Electronic Supplementary Information

Electrostatically promoted dynamic hybridization of glucans with cationic polythiophene

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Experimental Section

Instruments. ^{1}H, NOESY, COSY, HMBC, and DOSY NMR spectra were recorded at 600 MHz on a Varian INOVA-600 instrument. UV/vis spectra were measured in a quartz cell (1 cm optical path length) on JASCO V-550, V-560, or Varian Cary 1 spectrometer, and CD spectra on JASCO J-720WI or J-820YH spectrometer, both equipped with an ETC-505T temperature controller. Fluorescence emission and excitation spectra were recorded on JASCO FP-6500 or PT1 QM-2 spectrofluorimeter. Dynamic light scatteirng and zeta potential values were recorded on an Otsuka ELSZ-2 instrument. Molecular weight of pyridinio-polythiophene (PyPT) was determined relative to polystyrene standards by using analytical GPC (UV detector) with a TOSOH TSKgel α-4000 column at 40 °C eluted with DMF at a flow rate of 0.5 mL min^{-1} under isocratic conditions, and those of curdlan (Cur) and schizophyllan (SPG) were determined relative to dextran standards by using analytical GPC with a TOSOH TSKgel α-M column and a refractive index (RI) detector at 40 °C eluted with DMSO at a flow rate of 0.5 mL min^{-1} under isocratic conditions. Solution pH values were measured by using a HORIBA standard ToupH electrode 9615-10D.

Materials. Spectrophotometric grade DMSO (Wako) and water (milli-Q) were used as solvents without further purification.

PyPT[1] and CDPT[2] were synthesized as reported previously and showed satisfactory agreement of the spectroscopic data with the literature values. The number average molecular weight (M_n) and the polydispersity index (PDI) of PyPT were determined as 3.65 x 10^4 and 2.4 at 280 nm, respectively. Cur, purchased from Wako, was dried at 80 °C under high vacuum prior to use and its M_n and PDI were determined as 1.3 x 10^6 and 1.5, respectively. Amylose was purchased from TCI (M_w = ca. 15000). SPG was supplied by Mitsui sugar Co., and its M_n and PDI were determined as 1.44 x 10^5 and 3.8, respectively.

Sample Preparation. A 1:9 mixture of DMSO and H_2O was used throughout the work and all polymer concentrations are in monomer unit. Stock solutions of Cur (10 mM), CDPT (0.45 mM), and amylose (6 mM) were prepared by dissolving the pure samples in DMSO. A stock solution of PyPT (1.8 mM) was prepared by dissolving the sample in water. The stock solutions of Cur were sonicated for 3 h to achieve transparent solutions, while the PyPT stock solution was similarly sonicated for 1 h.

Cur/PyPT and amylose/PyPT solutions were prepared as follows: A 325 μL portion of the PyPT stock solution was added to a 0.3 mL portion of the stock solution of polysaccharide as prepared or further diluted to the desired concentration. The resulting solution was diluted with water (2.375 mL) and the mixture was stirred for 10 min. Acidic or basic sample solution was prepared by adding an appropriate amount of aqueous HCl solution (10 mM) or aqueous KOH solution (0.2, 2.0, or 16.5 M). Cur/CDPT solutions were prepared as follows: A 133 μL portion of the CDPT stock solution was added to a 0.3 mL portion of the stock solution of Cur as prepared or further diluted to the desired concentration. The resulting solution was diluted with water (2.567 mL) and the mixture was stirred for 10 min.

Annealing experiments were run in a temperature-controlled cell with CD monitoring. The sample was heated to 90 °C at a rate of 1 °C min^{-1}, held at that temperature for 30 min, and then cooled down to 25 °C at a rate of 1 °C min^{-1}. The salt effect was examined by adding appropriate amounts of NaCl powder to the sample solution and stirring the resulting solution.

