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A Neutral Porous Organic Polymer Host for the Recognition of Anionic Dyes in Water

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Abstract

Neutral hosts for the recognition of anionic guests in water remain underdeveloped due to the inherent thermodynamic barrier for desolvation. As a new strategy to address this challenge, we have repurposed crosslinked porous organic polymers (POPs) as hosts. This polymer architecture affords a hydrophobic environment with a densely packed array of urea hydrogen bond donors to cooperatively promote anion desolvation and recognition in water. As a proof-of-concept, we demonstrate through adsorption assays that the resulting Urea-POP-1 can discriminate between structurally different dyes containing phosphonate, sulfonate, and carboxylate anions. Moreover, when compared to Methyl-POP-1, a control POP lacking hydrogen bond donors, we find that recognition is not exclusively driven by the hydrophobicity of the dyes but through selective hydrogen bonding interactions of the urea sidechains with the anionic functional groups. This starting point sets the stage to exploit the modularity of our design to build a family of neutral polymer hosts with tunable pore sizes and anion preferences for fundamental investigations and targeted applications.

Introduction

Molecular recognition of anionic guests in water remains a key challenge in synthetic supramolecular chemistry. Due to the hydrogen-bonding network around hydrated anions, hosts must overcome a large thermodynamic barrier. Drawing inspiration from Nature’s ability to recognize anions in water, abiotic polymer hosts offer an attractive solution by relying on a combination of cooperative interactions and the hydrophobic effect. To achieve anion binding in water, polymers can be functionalized with molecular receptors that are designed to be structurally rigid with densely packed arrays of donors tailored to the size, shape, and charge of a specific anion. Donors with positively charged atoms and Lewis acidic elements have been explored to form electrostatic or coordinate bonds with anions in water. Alternatively, charge-neutral donors capable of hydrogen, halogen, chalcogen, or
π-bonding can also be used; however, receptors with these neutral donors and resulting polymer hosts are fewer in number.32–46

As a complementary approach to address this challenge, we are developing neutral organic polymer hosts without predesigned receptors. Along these lines, we and others have recently demonstrated that a number of water soluble, neutral polyolefin polymers (e.g. PVP, PNIPAM, and PAAM) can be used for anion recognition in water.47–51 In parallel, we envisioned that neutral hydrogen bond donors could be encapsulated into rigidly structured polymer hosts, through the use of porous organic polymers (POPs) (Figure 1). POPs are highly crosslinked, amorphous, hydrocarbon frameworks that can maintain permanent porosity owing to their rigid and inflexible backbones.52–54 Even though POPs are insoluble, these properties have been exploited for binding a range of molecules and ions.55–69 As such, we hypothesized that a POP could provide a hydrophobic microenvironment with a densely packed array of neutral hydrogen bond donors to cooperatively promote anion desolvation and recognition in water.

![Figure 1](image.jpg)

**Figure 1.** Cartoon schematic illustrating the approach used in this study to bind anionic dyes (yellow sphere) in water with a porous organic polymer host (brown cube) with neutral hydrogen (H) bonding functional groups (pink receptor). Water molecules are shown as red and white spheres connected by gray lines in the blue background to represent the aqueous environment.

To test our approach, here we have prepared Urea-POP-1, a urea functionalized POP host. Urea is commonly used as a neutral recognition motif that effectively provides two parallel hydrogen bond donors for binding oxyanions but primarily in organic solvents or mixtures of organic solvents and water.45,70–79 However, through encapsulation in a POP, here we show in a rare instance that urea is capable of hydrogen bonding with anionic guests in pure water.70,80 As a proof-of-concept, we tested the ability of Urea-POP-1 to recognize organic dyes through adsorption assays that contain phosphonate (R-PO$_3^{2-}$), sulfonate (R-SO$_3^-$), and carboxylate (R-COO$^-$) anions. Moreover, when compared to Methyl-POP-1, a control POP lacking hydrogen bond donors, we demonstrate that anion recognition with Urea-POP-1 is not exclusively driven by the hydrophobicity of the dyes but through selective hydrogen bonding interactions with the anionic functional groups.

**Experimental**

**General synthetic materials and methods.** Reagents and chemicals were purchased from Alfa Aesar, Electron Microscopy Sciences, Matrix Scientific, Oakwood Chemicals, Sigma-Aldrich, TCI America, Strem Chemicals, or Thermo Fisher Scientific and were used as received. The syntheses of the starting materials for the POPs are shown in Scheme S1. All compounds were purified using SiliaFlash Irregular Silica Gel P60 (SiliCycle) or with preloaded cartridges on a CombiFlash NextGen 300 flash chromatography system (Teledyne ISCO Inc.). NMR spectra were collected at 25 °C on a Bruker AVANCE III HD spectrometer in the Molecular and Protein Analysis Core Facility at The University of Texas at Dallas. Chemical shifts (δ) are reported in parts per million versus the residual proton and carbon signals of the deuterated NMR solvent.
High-resolution electrospray ionization mass spectra (HR-ESI-MS) were acquired on a Waters SYNAPT G2-Si mass spectrometer.

