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Efficient Inhibition of Human Papillomavirus 16 L1 Pentamer Formation by a Carboxylatopillarene and a *p*-Sulfonatocalixarene

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1. Materials and Methods

1.1. Protein Expression and Purification

Recombinant full length HPV16 L1, which combines a GST tag at the N-terminus, was purified from *E. coli* strain BL21 containing the overexpression vector pGEX-6p-1. The protein expression and purification were carried out essentially as described previously.[1] Briefly, cells from one liter culture were resuspended in lysis buffer L (50 mM Tris-HCl, pH = 8.0, 0.2 M NaCl, 1 mM DTT, 1 mM EDTA) and then were lysed by sonification. Thereafter, ATP, $MgCl_2$ and 3.5 M urea were added to the cell lysate to release tightly bound GroEL. The obtained GST-L1 was purified by the glutathione affinity column, after which it was eluted out by 10 mM GSH. Finally, further dialysis was used to remove GSH and the eluted GST-L1 was used for monitoring pentamer formation.

1.2. *In Vitro* Monitoring of Pentamer Formation from GST-L1 by Static Light Scattering (SLS) Measurements

The purified GST-L1 dissolved in buffer solution was used for the time-dependent *in situ* SLS measurements on a fluorescence spectrophotometer, SHIMADZU (Japan) RF-5301 at 25 ºC. GST-L1 fusion proteins (0.1 mg/mL, 2.0 mL) in buffer L (50 mM Tris-HCl, 0.2 M NaCl, 1 mM EDTA, 1 mM DTT) was treated with carboxylatopillar[5]arene (CP5A) or sulfonatocalix[4]arene (SC4A) at different concentrations for 30 min. The solutions were passed through a 0.2 μm filter unit to remove aggregates and dust particles before added into a 10 × 10 mm quartz cuvette. Static fluorescence intensity was recorded with an excitation wavelength of 350 nm. Under constant stirring, PreScission™ Protease (PPase, 200 IU/100 μL) was added into the cuvette and the moment was treated as the starting point (t = 0) for the measurements, continuously recording the SLS intensities by the spectrophotometer at 10 s intervals for 12 h.

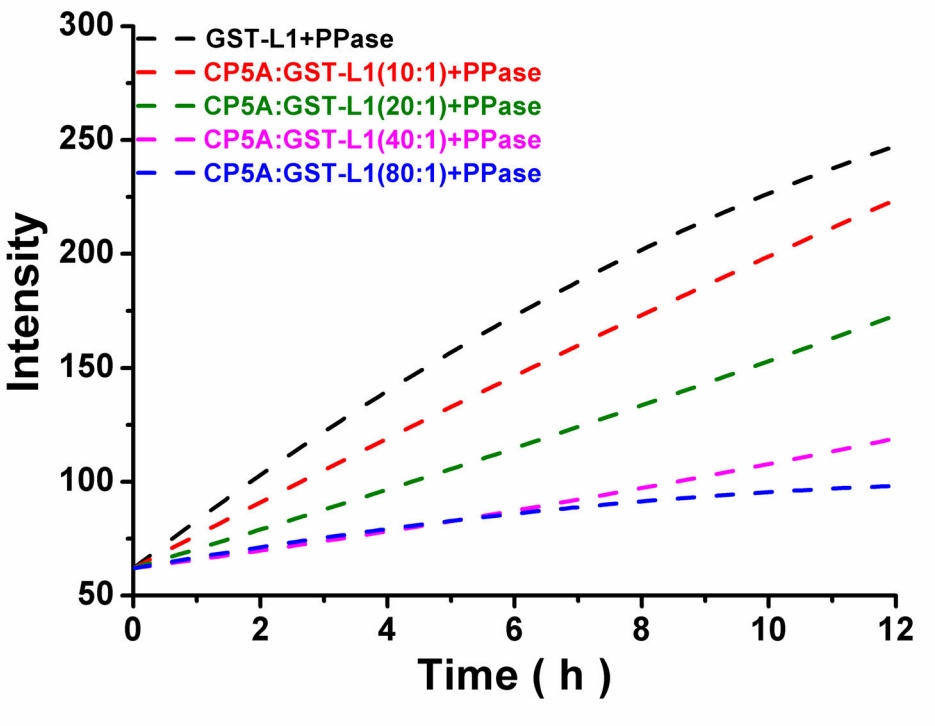


Fig. S1 Curve fitting plots of the SLS ones in **Fig. 1**, which demonstrated more clearly the differences in the increase rate of each line under the inhibition of CP5A.

