doi:10.1246/cl.2012.1689
Published on the web December 8, 2012

Microflow-driven Temporal Self-assembly of Amphiphilic Molecules

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A dynamic oil–water interface generated in a double-Y-shaped microfluidic device allowed amphiphilic molecules to self-assemble spontaneously for a set period of time, leading to the creation of discrete supramolecular coordination polymers. After elution from the device, these 1-D structures gradually dissociated into their monomer units, regenerating the initial state. The self-assembled structures were maintained only under the flow conditions in the device.

From a limited set of components, nature generates a diverse range of self-assembled architectures depending on the physiological environment. These architectures are created in a desired space and moment and exert their functionalities within a regulated time; subsequently, they dissociate into their initial components, thereby acting as renewable molecular sources for further self-assembly. In this sense, nature's self-assembled architectures can be characterized in terms of both temporal and discrete structures; they emerge spontaneously at a desired space but maintain their forms only through the consumption of energy supplied from external systems (i.e., far from equilibrium conditions). One of the goals of supramolecular chemistry is to reconstruct such a nonequilibrium self-assembling system, as characterized by flash assembly, a temporal assembled structure, and circular chemical conditions.[1] Inspired by nature, herein we propose a novel self-assembling system, based on a dynamic oil–water interface generated temporally in a microfluidic device in which amphiphilic molecules could self-assemble within a controlled period of time (Figure 1).[2,3]

To provide a dynamic interface, we developed microfluidic devices having a channel pattern (width, 160 μm; depth, 40 μm) with Y-shaped junctions at both the up- and downstream ends, separated by 80 mm. When an organic solvent and water were charged continuously into the two channels at a constant flow rate, a dynamic liquid–liquid (oil–water) interface was generated at the upstream junction; it was maintained through the channel and disappeared at the downstream junction. Therefore, molecules flowing through the device would experience the interface only temporarily. If an appropriately designed amphiphilic molecule having 1-D self-assembling ability was to be injected into the channel, it would begin to self-assemble at the upper junction to form 1-D assemblies that would separate from the interface after a period of time at the downstream junction, losing the self-assembling ability.[4] This self-assembly event would be influenced by the local concentration at the upper junction and the flow time required to reach the downstream junction.

For this study, we designed Zn–chlorophyll-based amphiphilic molecules featuring dendritic tetra(ethylene glycol) (TEG) units at position 17 and an isonicotinic acid (Chl-4Py, Figure 1)

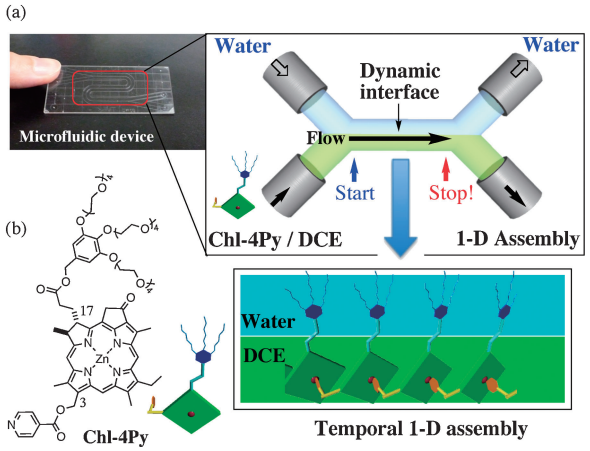

Figure 1. (a) Photograph and schematic representation of the microflow system. (b) Chemical structure of Chl-4Py and schematic representation of the 1-D self-assembly of injected amphiphilic molecules at the dynamic interface.

or nicotinic acid (Chl-3Py, Scheme S1)[11] moiety at position 3 of the chlorophyll ring.[3b,5] These amphiphilic molecules, which feature a perpendicular orientation between the TEG units and the coordination moieties, were suitable for 1-D self-assembly—formed mainly through Zn–pyridine coordination—at the dynamic interface (Figure 1). In addition, we prepared Chl-OMe and Chl-OH, which could form somewhat stiffer 1-D assembled structures through strong π–π stacking in addition to Zn–oxygen coordination (Scheme S1).[5b,5d,11]