1,4-dibromo-2-(bromomethyl)benzene (1a). The synthesis of compound 1a was adapted from a previously reported procedure for 5-bromo-2-chlorotoluene. A solution of N-bromosuccinimide (7.76 g, 43.6 mmol) in MeCN (150 mL) was purged with a stream of nitrogen for 15 min, followed by the addition of 2,5-dibromotoluene (4 mL, 29.1 mmol) and benzoyl peroxide (1.41 g, 5.81 mmol). The reaction mixture was heated to reflux overnight under a nitrogen atmosphere. The next day, the reaction mixture was cooled to room temperature, quenched with sodium sulfite (ca. 1 g), and concentrated in vacuo. The crude reaction mixture was dissolved in CH₂Cl₂ (50 mL) and washed with water (3 x 50 mL) and brine (3 x 50 mL). The organics were combined, dried over anhydrous Na₂SO₄, and concentrated in vacuo. The resulting crude solid was recrystallized from methanol to furnish a white powder (1.30 g, 45%). The purity was determined with NMR spectroscopy and was consistent with that previously reported.

2-(2,5-dibromobenzyl)isoindoline-1,3-dione (1b). The synthesis of compound 1b was adapted from a previously reported procedure. Potassium phthalimide (3.59 g, 19.4 mmol) was added to a solution of compound 1a (4.25 g, 12.9 mmol) in anhydrous DMF (15 mL) under a stream of nitrogen. The reaction mixture continued to stir overnight at room temperature under a nitrogen atmosphere. The next day reaction mixture was quenched with water (20 mL), and the resulting precipitate was collected by vacuum filtration, washed with water (50 mL), and dried to yield a white powder (5.06 g, 99%). The purity was determined with NMR spectroscopy and was consistent with that previously reported.

(2,5-dibromophenyl)methanamine (1c). The synthesis of compound 1c was adapted from a previously reported procedure. Compound 1b (4.98 g, 12.6 mmol) was dissolved in absolute EtOH (30 mL) at 90 °C under a nitrogen atmosphere. A solution of anhydrous hydrazine (25 mL, 80.3 mmol) was added dropwise to the reaction. The reaction mixture continued to stir for 6 h at reflux under a nitrogen atmosphere. The resulting golden yellow solution was cooled to room temperature, quenched slowly with a saturated solution of NaHCO₃, and extracted with CH₂Cl₂ (3 x 20 mL). The organics were combined, washed with water (2 x 50 mL), dried over anhydrous Na₂SO₄, and concentrated in vacuo to generate a light yellow solid (2.76 g, 82%). The purity was determined with NMR spectroscopy and was consistent with that previously reported.

[(2,5-dibromophenyl)methyl]urea (1). The synthesis of compound 1 was adapted and modified from a previously reported procedure for the preparation of benzylurea derivatives. Concentrated HCl (ca. 1 mL) was added dropwise to a suspension of compound 1c (2.00 g, 7.55 mmol) in water. The reaction mixture was heated to 90 °C to dissolve all of the solids. To the resulting solution, potassium isocyanate (0.92 g, 11.32 mmol) in water (5 mL) was added dropwise, and the reaction mixture continued to stir for 4 h at 90 °C. The reaction mixture was cooled to room temperature, and the resulting precipitate was collected by vacuum filtration, washed with water (100 mL), and dried to yield a white powder (2.15 g, 92%). ¹H NMR (600 MHz, DMSO-d₆): δ 7.54 (d, J = 8.34 Hz, 2H), 7.41 (s, 1H), 7.39 (d, J = 8.34 Hz, 2H), 6.56 (t, J = 5.82, 5.88 Hz, 1H), 5.72 (s, 2H), 4.17 (d, J = 6.06 Hz, 2H). ¹³C NMR (150 MHz, DMSO-d₆): δ 158.53, 142.18, 134.20, 131.28, 130.89, 120.99, 120.86, 43.04. HR-ESI-MS calculated for [C₈H₆Br₂N₂O + H]⁺ m/z = 306.9076, found m/z = 306.9157.

1,3,5-tris((trimethylsilyl)ethynyl)benzene (3a). The synthesis of compound 3a was adapted from a previously reported procedure. Triethylamine (9 mL, 57.5 mmol) in a heavy-walled pressure flask was purged with a stream of nitrogen for 20 min, followed by the addition of
1,3,5-tribromobenzene (0.500 g, 1.59 mmol), bis(triphenylphosphine)palladium(II) dichloride (Pd(PPh₃)₂Cl₂) (33 mg, 0.05 mmol), and copper iodide (CuI) (9.1 mg, 0.05 mmol). The reaction mixture was purged with a stream of nitrogen for an additional 15 min prior to the addition of trimethylsilylacetylene (0.88 mL, 6.35 mmol). Following this, the pressure flask was immediately sealed, and the reaction mixture continued to stir for 16 h at 65 °C. The following day, the reaction mixture was cooled to room temperature, filtered, washed with hexanes (50 mL) on a silica plug, collected, and concentrated in vacuo. The resulting residue was purified by flash chromatography (silica, hexanes) to afford a pale-yellow solid (0.50 g, 86%).

1,3,5-triethynylbenzene (3). The synthesis of compound 3 was adapted from a previously reported procedure. A solution of K₂CO₃ (0.108 g, 0.78 mmol) in water (0.4 mL) was added to a solution of compound 3a in a mixture of THF and MeOH (7:2 ratio, 9 mL). The reaction mixture continued to stir for 6 h at room temperature. Water (10 mL) was added to dilute the reaction mixture, and the product was extracted with CH₂Cl₂ (3 x 10 mL). The organics were combined, washed with water (20 mL), dried over anhydrous Na₂SO₄, and concentrated in vacuo to furnish an off-white solid (0.203 g, 98%).

