Supplementary Information (SI) for Materials Chemistry Frontiers.
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Supporting Information

Supramolecular Enantioselective Transfer Amino Acid @pillar[5]arene Bio-interface for Effective Tumor Cells Suppression

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1.Experimemtal

1.1 Chemical reagent

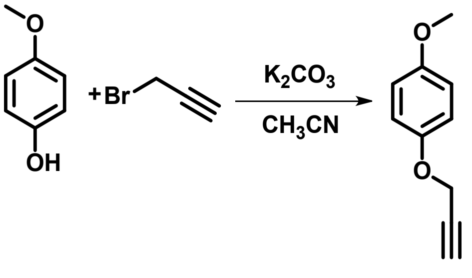
All chemicals were purchased from local sources in A.R. grade and were used without further purification. Dulbecco's modified Eagle medium (DMEM) was purchased from Irvine Scientific and was supplemented with 3700 mg/L sodium bicarbonate, 1% (v/v) penicillin, streptomycin, and amphotericin B (Sigma-Aldrich). Fetal bovine serum (FBS) was obtained from Hyclone. Phosphate-buffered saline (PBS; 8 mM phosphate, 150 mM NaCl, pH = 7.4) was sterilized by autoclaving at 120°C for 0.5 h. Deionized water (18.3 MΩ-cm) was obtained using a Nanopure Infinity water purification system (Barnstead). All other chemicals were of the highest quality available and were used as received.

1.2 Analysis apparatus

^{1}H NMR and ^{13}C NMR were recorded on Varian Mercury VX600 instrument at ambient temperature with TMS as the internal standard. MALDI-TOF mass spectra were recorded with an Autoflex time-of-flight instrument (Bruker, Germany) with 2,5-dihydroxybenzoic acid (DHB) as the matrix. Transmission electron microscopy (TEM) investigations were carried out on a JEM-1200EX instrument. XPS was recorded on a KRATOS XSAM800 Electron spectrometer (FRR mode). The contact angle was measured at 25 °C by means of an OCA 20 contact angle system (Dataphysics, Germany). Mass spectrometer, UltrafleXtreme, Bruker Daltonics Inc, Germany. UV-Visible spectra were measured in PHI Quantera SXM UV-Visible spectrometer. Fluorescent images of cells were recorded using Olympus inverted fluorescent microscope. Flow cytometry was performed with Gallios flow cytometer of Beckman kurt USA.

2. Preparation of MP5.

2.1 Synthesis of compound 1.



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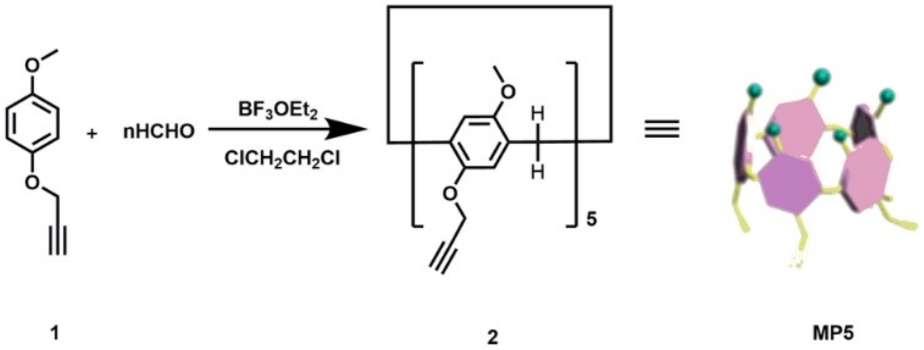
Scheme S1. Synthesis route of compound 1.

As shown in Scheme S1, p-methylaminophenol (7.44g, 60 mmol), 120 mL anhydrous acetonitrile, and K2CO3 (11.04 g, 80 mmol) were dissolved in a 250 mL three-neck flask. The mixture was heated and refluxed for 30 min, then propargyl bromide (4.7ml, 60 mmol) was added, heated at 90 ℃, and refluxed for 10 h. After the reaction was completed, acetonitrile was removed by rotary evaporation, and the product was washed three times with saturated salt water, followed by extraction with chloroform three times. At last, the organic phase was washed with 10% NaOH and water three times, dried with anhydrous sodium sulfate, filtered, and then evaporated using a vacuum rotary evaporator (8.4 g, 87% yield). 1H NMR (400 MHz, CDCl3) δ (ppm): 6.94-6.83 (m, 4H,), 4.65 (s, 2H,), 3.77 (s, 3H,), 2.51 (t, J = 6.0 Hz, 1H,). Anal. calcd for C10H10O2: C, 74.06%; H, 6.21%; found: C, 74.00%; H, 6.28%.

2.2 Synthesis of MP5 (compound 2).



Scheme S2. Synthesis route of compound 2.

As shown in Scheme S2, to the stirring solution of 1-methoxy-4-(prop-2-ynyloxy)benzene (1.7g, 11 mmol) and paraformaldehyde (0.63g, 21mmol) in 1,2-dichloroethane (50 mL) under nitrogen, boron trifluoride etherate (1.31 mL, 11 mmol) was added slowly, the reaction mixture was stirred at room temperature for about 30min, after confirming the completion of the reaction with TLC (PE: DCM = 1: 1), 20mL of methanol was added to stop the reaction, then solvent was removed under reduced pressure further diluted with dichloromethane (40mL), then washed with water thrice, then organic phase was dried over anhydrous sodium sulfate then solvent was removed under reduced pressure to attain the crude product, further it was purified by column chromatography using petroleum ether and dichloromethane (PE:DCM=1:1) as eluant, to obtain the pure propargyl pillar[5]arene (MP5) as white solid (1.01 g, yield 62%). ^{1}H NMR (600 MHz, DMSO-D$_6$) δ 6.87 (s, 10H, Ar-H), 4.78 (s, 10H, OCH$_2$), 4.17(s, 15H, OCH$_3$), 3.75 (m, 2H, BrCH$_2$), 2.52(s, 5H, C≡CH), ^{13}C NMR (150 MHz, CDCl$_3$) δ 147.3, 144.4, 123.6, 123.1, 110.7, 108.8, 75.3, 69.3, 52.5, 51.0 ppm.

