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Ion Transportation by Prussian Blue Nanoparticles Embedded in a Giant Liposome

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A new type of artificial giant liposome incorporating ion transport channels and using nanoparticles of metal organic frameworks was demonstrated. The micropores of Prussian blue nanoparticles served as ion transport channels between the outer and inner phases of liposomes.

Giant unilamellar vesicles (GUV), called giant liposomes, have been studied actively to construct artificial cell-mimic systems because they have a closed bilayer structure with the same size of living cells.[1-4] Several materials have been introduced to control GUV functions. One typical function of lipid membranes is morphological changes in response to external stimuli. Photosensitive amphiphiles embedded in the bilayer can induce the transformation of GUV.[5-7] Some functional proteins to force membrane interfaces were also introduced inside GUVs.[8-10] Another function is the transport property for specific molecules across the membrane. The lipid bilayer, because of its insulating properties, does not allow electrolytes / ions to pass through the plasma membrane. Creating a hybrid liposome by incorporating the particular ion channels into the lipid bilayers has persisted as an important challenge. Artificial materials with good stability present opportunities to design new hybrid GUV systems to replace proteins that are sensitive to environmental changes such as heat.

To synthesize ion channels other than biological ion channels by peptides, Tabushi and coworkers reported using a cyclodextrin derivative in 1982.[11] Kobuke and coworkers demonstrated Na^+ and K^+ transport using abiotic glycolate ethers with dioctadexyldimethylammonium using a patch clamping method in 1992.[12] Synthetic ion channels using abiotic scaffolds have been developed by many researchers.[13-17] In 2001, Fyles and coworkers demonstrated synthetic ion channels from low-molecular-weight alkoxy-substituted isophthalic acid derivatives in planar and vesicle bilayer membranes without metal ions.[18] In recent years, embedding of nanosized metal-organic cages and metal-organic polyhedra into the liposome has been demonstrated.[19, 20]

The study described herein specifically examined a nanoparticle of metal-organic frameworks (MOFs) as an introducing material. Actually, MOFs have a three-dimensional regular structure with high stability.[21, 22] Some MOFs are anticipated for use as ion-conductive materials.[23, 24] Based on this concept, as a kind of MOF, we are inspired to examine Prussian blue nanoparticles (PB NPs) specifically, which reportedly have high ionic conductivities at room temperature.[25, 26] To produce hydrophobicity, we modified PB NPs (m-PB NPs) with long alkyl chain oleylamine. Interactions between the alkyl chain of m-PB NPs and hydrophobic core of GUVs are expected to facilitate incorporation of m-PB NPs into the lipid bilayer.

Figure 1 presents a schematic view of m-PB NPs-embedded hybrid liposomes (m-PB NP-liposome), in which the aqueous solution of pyranine dyes was enclosed in the lipid bilayer. Pyranine dye has been used widely as a fluorescent dye to measure intracellular pH change.[27] Because pH is a fundamental parameter that expresses the concentrations of H^+ and OH^- ions in the solution, the pH change in the inner phase of liposomes can be monitored using pH-sensitive fluorescent dye. The difference of changes in fluorescence intensities that occur with pH in m-PB NP-liposome and normal liposome can suggest their respective ion transport properties.

The m-PB NPs and their dispersion were prepared according to an earlier report.[28] Insoluble PB NPs were synthesized by a simple mixture of aqueous solutions of $Fe(NO_3)_3$ and $Na_4[Fe(CN)_6]$ (extra pure grades; Kanto Chemical Co Inc.). As-synthesized PB NPs (0.791g, 0.700 mmol) were stirred with water (1 mL) for 30 min. A chloroform solution (10 mL) of long alkyl chain oleylamine ($CH_3(CH_2)_7CH{=}CH(CH_2)_8NH_2$, 98.3 mg,

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† Electronic Supplementary Information (ESI) available: Materials, X-ray diffraction pattern of PBNPs, Ion transport check by fluorescence microscopy, and Chemical stability test for PBNPs. See DOI: 10.1039/x0xx00000x