Urea-POP-1 (4). A solution of triethylamine (1 mL, 7.17 mmol) and toluene (3 mL, 56.5 mmol) in a heavy-walled pressure tube was purged with a stream nitrogen for 20 min followed by the addition of 1 (154 mg, 0.50 mmol), 3 (50 mg, 0.33 mmol), CuI (13 mg, 0.07 mmol), and tetrakis(triphenylphosphine)palladium (Pd(PPh₃)₄) (0.38 g, 0.03 mmol). The pressure tube was immediately sealed, and the reaction mixture continued to stir overnight 95 °C. The following day, the reaction mixture was cooled to room temperature, and the resulting solid was filtered and washed sequentially with hexanes (100 mL), CH₂Cl₂ (100 mL), MeOH, 2M HCl (20 mL), water (100 mL), and acetone (100 mL). Impurities from the remaining solid were removed through Soxhlet extractions with THF (2 d) and MeOH (1 d). The resulting solid was collected by filtration to produce Urea-POP-1 as a brown powder (70 mg, 57%).

Methyl-POP-1 (5). Following the procedure described above for Urea-POP-1, 2,5-dibromotoluene (2) (0.68 mL, 0.50 mmol) was used in place of compound 1, and Methyl-POP-1 was isolated as a dark brown powder (75 mg, 82%).

General methods to characterize Urea-POP-1 and Methyl-POP-1. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra of starting materials 1, 2, and 3 and finely ground Urea-POP-1 and Methyl-POP-1 were collected at 25 °C on an Agilent Cary 660 FTIR Spectrometer (Agilent) in the Molecular and Protein Analysis Core Facility at The University of Texas at Dallas. Representative FTIR spectra are shown in Figure S2. The Micromeritics ASAP 2020 Accelerated Surface Area and Porosimetry System (Micromeritics Instrument Corporation) was used to characterize five independently prepared batches of Urea-POP-1 and Methyl-POP-1. Briefly, at least 30 mg of each POP was degassed under dynamic vacuum for 12 h at 120 °C, followed by exposure to nitrogen (up to 760 torr, 99.999%, Airgas Corporation) in a liquid nitrogen bath at 77 K. Based on these nitrogen adsorption isotherms, the surface areas of each POP were determined using Brunauer-Emmett-Teller (BET) model, and the pore size distributions were determined using the Dubinin-Raduskevich (DR) model and non-localized density functional theory (NLDFT) carbon slit-pore model in the Micromeritics software package. The surface areas of each POP batch are summarized in Table S1, and the nitrogen isotherms and pore size distributions are shown in Figure S3.
Scanning electron microscopy (SEM) was performed on a Zeiss Supra 40 Scanning Electron Microscope (Carl Zeiss Microscopy) in the Cleanroom Research Laboratory at The University of Texas at Dallas. Briefly, the samples were ground into fine powder and transferred onto double-sided copper tape on 15 mm aluminum stubs. The samples were imaged at an approximate working distance of 8 mm with a voltage of 5 kV for Urea-POP-1 and 10 kV for Methyl-POP-1. Representative SEM images for both POPs are shown in Figure S4.

**Determination of Urea-POP-1 and Methyl-POP-1 yield and repeating units.** The yield of both POPs were determined by dividing the mass of POP synthesized by the molecular weight of each repeating unit and then divided by the total molar amounts of the starting materials used. Each repeating unit was defined as the molar ratio of starting materials added into the reaction – two units of 3 and three units of 1 or 2 as shown in Figure S5.

**Anionic dye adsorption assays with Urea-POP-1 and Methyl-POP-1.** The anionic dyes used were: adenosine monophosphate (AMP), adenosine triphosphate (ATP), Alizarin Yellow G, bromophenol blue, flavin mononucleotide (FMN), fluorescein, Lucifer Yellow CH, methyl orange, methyl red, and Rhodamine B. Stock solutions of each dye were prepared at 500 μM in ultrapure water (ca. pH 7). Stock solutions of each POP were prepared in ultrapure water at 1 mg/mL or 2 mg/mL and sonicated for 2 h in an ultrasonic bath (Branson Ultrasonics). For each assay, the dye and POP were combined in a 1.5 mL microcentrifuge tube to a final volume of 500 μL. The dye stock solutions were diluted to a final concentration of 40 μM, and the POP stock solutions were diluted to 0.05, 0.1, 0.2, 0.4, 0.6 and 0.8 mg/mL for the carboxylate and sulfonate dyes and 0.1, 0.2, 0.4, 0.8, 1.2, and 1.6 mg/mL for the phosphonate dyes. After incubation for 30 min at room temperature, each sample was centrifuged for 5 min at 20000g at room temperature (5810 R, Eppendorf). For all of the dyes, except AMP and ATP, a 100 μL aliquot of each supernatant was transferred to a 96-well half-area microtiter plate (Greiner Bio-One). Absorbance spectra were collected for all of the dyes on a plate reader (Tecan). However, due to low extinction coefficients, fluorescence spectra were collected for FMN and Lucifer Yellow CH. Plate reader settings for each dye are summarized in Table S2. Due to background interference in the UV region from fine POP particulates, absorbance spectra for AMP and ATP were measured on the Agilent Cary 7000 Universal Measurement Spectrophotometer (Agilent) in the Molecular and Protein Analysis Core Facility at The University of Texas at Dallas. Briefly, the AMP and ATP samples were filtered via centrifugation for 2 min at 2000g at room temperature through Zymo-Spin I Columns (Zymo Research). The filtered samples were transferred to a 0.2-cm x 1-cm quartz cuvette (0.4 mL, Hellma USA) for measurement. Absorbance and emission spectra are shown in Figure S5–S14. To determine the percentage of dye adsorbed to each POP (A/A₀ or F/F₀), the measured absorbance (A) or fluorescence (F) value of each sample was divided by the absorbance (A₀) or fluorescence (F₀) value of the dye alone at a particular wavelength that was within the linear limit of detection as specified in Table S2. For each dye, the average of three technical replicates with standard deviation is presented in Figure 3.

