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Characterization of the preparation process and the photochemical control of electrical properties of bilayer lipid membranes containing azobenzene chromophores

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We prepared photoresponsive bilayer lipid membranes (BLMs) containing azobenzene derivatives (4′-octylazobenzene-4-oxybutyric acid (AZ)) and observed the rapid and reversible changes in their electrical properties when irradiated with light. The BLMs consist of AZ (8 mol%) and glyceryl monooleate. The changes in capacitance and conductance upon irradiation by light were found to be 10 and 20%, respectively. The changes in the electrical properties of the membrane and in the structure of AZ under light irradiation were analyzed simultaneously by in-situ spectroscopic, electrical and microscopic measurements. These measurements showed that the electrical changes induced by exposure to light resulted from reversible changes in the membrane structure initiated by the photoisomerization reaction of AZ. This structural change in the membrane occurred within 1 s.

Introduction

Biological membranes play an important role in many of the physiological and biological activities of cells, including the detection of external stimulation and the selective transmission of molecules and ions [1–3]. If the function of such membranes could be mimicked, new artificial sensors and devices with biological functions would become possible. It is known that planar bilayer lipid membranes (BLMs) provide optimum conditions for the production of artificial biological membranes, which are essential for this kind of study [4–8]. One of the reasons for this is that BLMs have the closest similarities to the structure of biological membranes. The other is that BLMs have advantages as regards physiological measurements of the membrane characteristics, since both sides of the membrane are easily accessible to electrodes. However, in spite of their excellent characteristics, BLMs have been studied only in a limited area due to the difficulties in preparing and evaluating the membranes.

Of the various functions of biological membranes, the authors focused on light conversion, which is the first step in visual processing. An azobenzene amphiphilic derivative, AZ, was chosen as an artificial light receptor molecule. Azobenzene is a well-known photochromic compound, which undergoes *cis-trans* photoisomerization similar to the chromophore in rhodopsin residing in the photoreceptor cell. The regulation of photoisomerization reactions in azobenzene derivatives has been studied in many membrane types such as monolayers [9,10], Langmuir-Blodgett (LB) films [11–14], vesicles [15–22], capsule membranes [23,24], polymer membranes [25–28] and BLMs [29,30]. However, there has been hardly any research reported clarifying the relation between the structure of the chromophores and characteristics of the membranes.

In this study, we not only prepared BLM containing AZ, but also measured the changes of the characteristics of the membrane during the forming process, as well as the response of the membrane to light irradiation. A multi-observation method was employed, in which spectroscopic, electrical and microscopic measurements were combined. Spectroscopic measurements of microcrystalline semiconductors deposited on BLMs have been reported previously [31–33], but simultaneous spectroscopic, microscopic, and electrical measurements of BLMs themselves have never been tried before.

Materials and Methods

4′-Octylazobenzene-4-oxybutyric acid ((AZ) Dojin Kagaku), glyceryl monooleate ((GMO) Tokyo Kasei

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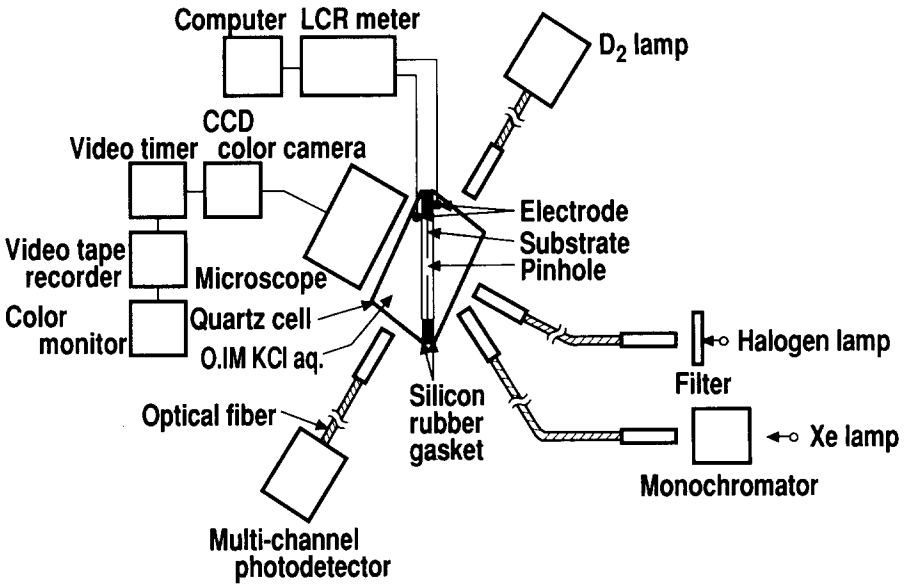

