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Flowing microenvironments regulate the helical pitch of a semi-artificial polymer

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In a microflow environment, the sizes of guest-binding pockets constructed by chlorophyll units can be regulated precisely through linking to the spring-like dynamic motion of a polymer backbone. Very rapid host/guest interactions at the polymer surface enabled isolation of metastable conformers having different helical pitches.

Because a polymer's function can be correlated directly to its conformation, the challenge remains to develop versatile strategies for regulating polymer conformations. In natural systems, the conformations of biopolymers (e.g., proteins, DNA) are dynamically regulated under physiological conditions and linked to microenvironmental changes, giving rise to diverse functions. To amplify the functions of a single polymer, such conformational changes should occur simultaneously for many polymers—that is, in a synchronized manner. This inherent conformational dynamism endows such biopolymers with unique molecular recognition behavior, reactivity, and self-assembling ability in extensive spaces and at appropriate times in response to external stimuli.¹ An example of dynamic self-assembly is that of the biopolymeric protein tubulin, which displays dynamic assembly/disassembly behavior at the end of microtubules, caused by conformational changes driven by GTP.^{1a} In addition, spider silk proteins switch their conformations

simultaneously from storage to assembly forms, leading to spontaneous self-assembly and the creation of extremely stable fibers on demand.^{1b,c} Appropriate functions can emerge from such spontaneous and synchronous conformational changes that are linked to the environment. The switching of these functions is generally an energy-consuming process, where micrometer-scale non-equilibrium phenomena are eventually translated to functions at molecular level. Controlling the dynamic motion of artificial polymers on demand through spontaneous responses to

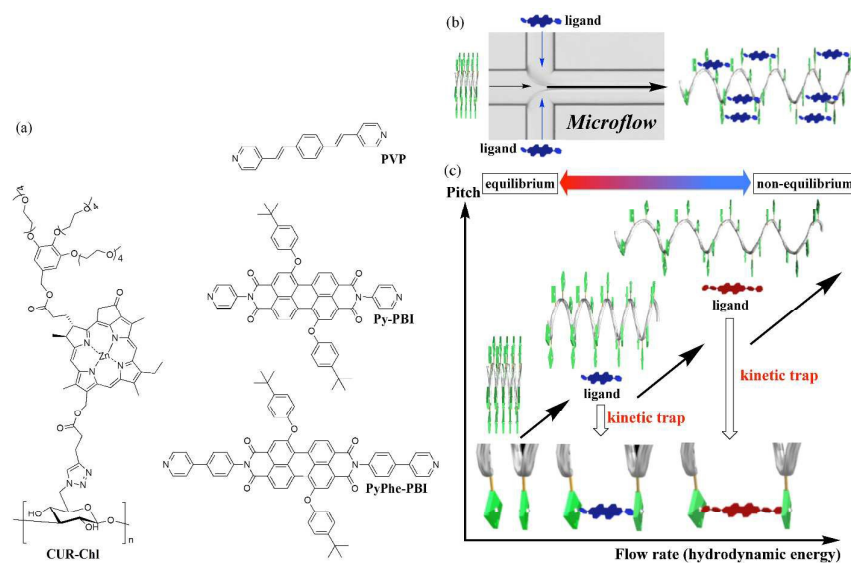


Fig. 1 (a) Chemical structures of CUR-Chl and the bipyridyl-type ligands PVP, Py-PBI, and PyPhe-PBI. (b) Schematic representation of the extension of CUR-Chl in a microflow channel. (c) Present concept described herein: adjustable molecular recognition ability depending on degree of energy input (flow rates), and kinetic trapping of temporally extended helical structures.

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environmental changes remains difficult, due to the lack of controlled non-equilibrium conditions.

The orientations of polymers under laminar flow—a non-equilibrium environment on the microscale—are strongly affected by the shear force generated, regardless of their chemical structure.^{2,3} Recently, we demonstrated the unique self-assembly behavior of a natural polysaccharide having a regular helical structure aligned

