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Electronic Supporting Information

Discrimination of Enantiomers and Constitutional Isomers by Self-generated Macroscopic Fluid Flow

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Experimental Section

Reagents and Materials

Boron trifluoride diethyl etherate ($BF_3{\cdot}(OC_2H_5)_2$, Branched polyethyleneimine (bPEI, 25kDa), polyacrylicacid (PAA 100kDa, 35 wt%), p-toluenesulfonyl chloride (TsCl), L-tryptophan, and sulfate latex particles (5µm in diameter) were purchased from Sigma-Aldrich. β-CD, D-tryptophan, 1,4-bis(2-hydroxyethoxy)benzene, paraformaldehyde, trimethylamine, ortho-nitrophenol, meta-nitrophenol and para-nitrophenol were obtained from TCI chemicals. Acetonitrile, 1,2-dichloroethane, hexane, dichloromethane, methanol, ethanol, dimethyl sulfoxide (DMSO), hydrochloric acid and sodium hydroxide were purchased from Merck. DC/GEN/184 SYLGARD (PDMS Elastomer KIT) was supplied by from Kevin Electrochem. Millipore water (18.2 MΩ•cm at 25 °C) was used in all experiments.

Substrate

The multilayer films were made on glass slides and silicon wafers. The thickness of the film was measured using silicon wafers as a substrate. Sylgard 184 was mixed with curing agent in a 10:1 weight ratio and cast onto a plastic petri plate to make PDMS elastomer. In a convection oven at 70° C, the prepolymer PDMS is allowed to settle for 2 hours. The piranha solution was used to clean all of the glass slides and silicon wafers. Glass slides were soaked for 45 minutes in a solution of H_2SO_4/H_2O_2 at a ratio of 7:3 (v/v), then rinsed with deionized water and dried for 5 minutes using nitrogen jet. Beware! Piranha solution reacts aggressively to organic compounds and must be handled with caution.

Fabrication of multilayer film

The multilayer films were made with a polyelectrolyte pair of PEI-β-CD and polyacrylic acid (PAA) (Figure S8). An automated rotatory Dip Coater was used to fabricate the PAA/PEI-β-CD (host multilayer films) on microscope glass slides. Figure S8 depicts the full deposition process for making multilayer films using LbL assembly. The formation of the films was predominantly governed by electrostatic interaction between the pairs. Prior to multilayer deposition, a cleaned substrate was first treated with a priming solution (1 wt% branched PEI solution; pH 10) for 5 minutes. Next, the multilayer films were prepared by dipping the primer coated substrate in a PAA (0.2 wt%; pH 4) solution for 5 minute and then washing it with MilliQ water;

the substrates were then dipped in PEI (0.1 wt%; pH 10) solution for 5 minutes before being washed with MilliQ water once more. The assembly of one PAA/PEI-β-CD double layer is described in this procedure. These steps with 1 minute dipping and washing in each solution were repeated until the desired number of bilayers have been deposited on the glass substrate. In the same way, the (PAA/CP[5]A)2BLs were made with a layer by layer assembly with consecutive adsorption of CP[5]A, and PAA (Figure S9). The primer coated substrate was immersed in an aqueous solution of PAA (0.2 wt%; pH 4) for 5 minutes and then immersed in CP[5]A (0.5 wt%) for 30 minutes with an intermediate steps of rinsing with water. By repeating the alternate immersion processes in PAA and CP[5]A solutions, multilayer films (nL, n being the number of deposited layers) were obtained.

Multilayer film pattern and tracking fluid flow

A thin layer of PDMS sheet with a small circular aperture in the centre (d=4.6mm) was first made and put on the glass slide in such a manner that the circular opening remained in the middle of the slide. Then the same glass slide with PDMS sheet was employed as a substrate for LbL deposition for required number of cycles. The PDMS sheet was peeled off to leave the film pattern after acquiring desired bilayers, maintaining the covered region untreated. The surface was carefully cleaned with water and sprayed with nitrogen gas. After that, an airtight imaging chamber (l= 1 cm, b= 1 cm, h = 1.8 mm) was used to cover the pattern, and an aqueous suspension of guest molecules was loaded into the chamber, along with 5 µm diameter neutral charged non-interacting polystyrene particles (tracer particles). Under an optical microscope, the complete setup was analysed, and movies were recorded for subsequent study. In each experiment, 20 tracer particles were monitored over a 15 second time interval using Tracker software to calculate fluid pumping velocity.

Characterization

A 400 MHz Bruker AvanceII spectrometer was used to record ^{1}H nuclear magnetic resonance (NMR) spectra of the products in DMSO-d_6 and D_2O. Based on proton integration, the grafting level of CD was estimated using the ^{1}H NMR spectra. Spectroscopic Ellipsometer (Angstrom Sun Technology Inc. USA) was used to measure the thickness of the host multilayer films. The binding of the “host-guest” molecules

were determined by isothermal titration calorimetry (Malvern MicroCal PEAQ-ITC). Optical microscope (OLYMPUS IX73) with a high resolution coloured cooled camera (DP74) and X-Cite 120 LED Boost were used to record all the videos at 15 frames per second. In each experiment, 20 tracer particles were monitored over a 15 second time interval using Tracker software (Motion Analysis Software) to determine fluid pumping velocity.

Synthesis of PEI-β-CD

Cyclodextrin functionalized polyethyleneimine (PEI-β-CD) was prepared according to the following two-step reaction procedure (Figure S1).

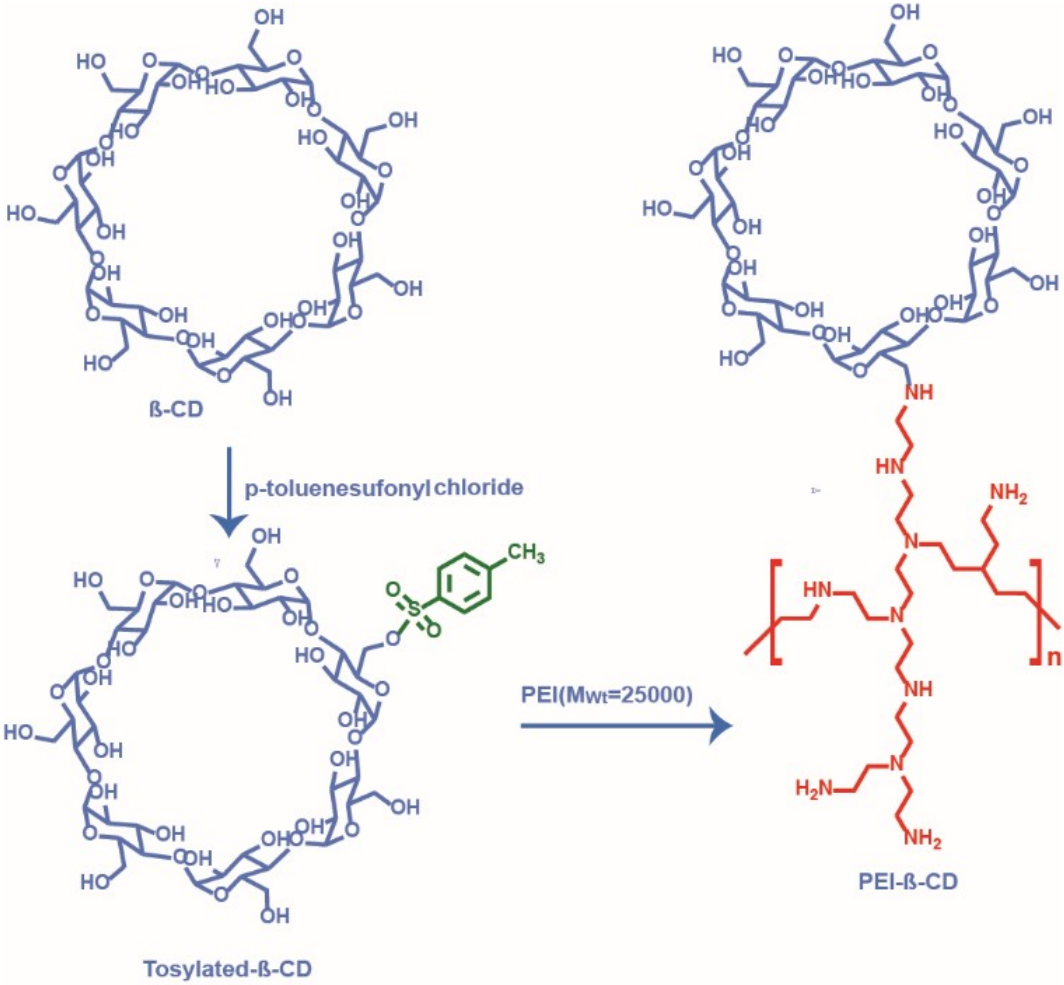

Figure S1. Synthesis of cyclodextrin functionalized polyethyleneimine (PEI-β-CD).

