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Nonlinear Self-Excited Oscillation of a Synthetic Ion-Channel-Inspired Membrane

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Nonlinear phenomena are crucially important in future science and engineering. Some researchers have achieved nonlinear oscillations such as the Belousov–Zhabotinskii reaction,^[1] chemical reactions on catalysts,^[2] rhythmic swelling phenomena of hydrogels,^[3] permeation phenomena of membranes,^[4,11] swinging motions of lipids,^[5] fluorescence oscillation of quantum dots,^[6] synthetic metabolators,^[7] and rhythmic drug-delivery systems.^[8] We have developed a synthetic ion-channel-inspired biomembrane, which we call a molecular recognition ion-gating membrane.^[9] This membrane could control both hydrostatic pressure-driven flow^[9b,c] and osmotic pressure^[9a] in response to Ba^{2+} ions. Herein, we report that our gating membrane generated nonlinear self-excited oscillation in response to Ba^{2+} ions (see Figure 1) by combining these two functions of the membrane.

The response mechanism of the gating membrane is described as follows. A copolymer of *N*-isopropylacrylamide (NIPAM) and benzo[18]crown-6-acrylamide (BCAm) was grafted onto the pore surface of the porous substrate membrane. Poly(NIPAM) is a thermosensitive polymer,^[10a] which acts as an actuator of pore opening and closing through its volume phase transition. BCAM^[10b] has a crown ether receptor, which works as an ion sensor. Thus, the membrane forms a complex with Ba^{2+} , which has a high complex formation constant with crown ether receptors and causes its grafted copolymer to swell. This swelling controls the pore size and hydrostatic flow.^[9b,c] Furthermore, the complexed Ba^{2+} ions work as fixed membrane charges, which generate Donnan potential and osmotic pressure.^[9a,c]

The present oscillator was inspired by the so-called Teorell oscillator,^[11] in which hydrostatic and electroosmotic pressure-driven flow pass back and forth through a negatively charged, glassy, porous membrane, driven by both a constant ion concentration gradient and a constant electric current. Consequently, the oscillator (Figure 1) has a solution chamber and a solvent chamber, with the membrane placed between these two chambers. A capillary was attached to the solution chamber, which was filled with an aqueous ionic solution.

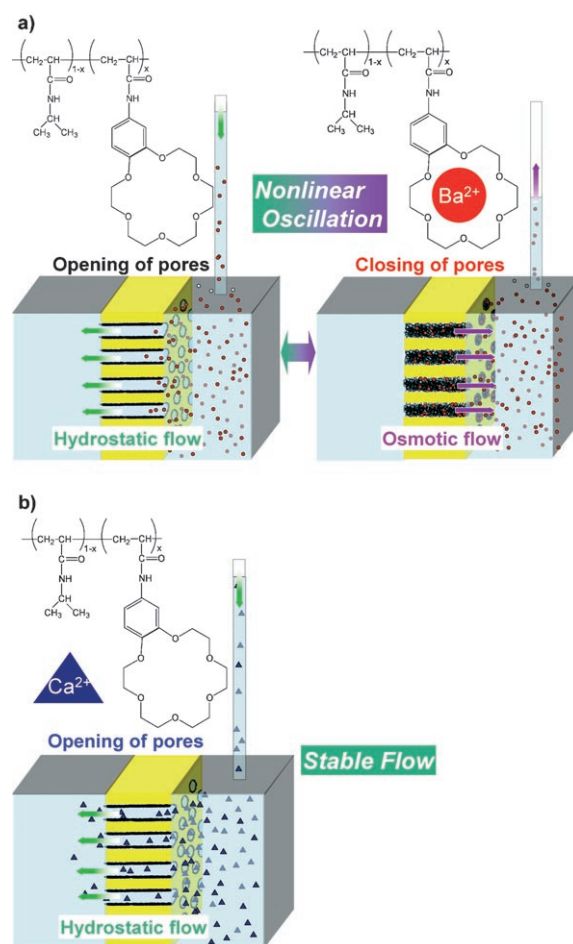


Figure 1. The concept of a nonlinear self-excited oscillator using a molecular recognition ion-gating membrane in response to a specific ion signal. a) The case of aqueous BaCl_2 solution. b) The case of aqueous CaCl_2 solution. A trace amount of free crown ether was loaded inside the membrane before experimentation.

