of the poly(NIPAAm) solution due to the hydrophilic effect of the MAPTAC component. On the other hand, in the oxidized state, the LCST disappeared because the polymer becomes highly hydrophilic.

Figure 5 shows the self-oscillating profiles of the MAPTAC-containing polymer solution at several temperatures under coexistence of only two BZ substrates (malonic acid and sulfuric acid). Since the polymer supplies the oxidizing agent (BrO$_3^-$) by itself as a counter ion of MAPTAC site, the self-oscillation was achieved without adding oxidizing agent. That is, the aggregation-disaggregation self-oscillation caused under oxidant-free conditions. In order to cause the self-oscillation, enough amount of BrO$_3^-$ is necessary. Therefore, the self-oscillation was not observed when the polymer concentration was below 5.0 wt%. Moreover, since the poly(NIPAAm-*co*-Ru(bpy)$_3$-*co*-MAPTAC) has the LCST around 45 °C in the reduced state, the polymer has an advantage to cause self-oscillation around body temperature as shown in Figure 5.
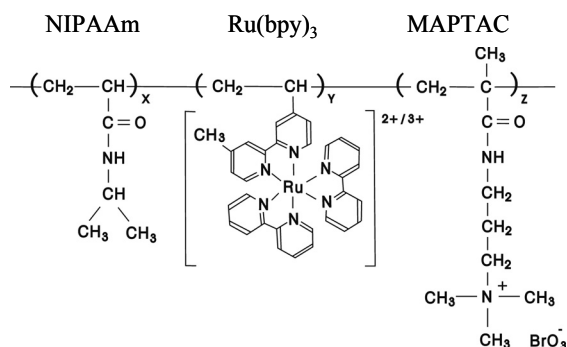
NIPAAm   Ru(bpy)₃   MAPTAC
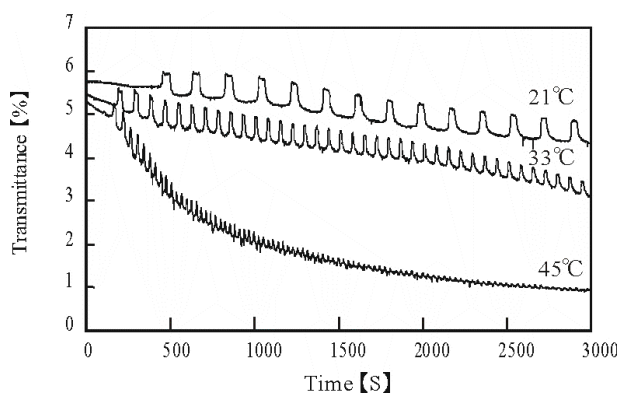

Figure 4. Chemical structure of

Figure 5. Oscillating profiles of optical transmittance for
poly(NIPAAm-*co*-Ru(bpy)₃-*co*-MAPTAC) solutions at several
constant temperatures.

This characteristic is significantly important for applications to biomimetic soft robot and soft actuator such as artificial muscle, etc. Further, it is a special tendency for the MAPTAC-containing polymer that the self-oscillation continues for a longer time compared to the other polymers. For example, the self-oscillation lasted more than ten hours at 21°C. The longer duration of the self-oscillation is also attributed to the higher LCST of the polymer chain to avoid aggregation due to intermolecular hydrophobic interaction at reduced state. Further, the polymer has no anionic site differently from poly(NIPAAm-*co*-Ru(bpy)₃-*co*-AMPS), which also acts to prevent aggregation.As the temperature approaches to 45°C (the LCST in reduced state), the self-oscillation gradually damped as the reaction progress as shown in Figure 5. The polymer chains in the reduced state easily aggregate around the LCST. Once the polymer aggregates, they could not be easily disaggregated even in the oxidized state. This characteristic leads to the damping self-oscillating transmittance change.

Final step for the construction of the novel molecular robot: Experimental results for the poly(NIPAAm-co-Ru(bpy)₃-co-AMPS-co-MAPTAC) that cause the self-oscillation under the biological condition.

Figure 7 shows the aggregation-disaggregation self-oscillation of the poly(NIPAAm-*co*-Ru(bpy)₃-*co*-AMPS

-*co*-MAPTAC) (see Figure 6) solutions at the constant temperature under coexistence of only one bio-related BZ substrate (malonic acid), which is the biological condition. The novel polymer chain supplied H^+, BrO_3^- and Br^- ions by itself as a counter ion from the AMPS, the MAPTAC and Ru(bpy)₃ site, respectively. In order to cause self-oscillation induced by the BZ reaction under the biological condition, the enough amounts of H^+, BrO_3^- and Br^- ion for the BZ reaction was needed. Therefore, no self-oscillation for the novel polymer solution was observed in the concentration below 6.5wt%. The aggregation-disaggregation self-oscillation for the novel polymer solution was attributed to the different solubilities between in the reduced and oxidized state. As shown in Figure 7, the waveforms of the transmittance was gradually damping with time due to construction of the aggregation among the polymer chains. In the first step of this study, the damping behaviors of the self-oscillation for the poly(NIPAAm-*co*-Ru(bpy)₃-*co*-AMPS) solution were observed. The AMPS-containing polymer chain has the betain type polymer structure, which indicated that it has both anionic and cationic site in single polymer chain at same time. This structure attracts the intra- and inter-molecular interaction due to the electrostatic interactions between the cationic and anionic site in the polymer chain. Therefore, the novel polymer solution caused damping as well as the AMPS-containing polymer solution (See Figure 3).
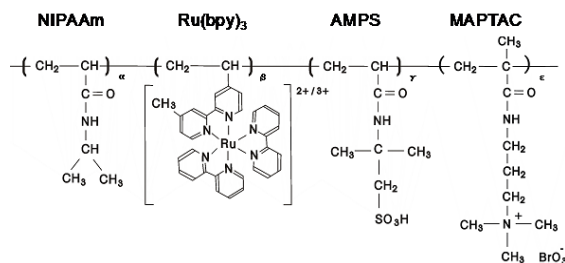