Synthesis of Mono-6-(p-Tolylsulfonyl)-β-Cyclodextrin (Ts-*O*-β-CD)

Ts-O-β-CD was synthesized using a previously described method.[1] At room temperature, 5 g of β-CD was dissolved in 240 mL of deionized water and 550mg of NaOH in 2ml deionized water was added to the mixed solution, the suspension turned to be homogenous. 21.26 g of p-toluenesulfonyl chloride in 2.5ml of acetonitrile was gently added into the β-CD aqueous solution under magnetic stirring to ensure that substitution occurred only at the C6 position. The solution was stirred for 2 hours at 25 °C after white precipitation appeared. The suspension was then cooled at 4 ° C overnight. The precipitate was then obtained using vacuum filtering The precipitate was recrystallized three times at 60^0 C to eliminate any remaining unreacted β-CD and p-TsCl. The product was obtained after vacuum drying at 50^0 C. ^{1}H NMR (DMSO-d_6) δ: 2.44 (s, 3H, CH_3), 3.41-3.74 (m, H-3,5,6) overlap with HOD, 4.21-4.53

(m, 6H, C6-OH), 4.80 (s, 7H, H-1 of CD), 7.43-7.77 (dd, 4H, aromatic tosyl) (see Figure S2).

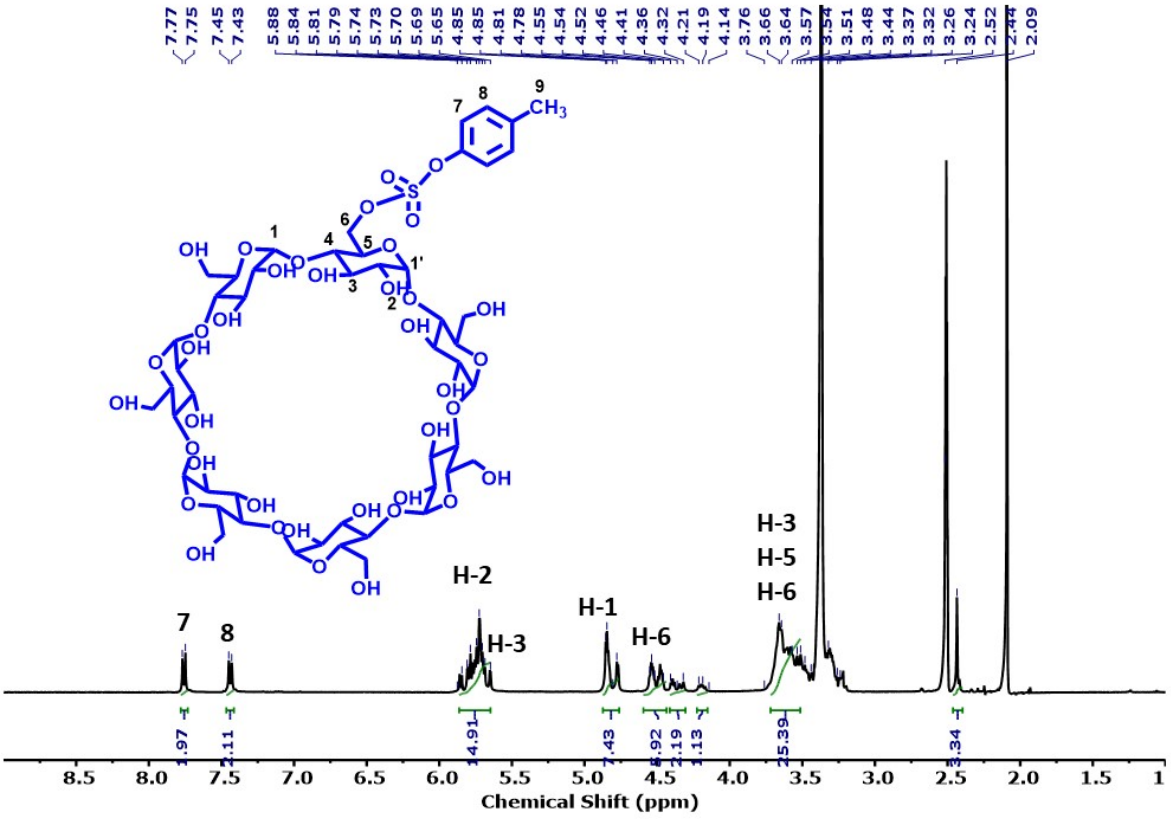

Figure S2. 400 MHz ^{1}H NMR spectra Tosyl-β-CD in DMSO-d_6.

Synthesis of PEI-β-CD

The PEI-CD was prepared in accordance with the previous report.[2] Briefly, 0.25 g of PEI was reacted with 1.9 g of Ts-O-β-CD synthesized as mentioned above in 10 mL dimethyl sulfoxide (DMSO). For 3 days, the solution was stirred in a nitrogen atmosphere at 70°C. After that, the product was dialyzed in water for 3 days with a MWCO 12kDa membrane. After 3 days of freeze drying, the light-yellow powder was obtained. According to ^{1}H NMR (D_2O), the β-CD grafting level per PEI chain estimated to be approximately 8.0%. δ: 4.99 (s,7H, H(e) of CD), 3.81 (m, 28H, H(a), H(c), H(f) of CD), 3.58 (m, 14H, H(b) and H(d) of CD), 2.33-2.76 (m, 78, methylene of $–CH_2CH_2$-) (see Figure S2). From the integrated area of (S1) of H1 of β-CD and the integrated peak area (S2) of PEI from 2.76 to 2.33, the degree of β-CD substitution (DS) to PEI was calculated according to the following formula[3]:

DS = (S1/7) [(S2-4S1)]