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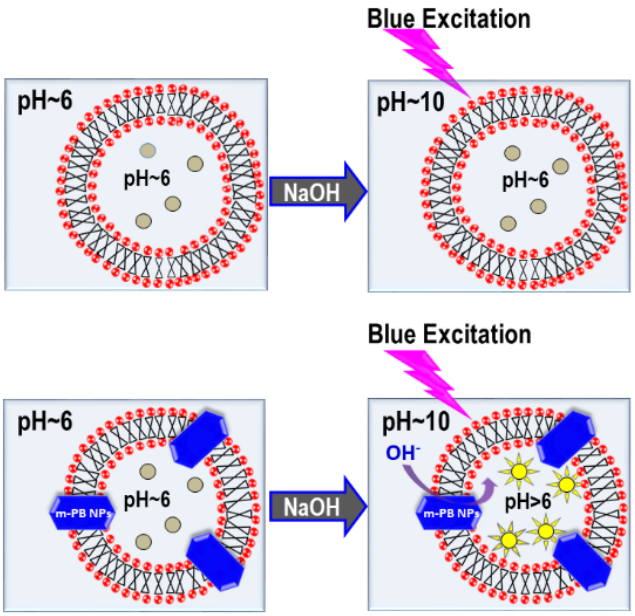

Fig. 1 Schematics showing the application of NaOH to pyranine-containing liposomes without (upper) and with (lower) PB NPs. Blue materials denote modified PB NPs. The blue arrow indicates the flow of OH^- ions. Gray and yellow spheres represent fluorescent pyranine dyes.

0.368 mmol, C18-content 80-90%; Acros Organics BVBA) was added to the PB suspension and was stirred for 1 h. After evaporation, blue powder was re-dispersed to chloroform (10 mL). The mixture was then centrifuged to remove large aggregations of PB NPs. Finally, we obtained the chloroform dispersion of m-PB NPs modified with oleylamine. The average particles of m-PB NPs with oleylamine were 23 nm in diameter, as determined by dynamic light scattering measurements.

Liposomes were prepared using the natural swelling method. A chloroform solution of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC, 6 mM, 13.4 µL) and a methanol solution of glucose (10 mM, 40 µL) were mixed in a glass tube for preparing liposome without m-PB NPs. A chloroform solution of m-PB NPs (50 or 130 µM, 40 µL) was added to prepare hybrid liposomes with m-PB NPs. The resulting mixture was dried gently under N_2 flow to produce a thin lipid film. It was subsequently dried under vacuum overnight. The lipid film was then hydrated for 4 hr at 50 °C with a 200 µL solution of 0.2 mM pyranine fluorescent dye of 8-hydroxy-1,3,6-pyrenetrisulfonate and 40 mM sucrose.

To remove the extra pyranine dye from the external phase of the liposomes, 1470 µL glucose solution with identical osmotic pressure was added to the 60 µL liposome suspension. After 1 hr, osmotic pressure was measured using a micro-sample osmometer (Fiske 210; Advanced Instruments, Inc., USA). Then, 1470 µL solution was taken out to remove the pyranine dyes in the external phase from the upper part of the solution mixture. The inner phase of the liposomes contains pyranine dyes. Finally, the sample was prepared for the measurement of ion transportation by the addition of 0.1 M NaOH solution (0.3 µL) into the liposome suspension (30 µL).

First, we investigated the response of control liposomes without m-PB NPs to the addition of NaOH. Liposomes containing pyranine dye were prepared in the inner aqueous phase, where the pyranine was removed from the outer phase by dilution. The pyranine is known to change fluorescence emission intensity under UV and blue excitation depending on the pH: at low and high pH, respectively, UV and blue excitation induce high emission. Figure 2 shows typical microscopic images of control liposomes without m-PB NPs with phase-contrast (Fig. 2a), UV (Fig. 2b) and blue (Fig. 2c) excitation modes. The control liposome shows high fluorescence at UV excitation, while little fluorescence was observed in the case of blue excitation. This indicates that the initial pH of the pyranine solution enclosed in the lipid bilayers is moderate. Subsequently, we added NaOH to the liposome suspension (final pH approx. 10), and observed the fluorescence images. No difference in the fluorescence response was observed when the pH of the control liposome suspension increased (Figs. 2e, f). This has occurred because of the insulating properties of lipid bilayers, which do not allow the electrolytes/ions to pass through the membrane.