Fig. 1. Schematic representation of the experimental setup used for the simultaneous spectroscopic, electrical and microscopic measurements of BLMs.

Kogyo) and n-decane (Aldrich) were used as the photoreceptor, membrane-forming lipid and solvent, respectively. The BLM-forming solution consisted of n-decane – including AZ and GMO (1:11 mol/mol) – and $CHCl_3$ (10:3 (v/v)). The solution (1 ml) contained 62 mg of AZ plus GMO.

Fig. 1 is a schematic representation of the experimental setup used for the simultaneous in-situ spectroscopic, electrical and microscopic measurements of the BLMs. Using this setup, simultaneous measurements to evaluate not only the electrical characteristics but also the molecular structure of the BLM are possible and the morphological change in the membrane can be observed through the microscope. BLMs were formed across a hole, 0.7 mm in diameter, punched in a 0.05-mm-thick teflon film. The film was sandwiched between two triangular quartz cells. The two quartz cells were electrically separated from each other by the BLM and were filled with 0.10 M KCl aqueous solution. In the case of absorption spectroscopy, a thin 0.01-mm-thick metal plate teflon-coated and with a 0.6 or 0.3-mm diameter hole was used instead of the teflon film to avoid the effects of the absorption of any AZ residue on the film surfaces.

Electrical measurements were performed with two Pt/Pt black electrodes immersed in the cells and connected to an LCR meter (4274A, Hewlett Packard). Operation of the LCR meter and the data processing were performed with a personal computer (PC-9801RA21, NEC). All electrical measurements using the LCR meter were carried out at a frequency of 1 kHz. The AC voltage across the BLM was kept at 7 mV (rms).

Absorption measurements were performed by using a multi-channel photodetecting system (MCPD-1000, Otsuka Electronics). Light from a 150 W D_2 lamp was

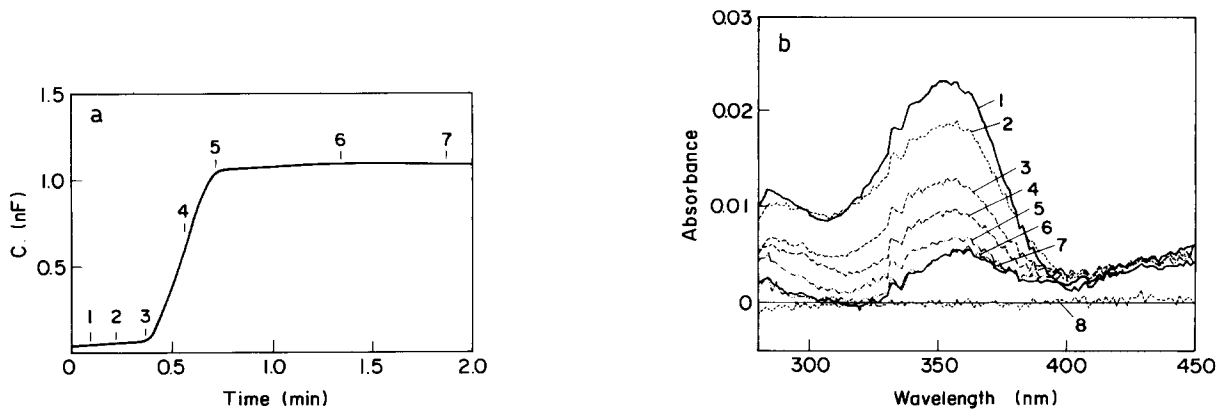

Fig. 2. Changes in membrane capacitance (a) and absorption spectrum (b) in the course of the AZ/GMO BLM formation. The numbers of the spectra correspond to those on the capacitance curve where each spectroscopic measurement was taken. Spectrum 8 was obtained after electrical breakdown of the BLM. The substrate is a thin metal plate with a 0.6-mm diameter hole.