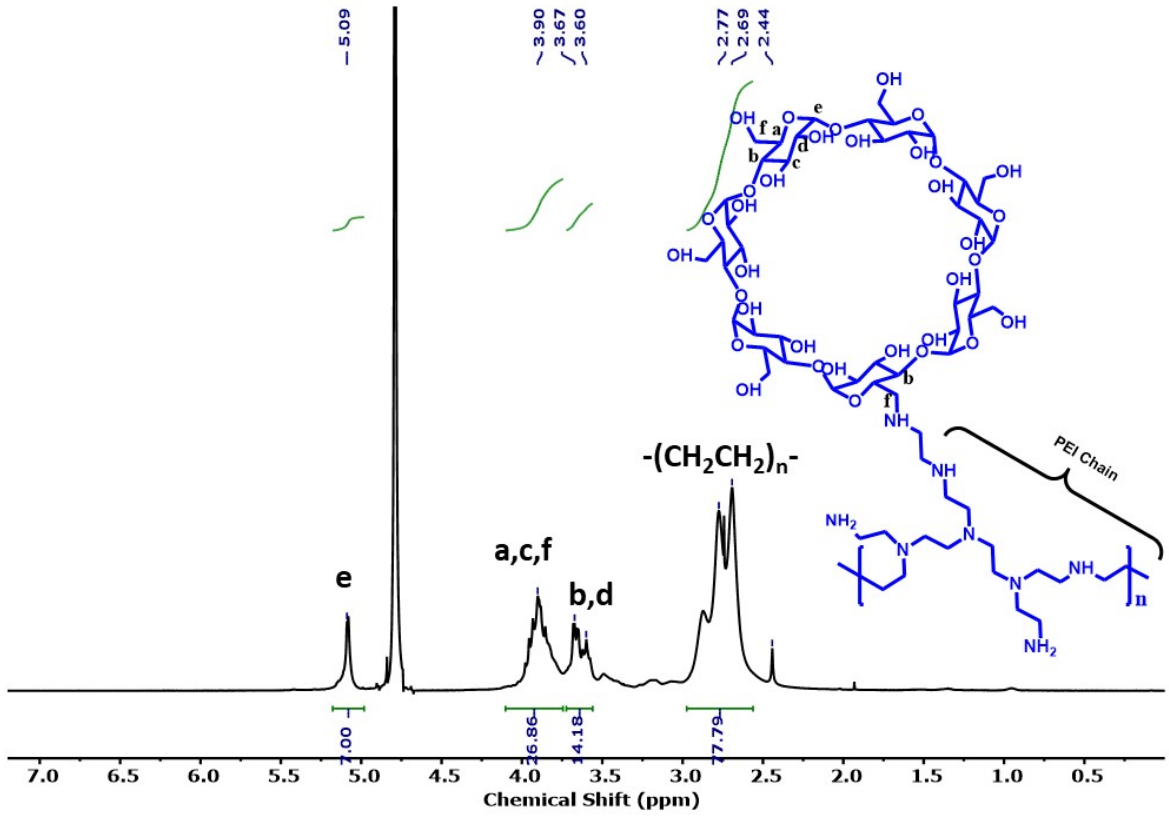

Figure S3. 400 MHz ^{1}H NMR spectra PEI-β-CD in D_2O.

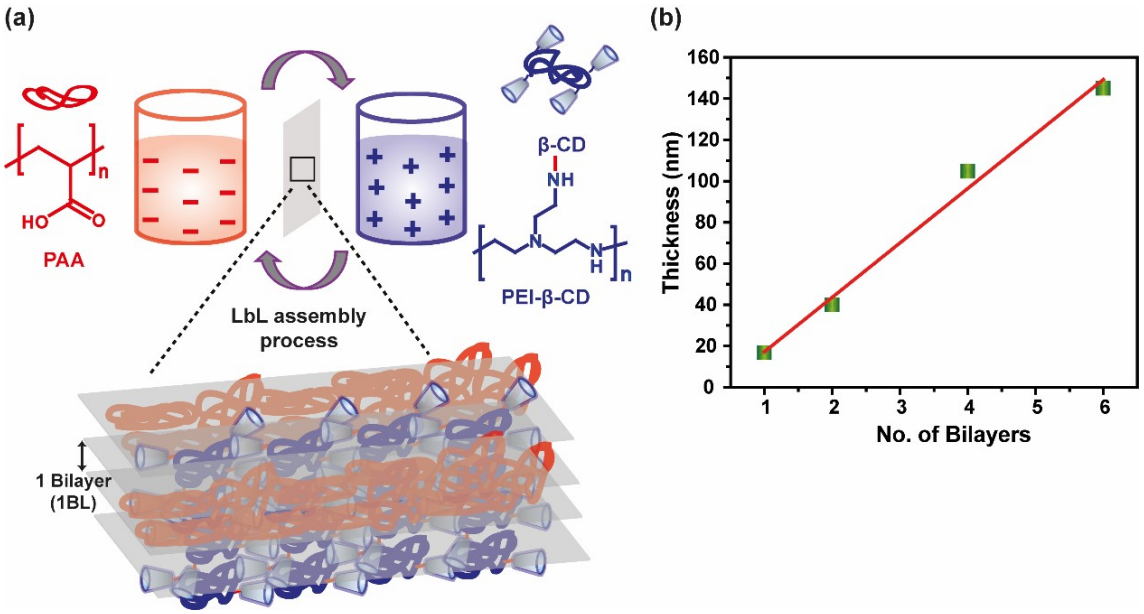

Figure S4. (a) Schematic of layer-by-layer (LbL) assembly using PAA and PEI-β-CD, (b) Thickness profile of the polymer multilayer film grown on a silicon wafer (dry state).

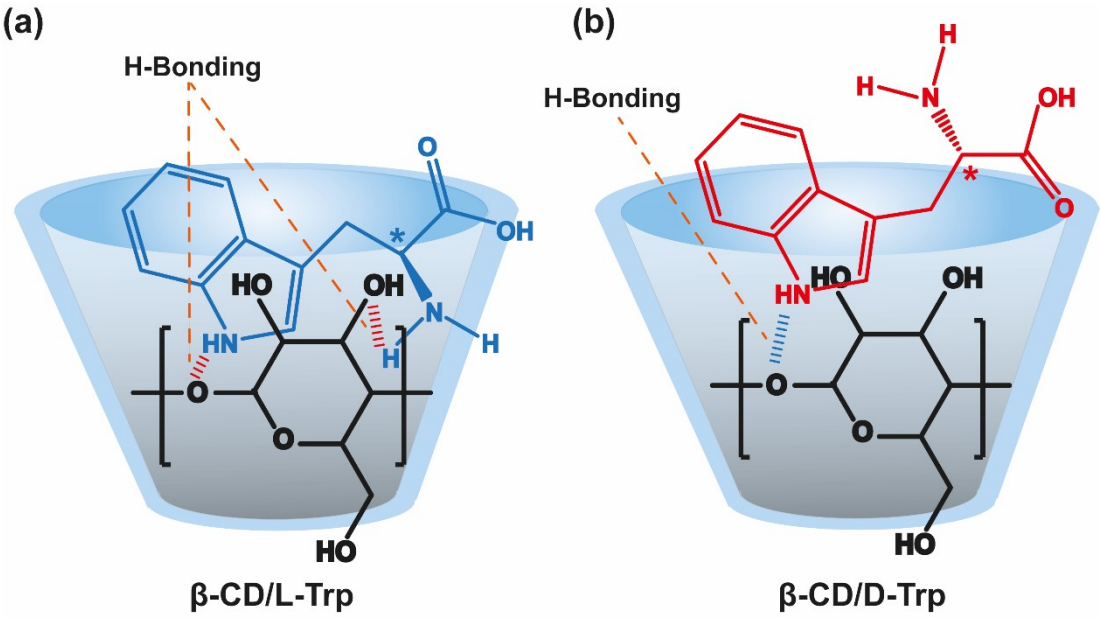

Figure S5. Schematic diagram showing the optimal orientation of L-Trp (a) and D-Trp (b) on the basis of the highest degree of hydrogen bonding and inclusion complexation.

Isothermal Titration Calorimetry (ITC)

The thermodynamic parameters of L- and D-Trp with β-CD complexations were measured at 25°C in phosphate buffer (pH 5.9) using a Malvern MicroCal PEAQ-ITC as shown in Figure S10. The 20 mM of L- and D-Trp solution in the syringe was titrated into the 250µM β-CD in the calorimeter cell with 25 (1.5µL) and 19 (1.5µL) consecutive injections every 150 seconds intervals respectively. The same injections into aqueous under the same conditions were used in all control experiments (for the heat of dilution). The curve fitting was done on the ITC instrument using a one-site model fitting based on the thermal effect per injection.

