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Spectral Shifts of the Environment-sensitive Fluorophore POLARIC™ in Heterogeneous Interfaces

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We have designed and synthesized amphiphilic environment-sensitive fluorophore, POLARIC™, from four building blocks using Suzuki–Miyaura cross-coupling. The absorption and fluorescence spectra were measured in various micelles and vesicles. The results show that the emission wavelengths of these probes respond to the surface charge and stability of the self-assemblies.

Lipid membranes are functionally important in enclosing and separating specific regions in many chemical and biological systems.[1] Furthermore, cell membranes play important roles in cell–cell interactions, material transport, and signal transduction.[2] However, the details of these mechanisms are poorly understood. Among the analytical methods used to clarify the membrane structure and dynamics, fluorescence techniques have particular advantages due to their relatively high sensitivity and high resolution under physiological conditions.[3] Membrane probes with environment-sensitive spectral shifts, such as laurdan,[4] Dapoxyl sulfonic acid,[5] 1,8-ANS (1-anilinonaphthalene-8-sulfonic acid),[6] and DCVJ (4-(dicyanovinyl)julolidine),[7] enable the discrimination of membrane compositions. Since the lipid membranes have a hydrophobic core and hydrophilic surfaces, we expected that fluorescent solvatochromic dyes, the emission wavelengths of which shift with solvent polarity, could act as a potential backbone for novel membrane probes.

Recently, we designed and synthesized a series of fluorescent solvatochromic dyes with four different heterocyclic rings (thiophene, furan, bithiophene, and 3,4-ethylenedioxythiophene).[8] All of these dyes were efficiently prepared from electron-donating, aromatic, and electron-withdrawing building blocks using Suzuki–Miyaura cross-coupling reactions. Bearing this synthetic strategy in mind, we have designed amphiphile membrane probes that resemble lipid molecules. The POLARIC™ (named from POLARity Indicating Chromophore) probe contains a pyridinium ring as the hydrophilic moiety and the others as the hydrophobic moiety (Figure 1). Hydrophobicity can be altered by substituting the electron-donating building blocks (R^1 and R^2). Moreover, hydrophilicity may easily be modified by exchanging the counter anion (X^-) using anion-exchange resin. As only the electron-withdrawing moiety is displaced in the dye structure, the same electron-donating and aromatic building blocks can be used for the membrane probes. The intermediate pyridine derivatives **2a**–**2e** were synthesized from 4-bromopyridine in the same manner as described in previous work (Scheme 1). The target fluorophores **1a**–**1e** were obtained by methylation of the pyridine derivatives using methyl triflate. The synthetic route was quite simple, and the products were obtained in moderate yields after three reaction steps starting from commercially available materials.

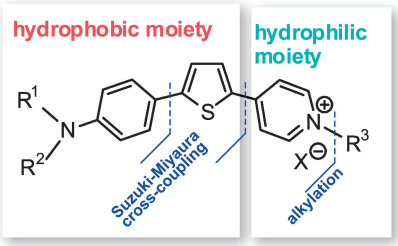
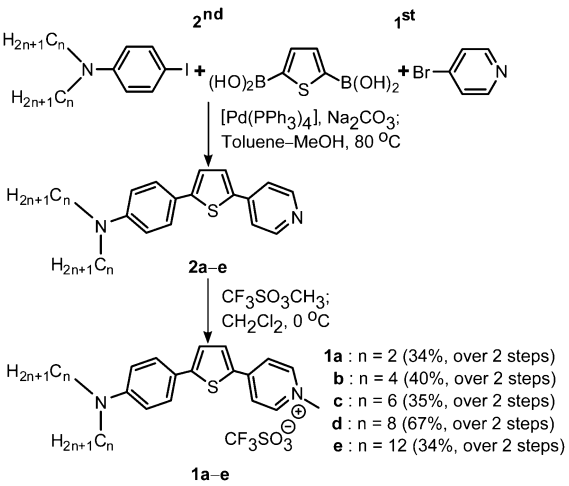


Figure 1. Synthetic strategy of POLARIC™.



Scheme 1. Synthesis of **1a**–**1e**.