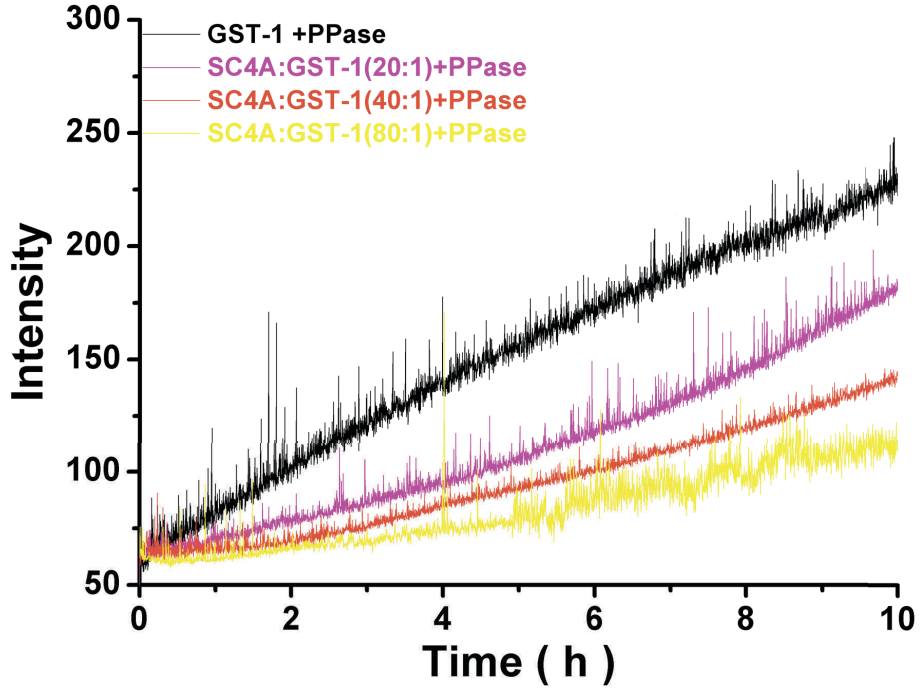


Fig. S2 Monitoring of the L1 pentamer formation from GST-L1 monomer (0.1 mg/mL) by time-dependent static light scattering (SLS) plots after the addition of PPase for GST cleavage. The proteins were incubated with SC4A in buffer L at different concentrations for 30 min before PPase addition and measurement. The different curves represent the molar ratios of SC4A to GST-L1 at 0:1, 10:1, 20:1, 40:1, 80:1, from up to bottom, respectively.

1.3. Nuclear Magnetic Resonance (NMR) Measurement

All NMR spectra were recorded at 298 K on a Bruker Avance 600-MHz spectrometer. NMR spectra of 1 mM peptide (Fig. S3 A, HPV L1 Helix 5), peptide with CP5A (Fig. S3 E) at different molar ratios (peptide / CP5A) of 5/1, 5/3, 5/15 (Fig. S3 B, C, D), respectively, were all measured in 90% H_2O / 10% D_2O / 20 mM sodium phosphate buffer at pH 7.4 at 25 °C. Simultaneously, the experiment of peptide and SC4A was conducted in the same way (Fig. S4), compared with that of peptide and CP5A. The TOCSY and NOESY experiments were performed using a mixing time of 100 ms and 200 ms, respectively; transients of 64 were acquired for each increment in t1. Water signal was suppressed using WATERGATE technique. The 2D NMR spectra were analyzed using SPARKY software.[5]