Thus, the resulting osmotic inflow from the solvent chamber increased the water level in the capillary. In the case of Ba^{2+} , the ions enter the membrane with the hydrostatic flow, and the membrane closes its pores and stops the hydrostatic flow. At the same time, the water level in the capillary decreases. After the pores close, the osmotic flow generated by the Donnan potential pours into the membrane in the opposite direction to the hydrostatic flow. This osmotic flow eliminates the complexed ions from the membrane; simultaneously, the water level in the capillary gradually increases. When the captured ions are fully eliminated, the pores of the membrane reopen and the hydrostatic flow reenters the membrane. This cycle repeats without external stimuli; therefore, a nonlinear self-excited oscillation occurs. In comparison, other ions such as Ca^{2+} do not form complexes with the membrane. Consequently, the membrane does not generate an osmotic flow, and oscillation does not occur.

The membrane was prepared by a peroxide plasma graft polymerization method, as described in an earlier publication^[2b] and the Supporting Information. SEM images of the membrane prepared in the present study are shown in Figure 2. Nanosized, ionic-stimulus-responsive pores were

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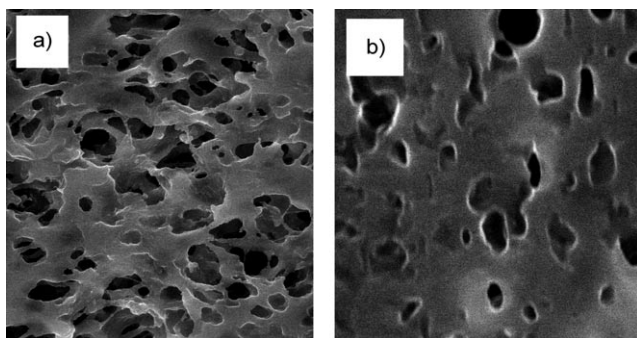


Figure 2. SEM images of membrane surfaces a) before plasma graft polymerization, b) after plasma graft polymerization.

formed successfully. Then, filtration experiments were performed using previously reported methods.^[9b,c] Osmotic flow was measured without attaching the capillary. The concentration of Ba^{2+} ions was increased from 0 to 100 mM in both filtration experiments and osmotic flow measurements. Finally, oscillation experiments were performed using a pressure sensor (Keyence, AP-12A) to measure the hydraulic pressure resulting from changes in the water level, which was recorded by a personal computer at intervals of 1 s. The cross-sectional area of the attached capillary was 0.024 cm^2 . The solvent chamber and the solution chamber were filled with pure water and aqueous BaCl_2 , CaCl_2 , or KCl solution of different concentrations between 0 and 100 mM, respectively. In some of the experiments, the membrane was immersed in aqueous benzo[18]crown-6 (10 mM) and then washed thoroughly with pure water. The temperature was maintained at 41°C in all the experiments. If a fluctuation of the water level was observed, it meant that the intended nonlinear oscillation was obtained.

Figure 3 shows the dependence of both the hydrostatic flow (\blacksquare), measured by filtration experiments, and the osmotic flow (\circ) on BaCl_2 concentration. This relationship indicates that both the pore-opening and pore-closing states can exist, depending on the value of the hydrostatic pressure, when the

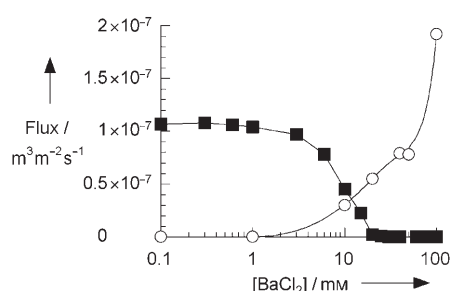


Figure 3. The hydrostatic and osmotic volume flux through the molecular recognition ion-gating membrane. The permeation volume fluxes through the membrane of aqueous BaCl_2 solutions of different concentrations were measured at different applied pressures at 41°C . At $\Delta P = 30 \text{ kPa}$ (306.1 cm H_2O) (\blacksquare), hydrostatic pressure permitted the flow from the ion-fed side into the membrane despite the closure of the pores of the membrane. In contrast, at $\Delta P = 0 \text{ kPa}$ (0 cm H_2O) (\circ), osmotic pressure generated the volume flux from the pure water side into the ion-fed side. Hence, the direction of volume flux depended on the applied hydrostatic pressure.