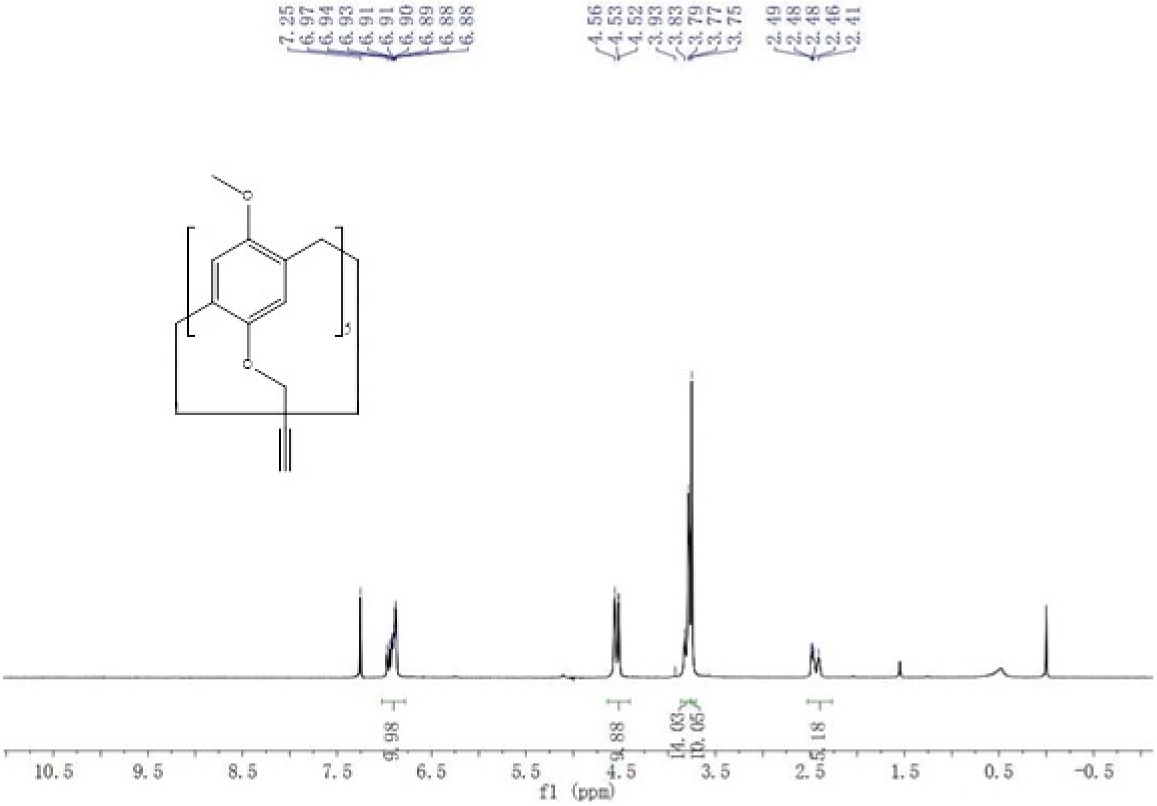


Figure S1. ^{1}H NMR ($CDCl_3$, 600M Hz, 298K) of MP5.

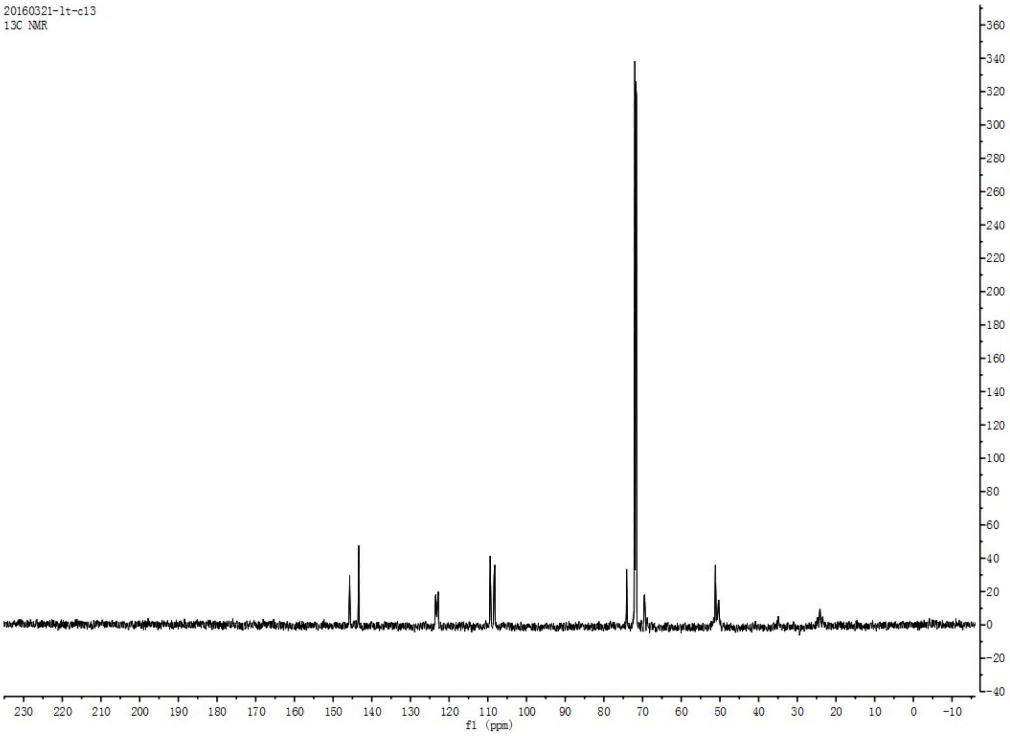


Figure S2. ^{13}C NMR ($CDCl_3$, 600 MHz, 298K) of MP5.

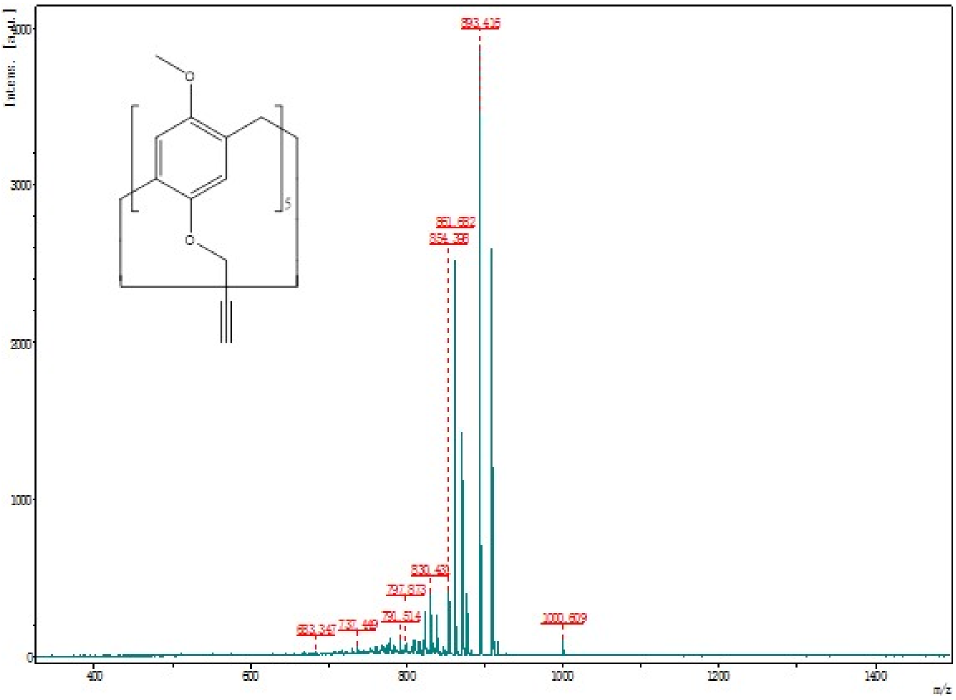
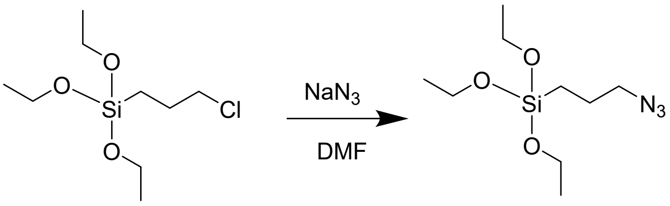


Figure S3. Mass spectrum of MP5.

3. Preparation of MP5-SAM.

3.1 Synthesis of azide silane SAM.

Sodium azide (5 g, mmol) was cautiously added to 60 mL DMF and 5 mL chlorosilane, and the mixture was stirred at 80 °C for 5 hours under N_2 atmosphere. Distillation at 60 °C was then performed to further remove low-boiling impurities. The reaction mixture was diluted with diethyl ether, resulting in the precipitation of sodium azide, which was removed by filtration. The filtrate was subsequently subjected to low-pressure distillation to remove the solvent, yielding azide silane as a pale yellow liquid product.[2]



3 **4**

Scheme S3: synthesis of azide silane (**4**).

3.2 Synthesis of MP5-SAM.

The alkyl azide–functionalized silicone interface (Si–N_3), prepared according to the procedure described in the ESI, was immersed in a solution of MP5 (871 mg, 1×10^{-3} mol) in 10 mL of dimethyl sulfoxide (DMSO). Subsequently, $CuSO_4 \cdot 5H_2O$ (15 mg, 6×10^{-5} mol) and sodium ascorbate (140 mg, 8×10^{-4} mol) were added, and the mixture was heated to 80 °C for 8 h. The resulting silica interface was then washed successively with DMSO and acetonitrile, followed by drying under nitrogen to afford the pillar[5]arene-functionalized silicone interface (MP5-SAM). The obtained MP5-SAM was further characterized by XPS and contact angle (CA) measurements.[18]

3.3 Synthesis of [MP5+D/L amino acid]-SAM.

The MP5-SAM surfaces were individually incubated with 10 mM aqueous solutions of D/L-Arg, D/L-Lys, and D/L-His at 25 °C overnight, followed by drying under nitrogen to obtain [MP5+D/L-Arg]-SAMs, [MP5+D/L-Lys]-SAMs, and [MP5+D/L-His]-SAMs, respectively.