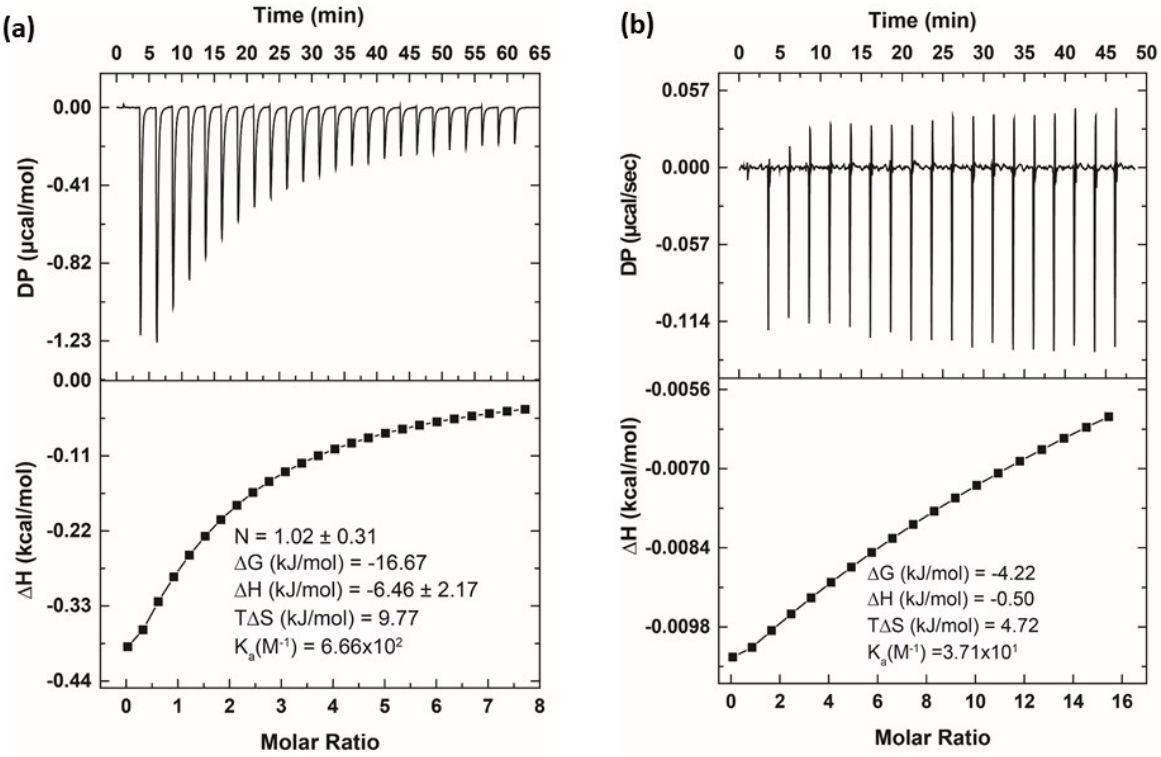

Figure S6. Isothermal Titration Calorimetry (ITC) isotherm of (a) L-Trp (20 mM), and (b) D-Trp with β-CD (250µM) at 25 °C.

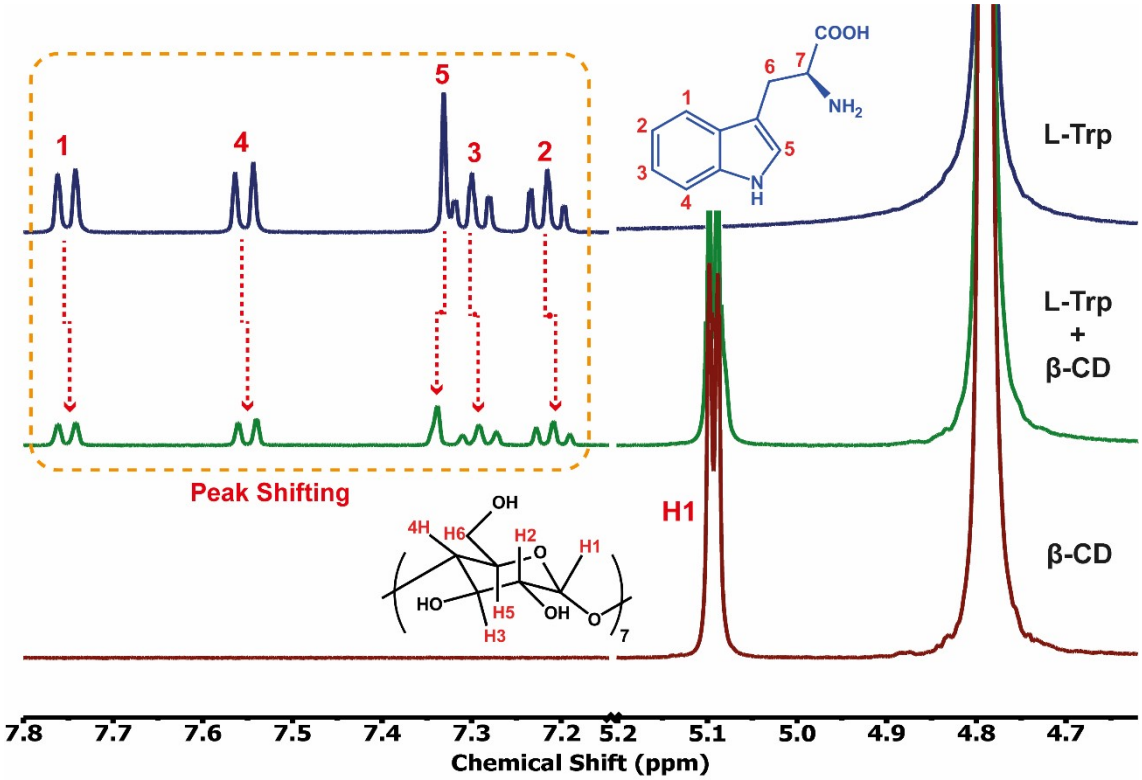

Figure S7. 400 MHz ^{1}HNMR spectra of (a) β-CD with L-Trp. The concentration for each component was 5 mM.

^{1}H NMR of L-/D-Trp: β-CD complex

Theoretical Modeling of Density Driven Flow

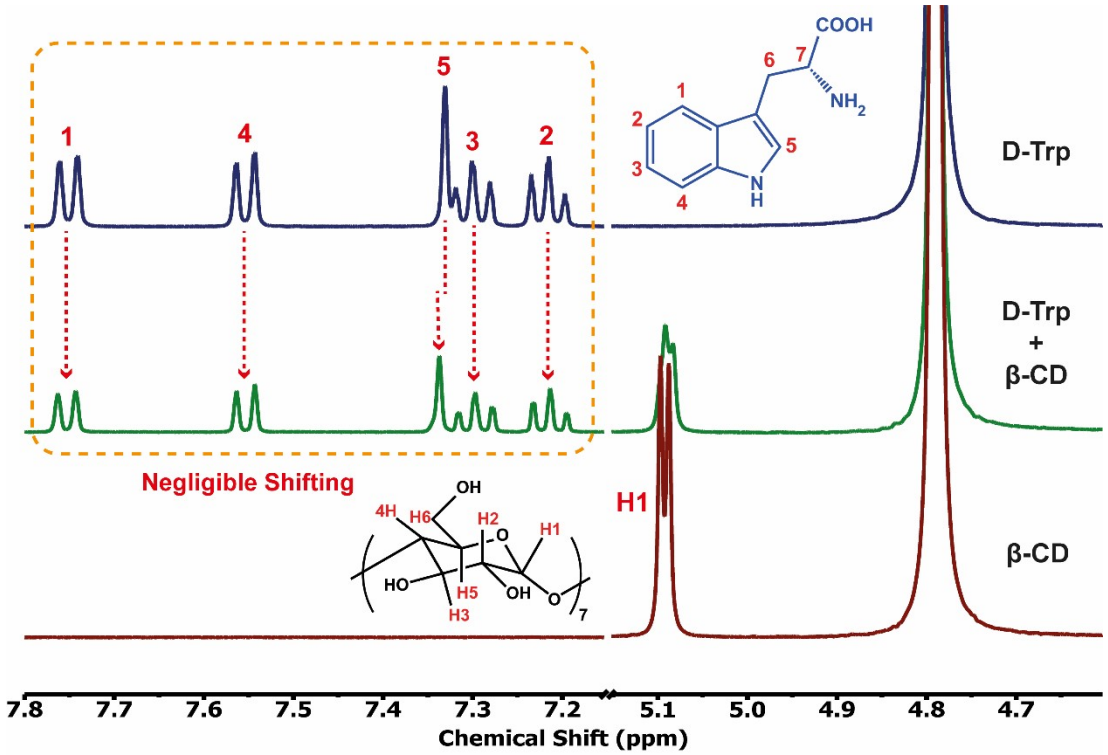

Figure S8. 400 MHz ^{1}HNMR spectra of (a) β-CD with L-Trp. The concentration for each component was 5 mM.

Theoretical modeling was used to get a better understanding of how solutal and thermal buoyancy impact fluid velocity. Thermal mechanisms were previously assumed to adequately explain the direction and approximate amplitude of the observed pumping, however the results of the numerical modelling revealed that the observed fluid flows were predominantly driven by solutal buoyancy.