BaCl_2 concentration is around 20 mM. Thus, both hydrostatic and osmotic flow can be generated, and the water level of the attached capillary can increase or decrease. This finding indicates that the proposed oscillation phenomena can occur in this region.

Following from the above results, oscillation experiments were performed using aqueous BaCl_2 solutions with concentrations between 20 and 50 mM. However, the water level in the capillary increased monotonically. In the case of CaCl_2 , the water level decreased monotonically, and became constant at zero. As a result, the nonlinear self-excited oscillation did not occur.

To trigger the oscillation, we added a 10 mM solution of benzo[18]crown-6 to the pure water chamber. The oscillation occurred against 100 mM BaCl_2 solution. After this oscillation, the membrane was cleaned carefully with pure water several times; oscillation experiments against a 40 mM BaCl_2 solution were performed on the sixth, eighth, 25th, 26th, and 28th days after the addition of crown ether. Although only trace amounts of crown ether remained in the membrane because of the repeated washes in pure water at each time point, oscillations in water level occurred as shown in Figure 4. The oscillation disappeared on the 28th day. Over time, the water level gradually increased during the nonlinear oscillation. The increases in water level indicate osmotic flow resulting from both the closing of the pores and the Donnan potential, while the decreases in water level indicate hydrostatic flow resulting from opening of the pores. The continual switch of the water level shows that opening and closing of the pores occurred autonomously and alternately without external stimuli.

To focus on the oscillation periods, they were random. Although not uniform, the oscillations on the sixth day were relatively regular (see Figure 4a). The period of osmotic flow was always about 1100 s. That of hydrostatic flow was initially about 250 s; it then decreased to about 1 s with increasing water level. This result indicates that the pore-closing mode is slow and the pore-opening mode is very fast; therefore, this oscillation is classified as a relaxation oscillation, which consists of a fast mode and a slow mode. The amplitude of the oscillation was almost constant (see Figure 4a–d). The quantity of ions that the hydrostatic flow supplied to the membrane in one wave can be estimated as about $10.5 \mu\text{mol}$ from the average amplitude of 1.8 cm H_2O in Figure 4a. In contrast, the total amount of crown ether receptor that was contained in the membrane was $7.3 \mu\text{mol}$, as estimated from the amount of grafted poly(NIPAM-co-BCAm). These values almost coincide; hence, it is speculated that the amplitude was determined by the mass balance of membrane crown ether receptors and signal ions.

In contrast, the water level decreased monotonically in the case of CaCl_2 or KCl (Figure 5), because the pores were always open at 41°C and a concentration of 40 mM.^[9b,c] Only the complex formation constant of Ba^{2+} with crown ether receptors was high enough to generate osmotic pressure and close the pores.

The reproducibility of these phenomena is an especially important point in the field of nonlinear science. After the procedure described above, we reloaded the free crown ether and repeated the same experiments, and the same types of