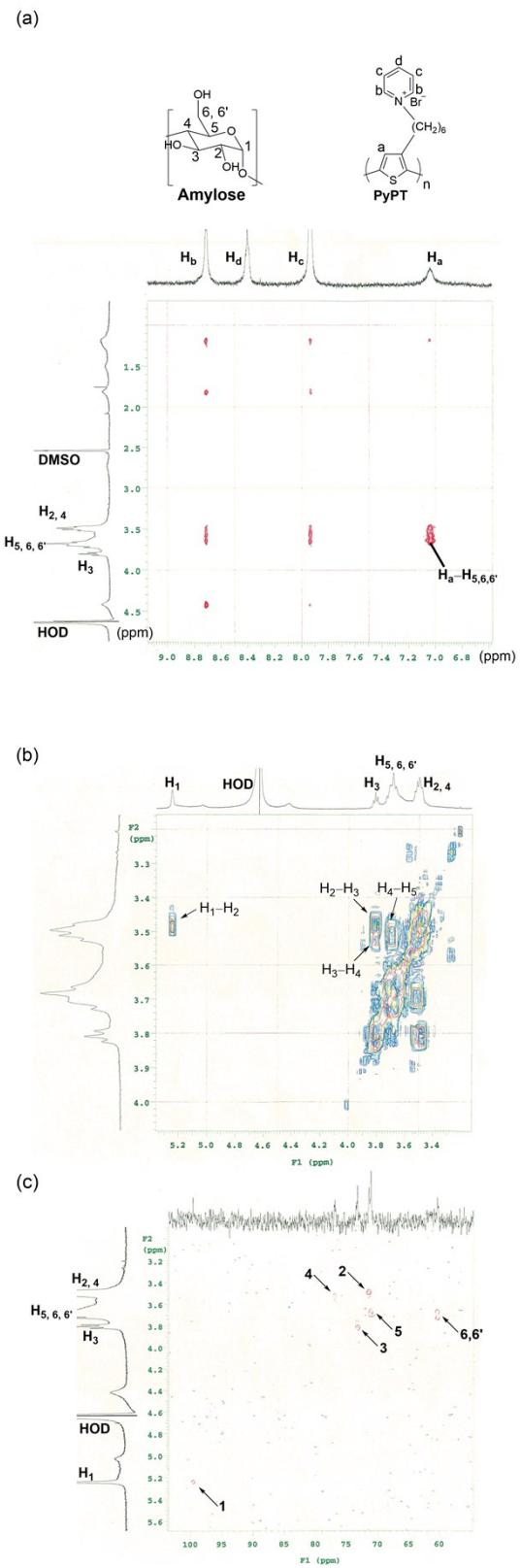

Fig. S1 (a) NOESY, (b) COSY and (c) HMBC spectra of PyPT (0.2 mM) and amylose (0.61 mM) in 1:9 DMSO-d_6/D_2O at room temperature.

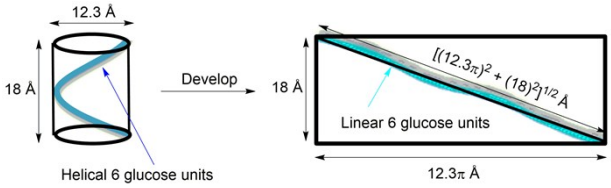

Fig. S2 Development of a 6_1 Cur helix.

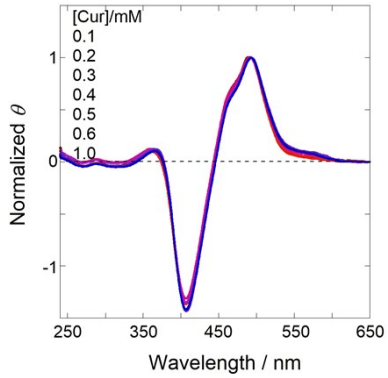

Fig. S3 Normalized CD spectra of PyPT (0.2 mM) in 1:9 DMSO/H_2O in the presence of varying amount of Cur (0.1-1.0 mM), measured at 25 °C in a 1 cm cell.

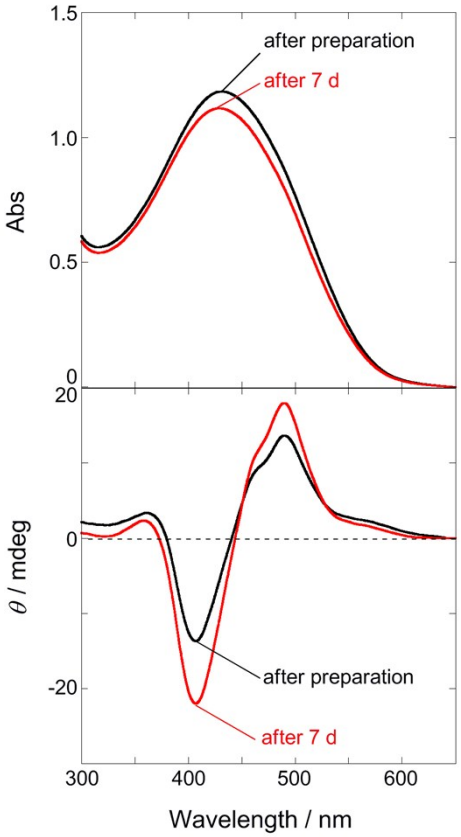

Fig. S4 UV-vis (top) and CD spectra (bottom) of PyPT (0.2 mM) and Cur (1.0 mM) in 1:9 DMSO/H_2O immediately after preparation (black) and after standing for 7 days at room temperature in the dark (red), measured at 25 °C in a 1 cm cell.

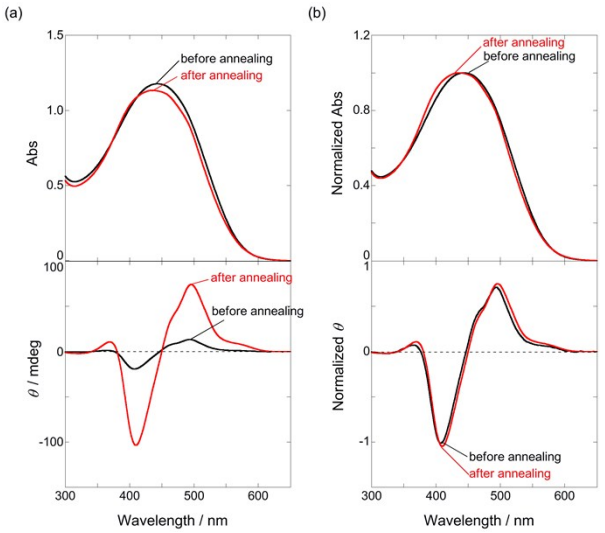

Fig. S5 (a) Original and (b) normalized UV-vis and CD spectra of PyPT (0.2 mM) and Cur (1.0 mM) in 1:9 DMSO/H_2O at 25 ºC before (black) and after repeated annealing (red).