We consider a 2-dimensional axisymmetric geometry (see SI Figure S10a), with height of 1.8 mm, width of 10 mm. A circular patch of radius *r* = 2.4 mm, which is considered as the reaction zone, is placed at the centre. We now solve for the radial velocity (*u*) and axial velocity (*w*) as a function of space coordinates (*r* and *z*) and time (*t*). The flow is governed by the continuity equation:

$$\frac{\partial u}{\partial r}+\frac{\partial w}{\partial z}=0$$

and the momentum balance equations in radial and axial directions, respectively:

$$\rho_0\left(\frac{\partial u}{\partial t}+u\frac{\partial u}{\partial r}+w\frac{\partial u}{\partial z}\right)=-\frac{\partial P}{\partial r}+\mu\left(\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial u}{\partial r}\right)+\frac{\partial^2 u}{\partial z^2}\right)$$

$$\rho_0\left(\frac{\partial w}{\partial t}+u\frac{\partial w}{\partial r}+w\frac{\partial w}{\partial z}\right)=-\frac{\partial P}{\partial z}-\rho_0 g\ -\rho_0 g\beta_c(c-c_0)+\ \mu\left(\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial w}{\partial r}\right)+\frac{\partial^2 w}{\partial z^2}\right)$$

where, P is the local pressure. In the above equation, we have applied the Boussinesq approximation to consider the solutal buoyancy effect, where the density variation is assumed to affect the gravity term only and ρ_0 is the fluid density at reference concentration c_0. β_c is the expansion coefficients due to solute concentration. No-slip condition is imposed at all the solid surfaces in the geometry. Due to the presence of the buoyancy terms in the momentum equation, it is coupled to the solute transport equation,

$$\frac{\partial c}{\partial t}+u\frac{\partial c}{\partial r}+w\frac{\partial c}{\partial z}=D\left(\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial c}{\partial r}\right)+\frac{\partial^2 c}{\partial z^2}\right)$$

The bulk diffusivity of the guest molecule in water, *D*, is assumed to be O (10^{-7} m/s^2). The solute is getting consumed at the reaction patch. The boundary condition at the patch is written as

$$D\frac{\partial c}{\partial z} = -kce^{-\gamma t}$$

indicating that the diffusive flux at the patch is balanced by the consumption due to the reaction. At all other surfaces of the domain, it is assumed that no flux can pass through. In the reaction kinetics term, the apparent rate constant *k* includes the resistance due to the polymer layer, and the binding constant of the "host -guest" molecule. The exponentially decaying terms accounts for the fact that as the "guest" molecules gradually occupy the cavity, the probability of finding a favourable site decreases. This form of the surface reaction has been proposed based on the experimental observations. The value of γ seems to be independent of the initial concentration of the "guest" molecule. Also, the apparent rate constant *k* may decrease with increase in the initial concentration, possibly due to the steric hindrance at the polymeric layer. The values of these constants seem to depend upon the relative orientation of the "guest" molecule with respect to the cavity of the β-CD. If the orientation is such that the insertion is not favored, then *k* can achieve a low value leading to smaller peak velocity. Also, once the sites get filled-up, the probability of finding another favourable site reduces drastically for D-Trp molecules indicated by a larger value of γ. This behaviour may help in sorting the chiral molecules.

The thermal contribution in the buoyancy is ignored due to the ratio of the Rayleigh numbers is small,

$$\frac{Ra_T}{Ra_s} = \frac{\beta_T \Delta H_{rxn}/\alpha}{\beta_s C_p \rho_0 / D} \sim O(10^{-3})$$

For this calculation, thermophysical properties of water were considered. Also, the enthalpy values presented earlier were used to estimate the order of magnitudes of ΔH_{rxn}. The change in density with concentration is simply given by, β_s = Mw (molecular weight of the molecule), which is the same for the chiral molecules considered here. The mesh for the computations is shown in SI Figure S10b. A mesh sensitivity test was done and a uniform mesh consisting of 50x1000 grids was found sufficient for the computations. Linear elements were used in the finite element method employed to solve the system of equations numerically. The velocity contours are shown in Figure S9a for 40 mM L-Trp at time, t = 1 minute and at Figure S9b for time t = 11 minutes.

The effective rate parameters for the "guest" molecules are listed in Table 1. The lower values of k for D-Trp indicate its lower affinity as compared to L-Trp for the “host” site supporting the preferential binding effect hypothesis due to favourable molecular orientation.

Table 1: Values of k and γ (effective rate parameters) used in the calculations of various cases of L-Trp and D-Trp molecule.

Conc (mM)	***k*(L-Trp)**	***k*(D-Trp)**	***γ(L-Trp)***	***γ(D-Trp)***
1	$2x10^{-8}$	$1.3x10^{-8}$	0.0015	0.0015
10	$4.3X10^{-9}$	$1.8x10^{-9}$	0.002	0.002
20	$2.5 X 10^{-9}$	$1.3 X 10^{-9}$	0.0022	0.004
40	$2.1 X 10^{-9}$	$1.3 X 10^{-9}$	0.0022	0.004

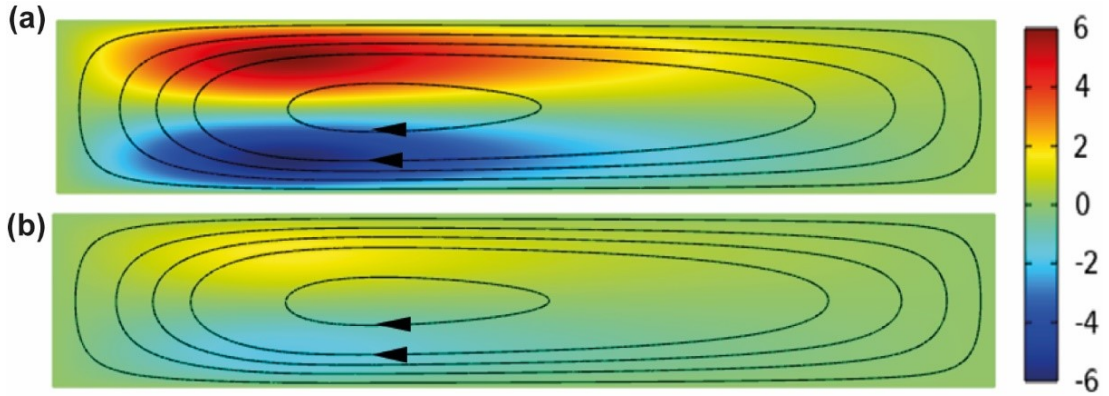

Figure S9. (a) Velocity contour and streamlines for 40 mM L-Trp at t=1 minute. The arrows indicate the direction of the flow, (b) Velocity contour and streamlines for 40 mM L-Trp at t=11 minutes. The arrows indicate the direction of the flow.