introduced via an optical fiber and focused on the hole. Then, the light transmitted through the hole was introduced into the photodetecting system through another optical fiber. A 300 W xenon lamp was used as an excitation source for the photoreaction. The light was focused on the BLM through a monochromator and an optical fiber. The intensity of light at the membrane was 1–3 mW/cm^2.

Besides electrical and spectroscopic measurements, observations were carried out simultaneously through a microscope (BHMJ-MB, Olympus). The BLM was illuminated by a 50 W halogen lamp through an optical fiber using a 550 nm cutoff filter and a water filter. The cutoff filter prevents AZ photoisomerization in the membrane. The light passed through an objective (ULWD MSPlan 20 ×, Olympus) with a working distance of 11 mm, and through a 0.44 × projection lens. The image was picked up by a CCD color camera (EC-202II, ELMO). The video signal was displayed on a video monitor (FCM-140A, Ikegami Tsushinki) via a video timer (VTG-33, For-A) and a video tape recorder (A-L72, Toshiba). All measurements were performed at 25 ± 2°C.

Results and Discussion

The formation of AZ/GMO BLMs was monitored simultaneously using three different techniques, spectroscopic, electrical and microscopic measurements. Fig. 2 shows the results of the capacitance measurements together with the change in the spectra as a function of time after smearing the BLM-forming solution across the hole. Microscope observations showed that the thinned region increased as the membrane capacitance rose (3 to 5 in Fig. 2a). Fig. 2b shows the change in absorption spectra. In this figure absorption at 355 nm, which is attributed to AZ, is seen, indicating the presence of AZ in the membrane. This fact is also supported from the disappearance of the absorption when the BLM suffers electrical breakdown (spectrum 8). The spectrum at 355 nm is the same as that of AZ in solution, suggesting that AZ is dispersed in the BLM on the molecular scale [34]. In the course of time (1 to 6 in the figure), absorption decreased, while the absorption maximum and the spectral shape did not change. The changes in capacitance paralleled those of the optical and spectroscopic measurements (Fig. 2b). As the peak absorbance decreased, the capacitance increased to a maximum and the absorption remained unchanged when the capacitance reached a plateau. The constant high value of the capacitance and the constant absorption spectrum indicates that the membrane is fully thinned at this stage. The specific capacitance of the BLM was determined to be 0.42 μF/cm^2. The BLMs prepared under these conditions were stable, and measurements had excellent reproducibility.

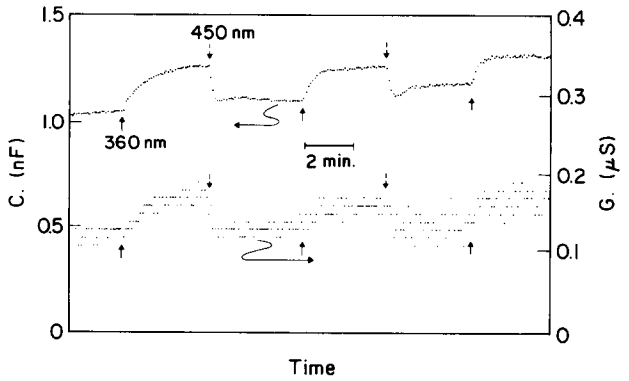

Fig. 3. Changes in capacitance and conductance of the AZ/GMO BLM upon alternating irradiation by 360 nm and 450 nm light. The arrows indicate the change from 360 nm to 450 nm and vice versa. The substrate is a teflon film with a 0.7-mm diameter hole.

