

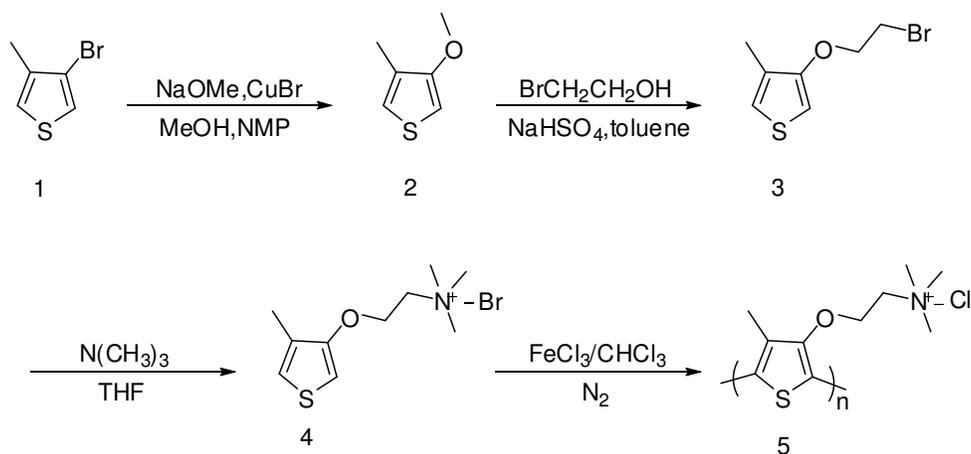
## Optically Active Supramolecular Complexes of Water-Soluble Achiral Polythiophenes and Folic Acid: Spectroscopic Studies and Sensing Applications

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### 1. Synthesis of the polymer PMTEA



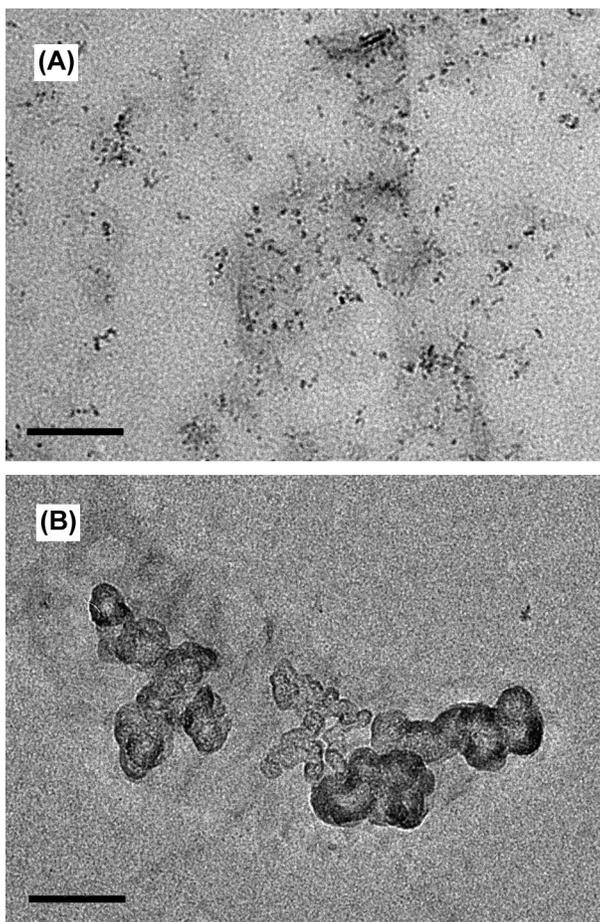
**Scheme 1.** Synthesis of the polythiophene derivative, PMTEA

**3-Methoxy-4-methylthiophene (2).** 3-Bromo-4-methylthiophene (5.72 g, 32.3 mmol) and CuBr (2.5 g, 17.4 mmol) were added to a mixture of 30 mL of sodium methoxide (28% in methanol) and 20 mL NMP and refluxed for 3 days under N<sub>2</sub> atmosphere. After cooling, the solid was filtrated, and the filtrate was extracted three times with diethyl ether. The organic phase was dried with magnesium sulfate and then evaporated. The final product was purified by chromatography (silica gel, hexane) (3.69 g, 89%).  
<sup>1</sup>H-NMR (CDCl<sub>3</sub>, TMS, 300 MHz) δ 6.84 (1H, d), 6.17 (1H, d), 3.86 (3H, s), 2.11(3H, s).