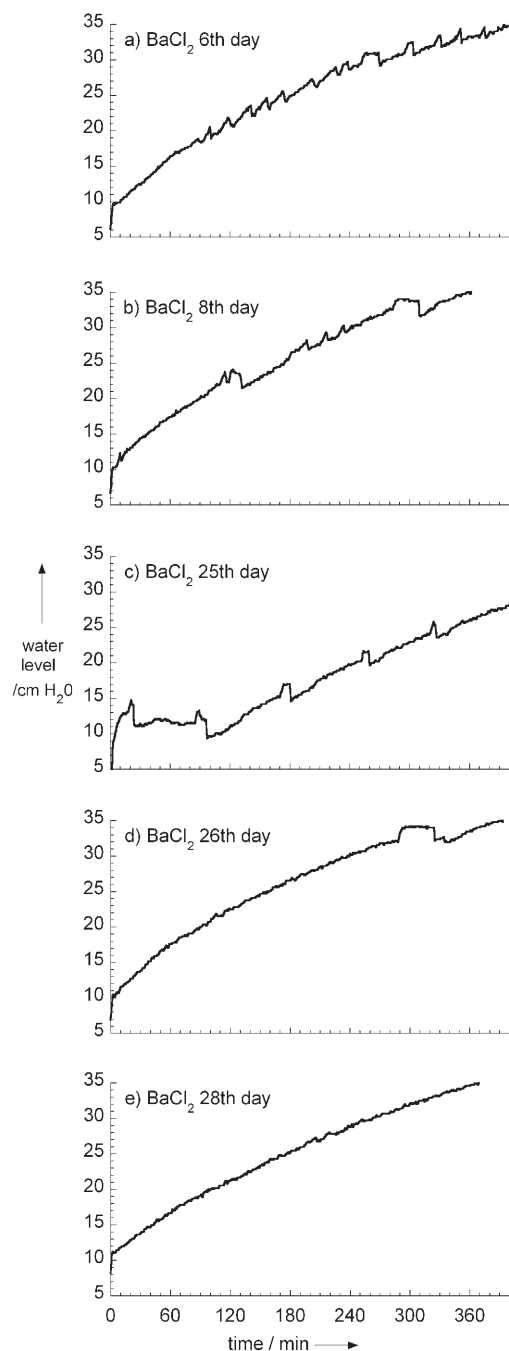


Figure 4. Nonlinear oscillations of the molecular recognition ion-gating membrane. The time course of the water level was measured at 41 °C. The concentration of BaCl₂ was 40 mM. The measurements were performed on the a) 6th day, b) 8th day, c) 25th day, d) 26th day, and e) 28th day after the addition of 10 mM crown ether.

oscillations were observed. However, we could not control the oscillation periods and the water level at which the oscillation started. The amplitude was the same as that seen in Figure 4. Consequently, these oscillations are thought to be very sensitive to the initial concentration distribution of the loaded crown ethers inside the gating membrane. We speculate that the loaded crown ethers were transported to the membrane by the osmotic flow, where they may have

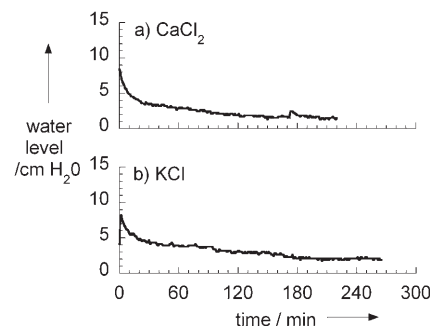


Figure 5. Monotonous decrease of the water level without oscillation measured at 41 °C. The concentration of a) CaCl₂ or b) KCl was 40 mM. The measurements were performed on the same day with the addition of 10 mM crown ether.

eliminated Ba²⁺ ions that were complexed with crown ether receptors in the grafted copolymer. The details of the oscillation mechanism are still unclear, so further research is ongoing.

In summary, the molecular recognition ion-gating membrane opened and closed its pores autonomously and alternately in response to Ba²⁺ ions; namely, the membrane generated a nonlinear self-excited oscillation like an ion channel of a biomembrane. This oscillation was a typical relaxation oscillation. By changing the receptor moiety of the grafted polymer,^[12] various gating membrane oscillation systems that respond to other molecular signals could be designed in the future. This type of oscillator is expected to lead to novel dynamic devices such as pulsatile drug-delivery systems, new affinity separation systems, microchips to handle information nonlinearly, and actuators that respond to various molecular signals.