**Determination of the amount of Urea-POP-1 at saturation points.** The saturation points at 50% and 95% were defined as where the relative absorbance or fluorescence signal of each dye had a 50% or less than a 5% change, respectively. These saturation points were then used to calculate the amount of Urea-POP-1. A representation of this is shown in Figure 4A. The saturation points were determined by plotting the relative absorbance A = [Aobs - Amin]/[Amax - Amin] or fluorescence F = [Fobs - Fmin]/[Fmax - Fmin] versus the concentration of Urea-POP-1 from Figure 3 in KaleidaGraph v4.5 (Synergy Software). Aobs or Fobs is the observed absorbance or fluorescence value of the supernatant in the presence of Urea-POP-1, Amax or Fmax corresponds...
to the absorbance or fluorescence value of the initial dye concentration in the absence of Urea-POP-1, and \( A_{\text{min}} \) or \( F_{\text{min}} \) corresponds to the absorbance or fluorescence value of the dye in the presence of the maximum concentration of Urea-POP-1 used. For each dye, three individual datasets were fitted to the following equation \( A \) or \( F = 1 - (m_1*[\text{Urea-POP-1}]) / (m_2 + [\text{Urea-POP-1}]) \) to determine the \( m_1 \) and \( m_2 \) constants. These constants were then used to calculate the amount of Urea-POP-1 at the 50% or 95% saturation points where \( A \) or \( F = 0.5 \) (m2) or 0.05, respectively. The average of three technical replicates with standard deviation is presented in Figure S15 and Table S3.

**Determination of partition coefficients.** A previously reported procedure was adapted to measure the partition coefficients or log \( D \) values of each dye. Each dye was prepared at 40 or 50 \( \mu \)M in ultrapure water (ca. pH 7). Equal volumes of each dye solution in water (750 \( \mu \)L) and octanol (750 \( \mu \)L) were combined into a 2-mL microcentrifuge tube, mixed for 1 min at maximum speed (Vortex 2 Shaker, IKA Works), and centrifuged for 5 min at 2900g at room temperature (5810 R, Eppendorf). For each dye, a 100 \( \mu \)L aliquot of the water layer was transferred to a 96-well half-area microtiter plate, but for ATP and AMP, a 200 \( \mu \)L aliquot of the water layer was transferred to a 96-well UV-Star UV-Transparent microtiter plate (Greiner Bio-One). The absorbance or fluorescence of each dye was measured as described above, compared to a standard curve, and used to determine the log \( D \) values as follows: log \( D = \text{log} \left( \frac{[\text{dye}]_{\text{octanol}}}{[\text{dye}]_{\text{water}}} \right) \) where \([\text{dye}]_{\text{water}}\) corresponds to the concentration of partitioned dye in water and \([\text{dye}]_{\text{octanol}}\) corresponds to the concentration of partitioned dye in octanol which was measured through mass balance. The average of three technical replicates with standard deviation is reported in Table S3.

**Results and Discussion**

Both Urea-POP-1 and Methyl-POP-1 were accessed through a modular synthetic route with simple building blocks (Figure 2A, Scheme S1). The [(2,5-dibromophenyl)methyl]urea monomer (1) was prepared in four high yielding steps with minimal chromatography. Briefly, 2,5-dibromotoluene was brominated with N-bromosuccinimide and benzoyl peroxide (45% yield), followed by substitution with potassium phthalimide and subsequent deprotection with hydrazine (81% yield over two steps), and finally treated with potassium isocyanate to generate the urea (92% yield). Compound 1 and 2,5-dibromotoluene (2) were polymerized with 1,3,5-triethynylbenzene (3), a commonly used monomer for the preparation of porous organic polymers, using standard Sonogashira coupling reaction conditions to furnish Urea-POP-1 and Methyl-POP-1 as insoluble powders.