HPV L1 helix 5 (DLDQFPLGRKFLLQ), a representative peptide in the L1 protein, has 14 basic amino acids and the chemical shifts of the backbone basic amide NH, amide protons in side chain of the lysine ammonium and arginine guanidinium are markedly affected upon addition of CP5A / SC4A (Fig. S3, S4 and Tables S1, S2 and S3). The differences between the chemical shifts of the backbone amide NH protons of the peptides in the absence and presence of CP5A / SC4A were plotted in Fig. S5. In peptide-CP5A system, the largest chemical shift change is for arginine (Fig. S5 A), compared with peptide-SC4A system, where the largest is for lysines (Fig. S5 B). In another word, the chemical shifts of NH in the backbone and side chains of AAs, especially Arg and Lys, are markedly affected by CP5A / SC4A (Fig. S6).

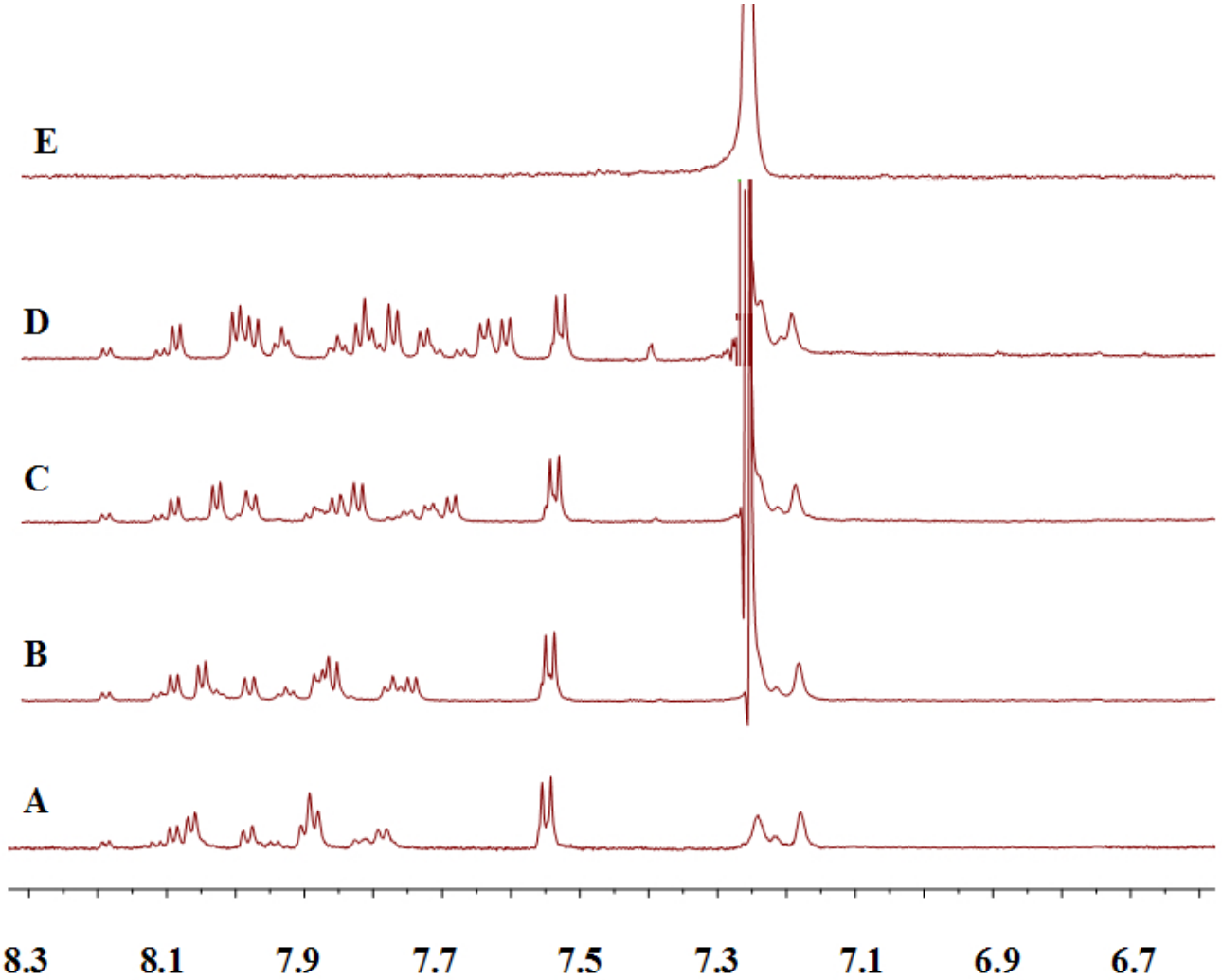


Fig. S3 Amide region of ^{1}H NMR spectra of 1 mM peptide (A, HPV L1 Helix 5) with CP5A (E) at the molar ratio (peptide/CP5A) of 5/1, 5/3, 5/15 (B, C, D), respectively, which are all measured in 90% H_2O / 10% D_2O / 20 mM sodium phosphate buffer at pH 7.4.