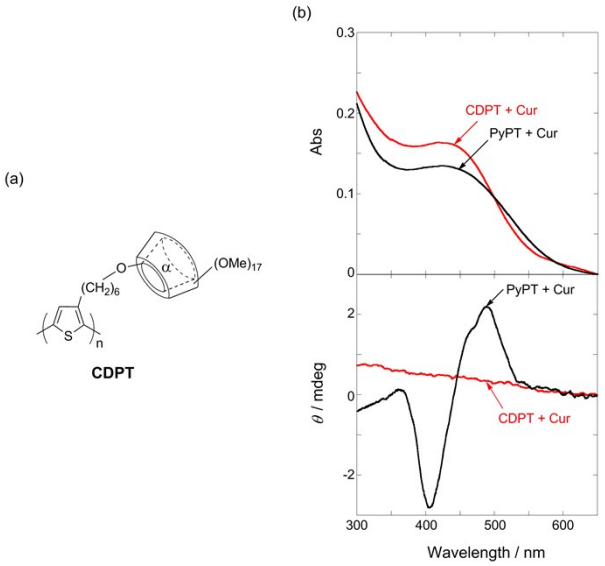

Fig. S6 (a) Structure of head-to-tail coupled poly(3-(6-(per-*O*-methyl-α-cyclodextrin-6-oxy)hexyl) thiophene) (CDPT) and (b) UV-vis (top) and CD (bottom) spectra of CDPT (0.02 mM; red) and PyPT (0.02 mM; black) in the presence of Cur (1.0 mM) in 1:9 DMSO/H_2O at 25 °C in a 1 cm cell.

Discussion: In order to assess the role of the cationic sidechain of PyPT in the hybridization with Cur, we employed α-cyclodextrin-appended PT (CDPT, degree of substitution = 1.0) (Fig. S6a)[2] as a potential hybridization partner (or a reference). The cyclodextrin pendant, though bulky, is electroneutral, highly water-soluble, and tethered to Cur with a long alkyl chain, and therefore will be sticking out of the helix when hybridized with PyPT in aqueous solution, without causing any serious steric hindrance. However, no appreciable Cotton effect was detected upon mixing aqueous CDPT solution with a DMSO solution of Cur (Fig. S6b, red line), indicating no hybridization. In keen contrast, the same amount of PyPT (0.02 mM) as the correct hybridization partner hybridized with Cur to give the apparent CD couplet (Fig. S6b, black line). This result reveals that the neutral sidechain introduced and the hydrophobic interaction between the PT main chain and Cur are not sufficient to promote hybridization, highlighting the positive role of the cationic sidechain in facilitating hybridization.

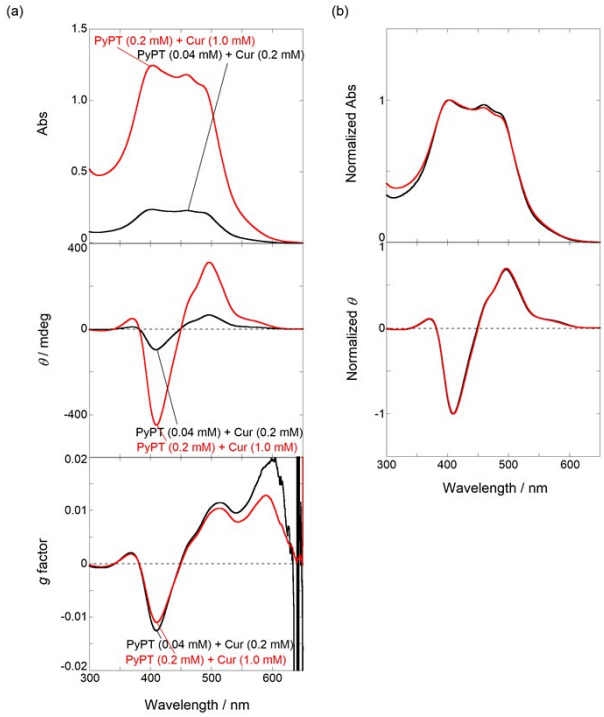

Fig. S7 Effects of 5-fold dilution on (a) original and (b) normalized UV-vis (top), CD (middle), and anisotropy (bottom) spectra of 0.2 mM PyPT and 1 mM Cur at pH 11 in 1:9 DMSO/H_2O, measured at 25 °C in a 1 cm cell.