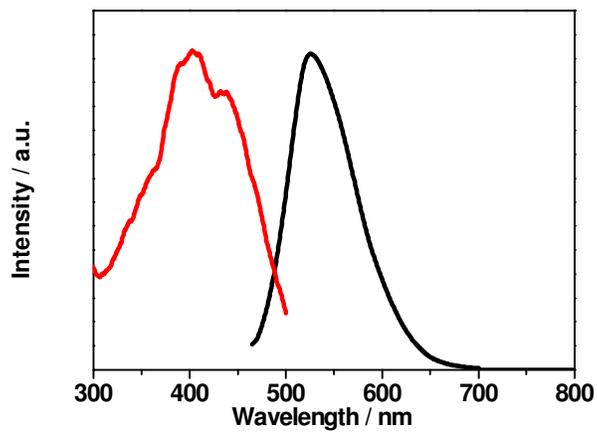
**3-(2-Bromo) ethyloxy-4-methylthiophene (3).** 3-Methoxy-4-methylthiophene (1.68 g, 13.1 mmol), 2-bromoethanol (5.00 g, 40.0 mmol), and NaHSO<sub>4</sub> (246 mg, 2.05 mmol) were added to 24 mL of toluene under N<sub>2</sub> atmosphere and heated at 100 °C until the produced methanol was distilled off. The reaction mixture was allowed to cool to room temperature and washed three times with water (20 mL each). The collected water phases were extracted with diethyl ether. The organic phase was combined and dried with MgSO<sub>4</sub>, filtered and evaporated to dryness. The crude product was submitted to column chromatography (silica gel, CH<sub>2</sub>Cl<sub>2</sub>/hexane (1:1)) to give 2.06 g (71%) **3** as colorless oil. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, TMS, 300 MHz) δ 6.84 (1H, d), 6.19 (1H, d), 4.26 (2H, t), 3.65 (2H,t), 2.11 (3H, s).

**3-(4-Methyl-3'-thienyloxy) ethyltrimethylammonium bromide (4).** Trimethylamine (30% aqueous) (25mL) was added into a solution of 3-(2-bromo) ethyloxy-4-methylthiophene (0.67 g, 3.03mmol) in THF (25 mL). The mixture was stirred 24 h at room temperature, and then evaporated to dryness. The crude product was washed with THF to give 0.63 g (74%) product **4**. <sup>1</sup>H-NMR: (D<sub>2</sub>O, 300 MHz) δ 6.93 (1H ,d), 6.41(1H, d), 4.37 (2H,m), 3.74 (2H, m), 3.14 (s, 9H), 1.96 (s, 3H).

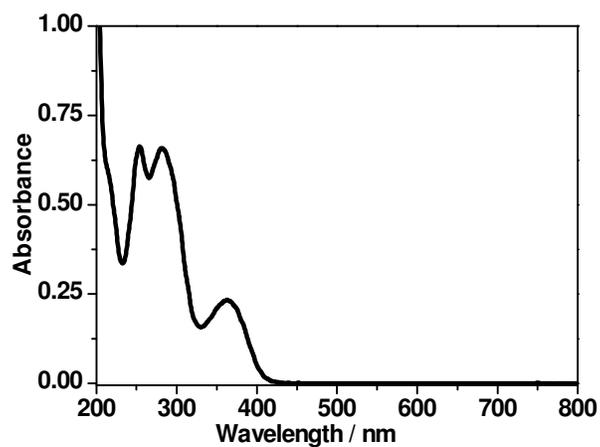
**Polymer PMTEA.** 244 mg (0.87 mmol) of **4** and 564 mg (3.48 mmol) of anhydrous FeCl<sub>3</sub> were mixed in 25 mL of dry CHCl<sub>3</sub>, and stirred for 24 h at room temperature under N<sub>2</sub> atmosphere, then evaporated to dryness. The solid was washed by methanol, filtered. The crude product was washed by acetone/Soxhlet extraction for 24 h. The insoluble fraction of the polymer was dried and then dissolved in methanol by adding a few drops of hydrazine. The resulting polymer was precipitated by addition of a saturated solution of tetrabutylammonium chloride in acetone and washed again with acetone, and then dried under reduced pressure to give polymer PMTEA (68 mg, 28%).



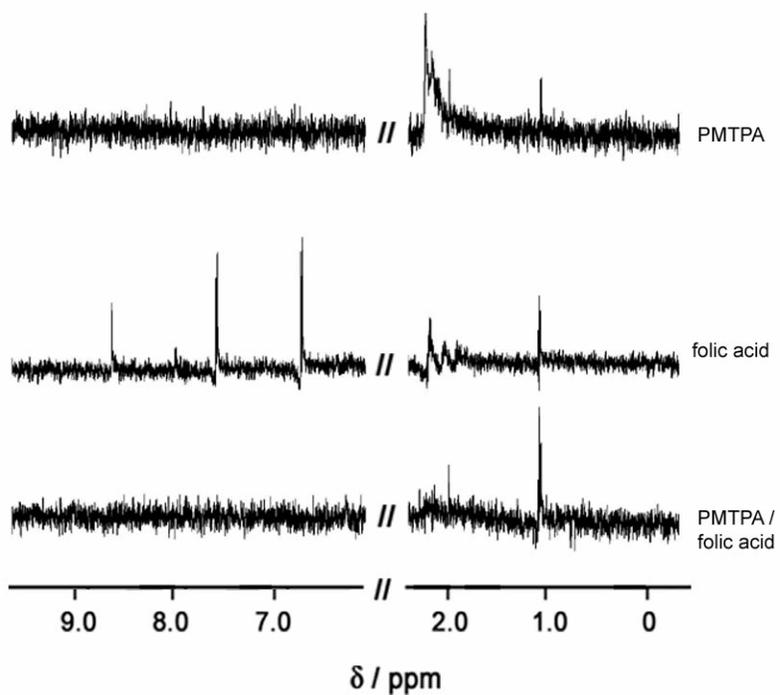
**Figure S1.** TEM images of the PMTPA (A) and PMTPA/folic acid complex (B). Scale bar: 100 nm.