Characterization with infrared spectroscopy provided evidence for the extent of polymerization (Figure S2). For both POPs, the disappearance of the alkyne C-H stretch at 3276 cm\(^{-1}\) confirms that the starting 1,3,5-triethynylbenzene co-monomer was consumed. Interestingly, the N-H stretches corresponding to the urea functional group are broadened and centered at 3398 cm\(^{-1}\) in Urea-POP-1 relative to the N-H stretches at 3429 and 3338 cm\(^{-1}\) in the starting urea monomer (1), suggesting the presence of hydrogen bonding between the urea units within the POP. To determine the accessibility of the urea sidechains, we measured the nitrogen adsorption isotherms of five independently prepared batches at 77 K (Table S1, Figure S3). Although both POPs are permanently porous, on average Urea-POP-1 (862 ± 120 m\(^2\)/g) has a larger BET surface area than Methyl-POP-1 (496 ± 69 m\(^2\)/g) (Figure 2B). Based on these isotherms, both POPs are microporous, as the average pore size distributions are predominately within 10–20 Å (Figure S3). Moreover, scanning electron microscopy does not reveal any uniform crystalline morphology indicating that both POPs are amorphous in nature (Figure S4).
We next tested if the urea sidechains in Urea-POP-1 could be used for the recognition of anionic guests in water. To do so, we selected dyes containing oxyanions commonly known to interact with urea-based hosts, including phosphonate (AMP, ATP, and FMN), sulfonate (methyl orange, Lucifer Yellow CH, and bromophenol blue), and carboxylate (Rhodamine B, methyl red, Alizarin Yellow G, and fluorescein) anions (Figure 3). Recognition of the dyes was evaluated through adsorption assays in water as monitored by absorption or fluorescence spectroscopy with varying concentrations of Urea-POP-1 and Methyl-POP-1, with the latter serving as a control for hydrophobic interactions (Figure 3). To ensure that recognition was driven by hydrogen bonding interactions, each dye was dissolved in ultrapure water (ca. pH 7) and diluted to 40 µM for detection within a dynamic, linear range. Moreover, at this concentration of dye, the number of urea sidechains available for binding was in 10 to 320-fold excess, corresponding to a range of 0.05 to 1.6 mg/mL of POP (Figure S5).

As can be seen in Figure 3, increasing concentrations of Urea-POP-1 promote greater adsorption of each dye, albeit to varying degrees depending on the anionic functional group. These data were fitted to a binding model as described in the methods section above to determine the amount of Urea-POP-1 at a saturation point where the relative absorbance or fluorescence signal of each dye decreased by 50% (Figure 4A, Table S3). To better explain the observed differences, we first considered the overall charge and any additional functional groups of each dye that could influence the hydrogen bonding interactions between the anionic functional groups and the urea sidechains. Within the phosphonate dyes, more Urea-POP-1 is required to adsorb AMP (0.667 ± 0.006 mg/mL) versus FMN (0.4 ± 0.1 mg/mL), even though both dyes have an overall charge of -2. Given that the anthracene-like backbone of FMN is more hydrophobic than the adenosine backbone of AMP, hydrophobic interactions could contribute to adsorption. For ATP the saturation point could not be determined due to weak binding. Based on this, more than 1.6 mg/mL of Urea-POP-1 would be required for adsorption of ATP, which correlates with its greater negative charge of -4.
In comparison, less Urea-POP-1 is required to adsorb both the sulfonate and carboxylate dyes. For the sulfonate dyes, the amount of Urea-POP-1 for adsorption can be ranked from lowest to highest as follows: methyl orange (0.119 ± 0.005 mg/mL) < Lucifer Yellow CH (0.20 ± 0.03 mg/mL) < bromophenol blue (0.33 ± 0.02 mg/mL). Indeed, the amount of Urea-POP-1 required for dye adsorption not only increases with the number of sulfonate groups but also with the overall negative charge. Specifically, methyl orange has one sulfonate group with an overall charge of -1, whereas Lucifer Yellow CH, with an additional sulfonate group, and bromophenol blue, with a phenolate group, both have an overall charge of -2. However, more Urea-POP-1 is needed to adsorb bromophenol blue than Lucifer Yellow CH, likely stemming from differences in the hydration and basicity of the phenolate versus the sulfonate anion.91 A similar observation can also be made for the carboxylate dyes albeit over a narrower range, with overlapping standard deviations. The amount of Urea-POP-1 for adsorption can be ranked from lowest to highest as follows: Rhodamine B (0.045 ± 0.007 mg/mL) ≈ methyl red (0.05 ± 0.02 mg/mL) < Alizarin Yellow G (0.07 ± 0.02 mg/mL) < fluorescein (0.11 ± 0.04 mg/mL). On one end,
Rhodamine B has a carboxylate and a protonated imine whereas fluorescein has a carboxylate and a phenolate, resulting in an overall charge of 0 and -2, respectively. Even though they are structurally similar, the difference in the overall charge of these two dyes likely contributes to the amount of Urea-POP-1 needed for adsorption. The amount of Urea-POP-1 for both methyl red and Alizarin Yellow G are comparable since both are not only structurally similar but also have an overall charge of -1. The differences observed in the dye adsorption within each anionic functional group are primarily linked to differences in the overall charge of each dye. Irrespective of the identity of the anionic functional group, adsorption is observed, suggesting that hydrogen bonding interactions do occur with the urea sidechains.

We next evaluated dye adsorption to Methyl-POP-1 to isolate any effects arising from hydrophobic interactions. While the average amount of Urea-POP-1 at a 50% saturation point for each dye based on the anionic functional group can be compared and ranked as follows: phosphonate > sulfonate > carboxylate, this trend is not observed with Methyl-POP-1. None of the sulfonate dyes adsorbed, and of the three phosphonate dyes, FMN is the only dye that adsorbed to the hydrophobic pores of Methyl-POP-1, supporting the observations described in Figure 4.