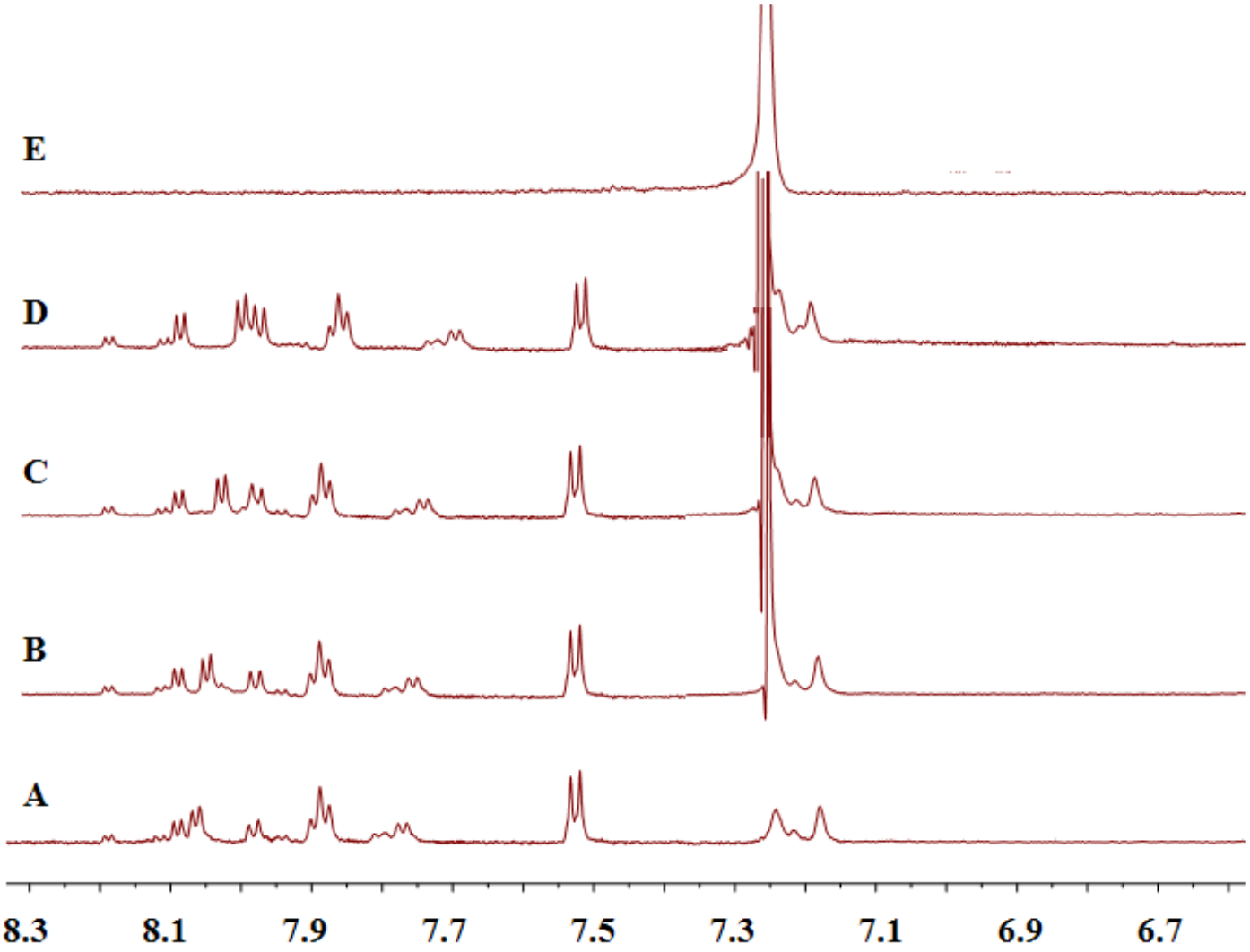


Fig. S4 Amide region of ^{1}H NMR spectra of 1 mM peptide (A, HPV L1 Helix 5) with SC4A (E) at the molar ratio (peptide/CP5A) of 5/1, 5/3, 5/15 (B, C, D), respectively, which are all measured in 90% H_2O / 10% D_2O / 20 mM sodium phosphate buffer at pH 7.4.