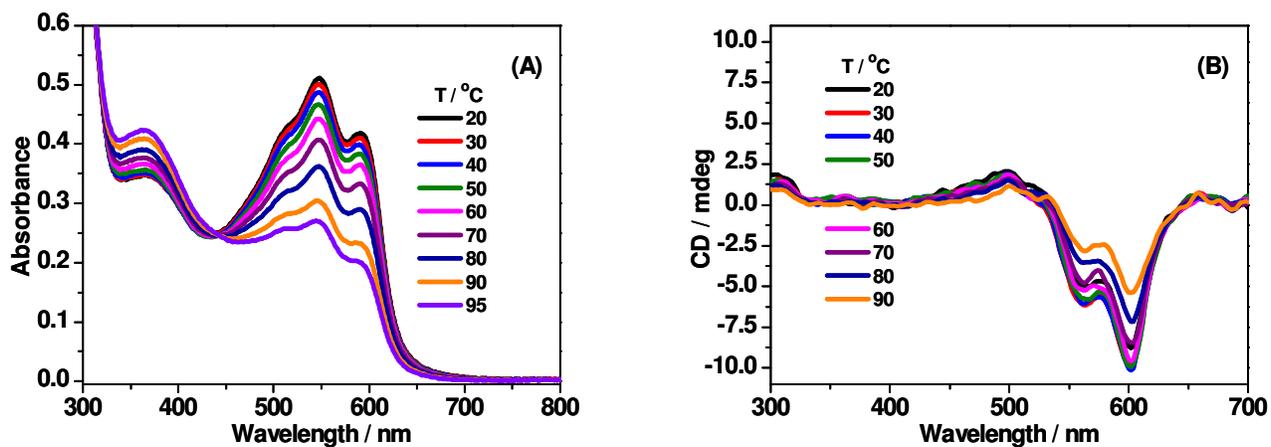
**Figure S2.** Emission and excitation spectra of PMTPA ( $1.0 \times 10^{-4}$  M) in water at 20 °C.  $\lambda_{\text{ex}} = 400$  nm;  
 $\lambda_{\text{em}} = 525$  nm.



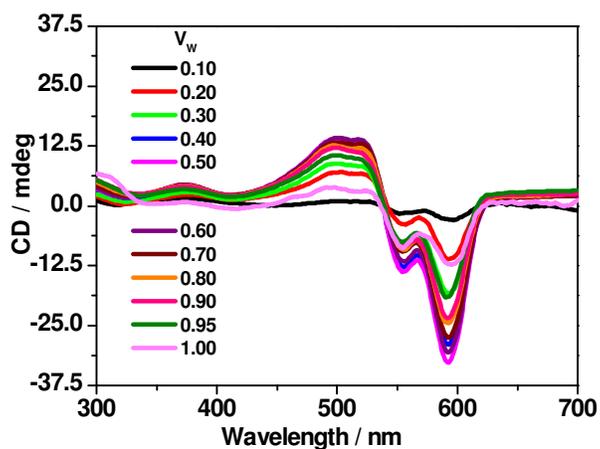
**Figure S3.** Absorption spectrum of folic acid in water (pH = 9.0) at 20 °C.