along the laminar flow.⁴ Thanks to their relative flexibility, such polysaccharides can also induce dynamic motion. Indeed, after appending appropriate peripheral molecular recognition moieties, based on chlorophyll units, onto this natural polymer, the resultant semi-artificial helix underwent spring-like contraction and expansion in a vial upon the addition of ligands capable of binding to the central metal atoms of the chlorophyll units.⁵ If this spring-like polymer were subjected to a microflow environment, we suspected it would align along the flow with its helical pitch varying in response to shear force effects, with the peripheral molecular recognition moieties aligning laterally to the flow. Considering the uniform microenvironments found in a microflow, synchronous expansion of many spring polymers should also occur; that is, all springs should adopt the same helical pitch at the same spatial point in the channel, exerting the same molecular recognition abilities on the polymer surface. In this paper, we demonstrate how precise regulation of the flowing non-equilibrium field on the micrometer-scale can induce continuous changes in the helical pitch of a semi-artificial polymer. Similar to temporal polymer alignment in a microflow, such induced conformational changes are maintained only under the effect of the laminar flow; therefore, the architectures formed can be regarded as examples of kinetic structures under non-equilibrium conditions. To fix the temporal conformations, appropriate ligands having molecular lengths comparable with the extended helical pitch can be inserted between the peripheral chlorophyll units through rapid molecular recognition.

We synthesized a semi-artificial curdlan (a β -1,3-glucan) featuring a dendritic amphiphilic Zn-chlorophyll unit attached to each glucose residue (CUR-Chl); we expected these units to act as molecular recognition sites for bipyridyl-type ligands (Figure 1a).⁵ Native curdlan adopts a right-handed helical structure having a pitch of approximately 1.8 nm, with six glucose residues per pitch.⁶ Accordingly, the attached chlorophyll units in CUR-Chl are aligned one-dimensionally along the long axis at 1.8 nm intervals. Previously, we reported that CUR-Chl can interact with PVP, a phenylenevinylene-derived bipyridyl ligand having a length of 1.6 nm, through Zn-pyridyl interactions.⁵ If CUR-Chl could also adopt extended forms under laminar flow, bipyridyl ligands longer than 1.8 nm might, therefore, interact with its chlorophyll units, leading to dramatic conformational changes in the helical main chain (Figure 1b). To test this hypothesis, we prepared two perylene bisimide-based bipyridyl ligands (Figure 1a) having lengths greater than 1.8 nm: Py-PBI (ca. 2.0 nm) and PyPhe-PBI (ca. 2.8 nm). We have confirmed that Py-PBI and PyPhe-PBI do not interact with CUR-Chl during conventional vial mixing, due to mismatches in molecular length.

We prepared a hydrodynamic flow focusing channel having a width of 100 μ m and a depth of 45 μ m (for details, see the Supporting Information). In this channel, at low Reynolds numbers, the central solution is squeezed into a narrow stream between the two adjacent streams. Because of the very short diffusion distance, solvent diffusion occurs rapidly across the stream line. We injected an aqueous solution [DMSO/water, 1:9 (v/v)] of CUR-Chl (0.5 mg/mL, 0.32 mM/repeating unit) from the central leg and squeezed it from the sides through the injection of THF (or DMF for PyPhe-PBI) solutions containing the bipyridyl ligands,⁷ which we expected to diffuse rapidly across the stream line and reach the chlorophyll units. Notably, CUR-Chl adopts a contracted form in mixed DMSO/water solutions, due to stacking of its chlorophyll units. We anticipated that dramatic conformational changes, from contracted to extended forms, would occur under the microflow conditions.

To determine the lower limit of the flow rate to induce an extended conformation for CUR-Chl, we employed PVP as the ligand; its length is almost identical to the original helical pitch of CUR-Chl. We injected an aqueous solution [DMSO/water, 1:9 (v/v)] of CUR-Chl (contracted form) from the central leg and a THF solution of PVP from the side legs. The eluted solution (200 μ L; injection time: 5.0 min) was poured directly into a vial containing distilled water (200 μ L) to give a solution having a concentration of 0.12 mg/mL (0.077 mM/repeating units) and a final solvent composition of DMSO/THF(DMF)/water of 1:10:29 (v/v/v). We confirmed that CUR-Chl itself could be solubilized in such a mixed solvent. We adjusted the flow rate ratio of the central and side flows to obtain total flow rates of 1, 5, 10, and 20 μ L/min. UV-Vis and circular dichroism (CD) spectra (Figure S1) of the resulting clear solutions confirmed that the CUR-Chl/PVP complex formed regardless of the applied flow rate. The absorption peak corresponding to PVP appeared near 350 nm. Notably, induced CD (i-CD) was clearly evident (Figure S1b) in the same wavelength region for each sample, implying that PVP was certainly incorporated within the π -space constructed by two chlorophyll units, stabilized through Zn-pyridyl coordination. These results suggest that CUR-Chl extended from its contracted conformation in the microflow even when the flow rate was as low as 1 μ L/min, providing suitable recognition pockets for PVP ligands.⁸ This result clearly indicates that CUR-Chl undergoes dramatic conformational changes, from globular to helical forms, and interacts with PVP ligands to form supramolecular complexes in the microflow. Considering the total flow rates and residence time, this dynamic event must have reached completion within a timeframe on the order of milliseconds.