Figure 4. The amount of Urea-POP-1 required for adsorption is correlated to the anionic functional groups. (A) Representative curve fit generated from the data in Figure 3 to determine the amount of Urea-POP-1 at saturation points (indicated by red arrows) where the relative absorbance or fluorescence signal of each dye has a 50% change or less than a 5% change. Curve fits for all dyes are shown in Figure S15. (B) The amount of Urea-POP-1 at the 95% saturation point for each dye versus the dye partition coefficient is shown. Dyes that contain carboxylate (R-COO⁻), sulfonate (R-SO₃⁻), phosphonate (R-PO₃²⁻) functional groups are grouped by the red, blue, and green boxes, respectively. The light blue background represents the preference of the dye to stay in water (hydrophilic), and the light red background represents the preference of the dyes to stay in octanol (hydrophobic). The dyes are abbreviated as follows: adenosine monophosphate (AMP), adenosine triphosphate (ATP), Alizarin Yellow G (AY), bromophenol blue (BB), flavin mononucleotide (FMN), fluorescein (FL), Lucifer Yellow CH (LY), methyl orange (MO), methyl red (MR), and Rhodamine B (RB).

*For ATP a saturation point could not be determined. The average of three technical replicates with standard deviation is reported for each dye (Table S3).
above for FMN adsorption to Urea-POP-1. The fluorescence signal for FMN decreases by only 20% with 1.6 mg/mL of Methyl-POP-1 but decreases by >95% with the same amount of Urea-POP-1. Notably, all of the carboxylate dyes adsorb to Methyl-POP-1, which is consistent with the fact that all of these dyes have more positive log D values, and thus partition more readily into octanol over water (Table S3). However, clear differences are observed between Urea-POP-1 and Methyl-POP-1, particularly at ~0.2 mg/mL, indicating that the carboxylate anions, in large part, preferentially adsorb to Urea-POP-1. These control experiments clearly show that hydrophobic interactions do occur between the dyes and the hydrophobic environment of the POP; however, it is not the primary driving force as the dyes do not adsorb to Methyl-POP-1 to the same extent as Urea-POP-1 – even when more hydrophobic dyes are used.

To further solidify that dye adsorption to Urea-POP-1 is primarily driven by hydrogen bonding interactions, we compared the partition coefficient (log D value) of each dye to the amount of Urea-POP-1 at a saturation point where the relative absorbance or fluorescence signal of each dye decreased by 95% (Figure 4A, Table S3). At this saturation point, the adsorption of each dye to Urea-POP-1 was likely maximized given the number of excess urea sidechains, thus allowing for a better comparison. This is indeed the case because three groups based on the identity of the anionic functional group emerge (Figure 4B). As expected, the phosphonate dyes have negative log D values (-1.5 ± 0.4 to -1.08 ± 0.03) and readily partition into water over octanol, independent of the overall charge. On average, more than 1 mg/mL of Urea-POP-1 is needed for dye adsorption. Similarly, the sulfonate dyes have negative log D values within a comparable range (-1.6 ± 0.1 to -1.14 ± 0.03) but require ~60% less Urea-POP-1. On the other end, the carboxylate dyes have log D values ranging from -0.22 ± 0.06 and 1.88 ± 0.04 and partition more favorably into octanol. However, the average amount of Urea-POP-1 required for adsorption of the carboxylate dyes is within ~30% and comparable with overlapping standard deviations to the amount required for adsorption of the sulfonate dyes. The extent to which the phosphonate, sulfonate, and carboxylate groups of the dyes are hydrated could provide an explanation for the observed selectivity of Urea-POP-1. In fact, the adsorption behavior of Urea-POP-1 correlates well with the Gibbs free energy of hydration ($\Delta_{\text{hyd}}G$) and the basicity, particularly on the extreme end with the hydrogen phosphate anion ($\Delta_{\text{hyd}}G$: HPO$_4^{2-}$ (-1366 kJ/mol) > HCO$_2^-$ (-395 kJ/mol) > HSO$_3^-$ (-330 kJ/mol)),$^{91}$ basicity: RPO$_4^{2-}$ > RCO$_2^-$ > RSO$_3^-$ where R corresponds to a hydrogen or a phenyl group).$^{72,92}$ Taken together, hydrophobic interactions with the POP backbone and polar interactions with the urea groups do contribute. However, this observed behavior would not emerge if selective hydrogen bonding interactions between the anionic functional groups of the dyes and the urea sidechains of Urea-POP-1 did not occur to promote desolvation and adsorption.

Conclusions

We have demonstrated that Urea-POP-1 is a neutral organic polymer host capable of recognition through adsorption of anionic dyes in water. In the absence of a predesigned receptor, Urea-POP-1 can discriminate between structurally different dyes containing phosphonate, sulfonate, and carboxylate anions, whereas Methyl-POP-1, a control lacking hydrogen bond donors, cannot. Even though hydrophobic interactions do occur between the POP backbone and the dyes, our data indicate that the urea sidechains can cooperatively hydrogen bond to the anionic functional groups even in the presence of water. Since the precise contributions of enthalpy and entropy cannot be easily obtained using polymers of this type, we can only speculate as to what factors could play a role.$^2$ By design, the dense array of urea hydrogen bond donors and the hydrophobic pores should have positive effects on the enthalpy of adsorption. This would be achieved by forming primary interactions with the anionic functional groups and secondary interactions with the other components of the dyes. Moreover, the
desolvation of the anionic functional groups and the hydrophobic pores, although minimal for the latter since the POP host is insoluble, could provide significant entropic gains to the overall driving force for adsorption. To close, our study not only showcases a new approach to design neutral hosts for anionic guest recognition in water, but also highlights how well studied polymer architectures can be repurposed to expand our fundamental understanding of anion supramolecular chemistry. Along these lines, future efforts will exploit the modularity of our starting design to build a family of neutral polymer hosts with tunable pore sizes and anion preferences.