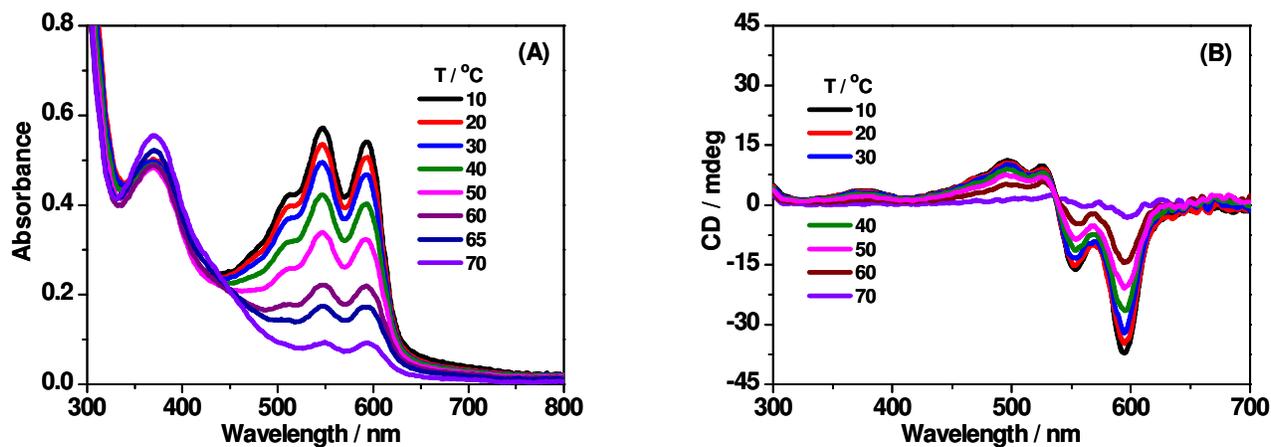
**Figure S4.**  $^1\text{H}$ -NMR spectra of the PMTPA/folic acid (4/1) complex along with the  $^1\text{H}$ -NMR spectra of PMTPA and folic acid. Solvent:  $\text{D}_2\text{O}$



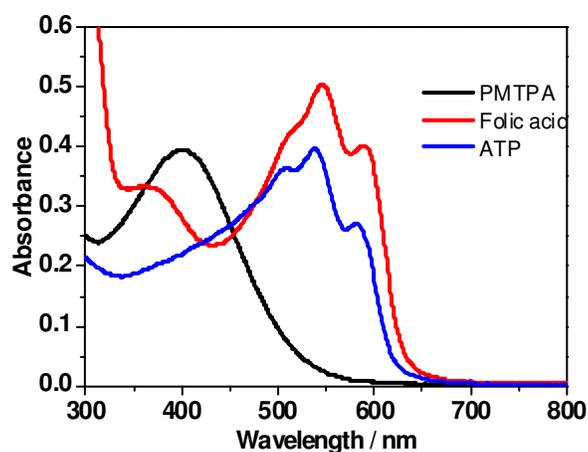
**Figure S5.** Temperature-dependent absorption (A) and CD (B) spectra of the PMTPA/folic acid complex in water. [PMTPA] =  $1.0 \times 10^{-4}$  M; [folic acid] =  $2.5 \times 10^{-5}$  M.



**Figure S6.** Composition dependent CD spectra of the PMTPA/folic acid complex in the water/methanol mixed solvent with the volume fraction of water ( $V_w$ ) as indicated. [PMTPA] =  $1.0 \times 10^{-4}$  M; [folic acid] =  $2.5 \times 10^{-5}$  M.



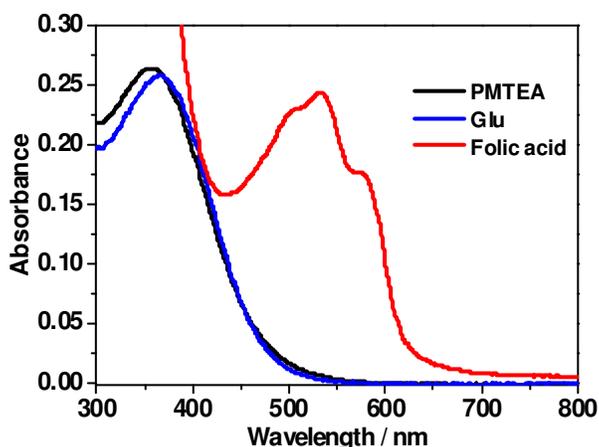
**Figure S7.** Temperature-dependent absorption (A) and CD (B) spectra of the PMTPA/folic acid complex in the water/methanol (1:1 by volume) mixed solvent. [PMTPA] =  $1.0 \times 10^{-4}$  M; [folic acid] =  $2.5 \times 10^{-5}$  M.



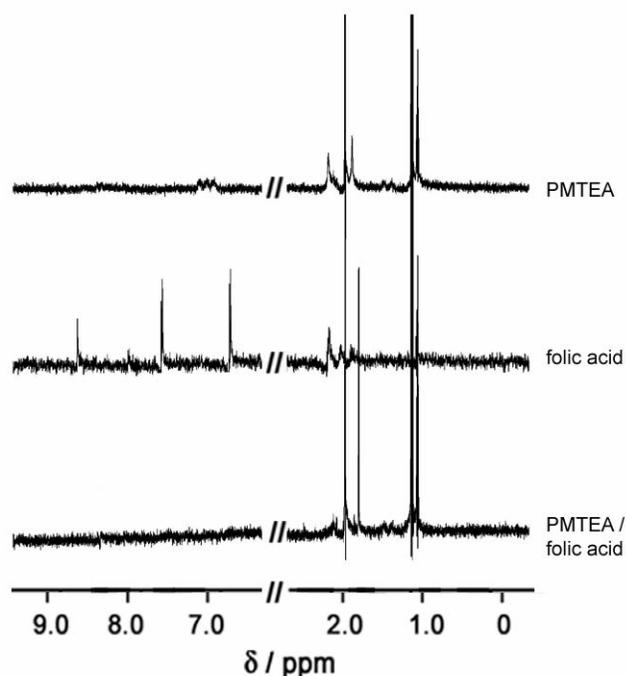
**Figure S8.** Absorption spectra of PMTPA ( $1.0 \times 10^{-4}$  M) in the absence and the presence of equimolar amounts of ATP and folic acid in water.