Discussion: In this experiment, we employed the concentrations of 0.2 mM for PyPT and 1.0 mM for Cur, at which the induced CD intensity was saturated (Fig. 3a). The 5-fold dilution of this solution led to a decrease of the CD intensity at 409 nm by a factor of 5, indicating that the PyPT in the solution was fully hybridized with Cur even under the dilute condition. Also, the anisotopy and the normalized UV-vis and CD specta of the two solutions at different concentrations are in nice agreement with each other, validating that the induced Cotton effects arise from the same species, i.e. hetero-triplex. This result is consistent with the discussion in the main text (based on the data in Figs. S4 and 4) that PyPT irreversibly hybridizes with Cur to give a kinetically stable hybrid complex and also that the aggregation does not appreciably affect the chiroptical properties of hetero-triplex and hetero-duplex at least under the conditions employed; see Fig. S16 for more detailed examinations of the dilution effects.

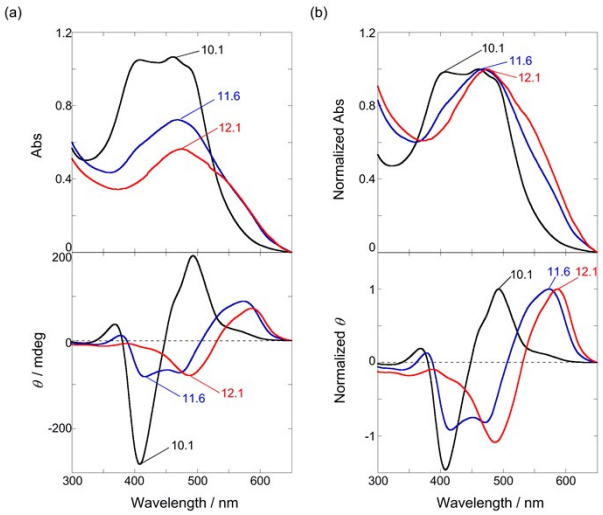

Fig. S8 (a) Original and (b) normalized UV-vis and CD spectra of PyPT (0.2 mM) and Cur (1.0 mM) in 1:9 DMSO/H_2O at pH 10.1 (black), 11.6 (blue), and 12.1 (red), measured at 25 ºC in a 1 cm cell.

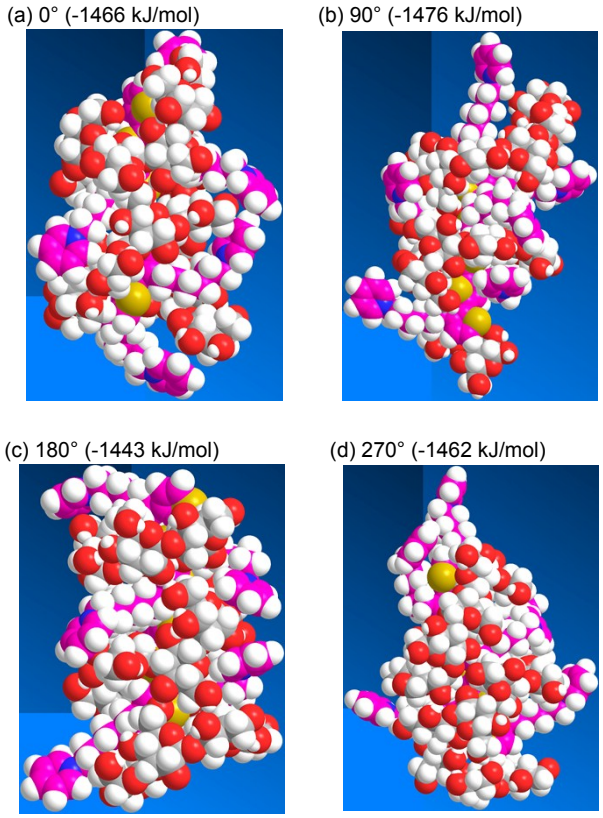

Fig. S9 Optimized structures (sideviews) and energies (in the parentheses) of PyST-glucose 18-mer complexes, obtained by the Monte-Carlo conformer search using the OPLS-2005/H_2O force field (1000 steps). The initial structures were constructed by arbitrally placing PyST at (a) 0°, (b) 90°, (c) 180°, and (d) 270° in the central cavity of glucose 18-mer sliced out of the crystallographic structure of Cur triplex.[3]

Discussion: The most acidic 2-hydroxyls[4] on the 2nd, 5th, 8th, 11th, 14th, and 17th glucose units in the glucose 18-mer were deprotonated to balance the charges of the pairing partner PyST and also to satisfy the 1:3 stoichiometry experimentally proven in the main text. The PyST was arbitrarily placed in the cavity of the glucose 18-mer at four different angles of 0, 90, 180, and 270° to make as the initial duplex structures. The four initial duplex structures thus prepared were geometry-optimized by the Monte-Carlo conformer search program using the OPLS-2005/H_2O force field,[5] which is known appropriate for electrostatically interacting systems in biomacromolecules. During the calculation, the sulfur atoms in PyST and the C-1 carbons in the glucose 18-mer were restricted to avoid the dethreading of the PyST from the cavity (for all searches; see Fig. S9).