Conflicts of Interest

There are no conflicts to declare.

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Supporting information for

A Neutral Porous Organic Polymer Host for the Recognition of Anionic Dyes in Water

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Scheme S1. Synthesis of (A) \([(2,5\text{-dibromophenyl})\text{methyl}]\text{urea} \ (1)\) and (B) \(1,3,5\text{-triethynylbenzene} \ (3)\).
Figure S1. (A) $^1$H-NMR and (B) $^{13}$C-NMR spectra of [(2,5-dibromophenyl)methyl]urea (1).
Figure S2. Stacked attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra of starting monomers and POPs.
Table S1. BET surface areas for five independently prepared batches of Urea-POP-1 and Methyl-POP-1. The average with standard deviation is reported.

| Batch       | 1      | 2      | 3      | 4      | 5      | Average       |
|-------------|--------|--------|--------|--------|--------|---------------|
| Urea-POP-1  | 1041 m²/g | 936 m²/g | 743 m²/g | 873 m²/g | 718 m²/g | 862 ± 120 m²/g |
| Methyl-POP-1| 530 m²/g  | 409 m²/g | 550 m²/g | 572 m²/g | 417 m²/g | 496 ± 69 m²/g  |
Figure S3. Comparison of the (A) nitrogen adsorption (closed circles) and desorption (open circles) isotherms and (B) differential (closed circles) and cumulative (open circles) pore volumes for Urea-POP-1 (red) and Methyl-POP-1 (black). Data is shown for five independently prepared batches of each POP.
Figure S4. Representative scanning electron microscopy (SEM) images of (A) Urea-POP-1 and (B) Methyl-POP-1.
Figure S5. Structure of the monomer unit for Urea-POP-1 (738.80 g/mol) and Methyl-POP-1 (564.68 g/mol).
Table S2. Plate reader settings for each dye.

| Dyes                | Absorbance<sup>c</sup> | Fluorescence<sup>c</sup> |     |     |     | Flashes | Gain |
|---------------------|-------------------------|---------------------------|-----|-----|-----|---------|------|
|                     | Range (nm)              | λ<sub>measured</sub> (nm) | Excitation (nm) | Emission (nm) | λ<sub>measured</sub> (nm) |     |     |
| FMN<sup>a</sup>     | 300–800                 | 445                       | 450 | 480–800 | 530 | 30      | 100  |
| ATP<sup>a,b</sup>   | 230–300                 | 255                       | N/D | N/D   | N/D | N/D     | N/D  |
| AMP<sup>a,b</sup>   | 230–300                 | 255                       | N/D | N/D   | N/D | N/D     | N/D  |
| Methyl orange       | 350–650                 | 465                       | N/D | N/D   | N/D | N/D     | N/D  |
| Lucifer Yellow CH   | 350–700                 | 425                       | 430 | 450–700 | 530 | 30      | 90   |
| Bromophenol blue    | 450–700                 | 560                       | N/D | N/D   | N/D | N/D     | N/D  |
| Rhodamine B         | 450–650                 | 530                       | N/D | N/D   | N/D | N/D     | N/D  |
| Methyl red          | 360–560                 | 435                       | N/D | N/D   | N/D | N/D     | N/D  |
| Alizarin Yellow G   | 330–480                 | 350                       | N/D | N/D   | N/D | N/D     | N/D  |
| Fluorescein         | 400–600                 | 475                       | N/D | N/D   | N/D | N/D     | N/D  |