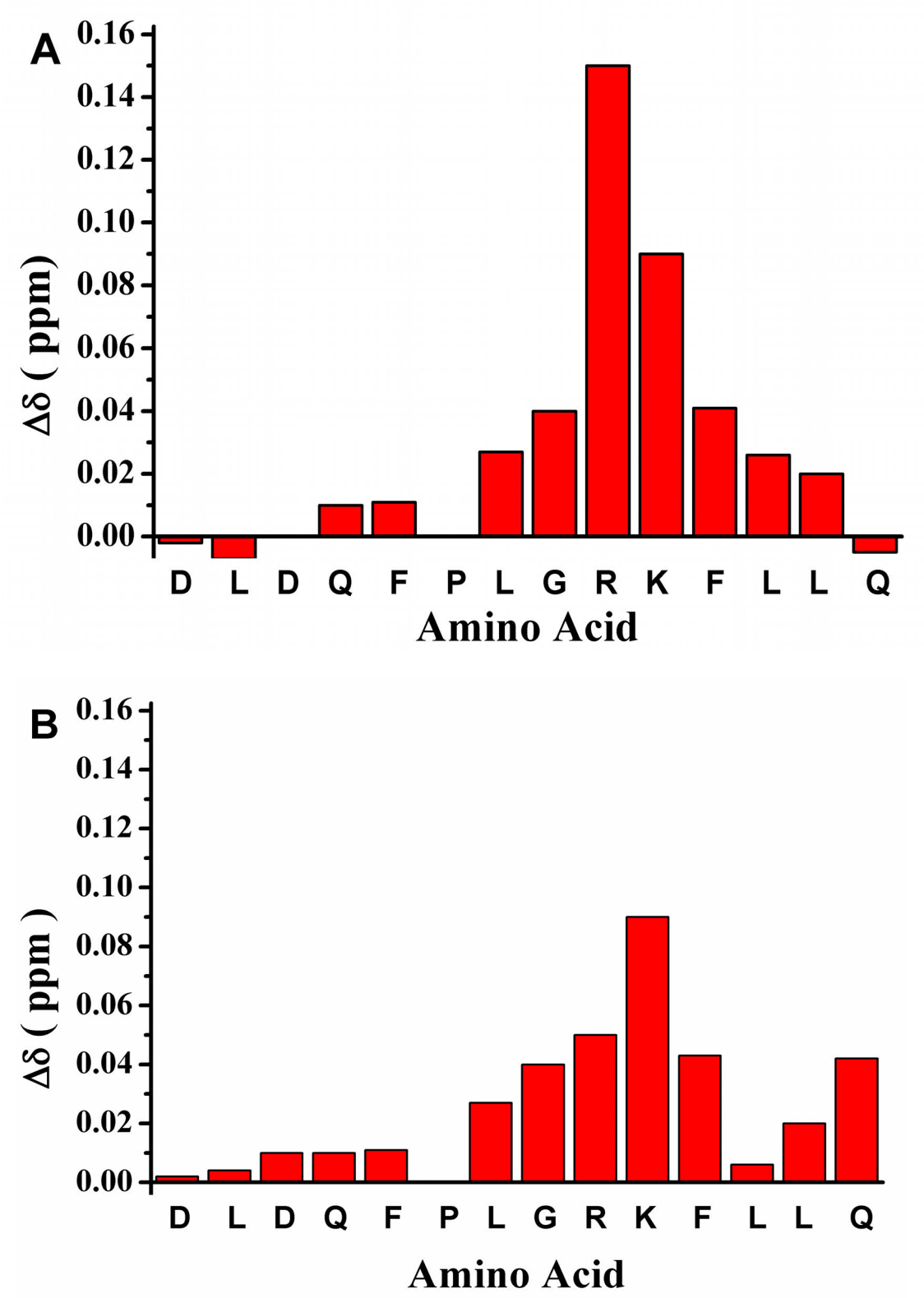


Fig. S5 The change in the chemical shift of the backbone amide NH resonances of HPV L1 helix 5 peptide upon binding to (A) CP5A and (B) SC4A in 90% H_2O / 10% D_2O / 20 mM sodium phosphate buffer at pH 7.4. $\Delta\delta = \delta_{\text{free peptide}} - \delta_{\text{with CP5A/SC4A}}$