## 2. Mechanism for the Supramolecular PMTEA/Folic Acid Complex Formation.

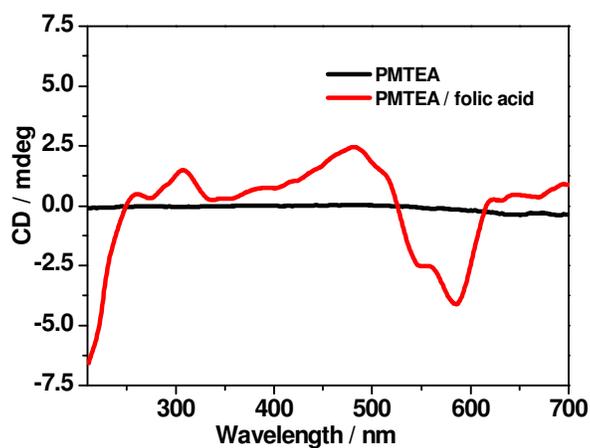
Figure S9 compares the absorption spectra of PMTEA in the absence and the presence of equimolar amount of Glu and folic acid in water. It was found that the presence of Glu does not induce a distinct spectral change and the solution color is kept as yellow, indicating that no supramolecular aggregate is formed in the mixture; whereas the addition of folic acid can induce a distinct color change of the solution of PMTEA, and the absorption maximum exhibits a red shift by 175 nm. These results indicate that the pterin moiety in the folic acid molecule is indispensable for accelerating the formation of a  $\pi$ -stacked supramolecular aggregate. Comparison of  $^1\text{H-NMR}$  spectra of folic acid, PMTEA and the PMTEA/folic acid complex further supports this conclusion (Figure S10). In the complex, peaks originated from the pterin moieties are broadened and disappeared with respect to those for “free” folic acid in solution, indicating the significant contribution of  $\pi$ - $\pi$  stacking between pterin moieties. PMTEA is intrinsically optically inactive, and no CD pattern in the  $\pi$ - $\pi^*$  transition region can be detected. The introduction of folic acid into aqueous PMTEA solution resulted in an intense ICD signal in the  $\pi$ - $\pi^*$  transition region of PMTEA (Figure 11), being characteristic of the presence of strong exciton coupling between PT backbones in the chiral  $\pi$ -stacked PMTEA/folic acid complex.



**Figure S9.** Absorption spectra of PMTEA ( $1.0 \times 10^{-4}$  M) in the absence and the presence of equimolar amounts of Glu and folic acid in water.



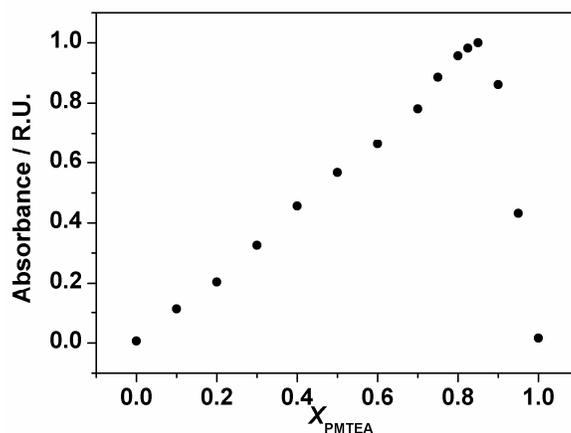
**Figure S10.**  $^1\text{H-NMR}$  spectra of the PMTEA/Folic acid (6/1) complex along with the  $^1\text{H-NMR}$  spectra of PMTEA and folic acid. Solvent:  $\text{D}_2\text{O}$ .