Next, we investigated the response of the pyranine dye inside m-PB NP-liposomes to an increase in pH of the bulk solution. Figure 3 shows typical microscopic images of m-PB NP-liposomes with phase-contrast (Fig. 3a), UV (Fig. 3b), and blue (Fig. 3c) modes before the application of NaOH. The molar ratio of m-PB NPs:lipids was 1:40. As we did for the control liposomes, we observed fluorescence not in blue but in UV excitation. When NaOH was added to the m-PB NP-liposome suspension, the fluorescence intensity decreased significantly or even lost in the case of UV excitation whether fluorescence response increased in the case of blue excitation (Figs. 3e, f). This result suggests that the pH of the pyranine solution enclosed in the lipid bilayers increased. Because no significant fluorescence change was observed with the control liposomes during increase of the suspension pH (Fig. 2), the ion transfer must be conducted through the m-PB NPs

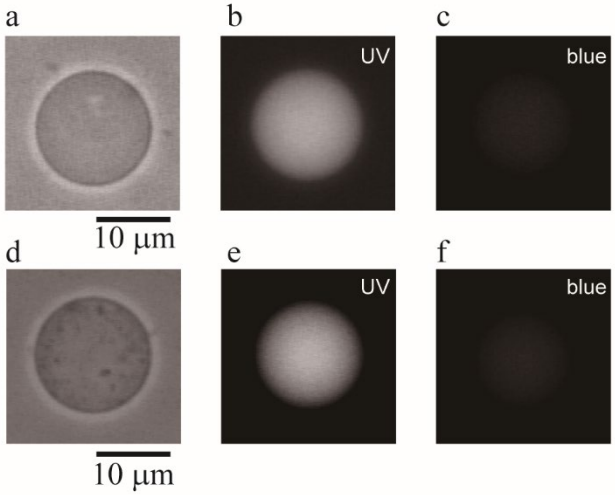

Fig. 2 Typical microscopic images of the control liposomes without m-PB NPs before (a-c) and after (d-f) the application of NaOH. Images are phase-contrast (a, d), UV (b, e) and blue (c, f) excitation modes.

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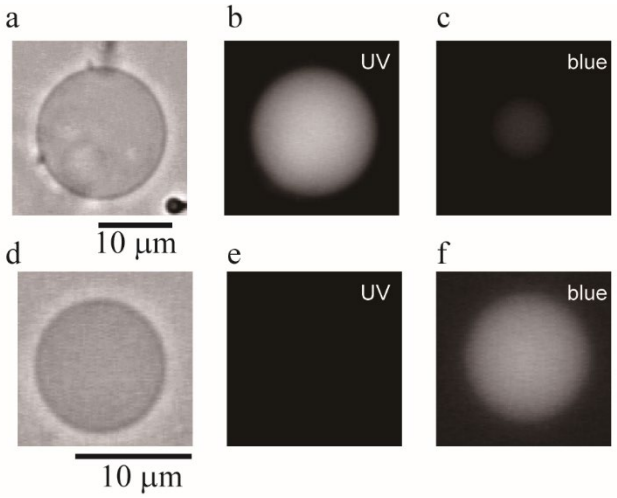

Fig. 3 Typical microscopic images of the liposomes with m-PB NPs before (a-c) and after (d-f) the application of NaOH. Images are phase-contrast (a, d), UV (b, e) and blue (c, f) excitation modes.

embedded into the lipid bilayers.

To clarify the ion transport property of m-PB NP-liposomes, we measured the fluorescence intensity from images taken using an optical microscope. Figure 4 presents datasets of the fluorescent intensities of m-PB NP-liposomes (the molar ratio of m-PB NPs:lipids was 1:15) by blue and UV excitation before (blue triangle) and after (red circle) NaOH application. The distribution of intensities shifted dramatically in response to the NaOH application. The variation of fluorescence intensity derives from differences of pyranine dye concentration inside the liposomes, which are determined randomly during liposome formation from lipid films. The difference in

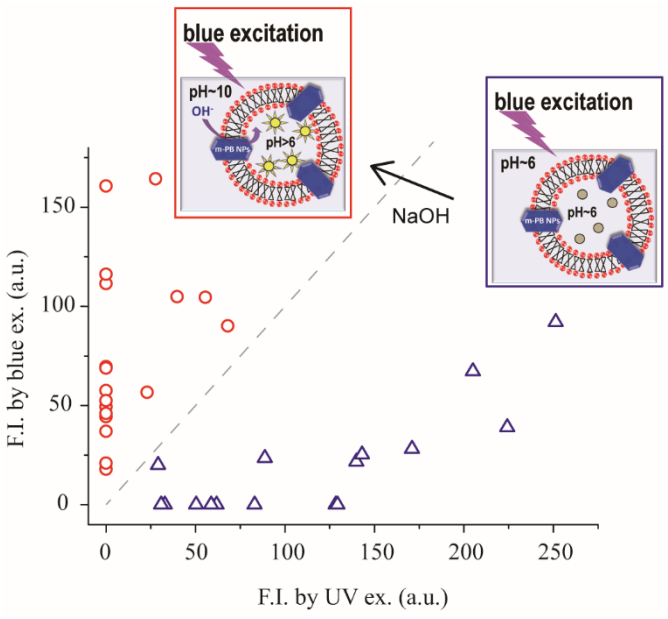

Fig. 4 Fluorescence intensity (F.I.) of m-PB NP-liposomes during UV and blue excitation before (blue triangle) and after (red circle) the application of NaOH. a.u. = arbitrary unit.