The resulting MP5-SAMs were then dried under a flow of N_2 and finally washed three times with water.[19]

4. Cell culture.

HeLa cells were purchased from the Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, and cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific) supplemented with 10% (v/v) fetal bovine serum (FBS, Thermo Fisher Scientific), 100 μg mL^{-1} streptomycin, and 100 U mL^{-1} penicillin (Thermo Fisher Scientific) at 37 °C in a humidified atmosphere containing 5% CO_2. HeLa cells in the logarithmic growth phase were harvested, and the cell concentration was adjusted to 1×10^6 cells mL^{-1}. Subsequently, 0.1 mL of the cell suspension was seeded into 6-well culture plates containing the modified glass coverslips, on which the cells were allowed to grow.

5. Cell viability of [MP5+D/L amino acid]-SAM

The cytotoxicity of the [MP5+D/L amino acid] complexes toward HeLa cells was evaluated using the MTT assay. HeLa cells were seeded in 96-well plates at a density of 5×10^3 cells per 100 μL of Dulbecco's modified Eagle's medium (DMEM, Invitrogen, USA) supplemented with 10% FBS and incubated for 24 h. The culture medium was then replaced with fresh medium containing the [MP5+D/L amino acid] complexes at varying concentrations (20–200 μg mL^{-1}), followed by incubation for an additional 24 h. Subsequently, 20 μL of MTT solution was added to each well, and the cells were further incubated for 4 h at 37 °C. The medium was then removed, and the formazan crystals were dissolved in 150 μL of dimethyl sulfoxide (DMSO). The absorbance was measured at 490 nm using a microplate reader (Bio-Rad 680, USA).[20]

6. Cell adhesion assays of [MP5+D/L amino acid]-SAM.

Cell adhesion assays were done: MP5-SAM, [MP5+D-Arg]-SAM, [MP5+L-Arg]-SAM, [MP5+D-Lys]-SAM, [MP5+L-Lys]-SAM, and [MP5+D/L-His]-SAM surfaces were sterilized by exposure to UV light for 30 min. Each functionalized silica surface was then placed separately in a 6-well cell culture plate. Subsequently, 1 mL of cancer cell culture medium containing approximately 5×10^4 cells mL^{-1} was added to each well, followed by incubation at 37 °C for 12 h. The cells adhered to the functionalized silicon wafers were fixed with 4% formaldehyde solution and gently washed with phosphate-buffered saline (PBS). Finally, the cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) and immediately observed using an inverted fluorescence microscope.[21] The cells adhered to the functionalized silicon wafers also treated with pancreatin, and were counted by Flow Cytometer.

Apoptotic cell death was determined using a dead cell apoptosis kit (Invitrogen, Carlsbad, CA) with FITC Annexin V and PI for flow cytometry according to the manufacturer's instructions. Cells were harvested after being treated with MP5-SAMs, [MP5+L-AAs]SAMs and [MP5+d-AAs]SAMs for 48 h and washed with cold PBS twice. After washing, cells were resuspended in 1 × Annexin V binding buffer. Then, 5 μL of FITC Annexin V and 1 μL of 100 μg mL^{-1} PI working solution were added to each 100 μL of cell suspension and incubated at room temperature for 15 min. After the incubation period, an additional 500 μL of 1 × Annexin binding buffer was added. Samples were then kept on ice until flow cytometry analysis.

7. NMR studies of host-guest interaction between MP5 and five AAs.

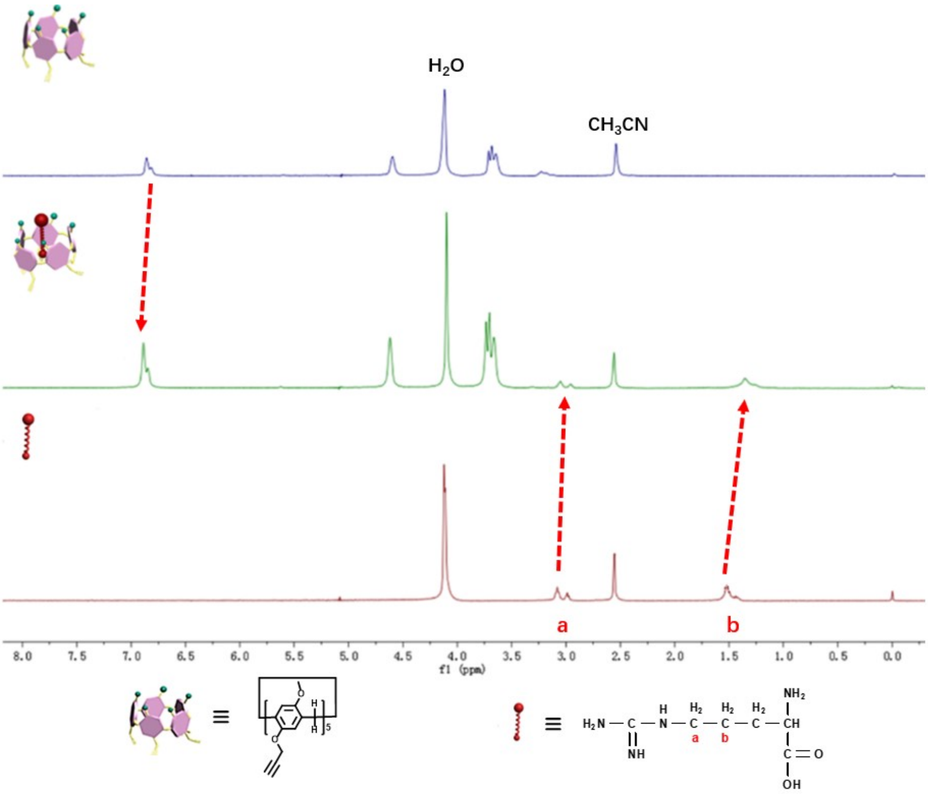


Figure S4: ^{1}H NMR spectra (CD_3CN/D_2O=1:1, 600 MHz, 298 K) of **L-Arg** (red curve); **MP5** and **L-Arg** (green curve); **MP5** (bule curve). Ha, Hb protons of **Arg** exhibited a substantial upfield shift of $\Delta\delta a$=0.07 ppm, δb= 0.13 ppm respectively. Concomitantly, the proton of benzene groups in **MP5** was also downshifted by $\Delta\delta$=0.03 ppm. These chemical shift effects suggested the weak host-guest interaction between **Arg** and **MP5**, caused by the π-π interaction between the cavity of pillar[5]arene and the imidazole group of **Arg**, which was in accordance with previous literature.

