

Supporting Information

Reversible logic gate modulated by nucleases based on cationic conjugated polymer/DNA assembly

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Materials. All Chemicals were of analytical grade and were used as received. All aqueous solutions were prepared with ultrapure water purified using a Millipore filtration. All DNA oligonucleotides and T4 DNA Ligase were ordered from Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. (China). Endonuclease PvuII and HaeIII in this study were custom-made by TAKARA Biotechnology CO. Ltd., Dalian, China. UV-vis absorption spectra were taken on a UV-2450 (Shimadzu Company) spectrophotometer. Fluorescence measurements were recorded in 3 mL quartz cuvettes using a Hitachi F-7000 spectrofluorometer equipped with a xenon lamp excitation source. All fluorescence spectra were measured at an excitation wavelength of 380 nm. The double stranded DNA (dsDNA) was obtained by annealing the mixtures of complementary strands in a buffer solution (10 mM tris-HCl, 1 mM EDTA, 50 mM NaCl, pH = 8.0) at 85 °C for 20 min and then slowly cooled to room temperature.

Assay of DNA cleavage by nucleases: The nonrestriction endonucleases (200 U for HaeIII, 200 U for PvuII) were added to a solution of total volume 100 μ L containing dsDNA (20 μ L, [dsDNA] = 1×10^{-5} M). After incubation at 37 °C for 2 h, 50 μ L of the solution was diluted with N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES) buffer (2 mL, 50 mM, pH = 7.4). After adding the PFPB ([PFPB] = 2×10^{-6} M), the fluorescence spectra were measured at 37 °C at an excitation wavelength of 380 nm.

Assay of DNA ligation by T4 DNA Ligase: HaeIII or PvuII in the left 50 μ L cleavage solution was inactivated by incubating the solution at 85 °C for 20 min, and then slowly cooled to room temperature. T4 DNA Ligase (100U) was added then the solution was incubated at 4 °C for 12 h. 50 μ L of the solution was transferred to 3 mL quartz cuvette and diluted with HEPES buffer (2 mL, 50 mM, pH = 7.4). After adding the PFPB ([PFPB] = 2×10^{-6} M), the fluorescence spectra were measured at the excitation wavelength of 380 nm.

Synthesis of PFPB

The compounds **1** and **2** were prepared according to the procedures in literatures.¹ The ¹H NMR and ¹³C NMR spectra were recorded on Bruker AV 300 NMR or Bruker AV 400 NMR spectrometer. Gel permeation chromatography (GPC) was performed using

DMF as the eluent. The GPC instrument was equipped with a Waters 717 plus autosampler, a Waters 1515 HPLC pump, three μ -Styragel columns, and a Waters 2414 refractive index (RI) detector. The columns were calibrated using polystyrene standards.

Compound 3: A solution of 20 mL acetone contains compound 2 (0.508 g, 1.0 mmol polymer2) was added dropwise to a flask containing a solution of a mixture of 1,3 - dibromo-propane (0.6 mL, 6.0 mmol) and potassium carbonate (2.76 g, 20 mmol) in 40 mL acetone. The resulting reaction mixture was refluxed at 70 °C for two days. After cooling to room temperature, the solvent was removed under reduced pressure. The residue was dissolved in chloroform and washed with water. Then the organic layer was washed successively with 10% potassium hydroxide solution, water and saturated sodium chloride solution for three times. The organic layer was dried over anhydrous MgSO_4 , filtered, and distilled the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography using petroleum ether/ dichloromethane (5:1) as eluent to give the product afford compound 3 as white solid (0.340 g, 45%). ^1H NMR (300 MHz, CDCl_3): δ =7.93 (d, J = 8.0 Hz, 2 H), 7.62 (d, J = 8.0 Hz, 2 H), 7.53 (s, 2 H), 7.02 (m, 4 H), 6.88 (m, 4 H), 4.03 (m, 4 H), 3.63 (m, 4 H), 2.19 (m, 4 H). ^{13}C NMR (100 MHz, CDCl_3) δ 157.8, 153.6, 137.9, 136.8, 133.3, 130.9, 129.3, 129.1, 121.6, 114.5, 65.3, 64.4, 32.4, 30.1. MS (ESI): m/z 772.8

([M+Na]⁺), 788.8 ([M+K]⁺).