Fig. 3 shows the changes in the BLM's electrical properties with time when irradiated at two different wavelengths, 360 nm and 450 nm. It is clear that the 360 nm irradiation increases the capacitance and the conductance, and that both of the properties are restored to the original level in dark conditions upon irradiation by 450 nm light. Such changes in capacitance and conductance dependent on the wavelength were not seen in the BLM formed from GMO alone. Therefore, the results indicate that the change in electrical properties can be attributed to the structural change of AZ in the BLM.

Fig. 4 shows the results of capacitance measurements during alternating irradiation by 360 nm and 450 nm light. A very rapid, reversible photoresponse is observed and it has good reproducibility. In the case shown in Fig. 4, each wavelength of light was used for irradiation up to 20 times over an interval of 90 min, and the capacitance changed alternated values throughout.

To investigate the mechanism of this change in electrical properties, simultaneous spectroscopic, elec-

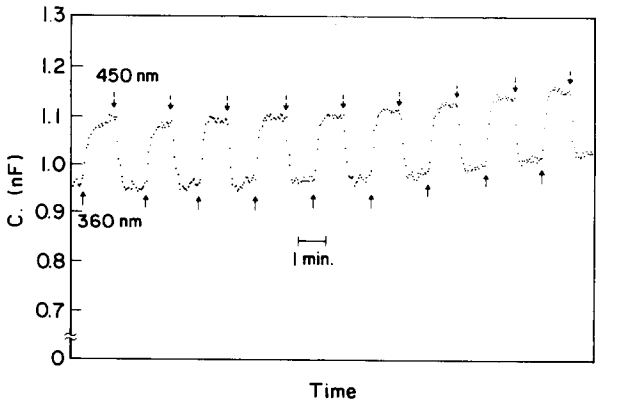

Fig. 4. Changes in capacitance of the AZ/GMO BLM upon alternating irradiation by 360 nm and 450 nm light. The arrows indicate the change from 360 nm to 450 nm and vice versa. The substrate is a teflon film with a 0.7-mm diameter hole.