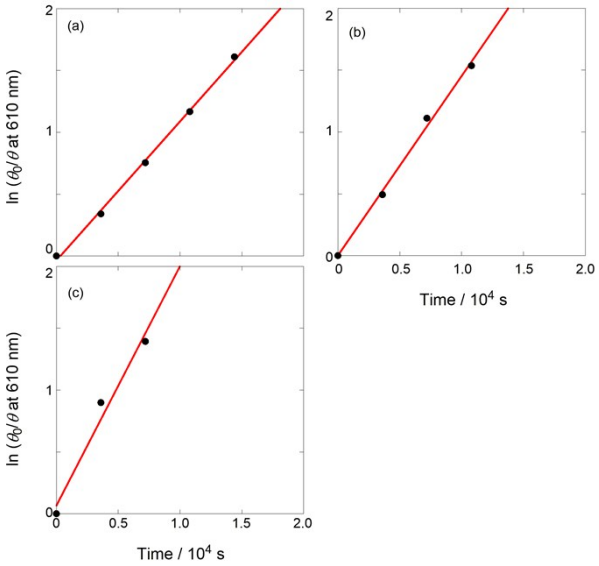

Fig. S10 Pseudo-first order kinetics plots for the rehybridization of Cur-PyPT hetero-duplex to hetero-triplex in 1:9 DMSO/H_2O (pH 10.5) at (a) 85 °C, (b) 90 °C, and (c) 95 °C; [PyPT] = 0.20 mM, [Cur] = 1.0 mM (correlation coefficient: (a) 0.999, (b) 0.998, and (c) 0.986).

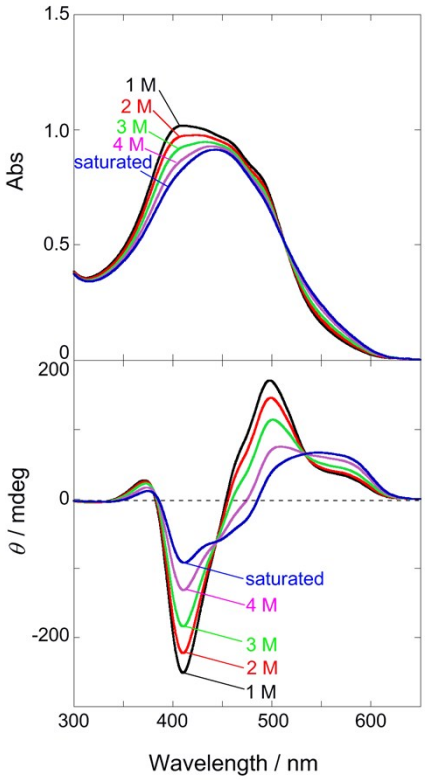

Fig. S11 UV-vis (top) and CD (bottom) spectral changes of a DMSO/H_2O (1:9) solution of PyPT (0.2 mM) and Cur (1.0 mM) at pH 10.1 upon addition of 1 M (black), 2 M (red), 3 M (green), 4 M (purple), and saturated NaCl (blue), measured at 25 ºC in a 1 cm cell.

Fluorescence spectral examinations. The effects of hybridization on the fluorescence behavior were evaluated by exciting PyPT in the presence and absence of Cur at an apparent isosbestic point (380 nm) (Fig. S12a). As shown in Fig. S12b, free PyPT emitted weak fluorescence at 570 nm (Fig. S12b, black). Upon addition of Cur, a new peak, assignable to the Cur-PyPT triplex, emerged at 530 nm in addition to the fluorescence of free PyPT at 570 nm (Fig. S12b, red). However, the direct subtraction of the black spectrum (free PyPT) from the red spectrum (free PyPT plus Cur-PyPT triplex) leaves a remnant shoulder at 570 nm assignable to free PyPT. The higher fluorescing efficiency is likely caused by the increased viscosity and decreased collisional deactivation by solvent upon addition of Cur. Hence, the fluorescence intensity of free PyPT was multiplied by a factor of 3.5 and then the subtraction was performed to give the fluorescence spectrum of Cur-PyPT triplex shown in Fig. S12b (purple). Significantly, the fluorescence intensity of the less-populated hetero-triplex was higher than that of free PyPT. The much enhanced fluorescence quantum yield upon hybridization is rationalized by the hindered rotational relaxation as well as the suppressed collisional deactivation by solvent molecules.

The fluorescence excitation spectra recorded at different monitoring wavelengths unequivocally revealed the origin and nature of the new emission. As shown in Fig. S12c, the excitation spectra monitored at 530 nm (black line), 570 nm (red line), and 600 nm (blue line) were distinctly different in shape from the UV-vis spectrum, with an extra peak being observed at 394 nm in the excitation spectra. This peak became more intense when monitored at shorter wavelengths, indicating that the Cur-PyPT hetero-triplex absorbs at 394 nm and fluoresces at 530 nm. The fluorescence and excitation spectral examination enabled us to unequivocally differentiate the Cur-PyPT triplex from free PyPT. The photophysical properties of the hetero-triplex, i.e. the narrower bandwidth and stronger fluorescence, are consistent with the shorter conjugation length of PT[6] in the triplex. As is often the case with conjugated polymers,[7] the fluorescence bandwidth of free PyPT was much narrower than the absorption bandwidth (Fig. S12d) and the Stokes shift was appreciably larger for free PyPT (63.1 kJ mol^{-1}) than for the triplex (42.9 kJ mol^{-1}) (Figs. S12a-c). These behaviors are likely to originate from the conformational flexibility that allows a wide distribution of the conjugation length in the ground state and the fast energy migration to lower energy segments over the PyPT main chain in the excited state, facilitating emission from the more conjugated, lower energy segments in the case of free PyPT.