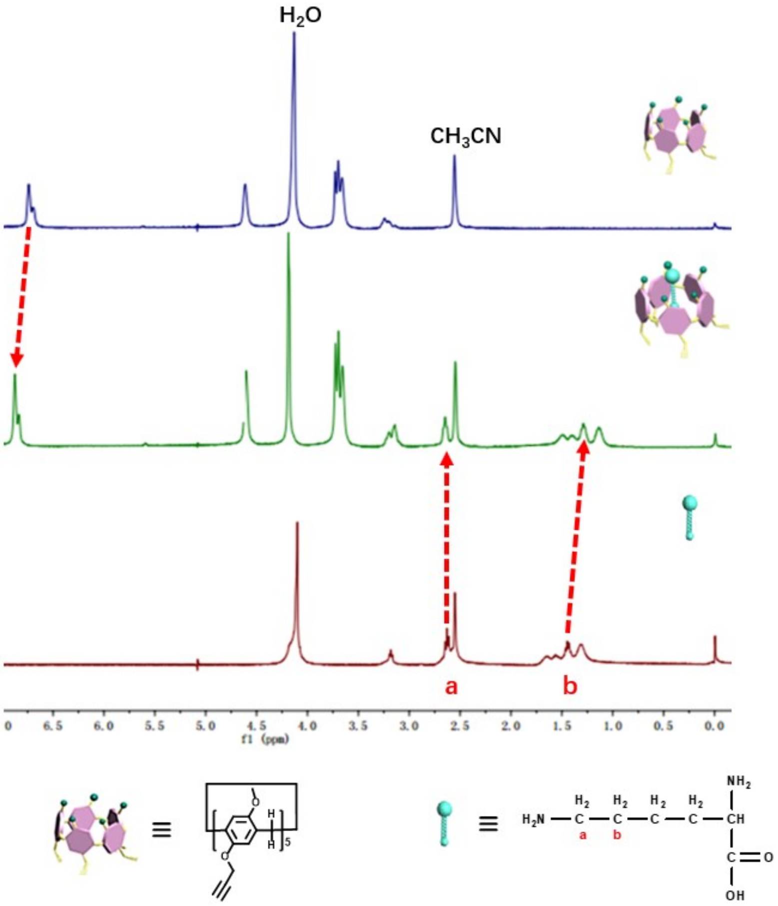


Figure S5: ^{1}H NMR spectra (CD_3CN/D_2O=1:1, 600 MHz, 298 K) of **L-Lys** (red curve); **MP5** and **L-Lys** (green curve); **MP5** (bule curve). Ha, Hb protons of **Lys** exhibited a substantial upfield shift of $\Delta\delta a$=0.07 ppm, δb= 0.1 ppm respectively. Concomitantly, the proton of benzene groups in **MP5** was also downshifted by $\Delta\delta$=0.03 ppm. These chemical shift effects suggested the weak host-guest interaction between **Lys** and **MP5**, caused by the π-π interaction between the cavity of pillar[5]arene and the imidazole group of **Lys**, which was in accordance with previous literature.

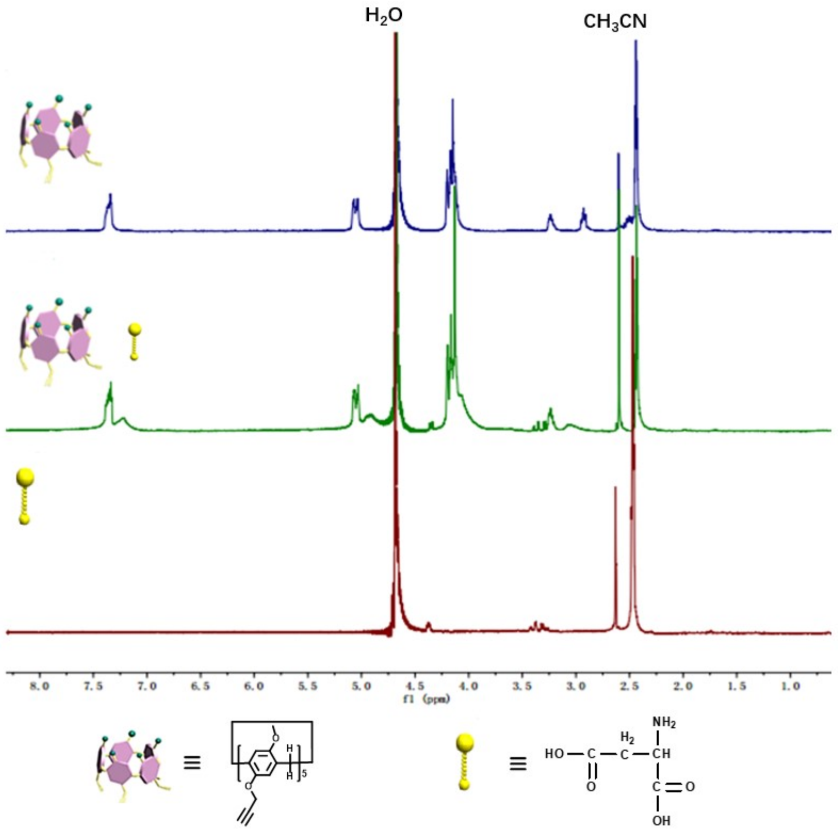


Figure S6: ^{1}H NMR spectra (CD_3CN/D_2O=1:1, 600 MHz, 298 K) of **L-Asp** (red curve); **MP5** and **L-Asp** (green curve); **MP5** (bule curve). No chemical shift was observed in ^{1}H NMR spectra, indicating that there was no host-guest interaction between **MP5** and **L-Asp.**

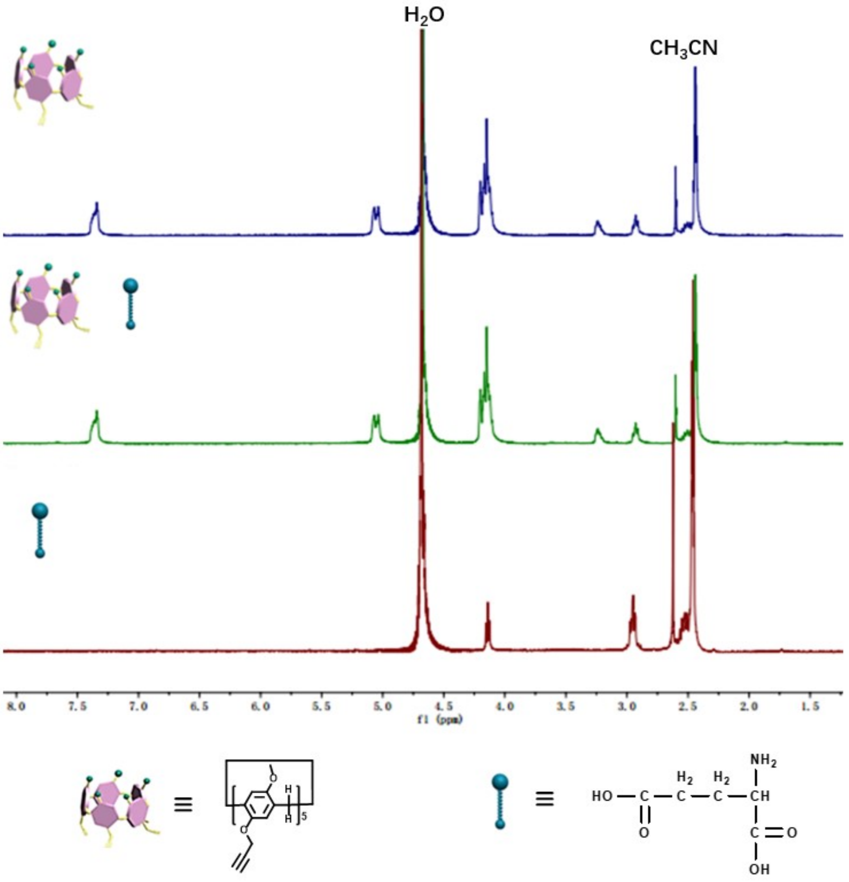


Figure S7: ^{1}H NMR spectra (CD_3CN/D_2O=1:1, 600 MHz, 298 K) of **L-Glu** (red curve); **MP5** and **L-Glu** (green curve); **MP5** (bule curve). No chemical shift was observed in ^{1}H NMR spectra, indicating that there was no host-guest interaction between **MP5** and **L-Glu.**