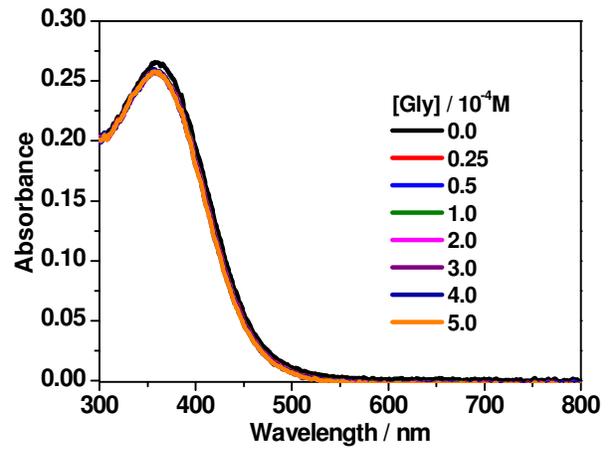
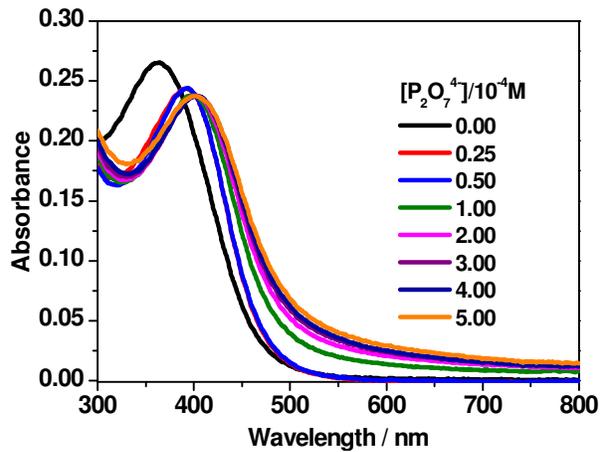
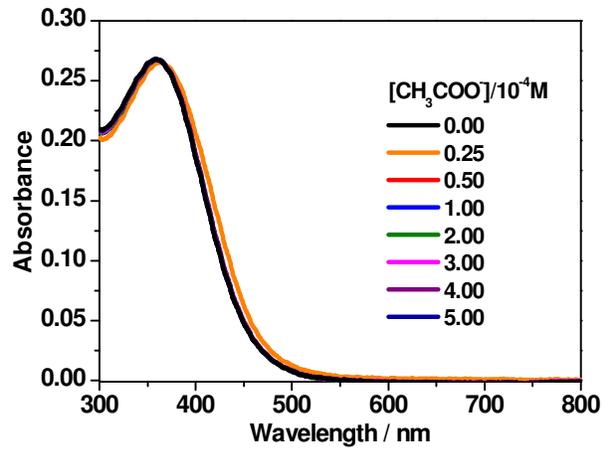
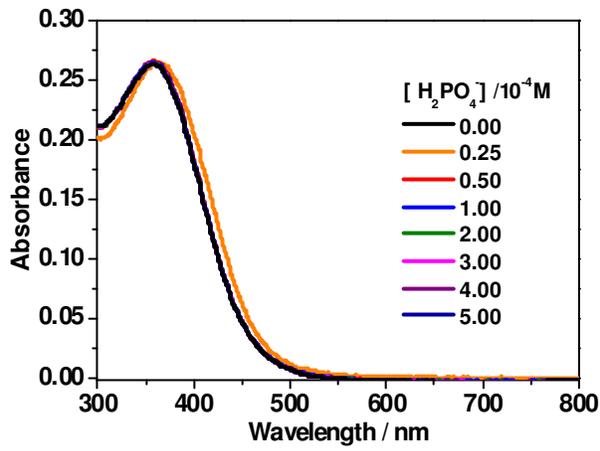
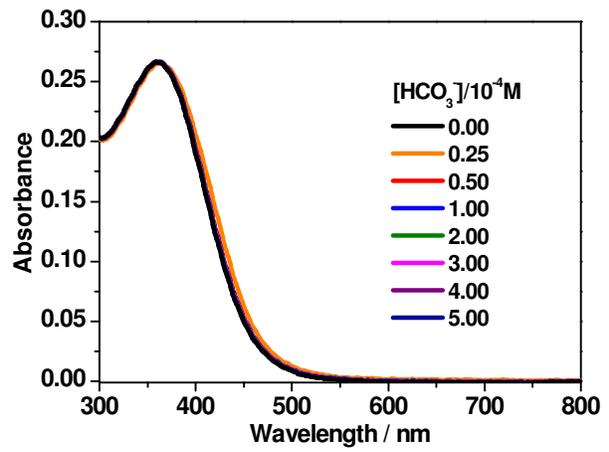
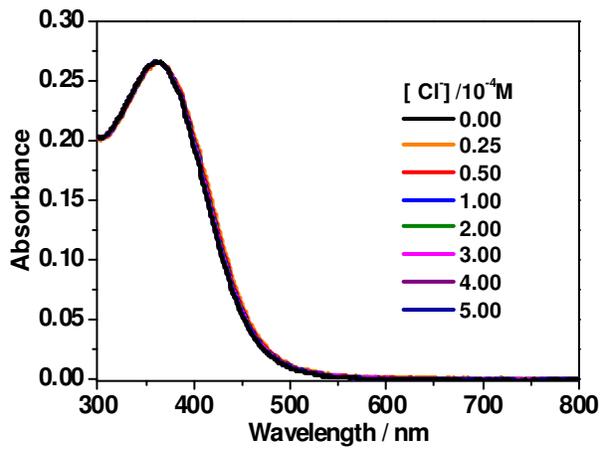
**Figure S11.** CD spectra of PMTEA ( $1.0 \times 10^{-4}$  M) in the absence and the presence of equimolar amounts of folic acid in water.