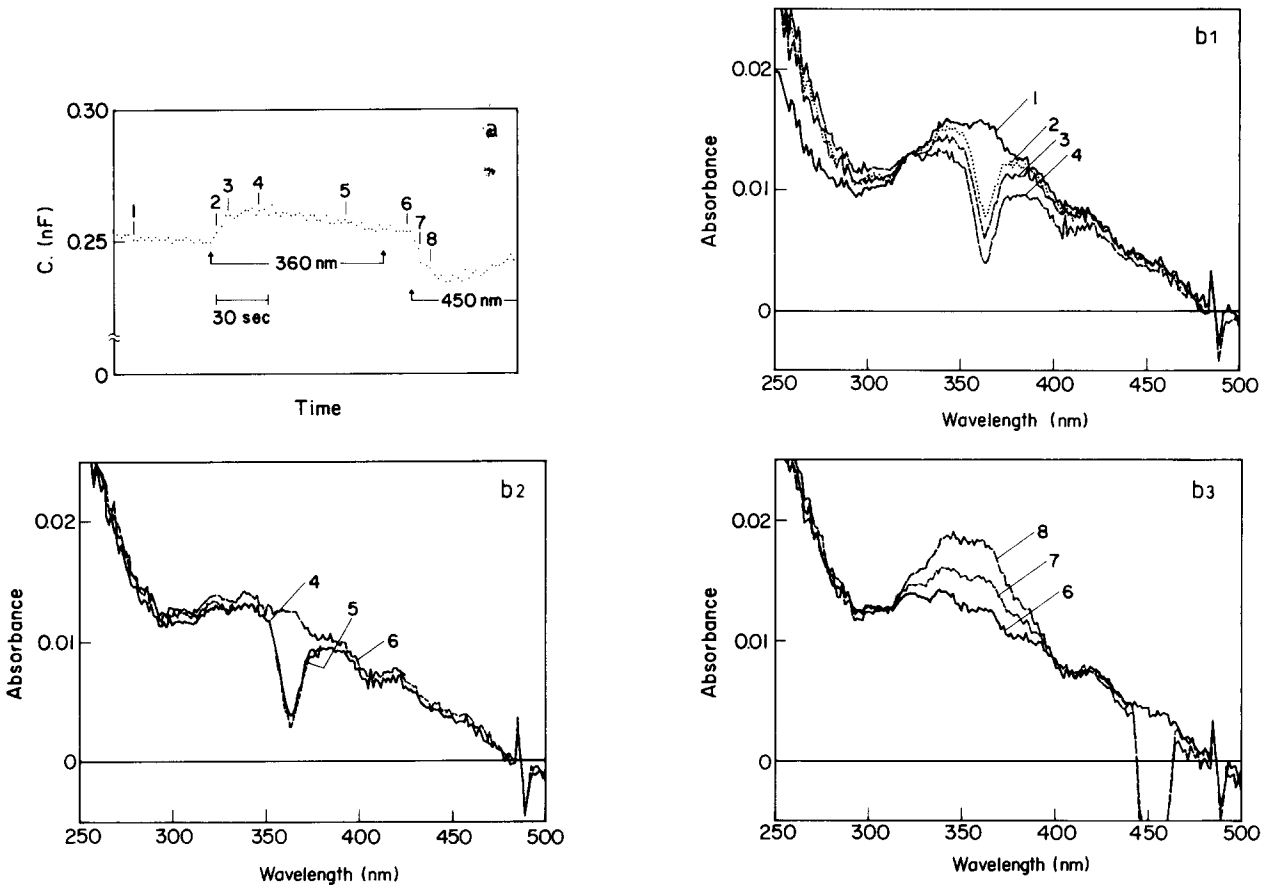

Fig. 5. Changes in capacitance (a) and absorption spectrum (b) of the AZ/GMO BLM upon alternating irradiation by 360 nm and 450 nm light. The numbers of the spectra correspond to those on the capacitance curve where each spectroscopic measurement was taken. The substrate is a thin metal plate with a 0.3-mm diameter hole.

trical and microscopic measurements were carried out. The results are shown in Fig. 5. The changes in membrane capacitance and absorption spectra with irradiation are shown in Fig. 5a and b, respectively. During the experiments, the bilayer area was cautiously monitored using an optical microscope to ascertain that the BLM area did not change morphologically. Valleys in the spectra near 360 nm and 450 nm are attributed to the excitation light. AZ in the BLM takes a *trans* form before the irradiation, showing a π-π * band near 355 nm (spectrum 1). As shown in Fig. 5b, the intensity of the 355 nm band decreases with the 360 nm irradiation, indicating a decrease of the trans form. Interestingly, Fig. 5a shows that the increase in membrane capacitance clearly corresponds to the change in the spectrum. When the spectrum stops changing, that is, when the reaction from *trans*-to-*cis* photoisomerization is completed, the membrane capacitance also reaches a constant value. The decrease in the 355 nm absorption band and the increase in capacitance are completely and synchronously restored by 450 nm irradiation. These results clearly indicate that the *cis–trans* photoisomerization of AZ can be controlled in the BLM. To investigate the relation between the structural changes of AZ and those of the membrane in detail, the membrane was irradiated with pulse-like light. The results are shown in Fig. 6. The irradiation period is 1 s. As shown in this figure, the capacitance changes stepwise, within 1 s after irradiation. Consequently, the electrical properties of the AZ/GMO BLM change synchronously with respect to the photoisomerization reaction of AZ.

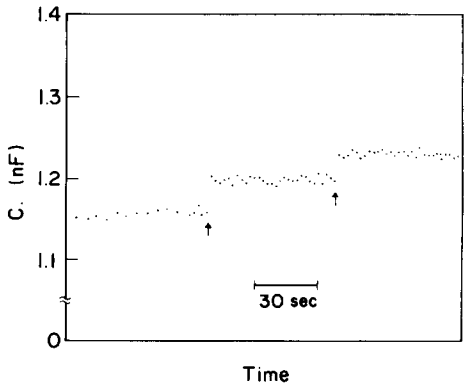

Fig. 6. Changes in capacitance of AZ/GMO BLM upon irradiation by 360 nm light. The arrows indicate light irradiation for 1 s. The substrate is a teflon film with a 0.7-mm diameter hole.