Next, we employed Py-PBI, having a length of 2.0 nm, as the ligand to investigate whether an even more extended form of CUR-Chl was isolatable. Here, we fixed the concentration ratio of [Py-PBI]/[chlorophyll unit] at 0.5. According to the procedure described above, we collected eluted solutions obtained at total flow rates of 2,

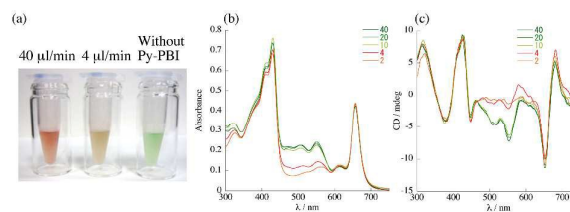


Fig. 2 Effect of flow rate on the molecular recognition ability of CUR-Chl; (a) UV-Vis (3-mm cell, r.t.) and (b) CD (10-mm cell, r.t.) spectra of CUR-Chl upon addition of Py-PBI; [Py-PBI]/[Chl unit] ratio: 0.5. Insets: Applied flow rates.

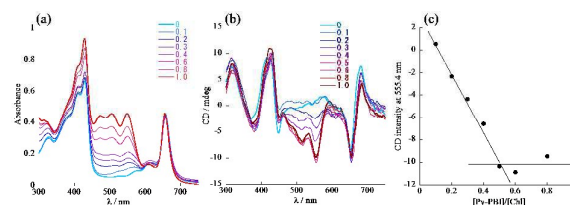


Fig. 3 (a) UV-Vis (3-mm cell, r.t.) and (b) CD (10 mm cell, r.t.) spectra of CUR-Chl in the presence of a stoichiometric amount of Py-PBI. Flow rate: 40 μ L/min.

4, 10, 20, and 40 $\mu\text{L}/\text{min}$. Microscopy images recorded inside the channel revealed that precipitation occurred at the cross-point under low-flow conditions (2 and 4 $\mu\text{L}/\text{min}$); in contrast, clear solutions formed at flow rates faster than 10 $\mu\text{L}/\text{min}$. Considering that CUR-Chl is soluble in DMSO/THF/water at 1:10:29 (v/v/v), it is likely that Py-PBI precipitated under the low-flow conditions in the absence of effective interactions with the chlorophyll units. In other words, the helical pitch of CUR-Chl would be shorter than 2 nm when the flow rate was less than 4 $\mu\text{L}/\text{min}$, whereas it extended to longer than 2 nm at higher flow rates. Color changes of the eluted solutions confirmed the different molecular recognition behavior of CUR-Chl in response to the flow rate. As displayed in Figure 2a, the original green solution of CUR-Chl changed dramatically upon complexation with Py-PBI in DMSO/THF/water at 1:10:29 (v/v/v); it became yellow after passing through the microflow at 4 $\mu\text{L}/\text{min}$ (where a less-than-stoichiometric amount of Py-PBI would interact with CUR-Chl), whereas, under a higher flow rate of 40 $\mu\text{L}/\text{min}$, the color of the solution changed from yellow to red, reflecting stoichiometric interactions. UV-Vis spectra of the eluted solutions provided more quantitative data. In the cases of flow rates of 2 and 4 $\mu\text{L}/\text{min}$, a weak absorption near 500–600 nm appeared, assignable to Py-PBI. In contrast, in the case of flow rates of 10, 20, and 40 $\mu\text{L}/\text{min}$, the intensity of this absorption band doubled, implying that CUR-Chl interacted with Py-PBI more effectively (Figure 2b, vide infra). Notably, i-CD was evident near 500–600 nm only for flow rates higher than 10 $\mu\text{L}/\text{min}$ (Figure 2c). These results support the notion that Py-PBI resided in the chlorophyll pockets only under higher flow rates. From these data, the lower critical flow rate required for complexation of Py-PBI was 10 $\mu\text{L}/\text{min}$, greater than that observed for the PVP ligand. These results support the view that a more extended form of CUR-Chl was temporally generated during the energy-consuming, non-equilibrium conditions, and that such a metastable polymer could be fixed through rapid interactions with Py-PBI.