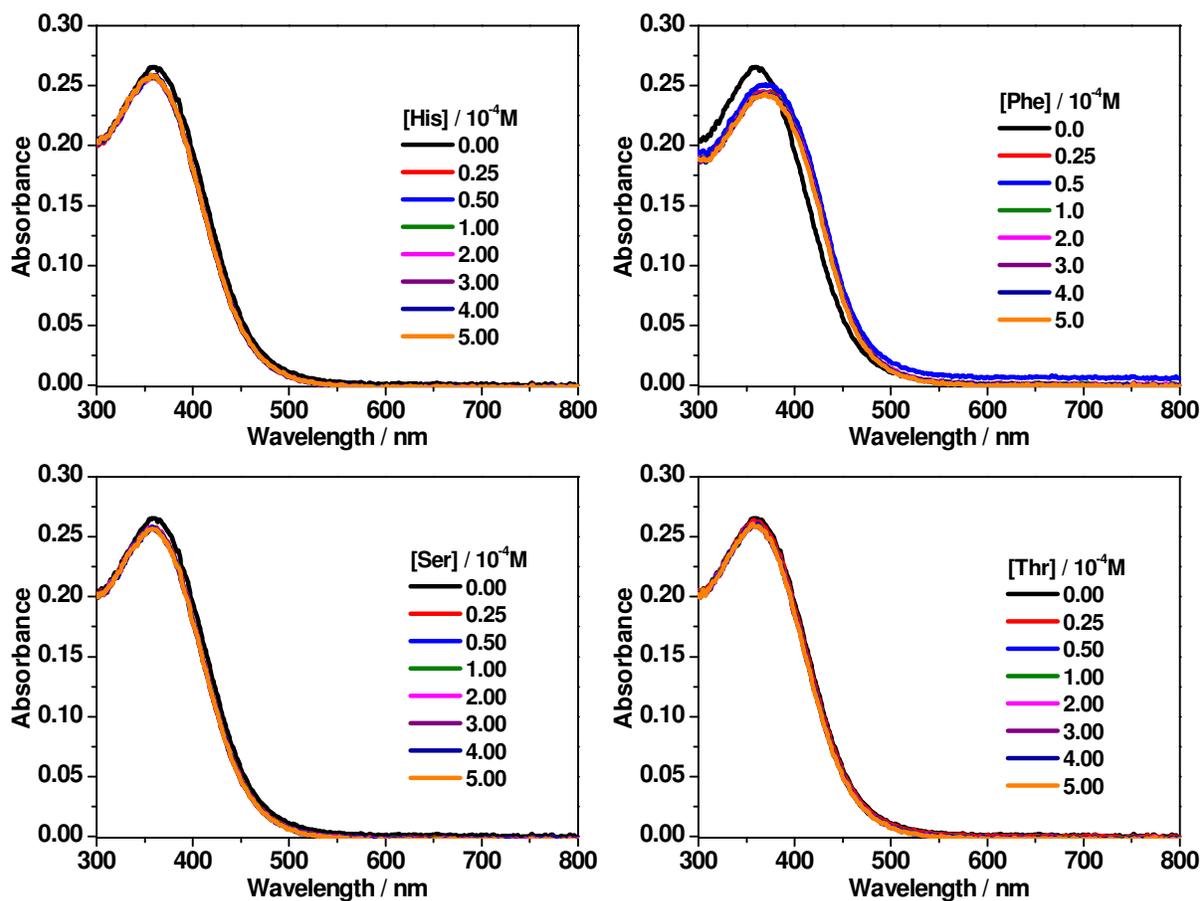
With a view to understanding the formation mechanism of PMTEA/folic acid aggregates, the stoichiometry of the aggregate formation was evaluated by means of continuous-variation plots from absorption studies (Figure S12), in which the total concentrations of PMTEA and folic acid were kept

constant at 0.2 mM. It was observed that the absorption intensity at 533 nm increases gradually with the increase in the molar fraction of PMTEA (repeating unit) and attains the maximal value at  $X_{\text{PMTEA}} = M_{\text{PMTEA}}/(M_{\text{PMTEA}} + M_{\text{Folic acid}}) = 0.85$ . This value corresponds to the molar ratio of 6/1 for PMTEA/folic acid, deviating from that simply expected from the complementary electrostatic interaction and giving the net positive charges to the aggregates. This finding allows us to conclude that stable homogeneous dispersion in water is maintained by these excess cationic charges. On the basis of the absorption spectroscopic results and the stoichiometry for the complex, one can draw a conclusion that not only do electrostatic interactions play an important role in the formation of complexes, but also some other secondary forces such as hydrophobic interactions, aromatic stacking, and hydrogen bonding are responsible for promoting the formation of the PMTEA/ folic acid supramolecular complex. Titration results have confirmed that electrostatic interaction between folate and PMTEA promotes planarization of the PT backbone upon addition of increasing amounts of folic acid, and above a critical concentration efficient  $\pi$ - $\pi$  stacking interaction between PT backbones is induced by the synergistic effect that arises from the hydrophobic interaction between pterin rings.