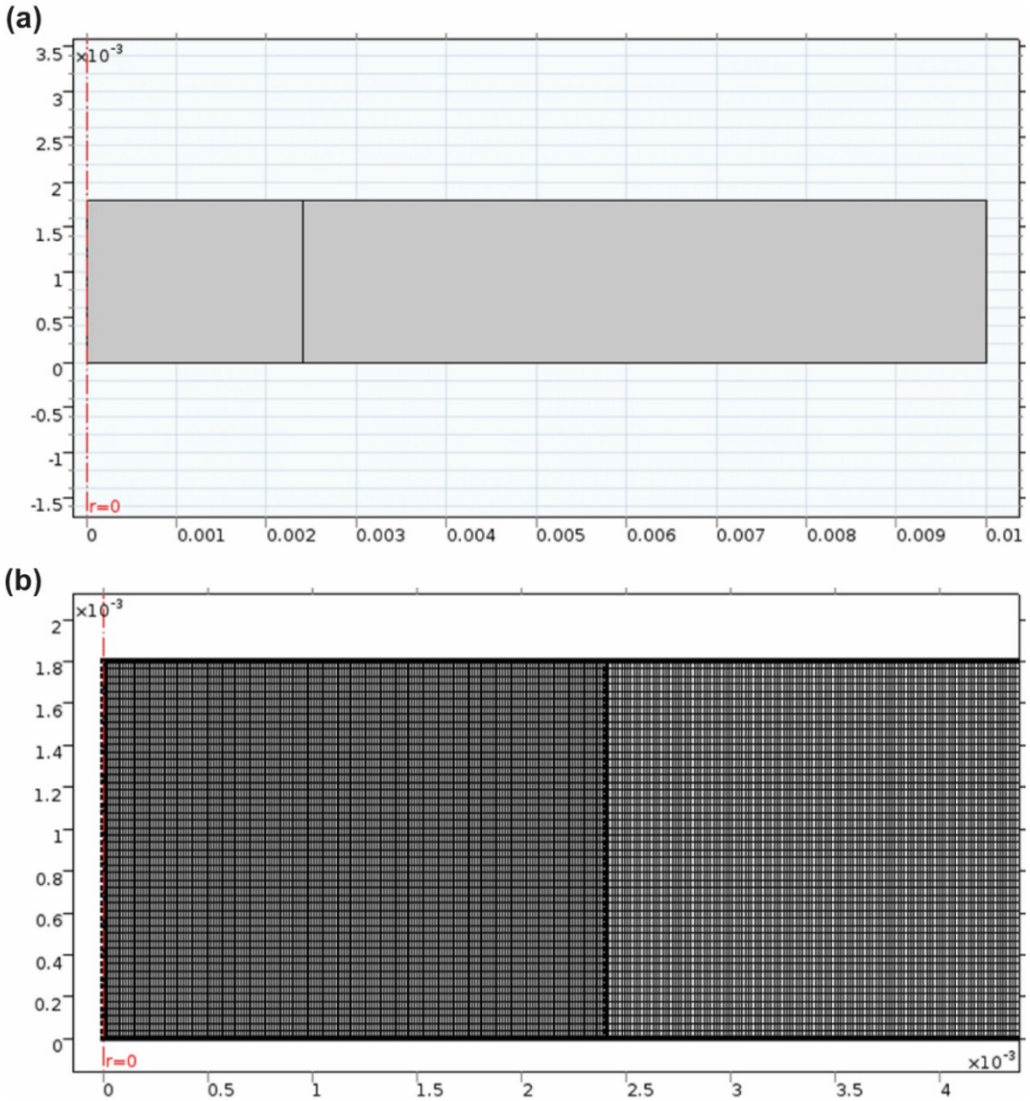

Figure S10. (a) Domain, and (b) Mesh used in the numerical study.

Synthesis of Cationic pillar[5]arene (CP[5]A)

The following three-step reaction was used to synthesize cationic pillar[5]arene according to the reported procedure (Figure S11).[4]

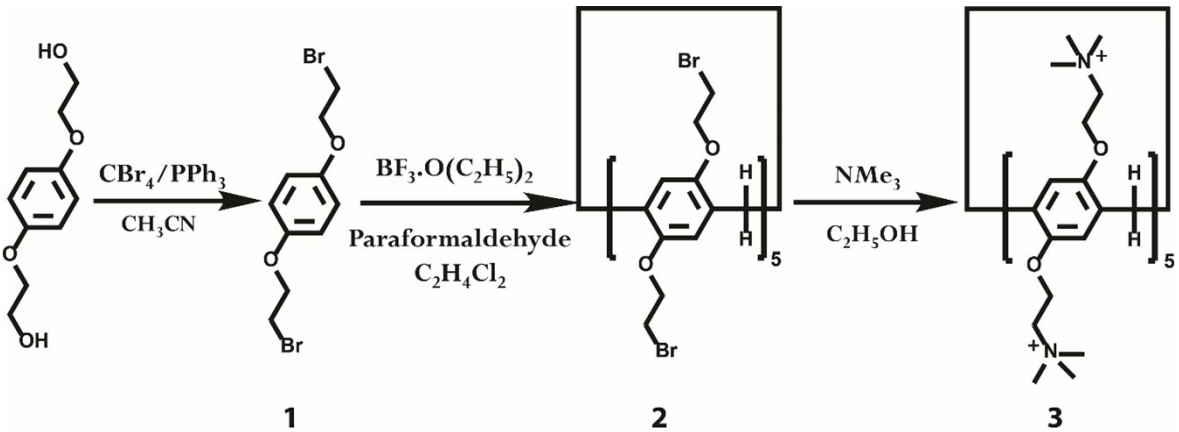

Figure S11. Synthesis of cationic Pillar[5]arene (CP[5]A).

Synthesis of 1

Carbon tetrabromide (39.8 g, 120 mmol) was gradually added in small amounts to a solution of 1,4-bis(2-hydroxyethoxy)benzene (10.0 g, 50.4 mmol) and triphenylphosphine (31.5 g, 120 mmol) in 300 mL dry acetonitrile at 0 °C with stirring. After allowing the reaction mixture to cool to ambient temperature, the clear solution was stirred for further 4 hours under N_2. Product 1 precipitated as a white solid when 200 mL of cold water was added to the reaction mixture. Vacuum filtration was used to collect the product, which was then completely cleaned in a 60:40 methanol/water solution before being recrystallized from methanol. High vacuum was used to dry the white flake-like crystals (13.8 g, 85 %). Figure S12 shows the ^{1}H NMR spectra of 1. ^{1}H NMR (400 MHz, $CHCl_3$-d, r): δ (ppm): 6.79 (s, 4H), 4.27 (t, J=6.3 Hz, 4H), 3.64 (t, J=6.3Hz, 4H).

Synthesis of 2

Paraformaldehyde (0.349 g, 11.5 mmol) was added to a solution of 1 (3.37 g, 11.5 mmol) in 1, 2-dichloroethane (200 mL) under nitrogen atmosphere. The solution was then mixed with boron trifluoride diethyl etherate ($BF_3(OC_2H_5)_2$, 1.63 g, 11.5 mmol), and the mixture was stirred at room temperature for 3 hours. A green solution was obtained. The obtained solid was purified by column chromatography on silica gel with hexane/dichloromethane (1:2 v/v) as the eluent after the solvent was removed, yielding a white powder (1.6 g, 41 %). ^{1}H NMR spectra of 2 is shown in Figure S13. ^{1}H NMR (400 MHz) δ (ppm): 6.83 (s, 10H), 4.15 (t, J=5.7Hz,

20H), 3.77 (s, 10H), 3.56 (t, j=5.7 Hz, 20H).

2.3 Synthesis of 3

Compound 3 (1.00 g, 0.595 mmol) and trimethylamine (33 % in ethanol, 6.43 mL, 23.8 mmol) were added to ethanol (50 mL). The mixture was refluxed overnight. The solvent was then evaporated, and deionized water (20 mL) was poured. A clear solution was obtained after filtration. The water was then evaporated completely to yield 1 as a colourless solid (1.28 g, 95 %). Figure S14 depicts the ^{1}H NMR spectrum of 3. ^{1}H NMR(400 MHz) δ (ppm): 6.94 (s, 10H), 4.44 (s, 20H), 3.93 (s, 10H), 3.80 (s, 20H), 3.22 (s, 90H).