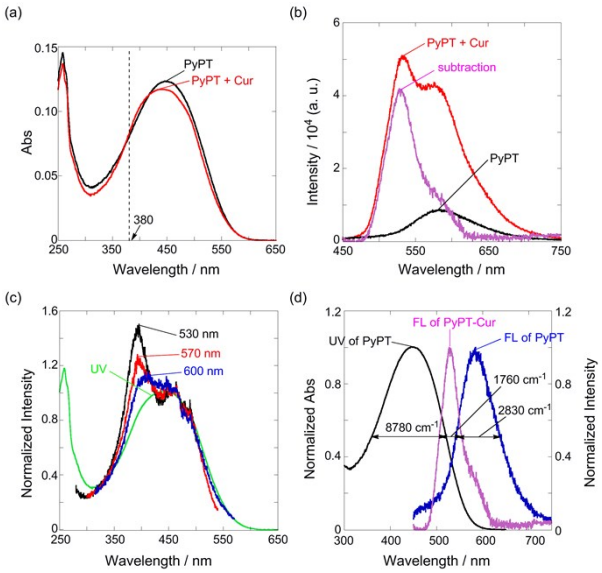

Fig. S12 (a) UV-vis spectra of PyPT (0.02 mM) in 1:9 DMSO/H_2O at pH 6.3 in the presence (red) and absence (black) of Cur (0.1 mM); (b) fluorescence spectra of the same solutions, excited at 380 nm (where the two solutions had the same absorbance); subtraction spectrum (red – black; multiplied by a factor of 3.5) in purple; (c) fluorescence excitation spectra of the same solutions monitored at 530 nm (black), 570 nm (red), and 600 nm (blue), and the UV-vis spectrum of the same solution (green), all normalized at 455 nm; (d) normalized UV-vis (black) and fluorescence spectra of free PyPT (blue) and Cur-PyPT triplex obtained by spectral subtraction (purple) with fwhm values; all spectra recorded at 25 ºC in a 1 cm cell.

Since annealing promoted the Cur-PyPT hybridization (Fig. 4), an aqueous DMSO solution of PyPT and Cur at pH 6.3 was heated up to 90 °C and the fluorescence spectral behavior was examined before and after the annealing process. As shown in Fig. S13 in ESI, the annealing significantly enhanced the hybrid fluorescence at 530 nm, but the free PyPT fluorescence at 580 nm was almost unaffected in intensity, appearing on the shoulder of the 530 nm peak.

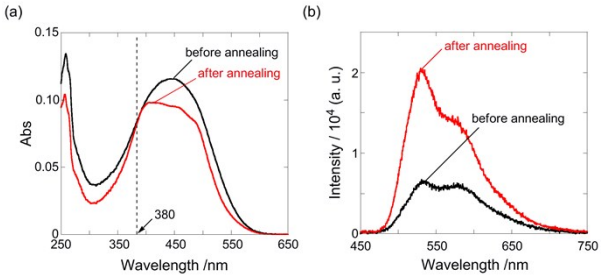

Fig. S13 (a) UV-vis and (b) fluorescence spectra of PyPT (0.02 mM) and Cur (0.1 mM) in 1:9 DMSO/H_2O before (black) and after (red) annealing at 90 °C; all measured at 25 ºC in a 1 cm cell with excitation at 380 nm.

Cur-PyPT hybridization was promoted also under basic conditions through deprotonation of the hydroxyl groups of the glucose units. Hence, we examined the fluorescence spectral behavior of Cur-PyPT hybrids in aqueous DMSO solutions at pH 11.0. As shown in Fig. S14a, a new band, which is assignable to Cur-PyPT duplex (Fig. 5), appeared at wavelengths >550 nm in the UV-vis spectrum, while the fluorescence intensity (Fig. S14b, red line) was enhanced over the entire wavelength range with an appreciable bathochromic shift of the low-energy peak from 580 nm to 590 nm, when compared with the behavior at pH 6.3 (black line). The global enhancement is accounted for in terms of the accelerated hetero-triplex formation, but the bathochromic shift cannot be rationalized without considering the fluorescence from hetero-duplex that absorbs and emits at longer wavelengths.