To obtain further evidence for the formation of the supramolecular complex only under higher flow rates, we changed the concentration ratio of [Py-PBI]/[chlorophyll unit] under a fixed flow rate (40 $\mu\text{L}/\text{min}$) at which the helical pitch of CUR-Chl would be greater than 2 nm. Figures 3a and 3b present spectra recorded upon changing the [Py-PBI]/[chlorophyll unit] ratio from 0.1 to 1.0. In the UV-Vis spectra (Figure 3a), the intensity of the absorption band near 450–600 nm increased linearly up to a [Py-PBI]/[chlorophyll unit] ratio of 0.5. At ratios from 0.5 to 1.0, a new absorption peak was present at 476 nm; its intensity also increased linearly. We attribute this blue-shifted new band that appeared at higher [Py-PBI]/[chlorophyll unit] ratios to the π -stacking of PBI that occurred during rapid solvent diffusion in the microflow.⁹ Because the eluted solution was clear, the unreacted Py-PBI or its aggregate was presumably trapped within the chiral space constructed by the chlorophyll units or polysaccharide helix. CD spectra provided evidence for the reacted Py-PBI existing within the chiral π -space constructed by adjacent chlorophyll units. A signal for i-CD was apparent near 555 nm (Figure 3b), indicating that Py-PBI ligands were certainly coordinated to the central Zn ions. Along this line, we also observed new i-CD signals near 510 nm for [Py-PBI]/[chlorophyll unit] ratios between 0.5 and 1.0, presumably arising from π -stacked PBI, thereby suggesting its incorporation within the chiral space. The wrapping of hydrophobic guests by β -1,3-glucans has been confirmed for various other molecules, polymers, and self-assembled structures, with similar i-CD signals recognized at their absorption wavelengths.⁶ Although the CD intensity at 555 nm increased initially upon increasing the [Py-

PBI]/[chlorophyll unit] ratio, it reached saturation at a ratio of 0.5. The plot of the CD intensity at 555 nm with respect to the [Py-PBI]/[chlorophyll unit] ratio clarified this point (Figure 3c): two chlorophyll units interacted cooperatively with one Py-PBI ligand in a stoichiometric manner. Note that such a stoichiometric interaction can be accomplished only when all CUR-Chl units adopt the same conformation at the same spatial point; that is, synchronous extension behavior of CUR-Chl would be indispensable for the effective incorporation of Py-PBI.

In a reference experiment, we mixed a DMSO solution of CUR-Chl with a THF solution of Py-PBI and water in a vial while changing the [Py-PBI]/[chlorophyll unit] ratio from 0.1 to 1.5 (Figure S2). Under such vial mixing conditions, we did not observe any i-CD signals in the PBI region similar to those in Figure 3b, implying no effective interaction between CUR-Chl and Py-PBI in the vial. Thus, temporal extension of the helix of CUR-Chl in DMSO under laminar flow (i.e., an energy-consuming process) was indispensable for its stoichiometric interactions with Py-PBI.

The formation of the CUR-Chl/Py-PBI complex encouraged us to examine the behavior of PyPhe-PBI, a ligand (2.8 nm) that is approximately 1 nm longer than the original helical pitch of native curdlan. While maintaining the [PyPhe-PBI]/[chlorophyll unit] ratio at 0.5, we gradually changed the flow rate from 10 to 80 $\mu\text{L}/\text{min}$.¹⁰ Microscopy images revealed that PyPhe-PBI precipitated at flow rates of less than 20 $\mu\text{L}/\text{min}$; no precipitation occurred at higher flow rates. Therefore, the lower critical flow rate required for quantitative complexation was 40 $\mu\text{L}/\text{min}$, significantly greater than that observed for Py-PBI (10 $\mu\text{L}/\text{min}$). UV-Vis and CD spectra (Figure S3) confirmed this boundary flow rate. At 10 and 20 $\mu\text{L}/\text{min}$, weak and broad absorption peaks assignable to PyPhe-PBI appeared near 550 nm in the UV-Vis spectrum; the intensities of these signals increased discontinuously upon increasing the flow rate to 40 $\mu\text{L}/\text{min}$ (Figure S3a). When we increased the [PyPhe-PBI]/[chlorophyll unit] ratio from 0 to 1.0 at a fixed flow rate of 80 $\mu\text{L}/\text{min}$, an i-CD signal assignable to PyPhe-PBI appeared at 550 nm (Figure S4b). In the range of [Py-PBI]/[chlorophyll unit] ratios from 0.5 to 1.0, a new i-CD band appeared at 510 nm, presumably arising