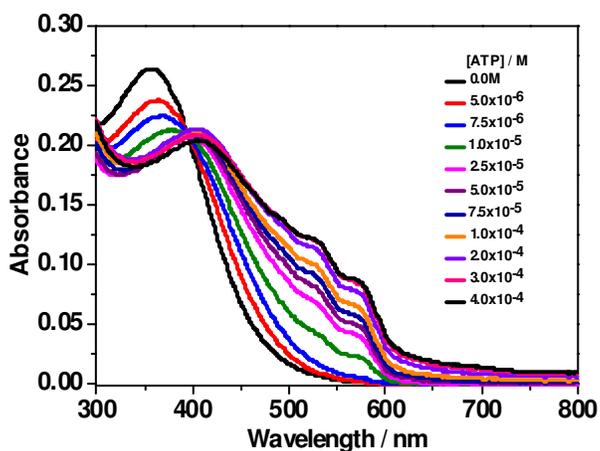


**Figure S12.** Job plots for the formation of PMTEA/FA complexes in water.  $[\text{PMTEA}] + [\text{folic acid}] = 0.2 \text{ mM}$ ;  $X_{\text{PMTEA}} = [\text{PMTEA}]/([\text{PMTEA}] + [\text{folic acid}])$ .

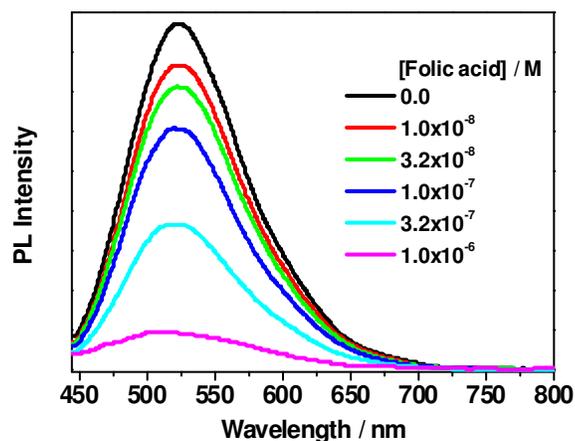




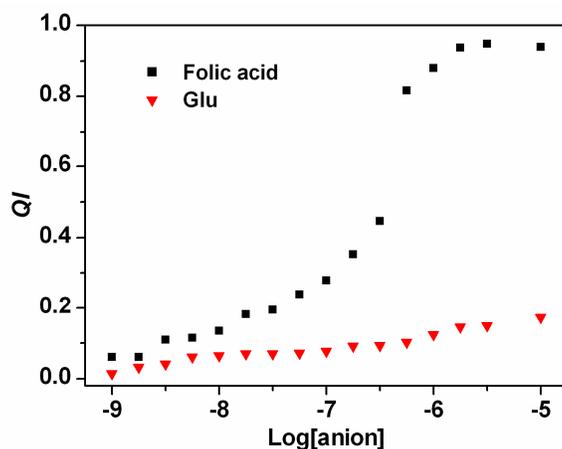
**Figure S13.** Titration of PMTEA ( $1.0 \times 10^{-4}$  M) with different anions in water.



**Figure S14.** Absorption spectra of PMTEA ( $1.0 \times 10^{-4}$  M) in the presence of different amounts of ATP in water.



**Figure S15.** Emission spectra of PMTEA ( $1.0 \times 10^{-5}$  M) in 10 mM HEPES buffer (pH = 7.4) in the presence of increasing concentrations of folic acid as indicated. ( $\lambda_{\text{ex}} = 415$  nm).



**Figure S16.** Fluorescence quenching of PMTEA ( $1.0 \times 10^{-5}$  M) by folic acid and Glu at various concentrations in water (pH = 9.0). The fluorescence quenching  $QI = [(I_0 - I)/I_0] \times 100\%$ ;  $I_0$  is the fluorescence intensity at 524nm of a solution of PMTEA ( $1.0 \times 10^{-5}$  M);  $I$  is the fluorescence intensity at 524nm of a solution of PMTEA ( $1.0 \times 10^{-5}$  M) in the presence of different amounts of analytes folic acid and Glu. Excited wavelength  $\lambda_{\text{ex}} = 415$  nm.