8. UV-Visible spectroscopy.

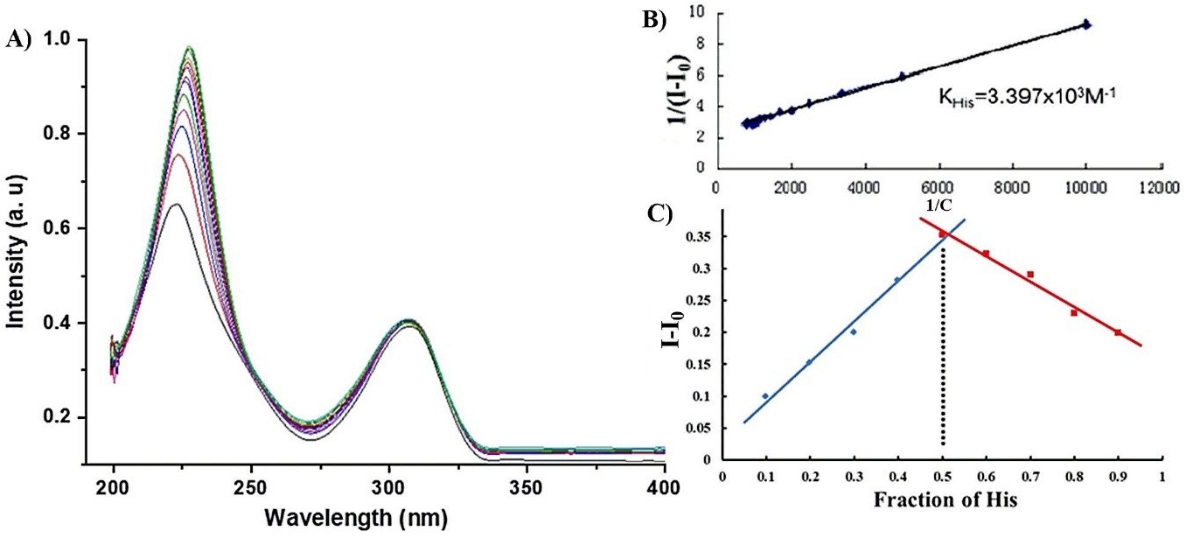


Figure S8. (A) UV spectra of **MP5** at a concentration of 1 × 10^{-4} M (2mL) upon addition of different concentrations of **L-His** (0-1.2 equivalent, dissolved in CH_3CN/H_2O at 1:1 ratio). (B) Fit plot for the absorbance upon addition of **L-His** against **MP5**. (C) Job plot analysis of **MP5+L-His**, proving **MP5** and **L-His** form a 1:1 complex.

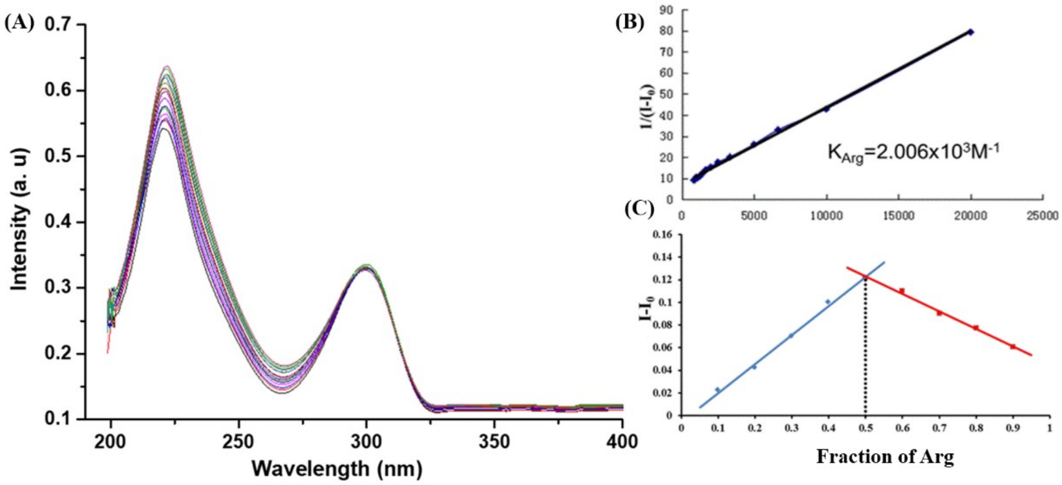


Figure S9: (A) UV spectra of **MP5** at a concentration of 1 × 10^{-4} M (2mL) upon addition of different concentrations of **L-Arg** (0-1.2 equivalent, dissolved in CH_3CN/H_2O at 1:1 ratio). (B) Fit plot for the absorbance upon addition of **L-Arg** against **MP5**. (C) Job plot analysis of **MP5+L-Arg**, proving **MP5** and L-Arg form 1: 1 a complex.

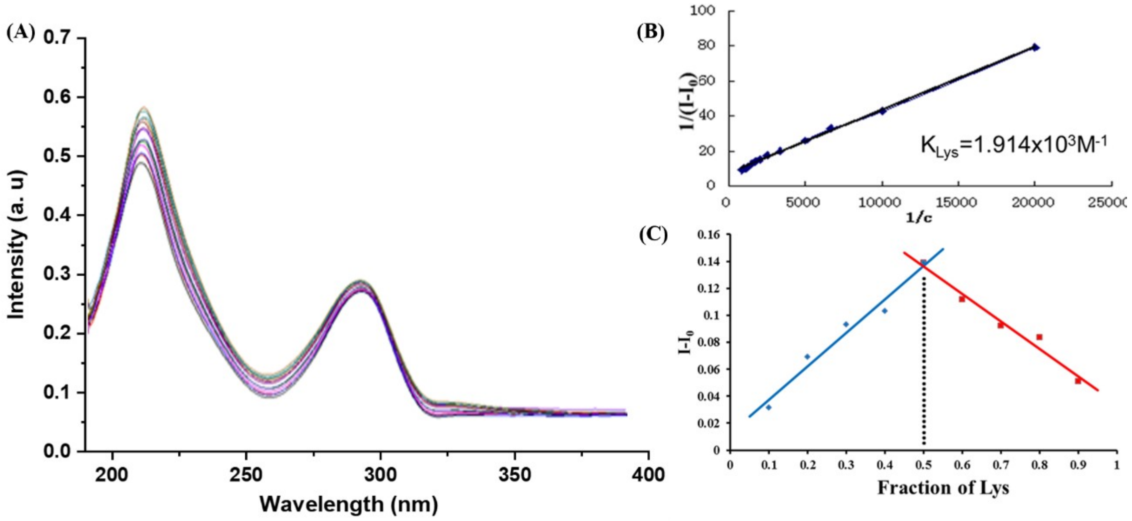


Figure S10. (A) UV spectra of **MP5** at a concentration of 1 × 10^{-4} M (2mL) upon addition of different concentrations of **L-Lys** (0-1.2 equivalent, dissolved in CH_3CN/H_2O at 1:1 ratio). (B) Fit plot for the absorbance upon addition of **L-Lys** against **MP5**. (C) Job plot analysis of **MP5+L-Lys**, proves **MP5** and **L-Lys** form a 1: 1 complex.

9. Gaussian simulation studies of MP5 and three AAs complexes.

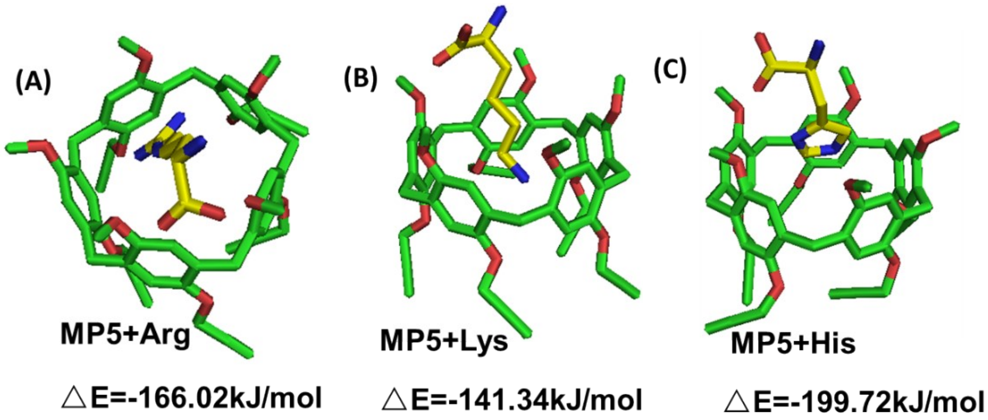


Figure S11. Molecular simulation for the complex of MP5 with Arg, Lys, and His, optimized at the HF/6–31G(d) level, respectively. This result shows that MP5 can strongly bind His through host-guest interaction.

Table S1: Binding constant and bonding energy of MP5-amino acids.