<sup>a</sup> Abbreviations: Flavin mononucleotide (FMN), adenosine triphosphate (ATP), adenosine monophosphate (AMP)
<sup>b</sup> UV-visible spectrometer was used instead
<sup>c</sup> Spectra were collected with a 5 nm step size
Figure S5. Fluorescence spectra of flavin mononucleotide (FMN) in the presence of increasing concentrations of (A) Methyl-POP-1 and (B) Urea-POP-1. The POP concentrations range from 0 (black, bold), 0.1, 0.2, 0.4, 0.8, 1.2 and 1.6 (red) mg/mL. All measurements were carried out with 40 µM of FMN in ultrapure water (ca. pH 7).
Figure S6. Absorbance spectra of adenosine triphosphate (ATP) in the presence of increasing concentrations of (A) Methyl-POP-1 and (B) Urea-POP-1. The POP concentrations range from 0 (black bold), 0.1, 0.2, 0.4, 0.8, 1.2 and 1.6 (red) mg/mL. All measurements were carried out with 40 µM of ATP in ultrapure water (ca. pH 7).
Figure S7. Absorbance spectra of adenosine monophosphate (AMP) in the presence of increasing concentrations of (A) Methyl-POP-1 and (B) Urea-POP-1. The POP concentrations range from 0 (black, bold), 0.1, 0.2, 0.4, 0.8, 1.2 and 1.6 (red) mg/mL. All measurements were carried out with 40 µM of AMP in ultrapure water (ca. pH 7).
**Figure S8.** Absorbance spectra of methyl orange in the presence of increasing concentrations of (A) Methyl-POP-1 and (B) Urea-POP-1. The POP concentrations range from 0 (black, bold), 0.05, 0.1, 0.2, 0.4, 0.6 and 0.8 (red) mg/mL. All measurements were carried out with 40 µM of methyl orange in ultrapure water (ca. pH 7).
Figure S9. Fluorescence spectra of Lucifer Yellow CH in the presence of increasing concentrations of (A) Methyl-POP-1 and (B) Urea-POP-1. The POP concentrations range from 0 (black, bold), 0.05, 0.1, 0.2, 0.4, 0.6 and 0.8 (red) mg/mL. All measurements were carried out with 40 µM of Lucifer Yellow CH in ultrapure water (ca. pH 7).
Figure S10. Absorbance spectra of bromophenol blue in the presence of increasing concentrations of (A) Methyl-POP-1 and (B) Urea-POP-1. The POP concentrations range from 0 (black, bold), 0.05, 0.1, 0.2, 0.4, 0.6 and 0.8 (red) mg/mL. All measurements were carried out with 40 µM of bromophenol blue in ultrapure water (ca. pH 7).
Figure S11. Absorbance spectra of Rhodamine B in the presence of increasing concentrations of (A) Methyl-POP-1 and (B) Urea-POP-1. The POP concentrations range from 0 (black, bold), 0.05, 0.1, 0.2, 0.4, 0.6 and 0.8 (red) mg/mL. All measurements were carried out with 40 µM of Rhodamine B in ultrapure water (ca. pH 7).
Figure S12. Absorbance spectra of methyl red in the presence of increasing concentrations of (A) Methyl-POP-1 and (B) Urea-POP-1. The POP concentrations range from 0 (black, bold), 0.05, 0.1, 0.2, 0.4, 0.6 and 0.8 (red) mg/mL. All measurements were carried out with 40 µM of methyl red in ultrapure water (ca. pH 7).
**Figure S13.** Absorbance spectra of Alizarin Yellow G in the presence of increasing concentrations of (A) Methyl-POP-1 and (B) Urea-POP-1. The POP concentrations range from 0 (black, bold), 0.05, 0.1, 0.2, 0.4, 0.6 and 0.8 (red) mg/mL. All measurements were carried out with 40 µM of Alizarin Yellow G in ultrapure water (ca. pH 7).
Figure 14. Absorbance spectra of fluorescein in the presence of increasing concentrations of (A) Methyl-POP-1 and (B) Urea-POP-1. The POP concentrations range from 0 (black, bold), 0.05, 0.1, 0.2, 0.4, 0.6 and 0.8 (red) mg/mL. All measurements were carried out with 40 µM of fluorescein in ultrapure water (ca. pH 7).
Figure S15. Average curve fits generated from the data in Figure 3 to determine the amount of Urea-POP-1 at 50% and 95% saturation points for (A) flavin mononucleotide, (B) adenosine triphosphate, (C) adenosine monophosphate, (D) methyl orange, (E) Lucifer Yellow CH, (F) bromophenol blue, (G) Rhodamine B, (H) methyl red, (I) Alizarin Yellow G, and (J) fluorescein. The average of three technical replicates with standard deviation is reported.
Table S3. Summary of the overall charge, partition coefficient, and concentration of Urea-POP-1 at 50% and 95% saturation point for each dye.

| Dye                | Overall charge at pH 7 | Partition coefficient (log D) | POP concentration at 50% adsorption saturation (mg/mL) | POP concentration at 95% adsorption saturation (mg/mL) |
|--------------------|------------------------|-------------------------------|--------------------------------------------------------|--------------------------------------------------------|
| FMN<sup>a</sup>    | -2                     | -1.4 ± 0.1                    | 0.4 ± 0.1                                              | 1.1 ± 0.2                                              |
| ATP<sup>a</sup>    | -4                     | -1.08 ± 0.03                  | N/D                                                   | N/D                                                   |
| AMP<sup>a</sup>    | -2                     | -1.5 ± 0.4                    | 0.667 ± 0.006                                         | 1.43 ± 0.09                                           |
| Methyl orange      | -1                     | -1.14 ± 0.03                  | 0.119 ± 0.005                                         | 0.45 ± 0.04                                           |
| Lucifer Yellow CH  | -2                     | -1.4 ± 0.1                    | 0.20 ± 0.03                                           | 0.58 ± 0.06                                           |
| Bromophenol blue   | -2                     | -1.6 ± 0.1                    | 0.33 ± 0.02                                           | 0.568 ± 0.007                                         |
| Rhodamine B        | 0                      | 1.88 ± 0.04                   | 0.045 ± 0.007                                         | 0.30 ± 0.06                                           |
| Methyl red         | -1                     | 0.46 ± 0.03                   | 0.05 ± 0.02                                           | 0.3 ± 0.1                                             |
| Alizarin Yellow G  | -1                     | -0.22 ± 0.06                  | 0.07 ± 0.02                                           | 0.5 ± 0.2                                             |
| Fluorescein        | -2                     | 0.21 ± 0.06                   | 0.11 ± 0.04                                           | 0.41 ± 0.05                                           |

<sup>a</sup> Abbreviations: Flavin mononucleotide (FMN), adenosine triphosphate (ATP), adenosine monophosphate (AMP)