The pyridine derivatives **2a**–**2e** showed a broad absorbance around 400 nm and fluorescence solvatochromism equivalent to the previous fluorophores in various organic solvents (Supporting Information; SI[13]). However, little fluorescence was shown by the pyridinium derivatives **1a**–**1e** in the solvents. This phenomenon could be explained by aggregation of **1a**–**1e** due to their amphiphilicity, so that the fluorescence was probably self-quenched. When surfactant or cyclodextrin was added to the aqueous solutions, the fluorescence shown by **1a**–**1e** was significantly stronger than that in the homogeneous solutions. The addition of the surfactants above the critical micelle concentration (CMC) led to a blue shift of the emission spectrum (SI[13]). Hence, it is likely that **1a**–**1e** localized in a certain area of the micelle and emitted a specific signal in response to the microenvironment. We, therefore, studied the photophysical properties of **1a**–**1e** in micelles and lipid vesicles to visualize the self-assembly dynamics. As an additional advantage, micelles and lipid vesicles serve as a simple model system for the cell membrane due to their ease of formation via

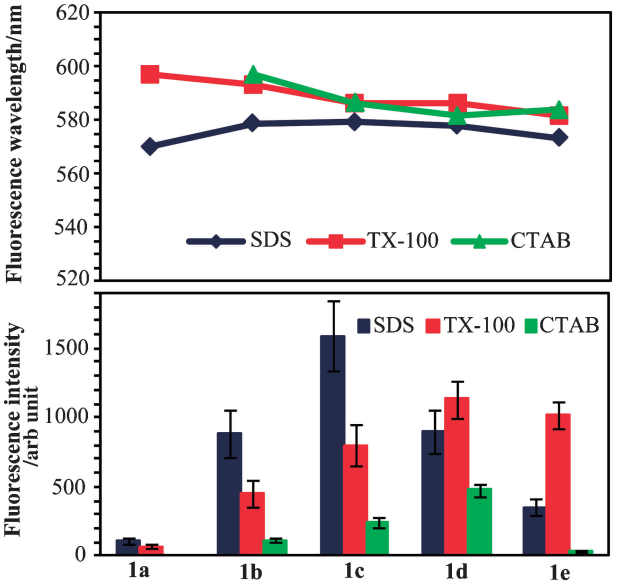


Figure 2. Fluorescence wavelength and intensity (peak height excited at the absorption maximum) of **1a**–**1e** in three different micellar solutions. $[\mathbf{1a}\text{–}\mathbf{1e}]_{final} = 6.8\,\mu M$, $[SDS]_{final} = 50\,mM$, $[TX\text{-}100]_{final} = 1\,mM$, and $[CTAB]_{final} = 5\,mM$. Error bars represent average ± standard deviations of $N = 3$ independent experiments.

self-assembly, structural integrity, large surfactant, and different surfactant environments.[9,10]

First, we measured the absorption and fluorescence spectra of **1a**–**1e** in three different micellar solutions; anionic surfactant (sodium dodecyl sulfate; SDS), nonionic surfactant (Triton X-100), and cationic surfactant (hexadecyltrimethylammonium bromide; CTAB) (Figure 2). The concentration of **1** was low enough to not significantly affect the CMC values. The emission maxima in SDS were shorter than those in Triton X-100 and CTAB. This observation suggests that the cationic **1a**–**1e** and the anionic SDS produce a hydrophobic and stable aggregate. The probes **1a**–**1e** seem to be sensitive to the surface charge of the micelle. The emission maxima also shifted to a slightly shorter wavelength upon increases in alkyl chain length. Longer alkyl chains may increase hydrophobicity and thus penetrate deeper into the micellar core. On the other hand, the fluorescence intensities varied regardless of the changes in wavelength. The intensities might result from the degree of dispersion due to the combination of the probe and surfactant.

We next measured absorption and fluorescence spectra in the vesicles, which more closely resemble cell membranes. Generally, vesicles are more stable than micelles, prepared from ionic phospholipids, such as phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), and sphingomyelin (SM), and nonionic cholesterol (CL). We prepared the vesicles using a general procedure from 4:1 PC/CL, 2:2:1 PE/PC/CL, 2:2:1 SM/PC/CL, and 2:2:1 PS/PC/CL, respectively. Figure 3 shows the absorbance and fluorescence spectra of **1c** in the vesicles. Similar to the fluorescent solvatochromic dyes, the absorption maxima changed little in the homogeneous solutions. The emission maxima in the vesicles were shorter than those in the micelles. This agreed with our results regarding the stability of the aggregates. Furthermore, the fluorescence maxima