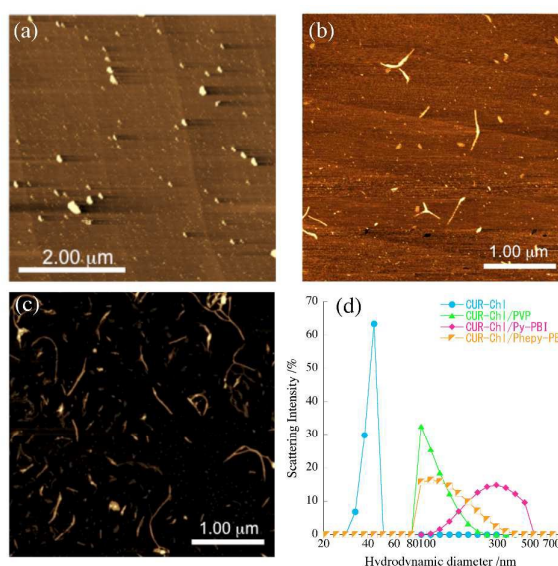


Fig. 4 (a, b) AFM images of the (a) Py-PBI/CUR-Chl and (b) PyPhe-PBI/CUR-Chl complexes; mica substrate. For other AFM images, see the Supporting Information. (c) DLS analytical data; x-axis represents the hydrodynamic diameter.

from self-aggregated PyPhe-PBI. A plot of the CD intensity at 549 nm with respect to the [PyPhe-PBI]/[chlorophyll unit] ratio (Figure S4c) confirmed the quantitative complex formation. The bend in the plot at a [PyPhe-PBI]/[chlorophyll unit] ratio of 0.5 strongly supports the notion of the formation of a 2:1 complex between the chlorophyll units and PyPhe-PBI. In terms of stoichiometry, the behavior is similar to that of the CUR-Chl/Py-PBI complex. We note, however, that a relatively high flow rate (high energy) was required for CUR-Chl to incorporate PyPhe-PBI quantitatively. The hydrodynamic energy regulated on the micrometer scale was directly converted to the helical pitch of CUR-Chl, which finally regulated the molecular recognition ability of the chlorophyll pockets on the nanometer scale.

We cast the eluted solutions containing the complexes onto mica substrates and observed their morphologies (Figures 4a–c) using atomic force microscopy (AFM). Although CUR-Chl itself formed globular aggregates in water-rich solvents, due to its strong π -stacking ability, we observed extended fine fibrous structures for both the CUR-Chl/Py-PBI and CUR-Chl/PyPhe-PBI complexes, with average lengths of 230 and 390 nm, respectively.¹¹ From the molecular weight of our CUR-Chl sample, we estimated an average length of approximately 200 nm in the neutral state.⁵ In addition, we used dynamic light scattering (DLS) to measure the lengths of the extended fibers formed from CUR-Chl/Py-PBI and CUR-Chl/PyPhe-PBI. Here, we fixed the [ligand]/[chlorophyll unit] ratio at 0.5, expecting the creation of stoichiometric complexes. As revealed in Figure 4d, the average hydrodynamic diameter of CUR-Chl was 48 nm in DMSO/THF/water at 1:10:29 (v/v/v), indicative of the formation of globular aggregates (vide infra). Quantitative interactions with the ligands in the microflow dramatically increased the average hydrodynamic diameters of the CUR-Chl/Py-PBI and CUR-Chl/PyPhe-PBI complexes to 173 and 200 nm, respectively. These results suggest that the helical structures arising from the natural polysaccharide are rather flexible, accommodating unusually extended conformations under high-energy conditions. These extended forms are isolatable (kinetically stabilized) after spontaneous interactions with appropriate ligands. We have confirmed that the obtained complexes were stable, even after elution, with no structural changes occurring in solution, indicative of kinetically trapped structures. Notably, in this present study, the initial structure formed prior to injection into the microflow would be a globular or coil structure. The creation of extended fibrous structures implies that CUR-Chl underwent dramatic conformational changes under the effect of the laminar flow. Height profiles of the AFM images provided consistent average diameters for these complexes of 1.8 nm, regardless of the ligand length. This value is less than the ideal fiber diameter expected for CUR-Chl, presumably because of the strong adsorption of CUR-Chl on the mica substrate. For comparison, we evaluated the relative fiber lengths. We estimated the ratio of the fiber lengths of the CUR-Chl/PVP, CUR-Chl/Py-PBI, and CUR-Chl/PyPhe-PBI complexes to be 1.00:1.15:1.95. CPK modeling suggested that the ratio of the lengths of the ligands PVP, Py-PBI, and PyPhe-PBI is 1.00:1.25:1.75. The good correlation between these ratios suggests that the fibrous structures were isolated as one-piece structures (not bundled fibers) with each ligand interacting quantitatively with two chlorophyll units in CUR-Chl to induce the different polymer conformations.