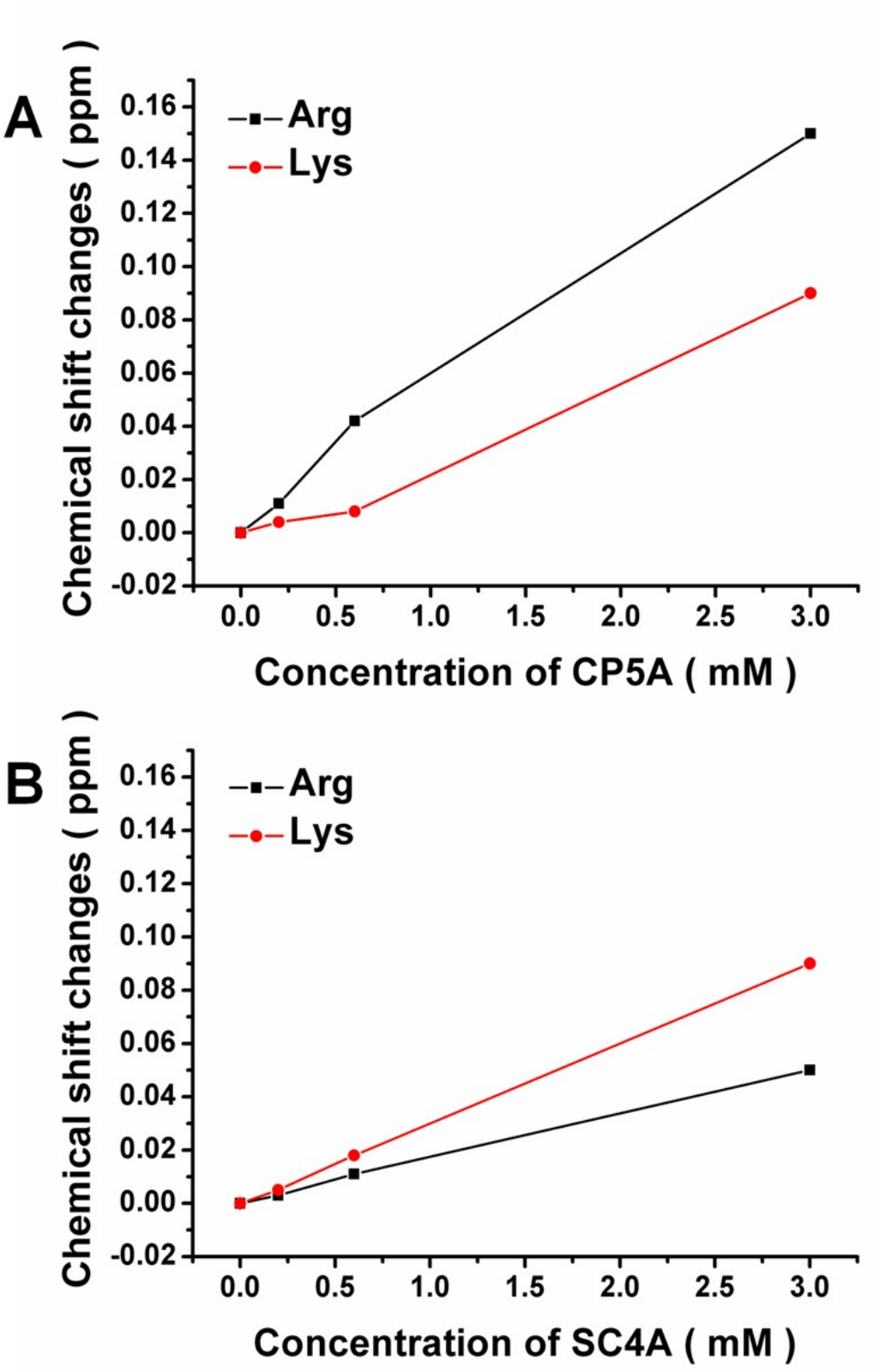


Fig. S6 The chemical shift of the backbone amide NH resonances of Arg and Lys in peptide (1 mM) affected by the increasing concentration of (A) CP5A and (B) SC4A, respectively.

Table S1. The ^{1}H NMR Chemical Shift (δ) of 1 mM peptide of HPV L1 helix 5 alone in 90% H_2O / 10% D_2O solution which containing 20 mM sodium phosphate (pH=7.4)

Residue	NH	áH	âH	ãH	Others
Asp[1]	8.093	4.791	2.872, 2.855		
Leu[2]	8.048	4.225	1.612	1.623	äH 0.913, 0.877
Asp[3]	8.121	4.772	2.853, 2.808		
Gln[4]	8.141	4.361	2.149, 2.035	2.377	äNH$_2$ 6.826, 7.584
Phe[5]	8.032	4.648	3.327, 2.955		2, 6 H 7.311; 3, 5 H 7.394; 4 H 7.342
Pro[6]		4.395	3.837, 3.665	2.034	äH 2.306, 1.903
Leu[7]	7.948	4.215	1.603	1.603	äH 0.916, 0.867
Gly[8]	7.889	3.971			
Arg[9]	7.917	4.293	1.824, 1.766	1.631	äH 3.202; NH 7.814, 7.882
Lys[10]	8.068	4.290	1.821	1.443	äH 1.691; åH 3.004; åNH$_3^+$ 7.653
Phe[11]	8.042	4.66	3.229, 2.991		2, 6 H 7.302; 3, 5 H 7.391; 4 H 7.348
Leu[12]	7.951	4.215	1.604	1.601	äH 0.915, 0.866
Leu[13]	7.948	4.215	1.603	1.603	äH 0.915, 0.867

Table S2. The ^{1}H NMR Chemical Shift (δ) of 1 mM peptide of HPV L1 helix 5 and 3 mM CP5A in 90% H_2O / 10% D_2O solution which containing 20 mM sodium phosphate (pH=7.4)