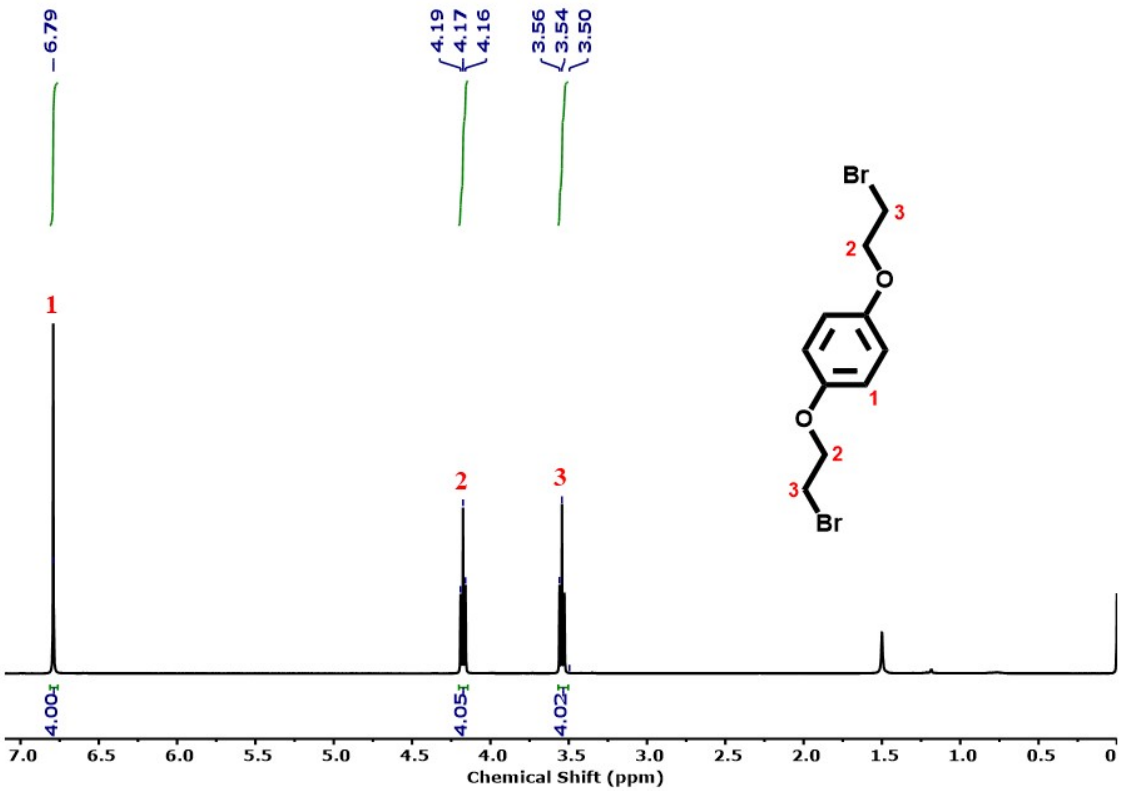

Figure S12. 400 MHz ^{1}H NMR spectra 1 in $CDCl_3$.

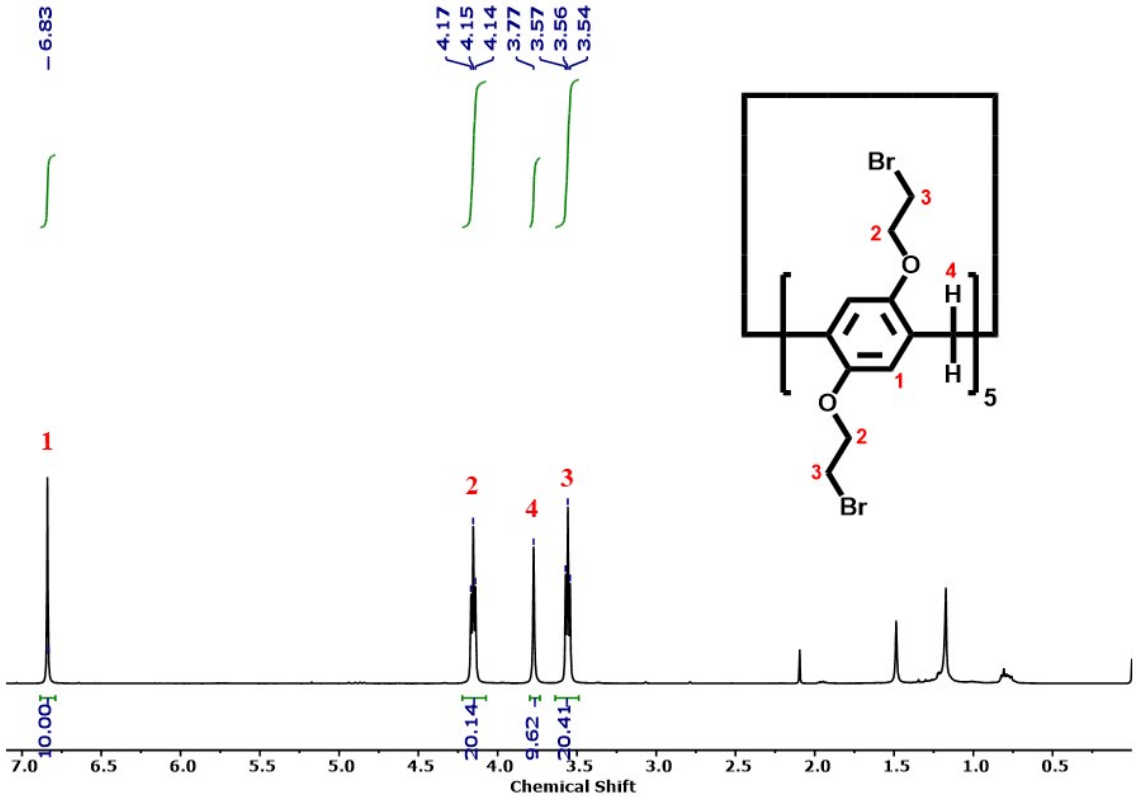

Figure S13. 400 MHz ^{1}H NMR spectra 2 in $CDCl_3$.

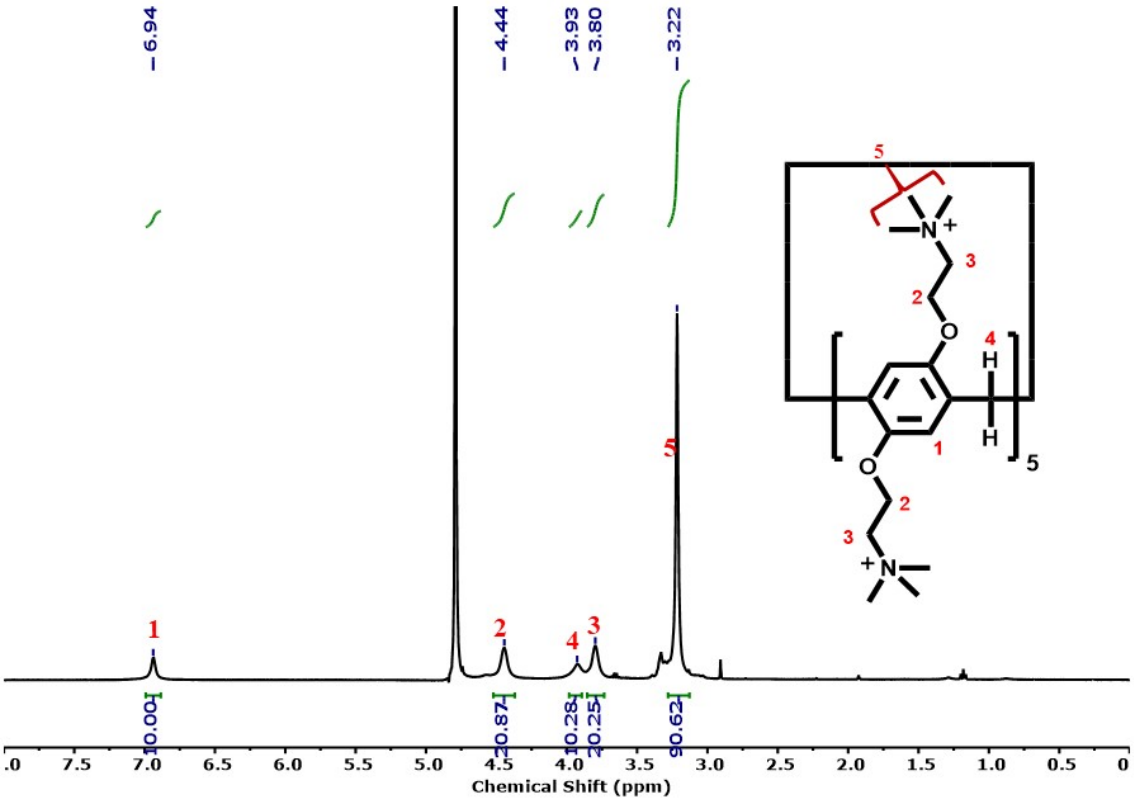

Figure S14. 400 MHz ^{1}H NMR spectra 3 in D_2O.