Figure 6. Chemical structure of
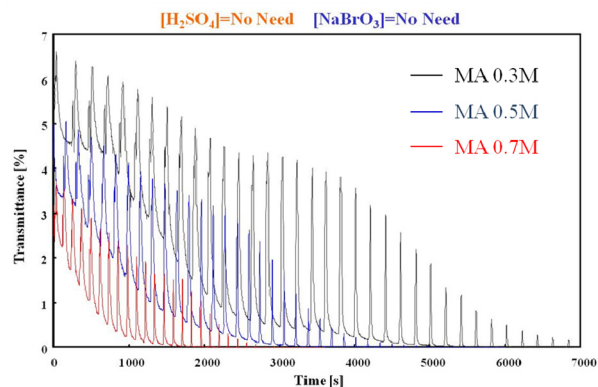poly(NIPAAm-*co*-Ru(bpy)₃-*co*-AMPS-*co*-MAPTAC)

Figure 7. Oscillating profiles of the optical transmittance for the
poly(NIPAAm-*co*-Ru(bpy)₃-*co*-AMPS-*co*-MAPTAC) solutions at
12°C for several concentrations of malonic acid ([MA]=0.3M, 0.5M,
and 0.7M).

III. Conclusion

In this study, we first succeeded in construction of the novel molecular robot that causes the self-oscillation under the physiological condition. The novel polymer chain was incorporated into the metal catalyst of the BZ reaction, the pH-control (AMPS) and the oxidant-supply (MATPAC) sites at the same time. The aggregation-disaggregation self-oscillation for the novel molecular robot was attributed to the different solubilities between in the reduced and oxidized state. Moreover, we clarified that the waveforms of the transmittance self-oscillation was gradually damping with time due to construction of the aggregation among the polymer chains as the same manner of the AMPS containing polymer chain.

References

[1] S. Maeda, Y. Hara, T. Sakai, R. Yoshida and S. Hashimoto, "Self-walking gel", Adv. Mater., 2007, 19, pp.3480-3484.

[2] S. Maeda, Y. Hara, R. Yoshida and S. Hashimoto : "Peristaltic motion of polymer gels", Angew. Chem. Int. Ed., 47, 2008, pp. 6690-6693.

[3] T. Fukushima, K. Asaka, A. Kosaka and T. Aida, "Fully plastic actuator by layer-by-layer casting using bucky gel", Angew. Chem. Int. Ed., 2005, 44, pp.2410-2413.

[4] T. Tanaka, "Collapse of gels and critical endpoint", Phys. Rev. Lett., 1978, 40, pp.820-823.

[5] R. Yoshida, K. Uchida, Y. Kaneko, K. Sakai, A. Kikuchi, Y. Sakurai, and T. Okano, "Comb-type grafted hydrogels with rapid de-swelling response to temperature changes", *Nature* 374, 1995, pp.240-242.

[6] Y. Yu, M. Nakano and T. Ikeda, "Directed bending of a polymer film by light", *Nature* 425, 2003, pp.145.

[7] T. Tanaka,"gels", *Sci. Am.* 244, 1981, pp.110-116.

[8] F. Llmain, T. Tanaka, E. Kokufta, "Volume transition in a gel driven by hydrogen bond", Nature 349, 1991, pp.400-401.

[9] A. Suzuki, T. Tanaka, "Phase taransition in polymer gels induced by visible r light", Nature 346, 1990, pp. 345-347.

[10] Y. Hirokawa, T. Tanaka, "Volume phase transition in a nonionic gel", J. Chem. Phys. 81, 1984 pp.6379-6380.

[11] A. N. Zaikin, A.M.Zhabotinsky, "Concentration Wave Propagation in Two-dimensional Liquid-phase Self-oscillating system", *Nature* 1970, 225, pp.535-537.

[12] Reusser, E. J.; Field, R. J.; "The transition from phase waves to trigger waves in a model of the Zhabotinskii reaction", *J. Am. Chem. Soc.* 1979, 101, pp.1063-1071.

[13] Field, R. J.; Noyes, R. M.; "Oscillations in chemical systems. IV. Limit cycle behavior in a model of a real chemical reaction", *J. Chem. Phys.* 1974, 60, pp.1877-1884.

[14] Gyorgyi, L. ; Turanyi, T. ; Field, R. J.; " Mechanistic details of the oscillatory Belousov-Zhabotinskii reaction", *J. Chem. Phys.* 1990, 94, pp.7162-7170.

[15] Turanyi, T. ; Gyorgyi, L. ; Field, R. J.; "Analysis and simplification of the GTF model of the Belousov-Zhabotinsky reaction", *J. Chem. Phys.* 1993, 97, pp.1931-1941.

[16] S. Maeda, Y. Hara, R. Yoshida, S. Hashimoto : " Chemical robot -Design of self-walking gel-", Proc.   IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS 2007), 2007, pp. 2150-2155.

[17] R. Yoshida, T. Sakai, S. Ito and T. Yamaguchi : "Self-oscillation of polymer chains with rhythmical soluble-insoluble changes", J. Am. Chem. Soc., 2002, 124, pp.8573-8578.