1,2-Dichloroethane (DCE) solutions of Chl-4Py at three different concentrations (0.5, 2.5, and 12.5 mM) were prepared. Each solution was charged into a channel, with distilled water charged into the other, using a syringe pump, at a flow rate of 10, 30, or 50 μL min^{-1}. In these cases, the residence time of the injected solution was calculated to be 5.48, 0.80, and 0.48 s, respectively. The collected DCE solutions were subjected to spectroscopic measurements and microscopic observations.

Resonance Raman spectroscopy is a sensitive tool for detecting intermolecular interactions between chlorophyll units.[6] Therefore, we used it to observe the self-assembly of Chl-4Py at the dynamic interface through analysis of the flowing DCE solution in the microfluidic device. For a bulk DCE solution containing Chl-4Py, the stretching modes of the chlorophyll rings appeared at 1554 cm^{-1} upon excitation at 405 nm, assignable to monomeric Chl-4Py. When the pyridyl group in Chl-4Py was coordinated to the central Zn atom, however, the Raman peak shifted to a higher wavenumber (by ca. 5 cm^{-1}). With focus on these signals, we recorded Raman spectra at the start, middle (40 mm from the Y-junctions), and end of the

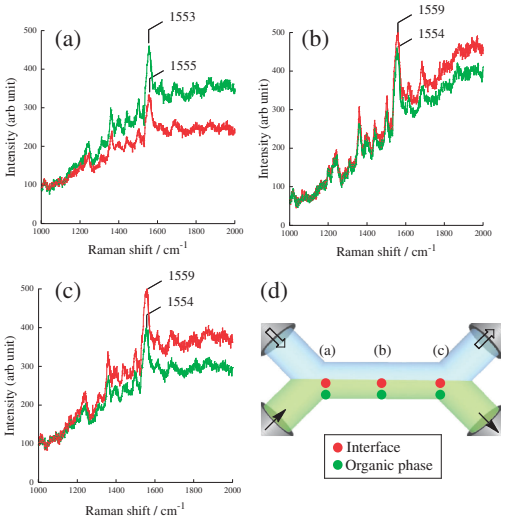

Figure 2. Resonance Raman spectra of Chl-4Py solutions (2.5 mM) flowing within the microchannel (10 μL min^{-1}) in (a) upstream, (b) midstream, and (c) downstream regions; red and green lines are spectra recorded at the interface and within the DCE phase, respectively; excitation at 405 nm. (d) Schematic representation of the data points; each letter (a)–(c) corresponds to the respective Raman spectrum.

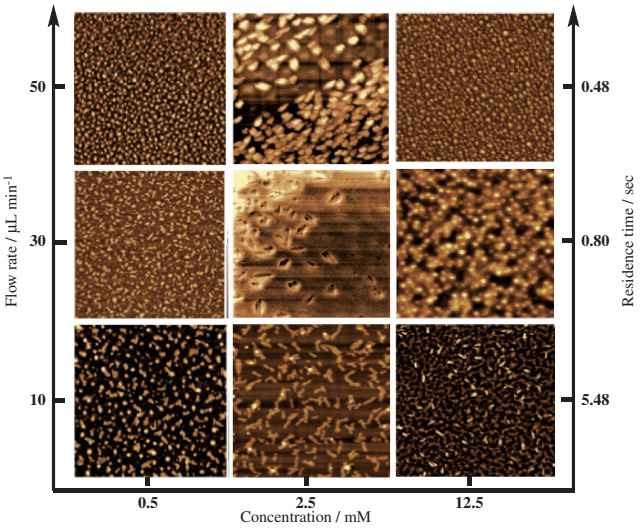

Figure 3. AFM images of the self-assembled structures of Chl-4Py; each sample was prepared immediately after eluting from the microchannel (5 μm × 5 μm, mica substrate, dynamic mode).