In conclusion, we have demonstrated that the helical pitch of a semi-artificial polymer can be tuned precisely on the nanometer scale upon regulating the hydrodynamic properties of its flowing solution on the micrometer scale (Figure 1c). This behavior can be

regarded as a type of top-down regulation of molecular function. Our results further indicate that non-equilibrium phenomena on the micrometer scale can directly influence supramolecular structures and their functions (molecular recognition abilities) on the nanometer scale. These findings provide an impetus into how to exploit non-equilibrium states within practical materials. Although stimuli-responsive dynamic motion of polymers linked to environmental changes remains rare outside of the cell, it has been achieved successfully under controlled non-equilibrium conditions in a microflow. The helical structures of the natural polysaccharide are rather flexible, accommodating unusual extended forms (almost twice as long as the natural state) when subjected to compressive stress. The resultant kinetic structures formed from CUR-Chl in the microflow can be immobilized through precise and rapid molecular recognition with appropriate ligands. Notably, the dramatic conformational changes and subsequent immobilization with ligands were complete within very short periods of time (generally milliseconds). In the microflow, all of the injected CUR-Chl molecules were presumably aligned in the same direction, allowing effective interactions with ligands diffused homogeneously and injected laterally toward the central solution. The orientation of CUR-Chl and the directional diffusion of ligands led to homogeneous and simultaneous interactions that played crucial roles in effectively immobilizing the elongated helical structures. We believe that this strategy will be applicable more generally to the structural manipulation of polymers, thereby providing a new field between supramolecular chemistry, micro- and non-equilibrium sciences.

Acknowledgment

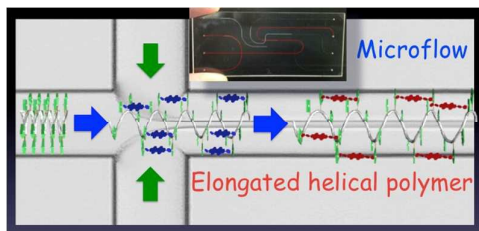
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- 7 When we injected a mixed solution containing both CUR-Chl and the ligand, we did not observe any interactions between them.
- 8 In our previous system, we performed a series of experiments beginning with the extended form of CUR-Chl in DMSO. Therefore, the interaction of CUR-Chl with the PVP ligands always occurred during the contraction process of Chl-Chl upon the addition of water (see reference 5). In the present system, however, CUR-Chl adopted the contracted form at the beginning and the extended form in the microflow.
- 9 We have previously confirmed that PBI derivatives spontaneously self-assemble to form aggregates under microflow conditions. In addition, the helical structure of CUR can provide a hydrophobic one-dimensional domain capable of incorporating insoluble aggregates; see M. Numata, *Chem. Asian. J.* 2015, **10**, 2574–2588 (Focus Review).
- 10 Here, we prepared a DMF solution of PyPhe-PBI, instead of THF, because of its poor solubility. In addition, the binding ability of CUR-Chl would decrease under the applied flow conditions because of its extreme elongation. Accordingly, we adjusted the final concentration of the final complex to be doubled (for details, see the Supporting Information).
- 11 We evaluated the average lengths of these complexes from measurements of more than 50 fibers.

Graphical Abstract



A novel strategy has been developed for tuning a polymer's conformation in a microfluidic system. The helical pitch of a semi-artificial polymer was controlled precisely in a top-down manner under the effect of shear forces. Furthermore, molecular recognition abilities of the polymer can be tuned under the non-equilibrium regulated in the microflow. The obtained temporal conformations were fixed at desired pitches through interaction with appropriate ligands.