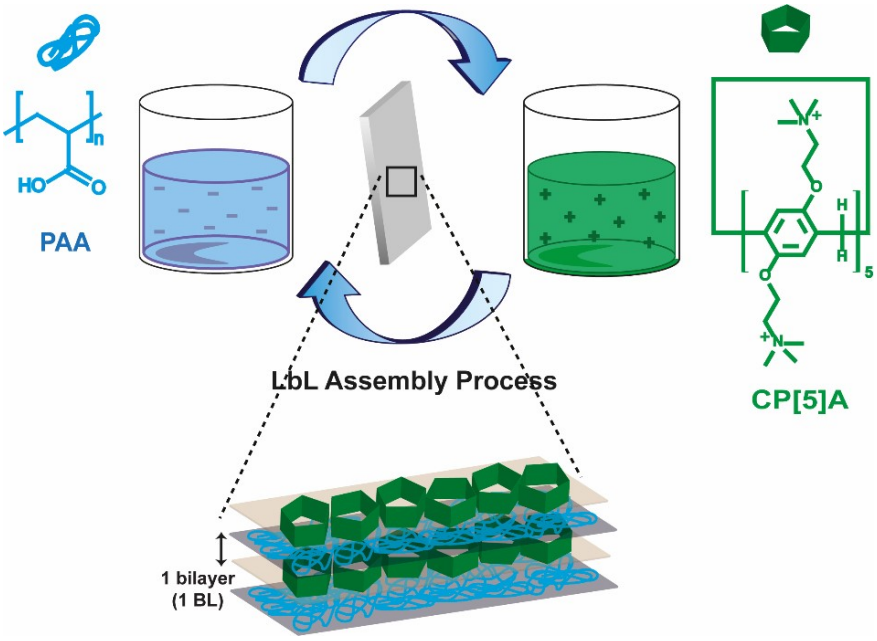

Figure S15. Schematic of layer-by-layer (LbL) assembly using PAA and CP[5]A.

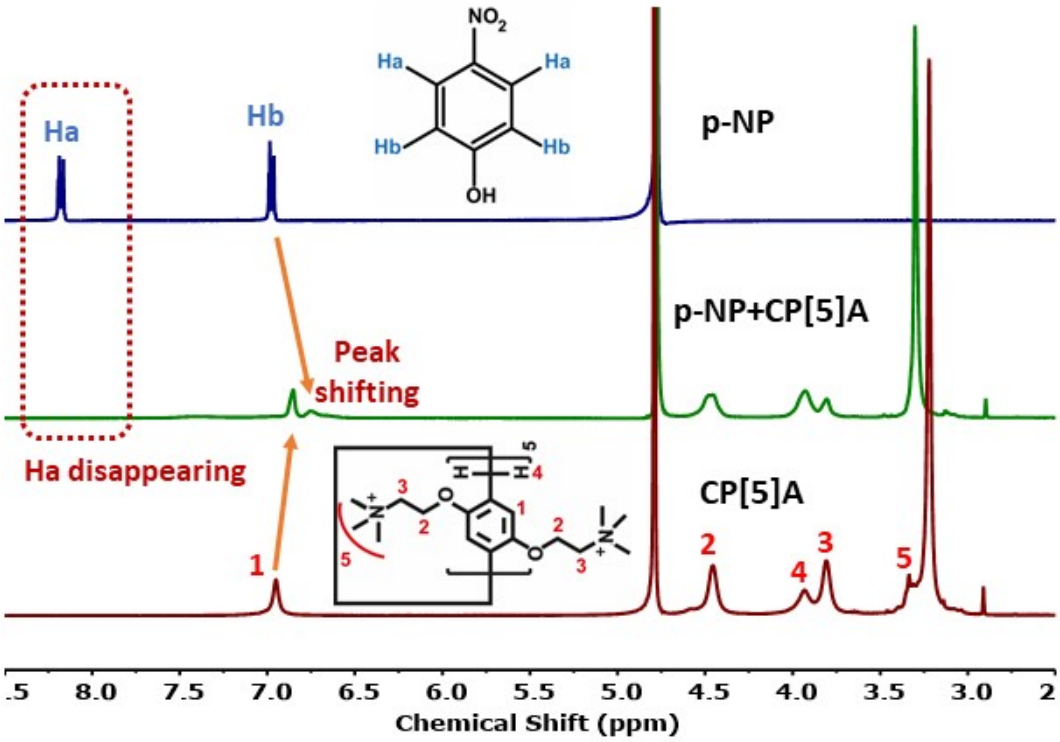

Figure S16. 400 MHz ^{1}H NMR spectra of (a) p-NP, CP[5]A + p-NP, and CP[5]A. The concentration for each component was 10 mM.

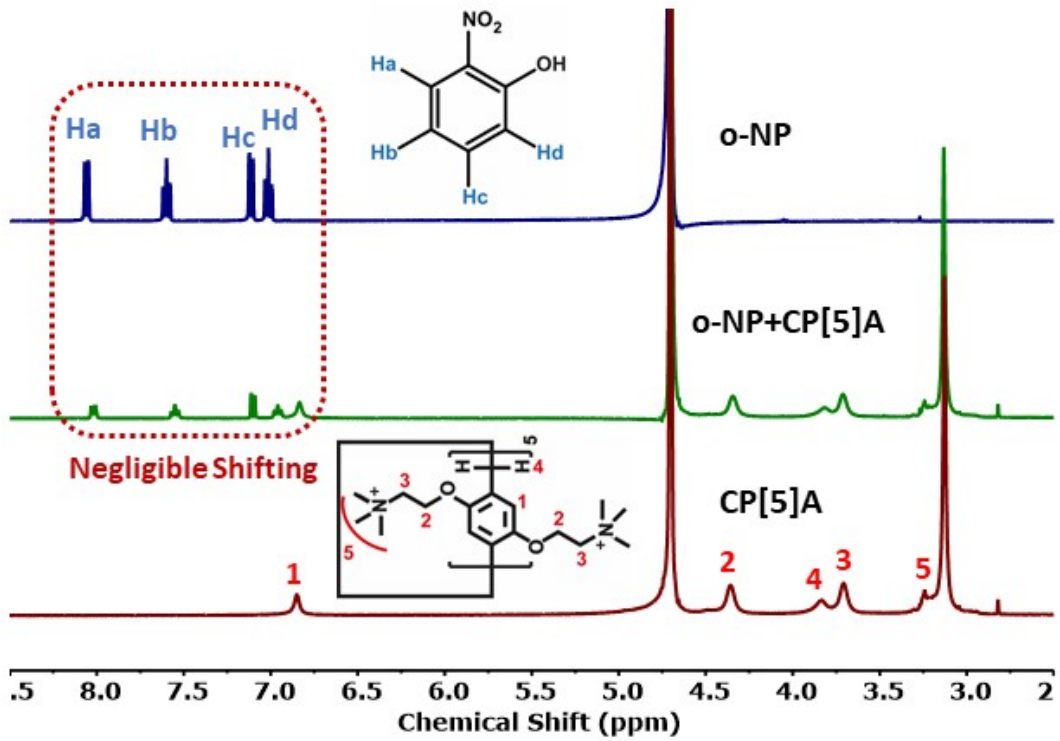

Figure S17. 400 MHz ^{1}H NMR spectra of (a) o-NP, CP[5]A + o-NP, and CP[5]A. The concentration for each component was 10 mM.

References

1. R. C. Petter, J. S. Salek, C. T. Sikorski, G. Kumaravel, F. T. Lin, *J. Am. Chem. Soc.* 1990, **112**, 3860–3868.
2. S. H. Pun, N. C. Bellocq, A. Liu, G. Jensen, T. Machemer, E. Quijano, T. Schluep, S. Wen, H. Engler, J. Heidel, M. E. Davis, *Bioconjug. Chem.* 2004, **15**, 831–840.
3. J. Wan, *Polymers* **2012**, *4*, 1084–1108. Y. Ma, X. Ji, F. Xiang, X. Chi, C. Han, J. He, Z. Abliz, W. Chen, F. Huang, *Chem. Commun.*, 2011, **47**, 12340-12342.