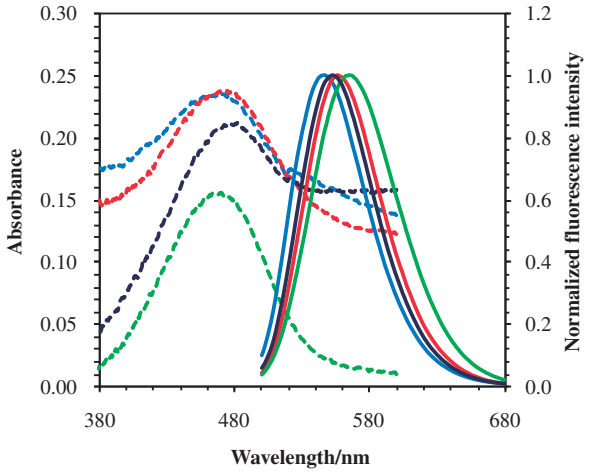


Figure 3. Absorbance (dashed lines) and fluorescence spectra (solid lines) of **1c** in lipid vesicles. The lipid vesicles were composed of —: PE/PC/CL 2:2:1, —: SM/PC/CL 2:2:1, —: PS/PC/CL 2:2:1, and —: PC/CL 4:1. $[\mathbf{1c}]_{final} = 6.8\,\mu M$ and $[lipid]_{total} = 0.5\,mM$. Excitation wavelength was 470 nm.

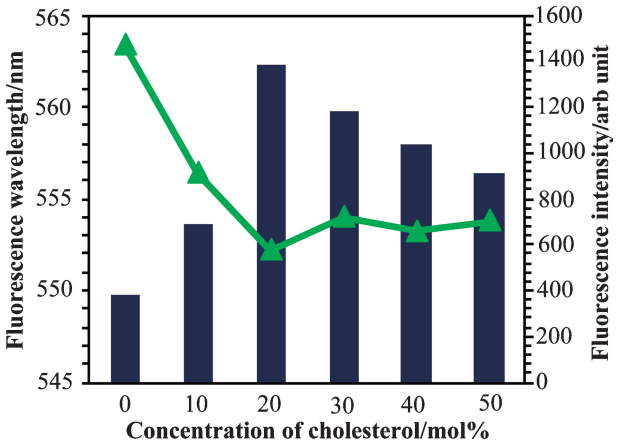


Figure 4. Fluorescence maximum (—▲—) and intensity (peak height excited at the absorption maximum) (▬) of **1c** in the PC vesicles with different CL concentrations. $[\mathbf{1c}]_{final} = 6.8\,\mu M$ and $[lipid]_{total} = 0.5\,mM$. Excitation wavelength was 480 nm.

changed markedly with changes in vesicle composition. The emission spectrum in a SM/PC/CL vesicular solution exhibits a slightly red-shifted maximum (4 nm) compared to that in PC/CL. It seems reasonable that SM and PC have the same ionic moiety. In contrast, that in PS/PC/CL shows a significantly red-shifted maximum (13 nm) relative to that in PC/CL. On the contrary, that in PE/PC/CL shows a modestly blue-shifted maximum (6 nm) relative to that in PC/CL. Collectively, these results suggest that the probe **1c** is sensitive to the surface charge of the vesicle.

To further characterize the photophysical properties of the probe **1c** in the vesicles, the effect of CL concentration was investigated. CL is known to be important in increasing membrane stability.[11] We, therefore, prepared vesicles from PC with different CL concentrations (0 to 50 mol %) and measured the emission spectra (Figure 4). The addition of CL up to 20 mol % enhanced the fluorescence intensity and shifted the

fluorescence spectrum to a shorter wavelength. However, the addition of CL over 20 mol % had little influence on fluorescence intensity or wavelength. This tendency was observed in other structural studies of PC/CL vesicles.[12] The probe **1c** may also be sensitive to the stability of the vesicle.

In conclusion, we have designed and synthesized amphiphilic fluorescent probes from four building blocks using Suzuki–Miyaura cross-coupling. The emission wavelengths of these probes respond to the surface charge and stability of the self-assemblies. The labeling moiety for biological molecules could be synthetically introduced at three alkyl chains (R^1–R^3). We believe that these probes have great potential for the visualization of various cellular reactions, such as proliferation, differentiation, signal transduction, and apoptosis. Cellular applications using POLARIC™ probes will be published soon.

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