Guest	Complex-ratio	Binding constant (L/mol)	Bonding energy ΔE (kJ/mol)
L-Arg	1	2.006×10^3	-166.02
L-Lys	1	1.914×10^3	-141.34
L-His	1	3.397×10^3	-199.72

10. Fabrication of the Si surface.

Silicon wafers with dimensions of 1 cm × 1 cm were processed using a combination of photolithography and inductively coupled plasma (ICP) deep etching to produce patterned silicon micropillar structures. The resulting surfaces contained ordered square pillars with a height of 20 μm, a width of 4 μm, and a spacing of 6 μm between them.[2]

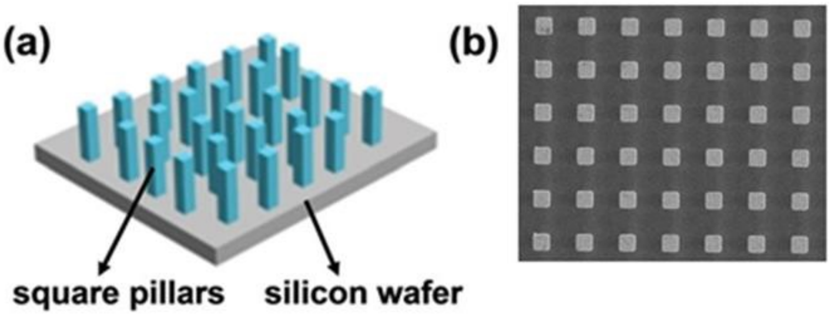


Figure S12. (a) The patterned silicon micropillar structure on a silicon wafer; (b) The SEM image of the silicon micropillar structure on a silicon wafer.

11. Introduction of the azide group over the silica surface.

The silicon wafers were immersed in chromic acid solution for 30 min to remove organic impurities from the surface. After washing several times with deionized water and drying under nitrogen, the wafers were sequentially soaked in 0.1 M NaOH solution for 10 min and 0.1 M nitric acid solution for 15 min, rendering the wafer surface rich in hydroxyl groups. The wafers were then incubated in a 5% (w/v) solution of azide silane in toluene at 80 °C for approximately 6 h to obtain azide-functionalized silicon surfaces (Si–N_3).

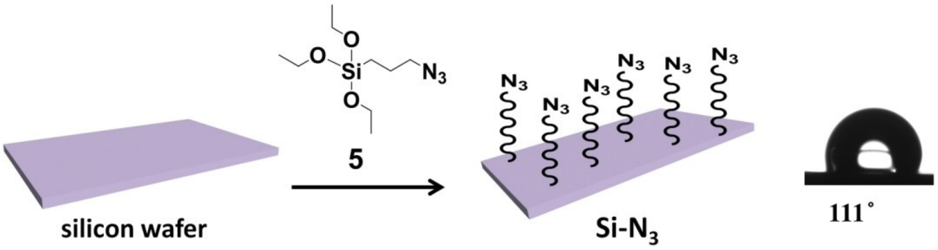
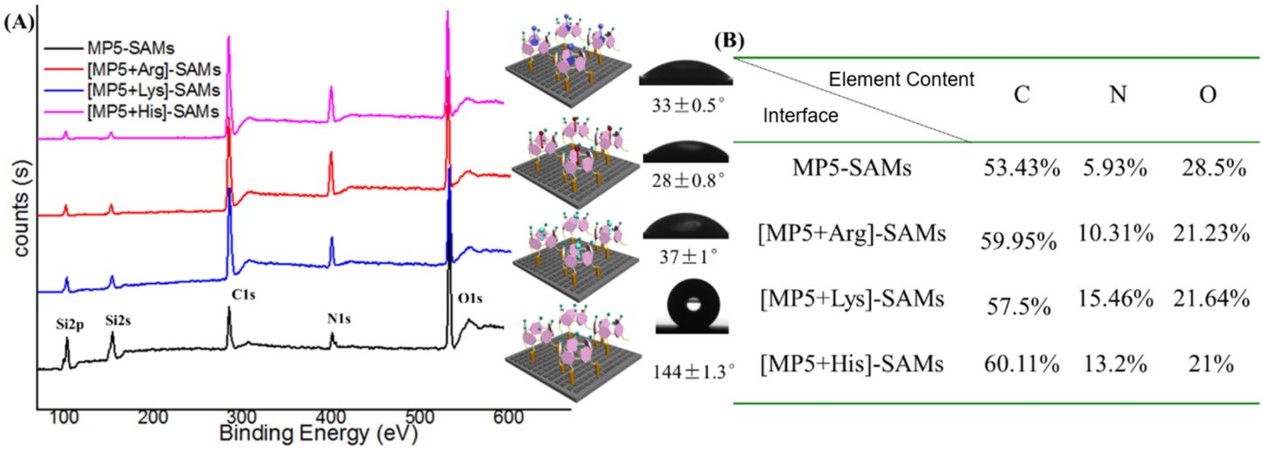


Figure S13. Construction of the azide-functionalized silica surface.

12. XPS analysis.



Element Content Interface	C	N	O
MP5-SAMs	53.43%	5.93%	28.5%
[MP5+Arg]-SAMs	59.95%	10.31%	21.23%
[MP5+Lys]-SAMs	57.5%	15.46%	21.64%
[MP5+His]-SAMs	60.11%	13.2%	21%

Figure S14: A) X-ray photoelectron spectra of **MP5-SAM** surface and **MP5-SAM+**Aminoacids (**L-Arg**, **L-Lys**, and **L-His**) surface; B) elementary analysis of the AAs functionalized surface.

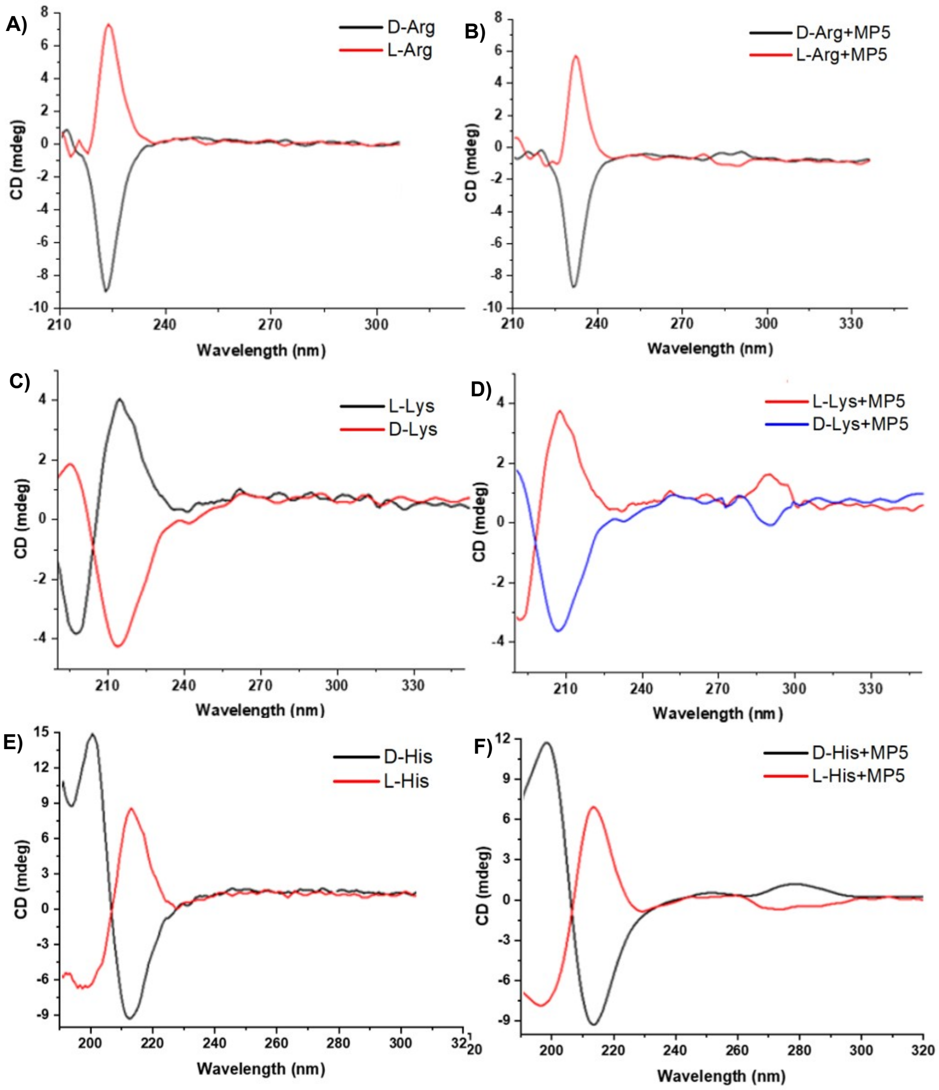


Figure S15. Circular dichroism spectra were recorded with A) D/L-Arg solution; B) [MP5+D/L-Arg]-SAM interface; C) D/L-Lys solution; D) [MP5+D/L-Lys]-SAM interface; E) D/L-His solution; D) [MP5+D/L-His]-SAM interface, respectively. These also indicate that MP5-SAM shows a high chiral performance.