water–DCE dynamic interface (Figure 2d). We found that Chl-4Py existed in its monomeric form at the upstream junction, whereas it tended to assemble at the middle and downstream junction. Figures 2a–2c (for magnified spectra, see: Figure S1)[11] display the spectra obtained from each position in the flow channel marked in Figure 2d. At the upstream junction, the Raman signal appeared at 1555 cm^{-1} at the interface, consistent with monomeric Chl-4Py. In contrast, in the middle and at the downstream junction, the Raman signal at the interface appeared at 1559 cm^{-1}, but that in the DCE phase had not undergone such a shift. The relative intensity of the Raman signal in the DCE phase decreased upon progressing from up- to downstream; the corresponding intensity at the interface increased, with the accompanying peak shift. These real-time measurements of Raman spectra strongly supported the view that Chl-4Py underwent self-assembly at the dynamic interface through Zn–pyridine coordination. It should be emphasized that, unlike in bulk solutions, the effect of the specific surface area becomes predominant in a microchannel. Furthermore, as characteristic features of the microflow, the diffusional mass-transfer properties of molecules are also enhanced in microspace. Thus, the enhanced self-assembling ability of Chl-4Py in the microspace would be essential for the effective intermolecular interactions among Chl-4Py at the interface, leading to the creation of a 1-D assembly.[7] As a reference experiment, when water was carefully added to the DCE solution of Chl-4Py, forming a centimeter-scale interface, we could not observe any Raman spectral changes in the DCE solution even after leaving it to stand for several days, supporting the view that the self-assembling event occurs rapidly in the microspace.

To obtain further evidence for Chl-4Py self-assembling at the interface, we recorded AFM images of the eluted DCE solutions. To avoid morphological transformations in DCE, we cast the eluting solutions directly onto fresh mica surfaces and dried them under reduced pressure immediately. The AFM images in Figure 3 (for selected magnified images, see Figures S2 and S8a)[11] reveal that Chl-4Py self-assembled to form unique 1-D structures only when we employed its 2.5 mM solution in DCE.[8] Importantly, the sizes of the 1-D structures were almost uniform in each of the samples, but their shapes were affected by the applied flow rate. In the case of a 10 μL min^{-1} flow rate, Chl-4Py self-assembled to form tape-like structures, with an almost-flat cross section over the range; higher flow rates (30 and 50 μL min^{-1}) gave 1-D structures having curved surfaces (Figures S2 and S3).[9,11] In contrast, other concentrations of Chl-4Py (0.5 and 12.5 mM in DCE) gave only dot-like structures. AFM observations of homogeneous DCE solutions cast onto mica surfaces revealed similar dot-like structures, implying that they were formed during the removal of the solvent from the mica surface. Thus, the self-assembly of Chl-4Py at the dynamic interface occurred within a narrow concentration window. At a concentration of 0.5 mM, intermolecular interactions were suppressed, even at the interface, owing to the low local concentration at the initial Y-junction; in contrast, at the higher concentration of 12.5 mM, most of the Chl-4Py units eluted from the channel without effective contact at the interface, leading to the creation of dot-like structures, arising from monomeric Chl-4Py.

To further characterize the self-assembly of Chl-4Py, we recorded UV–vis spectra of the DCE solutions immediately after they had eluted from the channel (Figure S4).[11] Unexpectedly, we did not observe any changes in the UV–vis spectra of any of the samples subjected to the flow conditions, suggesting the absence of strong π–π stacking interactions among the Chl-4Py molecules.[10] The small but obvious Raman shifts in Figure 2 indicated that coordinative interactions played a critical role in the 1-D self-assembly process. Similar Raman shifts were evident for the same DCE solutions subjected to UV–vis spectral analysis (Figure S5).[11] From these results, the unique 1-D structures observed in the AFM images appeared to maintain