This idea was proven by comparing the excitation spectra of Cur-PyPT solutions at pH 11.0 and 6.3. As shown in Fig. S14d, the excitation spectra of the pH 11 solution monitored at 530 nm (black line) and 570 nm (red line) did not greatly differ in shape from those of the pH 6.3 solution (Fig. S14c), with exception to the relative intensity of the two peaks. However, the excitation spectrum of the basic solution monitored at 600 nm (blue line) revealed significant differences from those of the slightly acidic solution, exhibiting a narrower peak at longer wavelengths in good agreement with the enhanced absorption by the hetero-duplex. It is summarized that the absorption and emission wavelengths, and hence the average conjugation length, increase in the order: Cur-PyPT triplex < free PyPT < Cur-PyPT duplex.

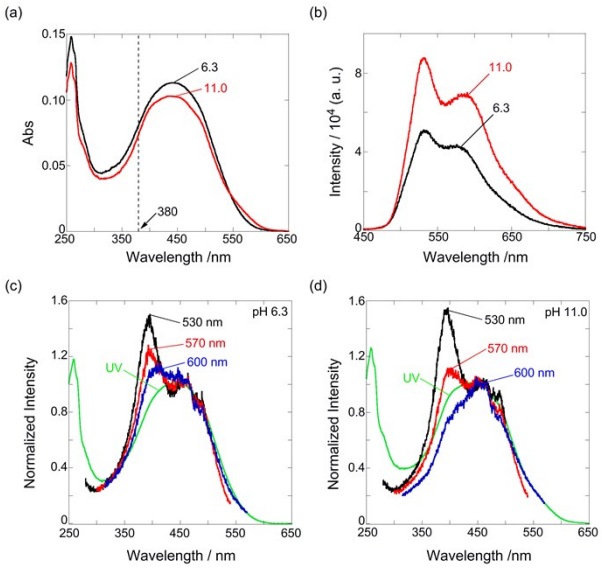

Fig. S14 (a) UV-vis and (b) fluorescence spectra of PyPT (0.02 mM) and Cur (0.1 mM) in 1:9 DMSO/H_2O at pH 6.3 (black) and pH 11.0 (red) recorded at 25 ºC in a 1 cm cell; the fluorescence spectra were measured with excitation at 380 nm to achieve comparable excitation. (c) and (d) Fluorescence excitation spectra of the same solutions at pH 6.3 (c) and 11.0 (d), monitored at 530 nm (black), 570 nm (red), and 600 nm (blue), and the corresponding UV-vis spectra (green); all normalized at 455 nm.

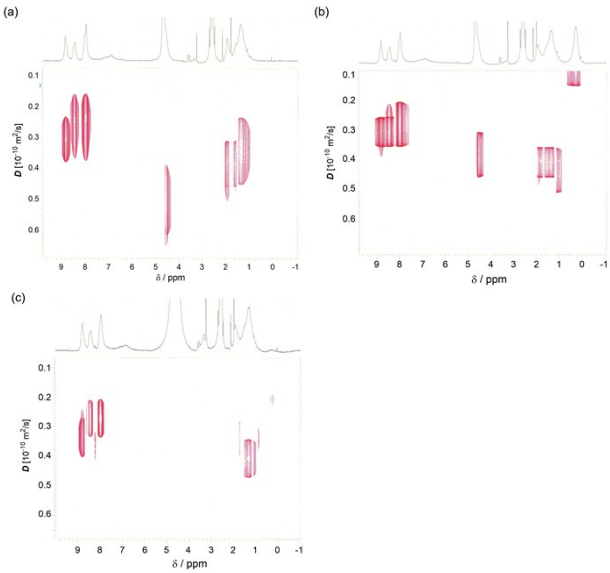

Fig. S15 DOSY spectra of (a) PyPT (1.0 mM), (b) PyPT (1.0 mM) + Cur (2.0 mM) at pH 6.3 (as prepared), and (c) PyPT (1.0 mM) + Cur (2.0 mM) at higher pH (>11) in 3:7 DMSO-d_6/D_2O at room temeparature.

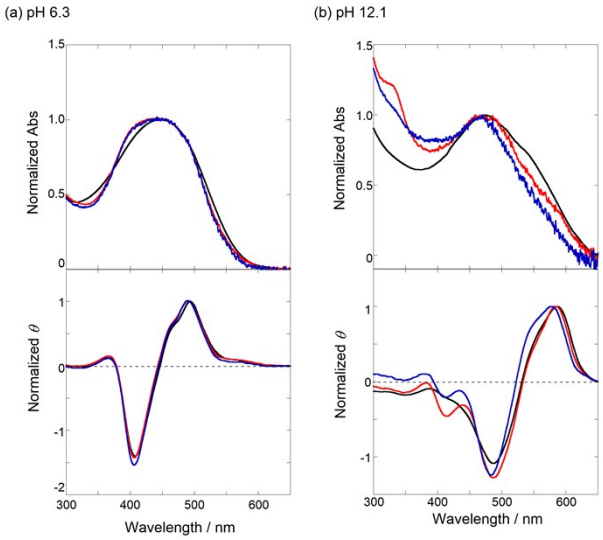

Fig. S16 Normalized UV-vis and CD spectra of PyPT and Cur at 0.2 and 1.0 mM (black), 0.02 and 0.1 mM (red), and 0.01 and 0.05 mM concentrations (blue) in 1:9 DMSO/H_2O at pH 6.3 (a) and 12.1 (b), measured at 25 °C in a 1 cm cell.