size might also affect the variation because the permeation of OH^- ion depends on the surface-to-volume ratio of liposomes. Furthermore, we examined the time-dependent fluorescent intensity of the m-PB NP-liposomes. Figure 5 portrays the development of fluorescent intensity at time intervals of 0-30, 30-60, and 60-90 min. The intensity in blue excitation increased gradually, indicating that the concentration of OH^- inside the liposomes increased with time.

Our results suggest that m-PB NPs can be adopted to serve an ion transfer function within the liposome bilayer. We speculated that m-PB NPs of smaller than average size were introduced into the liposome bilalyer because the average diameter of m-PB NPs was greater than the distance of the bilayer. No report has described a study of OH^- ion transport in bulk PB because it is known to be a chemically unstable material. A hybrid of liposome and long alkyl chains attached to PB NPs can contribute to improve the chemical stability of m-PB NP-liposome in the basic media. Our m-PB NPs were found to be stable for at least 45 min in the basic condition (Supplementary Information). It is noteworthy that we observed a change in fluorescence intensity with m-PB NPs to lipid molar ratios of 1:40 and 1:15 in our experiments. The high m-PB NPs ratio of 1:15 tended to show higher fluorescence for blue excitation, whereas the low m-PB NPs ratio of 1:40 tended to exhibit better formation of giant liposomes. Here, we demonstrated that OH^- ions were transported across the membrane with m-PB NPs, although pyranine dye (MW=524) did not penetrate across the membrane. Further experimental development will be necessary to identify other possible ions that pass through the hybrid m-PB NP-liposomes.

In summary, m-PB NPs were introduced into the bilayer of giant liposomes. Liposomes without m-PB NPs insulated ionic transport. However, pH change of the inner phase of liposomes was observed by the addition of NaOH solution in the case of liposome with m-PB NPs. Micropores of m-PB NPs served as ionic transport channels between the outer and inner phases of liposomes. We demonstrated a new type of hybrid liposome incorporating ion channels into the lipid bilayers. Results show that MOF has great potential for material design because its regular three-dimensional

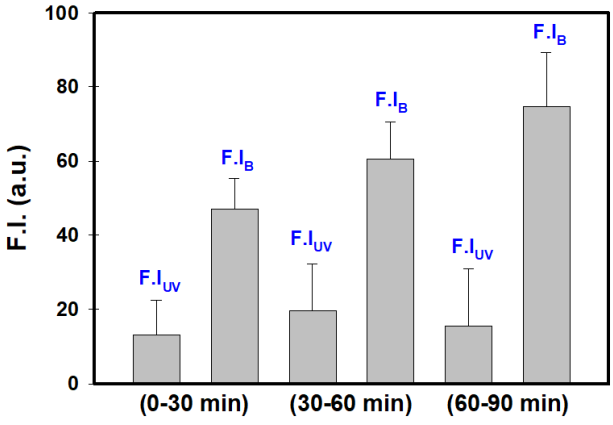

Fig. 5 Time-dependent fluorescent intensity (F.I.) of m-PB NP-liposomes after NaOH application. a.u. = arbitrary unit.

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structure with high stability is more useful to resist heat shock than conventional bio/soft materials are. Additional studies of MOF-liposome hybrid system development are underway.

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Conflict of interest

No author has any conflict to declare.