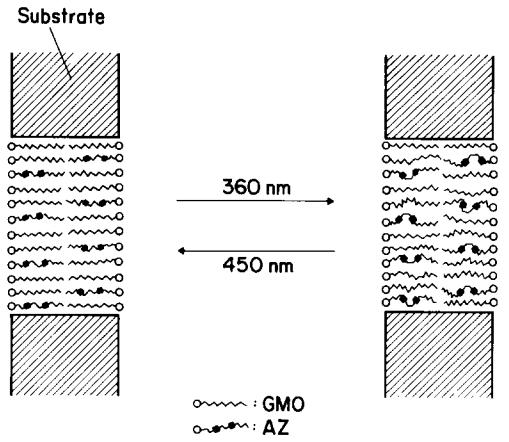

Fig. 7. Schematic illustration of the proposed mechanism by which the electrical properties of the AZ/GMO BLM change upon light irradiation.

The mechanism which gives these results can be explained using the schematic illustration shown in Fig. 7. In the dark, AZ takes a *trans* form in the membrane. When 360 nm irradiation occurs, the *cis–trans* photoisomerization reaction of AZ is triggered in the membrane. The induced change in the AZ structure disorders the arrangement of matrix molecules (GMO) around AZ, leading to a substantial change in the membrane structure. This phenomenon gives rise to the changes in capacitance and conductance. The reason for the conductance change is thought to be that the structural change makes it easier for ions in the solution to get through the membrane. The change in capacitance is thought to derive from a change in dielectric constant or the thickness of the membrane, although the actual mechanism at work cannot be clarified at this stage. It is noteworthy that the changes in electrical properties of the BLM occurred within 1 s, as soon as photoisomerization of AZ occurs (Fig. 6). Furthermore, the conversion to the *cis* isomer in the photostationary state is nearly perfect and the photoisomerization is completely reversible (Fig. 5). In other types of membranes containing azobenzene derivatives, such as monolayers [9,10], Langmuir-Blodgett (LB) films [11–13] and polymer membranes [25–28], changes in electrical properties are rather slow and the conversion ratio between two isomers is not so high. This indicates that the structural change of AZ in the BLM seems to be transmitted directly and swiftly to the neighboring molecules because of the fluid and ultimate thin structure of the membrane. This study shows that BLM research has possibility of leading to sensors or devices with the ability to convert light signals to electrical ones as rapidly as a biological system.

In this paper, we described the successful preparation of the BLMs containing azobenzene derivatives (8 mol%). The formative process was monitored by simultaneous spectroscopic, electrical and microscopic procedures. The electrical properties, capacitance and conductance, of the BLM changed reversibly and very rapidly upon irradiation by light. It was clarified, using simultaneous multi-measurement techniques, that these changes induced by light were caused by the reversible changes in the membrane structure initiated by the photoisomerization reaction of azobenzene derivatives. The structural changes in the membrane occurred within 1 s. The system developed here offers advantages in the dynamic characterization of BLMs in general, since it allows simultaneous evaluation of the molecules and corresponding electrical measurements.