Discussion: The DLS result urged us to examine the effects of dilution on the UV-vis and CD spectra, which were originally obtained for solutions containing 0.2 mM PyPT and 1.0 mM Cur. However, no essential changes were observed upon 5-fold dilution at pH 11, as can be seen from practically the same normalized UV-vis and CD spectra as well as the anisotropy spectra for the original and diluted solutions (see Fig. S7 and relevant discussion in ESI). Also, the normalized CD spectra of hetero-triplex (pH 6.3) (Fig. S16a, bottom) and hetero-duplex (pH 12.1) (Fig. S16b, bottom) did not show any significant changes over the concentration range employed, despite the progressively increasing hydrodynamic diameters at higher concentrations (Fig. 8). This means that the aggregation of hetero-triplex and -duplex does not affect their helical sense or pitch and hence the inherent chiroptical properties of PyPT-Cur hetero-triplex and hetero-duplex are preserved even in the aggregates.

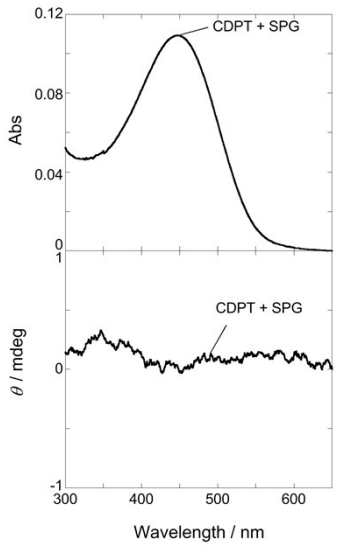

Fig. S17 UV-vis (top) and CD (bottom) spectra of CDPT (0.02 mM) and SPG (1.0 mM) in 1:9 DMSO/H_2O, measured at 25 °C in a 1 cm cell.

References

1. G. Fukuhara and Y. Inoue, *J. Am. Chem. Soc*., 2011, **133**, 768.
2. G. Fukuhara and Y. Inoue, *Chem. Eur. J*., 2012, **18**, 11459.
3. C. T. Chuah, A. Sarko, Y. Deslandes and R. H. Marchessault, *Macromolecules,* 1983, **16**, 1375.
4. Although no literature data was available for the pK_a value of Cur, the glucose's 2-OH of α- to γ-cyclodextrins are known to be relatively acidic as aliphatic OH (pK_a = 12.33, 12.20, and 12.08, respectively); W. Saenger, J. Jacob, K. Gessler, T. Steiner, D. Hoffmann, H. Sanbe, K. Koizumi, S. M. Smith and T. Takaha, *Chem. Rev.,* 1998, **98**, 1787; B. Gillet, D. J. Nicole and J.-J. Delpuech, *Tetrahedron Lett*., 1982, **23**, 65. It seems reasonable therefore to assume that the secondary OH of Cur is similarly acidic and the acidity is enhanced at higher temperatures to facilitate the hybridization with PyPT. Indeed, the acidity of aromatic alcohols is known to increase at higher temperatures; F. Rived, M. Rosés and E. Bosch, *Anal. Chimica Acta,* 1998, **374**, 309.
5. (a) K. H. DuBay, M. L. Hall, T. F. Hughes, C. Wu, D. R. Reichman and R. A. Friesner, *J. Chem. Theory Comput*., 2012, **8**, 4556; (b) C. Dong, L. Yong-Zhi, W. Zhi-Chao and L. Bo, *J. Mol. Model,* 2014, **20**, 2279.
6. The π,π* transition energy of PT is known to saturate at 461 nm for ≥20 mers in tetrahydrofuran; N. Sumi, H. Nakanishi, S. Ueno, K. Takimiya, Y. Aso and T. Otsubo, *Bull. Chem. Soc. Jpn*., 2001, **74**, 979. In our case measured in 1:9 DMSO/H_2O, the π,π* transition of free PyPT (113mer) appeared at 448 nm but was blue-shifted by 13 nm to 435 nm upon complexation with Cur, indicating that the effective conjugation length of PyPT is reduced to 9 mer in the hetero-triplex and ≥20 mer in the hetero-duplex on the basis of the estimation from the linear relationship of the conjugation length with the transition energy.
7. For examples, see: (a) N. T. Harrison, D. R. Baigent, I. D. W. Samuel and R. H. Friend, *Phys. Rev. B,* 1996, **53**, 15815; (b) D. A. V. Bout, W.-T. Yip, D. Hu, D.-K. Fu, T. M. Swager and P. F. Barbara, *Science,* 1997, **277**, 1074; (c) W. J. D. Beenken and T. Pullerits, *J. Chem. Phys*., 2004, **120**, 2490; (d) P.-I. Lee, S. L.-C. Hsu and P. Lin, *Macromolecules,* 2010, **43**, 8051; (e) L. Zou, Y. Liu, N. Ma, E. Maçôas, J. M. G. Martinho, M. Pettersson, X. Chen and J. Qin, *Phys. Chem. Chem. Phys*., 2011, **13**, 8838.