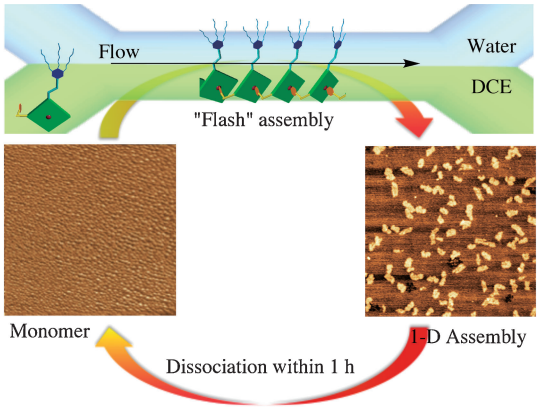

Figure 4. Schematic representation of the concept of temporal self-assembly at the flowing interface; AFM images of the self-assembled structures of Chl-4Py before and after contact with the interface (5 µm × 5 µm, mica substrate, dynamic mode). The image of 1-D assembly was recorded after reinjection of recycled DCE solution.

their structures mainly through Zn–pyridine interactions, with loose π–π stacking interactions merely supporting the 1-D molecular arrangement. Calculated models for the assembly of oligomeric Chl-4Py support this finding; π–π stacking interactions were weak in the 1-D structures because of the long pyridyl arms (Figure S6).[11] Along the same line, Chl-OMe and Chl-OH could not adopt such a 1-D molecular arrangement; they did not give any identifiable assembled structure through the microchannel accordingly.

Because Chl-4Py lacked the ability to form any self-assembled structures in DCE, time-course Raman spectra revealed that the shifted Raman peak assignable to the 1-D assemblies (1562 cm^{-1}) gradually returned to its original position (1554 cm^{-1}), assignable to monomeric Chl-4Py, within 1 h after eluting from the microchannel (Figure S5).[11] AFM images confirmed the dissociation of the assembled 1-D structures; all of the 1-D structures had transformed into dot-like structures after 1 h (Figure S8b).[11] Importantly, reinjection of the resultant DCE solutions into the microchannel led to reconstruction of the 1-D self-assembled structures, depending on the flow conditions (Figure 4). Thus, Chl-4Py displayed its inherent 1-D self-assembling ability at the dynamic interface but could maintain its resulting 1-D self-assembled structures only under the flow conditions; these architectures are temporal and renewable structures only under the flow conditions.

In summary, we have demonstrated that a microfluidic device can provide a novel self-assembling field within a flowing microspace, forming an oil–water interface that is indispensable for the temporal self-assembly of flowing amphiphilic molecules. One of the fundamental features of natural molecular systems—never before realized in an artificial system—is the use of time as a dimension in supramolecular assembly processes. We believe that this present system will open up new opportunities for the development of novel supramolecular structures.

We thank Dr. K. Sugiyasu and Dr. M. Takeuchi at NIMS for kindly supporting our SEM observations. This study was partially supported by Grants-in-Aid for Scientific Research on Innovative Areas "Reaction Integration," Scientific Research (B) (No. 22310069) and Exploratory Research (No. 23651118).

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8 We have investigated the effect of temperature on self-assembled property of Chl-4Py, with changing temperature of water phase at 4 and 65 °C. As a result, we could not observe any identical structures by AFM observation under such conditions.

9 To investigate the effect of water dissolving into DCE phase, we used water-saturated DCE for preparing a Chl-4Py solution. However, we could not observe any self-assembled structures in such "wet" DCE solution, implying that observed unique 1-D structures were certainly formed at the dynamic microinterface.

10 To avoid any transformations of the obtained self-assembled structures, we also injected the DCE solution directly into a flow cell immediately after its elution from the microfluidic device. Even under these conditions, however, we could not observe any shifts of the signals in the UV–vis spectra.

11 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