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- [1] a) A. N. Zaikin, A. M. Zhabotinsky, *Nature* **1970**, 225, 535–537; b) G. Nicolis, I. Prigogine, *Self-Organization in Nonequilibrium Systems*, Wiley, New York, **1977**.
- [2] a) G. Ertl, *Science* **1991**, 254, 1750–1755; b) H. H. Rotermund, W. Engel, M. Kordesch, G. Ertl, *Nature* **1990**, 343, 355–357.
- [3] a) V. E. Shashoua, *Nature* **1967**, 215, 846–847; b) A. Katchalsky, R. Spangler, *Q. Rev. Biophys.* **1968**, 1, 127–175; c) Y. Osada, H. Okuzaki, H. Hori, *Nature* **1992**, 355, 242–244; d) R. Yoshida, H. Ichijo, T. Hakuta, T. Yamaguchi, *Macromol. Rapid Commun.* **1995**, 16, 305–310; e) R. Yoshida, T. Takahashi, T. Yamaguchi, H. Ichijo, *J. Am. Chem. Soc.* **1996**, 118, 5134–5135.
- [4] a) K. Yoshikawa, S. Maeda, H. Kawakami, *Ferroelectrics* **1988**, 86, 281–298; b) R. Larter, *Chem. Rev.* **1990**, 90, 355–381; c) R. P. Rastogi, R. C. Srivastava, S. N. Singh, *Chem. Rev.* **1993**, 93, 1945–1990; d) S. Kihara, K. Maeda, *Prog. Surf. Sci.* **1994**, 47, 1–54; e) N. Minoura, S. Aiba, Y. Fujiwara, *J. Am. Chem. Soc.* **1993**, 115, 5902–5906; f) A. Higuchi, M. Hara, *J. Phys. Chem.* **1996**, 100, 2183–2188.
- [5] S. M. Nomura, T. Harada, K. Yoshikawa, *Phys. Rev. Lett.* **2002**, 88, 093903.

- [6] A. Komoto, S. Maenosono, Y. Yamaguchi, *Langmuir* **2004**, *20*, 8916–8923.
- [7] a) E. Fung, W. W. Wong, J. K. Suen, T. Bulter, S. G. Lee, J. C. Liao, *Nature* **2005**, *435*, 118–122; b) D. Sprinzak, M. B. Elowitz, *Nature* **2005**, *438*, 443–448.
- [8] a) R. A. Siegel, C. G. Pitt, *J. Controlled Release* **1995**, *33*, 173–188; b) G. P. Misra, R. A. Siegel, *J. Controlled Release* **2002**, *81*, 1–6; c) A. P. Dhanarajan, G. P. Misra, R. A. Siegel, *J. Phys. Chem. A* **2002**, *106*, 8835–8838.
- [9] a) T. Ito, T. Yamaguchi, *J. Am. Chem. Soc.* **2004**, *126*, 6202–6203; b) T. Ito, T. Hioki, T. Yamaguchi, T. Shinbo, S. Nakao, S. Kimura, *J. Am. Chem. Soc.* **2002**, *124*, 7840–7846; c) T. Yamaguchi, T. Ito, T. Sato, T. Shinbo, S. Nakao, *J. Am. Chem. Soc.* **1999**, *121*, 4078–4079; d) S. Okajima, Y. Sakai, T. Yamaguchi, *Langmuir* **2005**, *21*, 4043–4049; e) H. Ohashi, N. Miyaoi, T. Yamaguchi, unpublished results.
- [10] a) Y. Hirokawa, T. Tanaka, *J. Chem. Phys.* **1984**, *81*, 6379–6380; b) M. Irie, Y. Misumi, T. Tanaka, *Polymer* **1993**, *34*, 4531–4535.
- [11] a) T. Teorell, *J. Gen. Physiol.* **1959**, *42*, 831–845; b) T. Teorell, *J. Gen. Physiol.* **1959**, *42*, 847–863; c) Y. Kobatake, H. Fujita, *J. Chem. Phys.* **1964**, *40*, 2212–2218; d) Y. Kobatake, H. Fujita, *J. Chem. Phys.* **1964**, *40*, 2219–2222; e) R. P. Rastogi, G. Srinivas, R. C. Srivastava, P. C. Pandey, A. P. Mishra, A. R. Singh, *J. Colloid Interface Sci.* **1995**, *175*, 262–275; f) R. P. Rastogi, G. P. Mishra, P. C. Pandey, K. Bala, K. Kumar, *J. Colloid Interface Sci.* **1999**, *217*, 275–287.
- [12] a) K. Sumaru, M. Kameda, T. Kanamori, T. Shinbo, *Macromolecules* **2004**, *37*, 4949–4955; b) A. Matsumoto, T. Kurata, D. Shiino, K. Kataoka, *Macromolecules* **2004**, *37*, 1502–1510; c) Z. R. Lu, P. Kopeckova, J. Kopecek, *Macromol. Biosci.* **2003**, *3*, 296–300; d) E. Kokufata, Y. Q. Zhang, T. Tanaka, *Nature* **1991**, *351*, 302–304.