Notes and references

1. R. Dimova, S. Aranda, N. Bezlyepkina, V. Nikolov, K. A. Riske and R. Lipowsky, *J. Phys. Condens. Matter*, 2006, **18**, S1151-1176.
2. P. Walde, K. Cosentino, H. Engel and P. Stano, *Chembiochem.*, 2010, **11**, 848-865.
3. T. Hamada and K. Yoshikawa, *Materials*, 2012, **5**, 2292.
4. M. Weiss, J. P. Frohnmayer, L. T. Benk, B. Haller, J.-W. Janiesch, T. Heitkamp, M. Börsch, R. B. Lira, R. Dimova, R. Lipowsky, E. Bodenschatz, J.-C. Baret, T. Vidakovic-Koch, K. Sundmacher, I. Platzman and J. P. Spatz, *Nat. Mater.*, 2018, **17**, 89.
5. T. Hamada, R. Sugimoto, M. d. C. Vestergaard, T. Nagasaki and M. Takagi, *J. Am. Chem. Soc.*, 2010, **132**, 10528-10532.
6. Y. Suzuki, K. H. Nagai, A. Zinchenko and T. Hamada, *Langmuir*, 2017, **33**, 2671-2676.
7. C. Pernpeintner, J. A. Frank, P. Urban, C. R. Roeske, S. D. Pritzl, D. Trauner and T. Lohmüller, *Langmuir*, 2017, **33**, 4083-4089.
8. F. C. Keber, E. Loiseau, T. Sanchez, S. J. DeCamp, L. Giomi, M. J. Bowick, M. C. Marchetti, Z. Dogic and A. R. Bausch, *Science*, 2014, **345**, 1135-1139.
9. G. Rivas, S. K. Vogel and P. Schwille, *Curr. Opin. Chem. Biol.*, 2014, **22**, 18-26.
10. S. Tanaka, K. Takiguchi and M. Hayashi, *Commun. Phys.*, 2018, **1**, 18.
11. I. Tabushi, Y. Kuroda and K. Yokota, *Tetrahedron Lett.*, 1982, **23**, 4601-4604.
12. Y. Kobuke, K. Ueda and M. Sokabe, *J. Am. Chem. Soc.*, 1992, **114**, 7618-7622.
13. N. Sakai and S. Matile, *Angew. Chem. Int. Ed.*, 2008, **47**, 9603-9607.
14. S. Matile, A. Som and N. Sordé, *Tetrahedron*, 2004, **60**, 6405-6435.
15. G. W. Gokel and S. Negin, *Acc. Chem. Res.*, 2013, **46**, 2824-2833.
16. V. Gorteau, G. Bollot, J. Mareda, D. Pasini, D.-H. Tran, A. N. Lazar, A. W. Coleman, N. Sakai and S. Matile, *Biorg. Med. Chem.*, 2005, **13**, 5171-5180.
17. A. L. Sisson, M. R. Shah, S. Bhosale and S. Matile, *Chem. Soc. Rev.*, 2006, **35**, 1269-1286.
18. T. M. Fyles, R. Knoy, K. Müllen and M. Sieffert, *Langmuir*, 2001, **17**, 6669-6674.
19. M. Jung, H. Kim, K. Baek and K. Kim, *Angew. Chem. Int. Ed.*, 2008, **47**, 5755-5757.
20. R. Kawano, N. Horike, Y. Hijikata, M. Kondo, A. Carné-Sánchez, P. Larpent, S. Ikemura, T. Osaki, K. Kamiya, S. Kitagawa, S. Takeuchi and S. Furukawa, *Chem*, 2017, **2**, 393-403.
21. H. Li, M. Eddaoudi, M. O'Keeffe and O. M. Yaghi, *Nature*, 1999, **402**, 276.
22. S. Kitagawa, R. Kitaura and S. Noro, *Angew. Chem. Int. Ed.*, 2004, **43**, 2334-2375.
23. P. Ramaswamy, N. E. Wong and G. K. H. Shimizu, *Chem. Soc. Rev.*, 2014, **43**, 5913-5932.
24. T. Yamada, M. Sadakiyo, A. Shigematsu and H. Kitagawa, *Bull Chem. Soc. Jpn.*, 2016, **89**, 1-10.
25. S. Ohkoshi, K. Nakagawa, K. Tomono, K. Imoto, Y. Tsunobuchi and H. Tokoro, *J. Am. Chem. Soc.*, 2010, **132**, 6620-6621.
26. K. Ono, M. Ishizaki, K. Kanaizuka, T. Togashi, T. Yamada, H. Kitagawa and M. Kurihara, *Angew. Chem. Int. Ed.*, 2017, **56**, 5531-5535.
27. E. Damiano, M. Bassilana, J. L. Rigaud and G. Leblanc, *FEBS Lett.*, 1984, **166**, 120-124.
28. A. Gotoh, H. Uchida, M. Ishizaki, T. Satoh, S. Kaga, S. Okamoto, M. Ohta, M. Sakamoto, T. Kawamoto, H. Tanaka, M. Tokumoto, S. Hara, H. Shiozaki, M. Yamada, M. Miyake and M. Kurihara, *Nanotechnology*, 2007, **18**, 345609.

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