13. Cell culture studies.

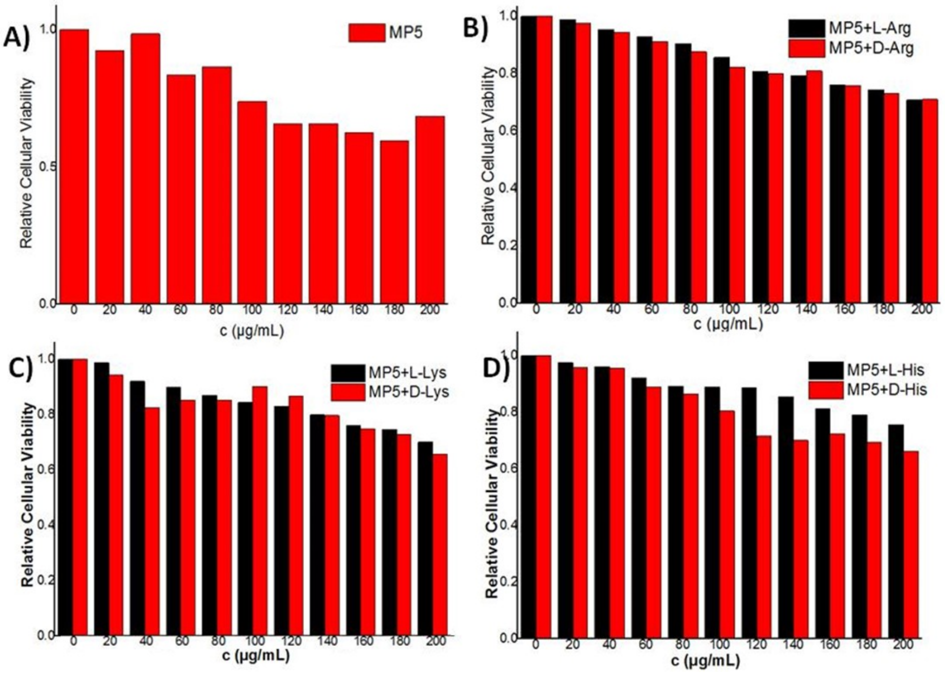


Figure S16 Cell viability of MP5-amino acids A) MP5, B) MP5-D/L Arg, C) MP5-D/L Lys and D) MP5-D/L His using MTT assay.

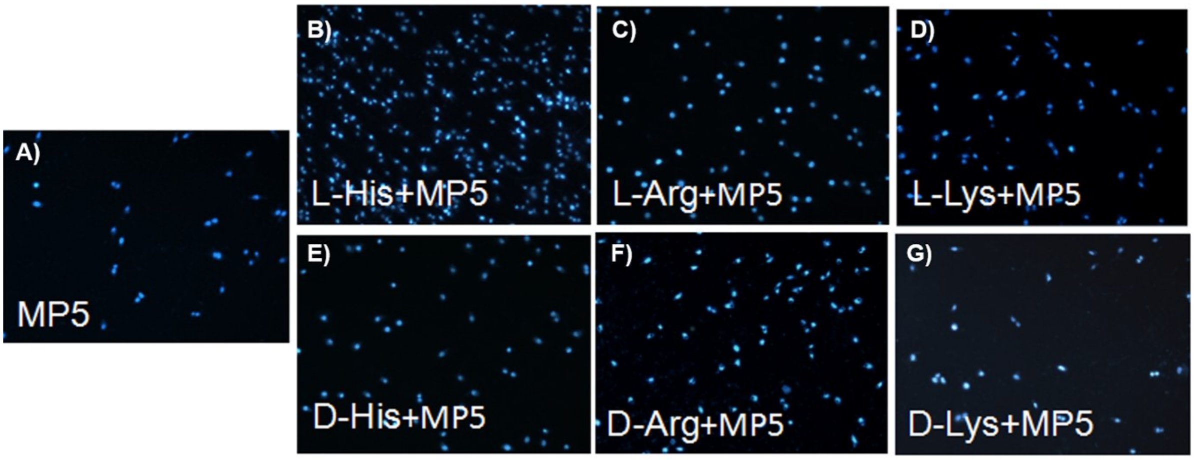
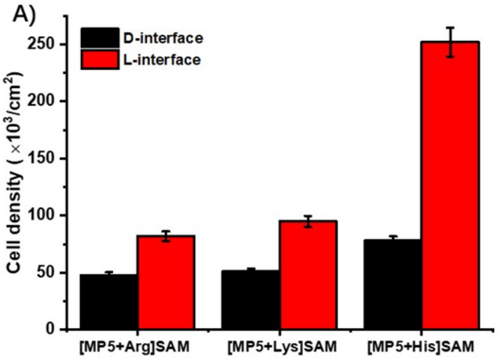


Figure. S17. LCMS images of HeLa cells adhered over A) **MP5-SAM, B**) [**MP5+L-His**] **SAM**, C) [**MP5+L-Arg**] **SAM,** D) [**MP5+L-Lys**] **SAM**, E) [**MP5+D-His**] **SAM, F**) [**MP5+D-Arg**] **SAM** and G) **[MP5+D-Lys] SAM** substrate, which was stained with DAPI.



B)

Bio-interface	Chiral discrimination ratio K_L/K_D
[MP5+Arg]-SAMs	1.87
[MP5+Lys]-SAMs	1.85
[MP5+His]-SAMs	2.91

Figure. S18. A) Relative cell density of representative images on the chiral MP5 surface confirmd by the cell-counting method, B) The table of related chiral discrimination ratios for the representative bio-interface.

14. Flow cytometry analysis.

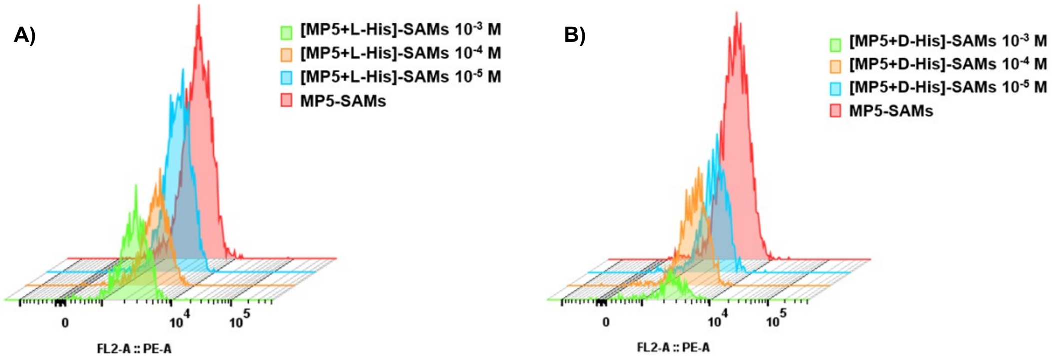


Figure S19. The quantity of adhered cells confirmed by flow cytometry analysis on the bionic surface of A) **MP5-SAMs**; B) **[MP5+L-His]-SAMs**, with different concentrations of His treatment, respectively**.**