Polymer 4: The solution of compound 3 (188 mg, 0.25 mmol) and 1,4-benzenediboronic acid bis(pinacol) ester (83 mg, 0.25 mmol) in 4 mL THF was degassed under nitrogen for 30 min. Then 2.0 mL potassium carbonate solution (2 M) and PdCl₂ (dppf) (20 mg) were added into the solution. The reaction mixture was heated to 80 °C and refluxed for 2 days. After cooling to room temperature, THF was removed under reduced pressure. The residue was dissolved in chloroform and washed with water twice. The organic phase was concentrated under reduced pressure. The residue was added dropwise to methanol solution and the precipitate was formed and then centrifuged. The residue was redissolved in CHCl₃ and precipitated in methanol. The compound 4 was obtained as light brown solid product after drying under vacuum. (138 mg, 82%). ¹H NMR (300 MHz, CDCl₃): δ=7.83-7.77 (m, 2H), 7.62-7.50 (m, 4H), 7.20-7.12 (m, 4H), 6.77 (m, 4H), 4.04 (m, 4 H), 3.57 (m, 4 H), 2.27 (m, 4 H). GPC: *M*_n = 7800 g mol⁻¹, PDI=2.88

Polymer PFPB (5): To a solution of polymer 4 (67 mg, 0.1 mmol) in CHCl₂ (5 mL) excess trimethylamine in methanol solution (3.2 M, 3 mL) was added. After stirring the reaction mixture for 5 h at room temperature, the precipitate was isolated, washed with CH₂Cl₂ and dried under vacuum to give compound 5 as brown solid (60 mg, 51%). ¹H NMR (400 MHz, DMSO) δ 8.07 (br), 7.71 (br), 7.19 (br), 6.88 (br), 4.00 (br), 3.18 (s), 2.67 (br), 2.33 (br). GPC: *M*_n = 14000 g mol⁻¹, PDI=1.08.

Logic Operation:

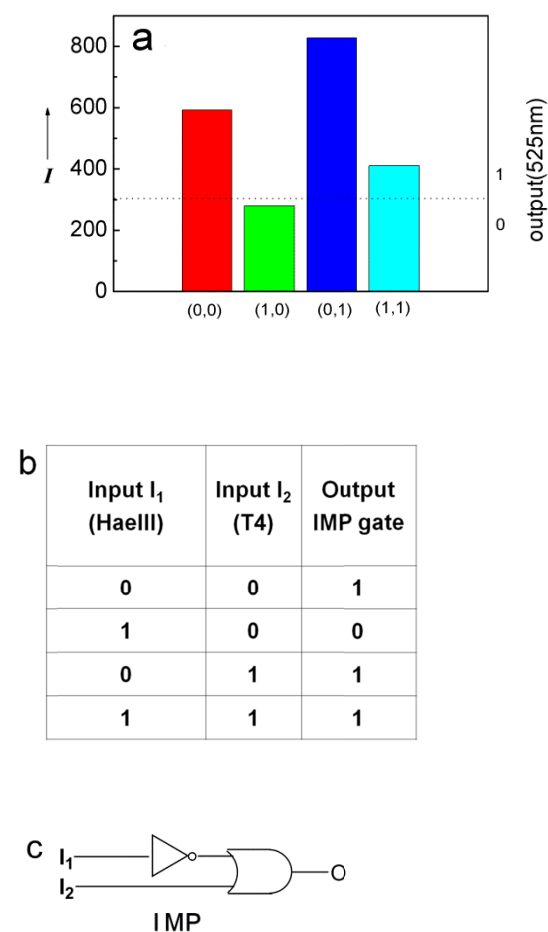


Fig. S1: Emission intensity of the output of the IMP logic gate at 525 nm from the input combinations (a), truth table (b) and logic scheme (c) for IMP logic gate. [PFPB] = 2.0×10^{-6} M in RUs, [dsDNA₁] = 5.0×10^{-8} M, [Hae III] = [T4 ligase] = 0.05 U/ μ L. The excitation wavelength is 380 nm.

Reference:

1. H. D. Burrows, V. M. M. Lobo, J. Pina, M. L. Ramos, J. S. de Melo, A. J. M. Valente, M. J. Tapia, S. Pradhan and U. Scherf, *Macromolecules*, 2004, **37**, 7425-7427.