Electronic Supplementary Information

Study on structural changes in supramolecular assemblies composed of amphiphilic nicotinamide and its dihydronicotinamide derivative by flow cytometry

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Experimental Methods

General

Fluorescent spectra were recorded on a JASCO FP777 spectrometer. HITACHI S-3000N was used for cryo-scaning electron microscopic (Cryo-SEM) observation. Differential interference contrast (DIC) and fluorescent micrographs were recorded using an Olympus IX71 inverted microscope equipped with a 3CCD camera (Toshiba Corp., JT-TU52H). The fluorescent micrographs were obtained using WUB filter units (Olympus) and a high pressure mercury lamp. HPLC was performed on a TOSOH 8020 series mounted with a ODS column (eluant: methanol and water; see fig. S3 and its caption).

Flow Cytometer and Cell Sorter

EPICS ALTRA (Beckman Coulter) was equipped with a water-cooled argon ion laser emitting at 350-360 nm for flow cytometry. As shown in fig. S1, the intensities of forward light scattering (FSC), side light scattering (SSC), and fluorescence at 430 nm and 525 nm from each object were collected. For analyzing these data, EXPO32 analyzing software (Beckman Coulter) and win MDI (written by J. Trotter) were used. The micrographs of separated self-assemblies are shown in fig. S2.

Materials

 N^{1} -dodecylnicotinamide was prepared from nicotinamide and *n*-bromoododecane according to previous reports.^[1] N^{1} -dodecyl-1,4-dihydronicotinamide was obtained by reduction of N^{1} -dodecylnicotinamide using sodium dithionite and purified by alumina column chromatography. Because of the instability of the compound, only fresh dihydronicotinamide derivative was used in measurements.

[1] K. Kalyanasundaram, T. Colassis, R. Humphry-Baker, P. Savarino, E. Barni, E. Pelizzetti and M. Grätzel, *J. Am. Chem. Soc.*, 1989, **111**, 3300-11.



Fig. S1 (a) Filter units of an optical system used in flow cytometric experiment. (b) Window-regions of band pass filters represented together with fluorescent spectrum of vesicle dispersion (1: 0.6 mM, 2: 0.4 mM). A Green solid line represents an emission spectrum ($\lambda_{ex} = 370$ nm, $\lambda_{max} = 505$ nm) and a green dotted line is the excitation spectrum ($\lambda_{em} = 505$ nm, $\lambda_{max} = 370$ nm).



Fig. S2 Fluorescent micrographs of various separated assemblies (bar = $10 \ \mu$ m): (a) separated assemblies belonging to region A, (b) separated assemblies belonging to region B.



Fig. S3 HPLC charts of A-separated dispersion (upper) and B-separated dispersion (lower) obtained 90 min after preparation; Retention times of **1** and **2** are 5.2 min and 19-20 min, respectively. It is clear that the molar ratios of **1**:2 were different between A-separated dispersion and B-separated dispersion. Because the concentrations of the samples were diluted by sorting, it was needed to turn the wavelength of UV detector from 270 nm to 365 nm at 8 min for calculating the molar ratio of **1** and **2**. The gradient protocol is to change the ratio of a mixed eluant from methanol and water (60% and 40%, respectively) to methanol (100%).



Fig. S4 Density plots for relative fluorescent emission intensities at 525 nm against at 430 nm of hybrid vesicles of **1** (0.6 mM) and **2** (0.4 mM): (a) immediately after preparation, (b) after 30 min, (c) after 60 min, (d) after 90 min.



Fig. S5 Phase diagram of 1 mM hybrid surfactant system of 1 and 2 in degassed triethanolamine-HCl bufferd solution (5 mM, pH = 7.0) in 25 °C.



Fig. S6 Emission spectra of 1 mM hybrid surfactant systems of 1 and 2 in different mixing ratio excited at 370 nm. The reason why the emission intensities of dispersion with the concentration of 1 and 2 in 0.3 mM : 0.7 mM and 0.2 mM : 0.8 mM were decreased from 0.4 mM : 0.6 mM is the molecules were not dispersed completely.