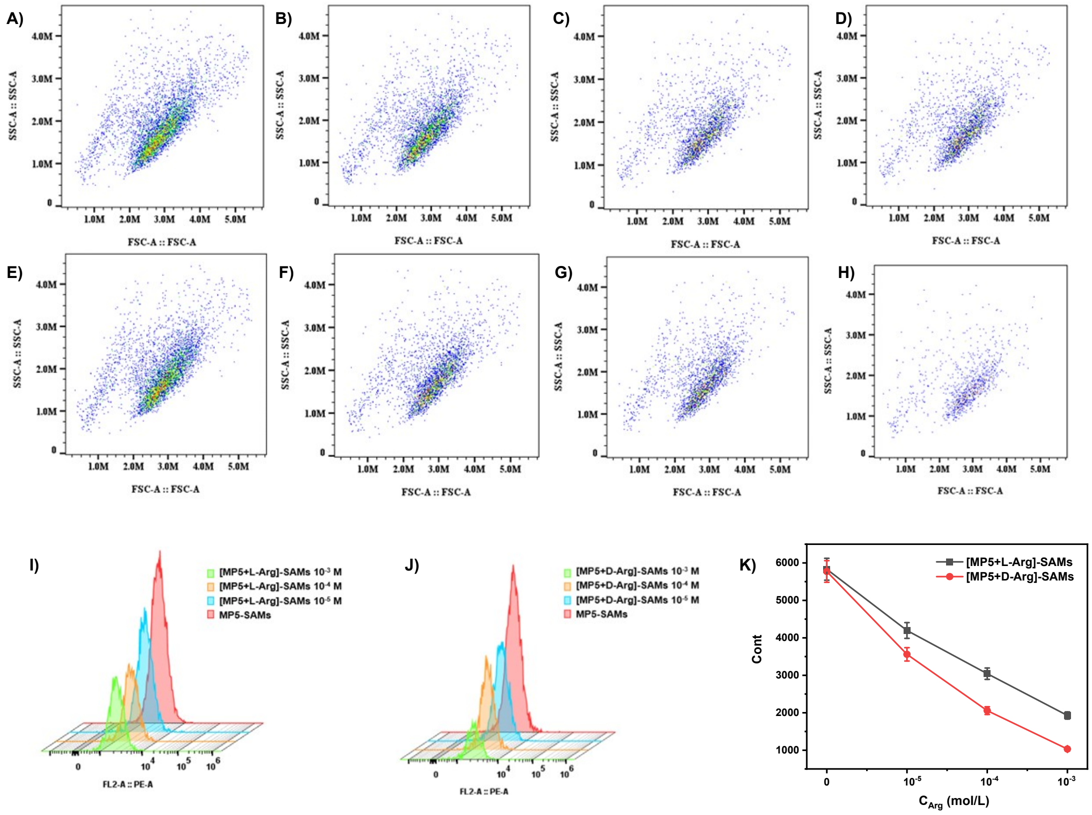


Figure S20. The flow cytometry results for quantitative detection of HeLa cells adhered to [MP5+D/L-Arg]-SAMs. The flow cytometry data of cell numbers on different MP5-SAMs assembled with L-Arg at the concentration of A) 0 mol/L, B) 10^{-5} mol/L, C) 10^{-4} mol/L, D)10^{-3} mol/L, and D-Arg at the concentration of E) 0 mol/L, F) 10^{-5} mol/L, G) 10^{-4} mol/L, H)10^{-3} mol/L, respectively. I) The flow cytometry data of cells incubating with [MP5+D-Arg]-SAMs I), and [MP5+ L-Arg]-SAMs J), at a series of concentrations of Arg treatment, separately. The cell number on different [MP5+D/L-Arg]-SAMs with a series concentration of Arg treatment. K) The gradual decrease in cell number on [MP5+D-Arg]-SAMs compared with [MP5+L-Arg]-SAMs, demonstrating a highly enantioselective-dependent for cells adhering behaviour on the chirality interface.

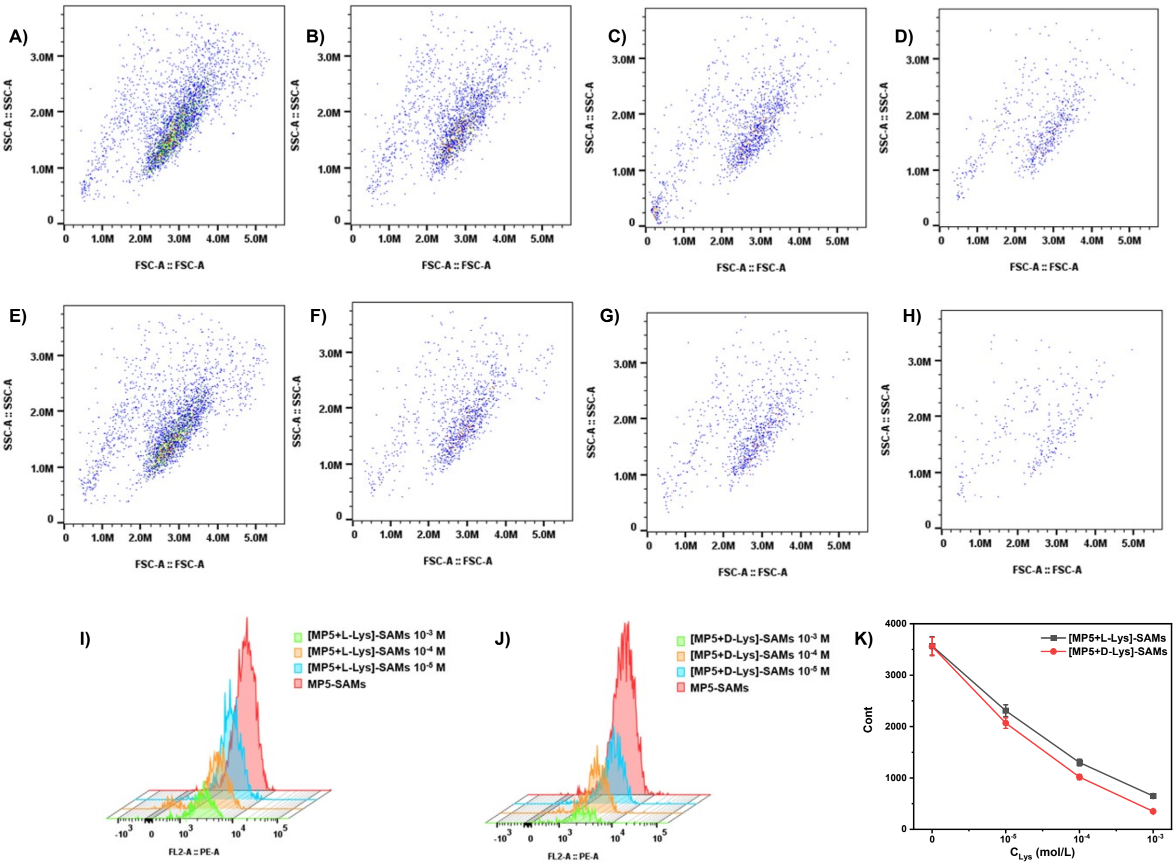


Figure S21. The flow cytometry results for quantitative detection of HeLa cells adhered to [MP5+D/L-Lys]-SAMs. The flow cytometry data of cell numbers on different MP5-SAMs assembled with L-Lys at the concentration of A) 0 mol/L, B) 10^{-5} mol/L, C) 10^{-4} mol/L, D)10^{-3} mol/L, and D-Lys at the concentration of E) 0 mol/L, F) 10^{-5} mol/L, G) 10^{-4} mol/L, H)10^{-3} mol/L, respectively. I) The flow cytometry data of cells incubating with [MP5+D-Lys]-SAMs I), and [MP5+ L-Lys]-SAMs J) at a series of concentrations of Lys treatment, separately. The cell number on different [MP5+D/L-Lys]-SAMs with a series concentration of Lys treatment. K) The gradually decrease in cell number on [MP5+D-Lys]-SAMs compared with [MP5+L-Lys]-SAMs, demonstrating a highly enantioselective-dependent for cells adhering behaviour on the chirality interface.

13. Reference.

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