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References

1 Jain, M.J. and Wagner, R.C. (1980) Introduction to Biological Membranes, Wiley, New York.
2 Chapman, D. (1984) Biomembrane Structure and Function, Verlag Chemie, Weinheim.
3 Yeagle, P. (1987) The Membranes of Cells, Academic Press, Orlando.
4 Tien, H.T. (1974) Bilayer Lipid Membranes (BLM). Theory and Practice, Marcel Dekker, New York.
5 Fendler, J.H. (1982) Membrane Mimetic Chemistry, Wiley, New York.
6 Miller, C. (1986) Ion Channel Reconstitution, Plenum, New York.
7 Cevc, G. and Marsh, D. (1987) Model Membranes. Phospholipid Bilayers, Wiley, New York.
8 Hille, H. (1992) Ion Channels of Excitable Membranes, 2nd Ed., Sinauer, Sunderland.
9 Nakahara, H., Fukuda, K., Shimomura, M. and Kunitake, T. (1988) Nippon Kagakukaishi, 1001–1010. (Japanese)
10 Iwamoto, M., Majima, Y., Naruse, H., Noguchi, T. and Fuwa, H. (1991) Nature 353, 645–647.
11 Yabe, A., Kawabata, Y., Niino, H., Tanaka, M., Ouchi, A., Takahashi, H., Tamura, S., Tagaki, W., Nakahara, H. and Fukuda, K. (1988) Chem. Lett., 1–4.
12 Komizu, H., Manda, E., Niino, H., Yabe, A. and Kawabata, Y. (1989) J. Am. Chem. Soc. 111, 3080–3081.
13 Nishiyama, K., Kurihara, M. and Fujihira, M. (1989) Thin Solid Films 179, 477–483.
14 Liu, Z.F., Hashimoto, K. and Fujishima, A. (1990) Nature 347, 658–660.
15 Kano, K., Tanaka, Y., Ogawa, T., Shimomura, M., Okahata, Y. and Kunitake, T. (1980) Chem. Lett., 421–424.
16 Kunitake, T., Nakashima, N., Shimomura, M., Okahata, Y., Kano, K. and Ogawa, T. (1980) J. Am. Chem. Soc. 102, 6642–6644.
17 Okahata, Y., Ihara, H., Shimomura, M., Tawaki, S. and Kunitake, T. (1980) Chem. Lett., 1169–1172.
18 Kano, K., Tanaka, Y., Ogawa, T., Shimomura, M. and Kunitake, T. (1981) Photochem. Photobiol. 34, 323–329.
19 Morgan, C.G., Thomas, E.W., Yianni, Y.P. and Sandhu, S.S. (1985) Biochim. Biophys. Acta 820, 107–114.

20 Shimomura, M. and Kunitake, T. (1987) J. Am. Chem. Soc. 109, 5175–5183.
21 Tazuke, S., Kurihara, S., Yamaguchi, H. and Ikeda, T. (1987) J. Phys. Chem. 91, 249–251.
22 Yamaguchi, H., Ikeda, T. and Tazuke, S. (1988) Chem. Lett., 539–542.
23 Okahata, Y., Lim, H. and Hachiya, S. (1983) Makromol. Chem., Rapid Commun. 4, 303–306.
24 Okahata, Y., Lim, H. and Hachiya, S. (1984) J. Chem. Soc. Perkin Trans. II, 989–994.
25 Shinkai, S., Kinda, H. and Manabe, O. (1982) J. Am. Chem. Soc. 104, 2933–2934.
26 Kumano, A., Niwa, O., Kajiyama, T., Takayanagi, M., Kano, K. and Shinkai, S. (1983) Chem. Lett., 1327–1330.
27 Anzai, J., Sasaki, H., Ueno, A. and Osa, T. (1983) J. Chem. Soc., Chem. Commun., 1045–1046.
28 Anzai, J., Ueno, A., Sasaki, H., Shimokawa, K. and Osa, T. (1983) Makromol. Chem., Rapid Commun. 4, 731–734.
29 Duchek, J.R. and Huebner, J.S. (1979) Biophys. J. 27, 317–321.
30 Huebner, J.S. and Varnadore, W.E. (1982) Photochem. Photobiol. 35, 141–148.
31 Basal, S., Zhao, X.K., Rolandi, R. and Fendler, J.H. (1987) J. Am. Chem. Soc. 91, 2701–2704.
32 Zhao, X.K., Baral, S., Rolandi, R. and Fendler, J.H. (1988) J. Am. Chem. Soc. 110, 1012–1024.
33 Zhao, X.K. and Fendler, J.H. (1988) J. Phys. Chem. 92, 3350–3355.
34 Shimomura, M., Ando, R. and Kunitake, T. (1983) Ber. Bunsenges. Phys. Chem. 87, 1134–1143.