Residue	*NH*	*αH*	*βH*	*γH*	*Others*
Asp[1]	8.095	4.760	2.84, 2.75		
Leu[2]	8.055	4.215	1.603	1.603	äH 0.916, 0.867
Asp[3]	8.121	4.757	2.84, 2.75		
Gln[4]	8.131	4.369	2.129, 2.001	2.378	äNH$_2$ 6.816, 7.587
Phe[5]	8.021	4.661	3.221, 2.989		2, 6 H 7.301; 3, 5 H 7.389; 4 H 7.334
Pro[6]		4.395	3.837, 3.665	2.034	äH 2.306, 1.903
Leu[7]	7.921	4.215	1.603	1.603	äH 0.916, 0.867
Gly[8]	7.849	3.967			
Arg[9]	7.767	4.187	1.704, 1.652	1.522	äH 3.102; NH 7.614, 7.687
Lys[10]	7.978	4.195	1.723	1.435	äH 1.675; åH 3.001; åNH$_3^+$ 7.636
Phe[11]	8.001	4.660	3.218, 2.989		2, 6H 7.30; 3, 5 H 7.39; 4 H 7.337
Leu[12]	7.925	4.215	1.603	1.603	äH 0.916, 0.867
Leu[13]	7.928	4.215	1.603	1.603	äH 0.916, 0.867
Gln[14]	7.998	4.367	2.123, 2.011	2.378	äNH$_2$ 6.815, 7.596

Table S3. The ^{1}H NMR Chemical Shift (δ) of 1 mM peptide of HPV L1 helix 5 and 3 mM SC4A in 90% H_2O / 10% D_2O solution which containing 20 mM sodium phosphate (pH=7.4)

Residue	*NH*	*áH*	*âH*	*ãH*	*Others*
Asp[1]	8.091	4.758	2.84, 2.75		
Leu[2]	8.044	4.215	1.603	1.603	äH 0.916, 0.867
Asp[3]	8.111	4.755	2.84, 2.75		
Gln[4]	8.131	4.364	2.13, 2.01	2.378	äNH$_2$ 6.816, 7.587
Phe[5]	8.021	4.658	3.22, 2.99		2, 6 H 7.301; 3, 5 H 7.391; 4 H 7.342
Pro[6]		4.395	3.837, 3.665	2.034	äH 2.306, 1.903
Leu[7]	7.921	4.215	1.603	1.603	äH 0.916, 0.867
Gly[8]	7.849	3.973			
Arg[9]	7.867	4.287	1.814, 1.746	1.611	äH 3.112; NH 7.614, 7.687
Lys[10]	7.978	4.110	1.623	1.415	äH 1.602; åH 3.001; åNH$_3^+$ 7.456
Phe[11]	7.999	4.66	3.22, 2.99		2,6H 7.296; 3,5H 7.384; 4H 7.334
Leu[12]	7.945	4.215	1.603	1.603	äH 0.916, 0.867
Leu[13]	7.928	4.215	1.603	1.603	äH 0.916, 0.867
Gln[14]	7.951	4.371	2.120, 2.01	2.381	äNH$_2$ 6.815, 7.596

1.4. Measurement of Fast Protein Liquid Chromatography (FPLC) Elution Profiles

The size-exclusion chromatography (SEC, Superdex-200, 26/60) analytical protocol based on FPLC system (GE, USA) was applied for the purification of GST-L1 and L1, and the simultaneous analysis of the relative molecular masses. For this essay, all samples and column washing were clarified by filtration through a 0.2 μm filter unit before sample loading and column washing with Buffer L (mobile phase). A flow rate of 3 mL/min was used in overall process and a 50 mL sample loop was employed for sample injection, all the steps of purification were conducted at 4 ºC.

Firstly, the GST-L1 (0.1 mg/mL) was treated at different molar ratios of CP5A (or SC4A) to GST-L1 (0:1, 10:1, 20:1, 40:1, 80:1) for 30 min, after that it was digested by PPase overnight to remove the GST-tag, then the sample was injected into a gel-filtration column to obtain the FPLC elution profile. The approximate sizes of L1 pentamer and monomer were determined on the basis of a comparison to three standard proteins, *i.e.*, Ferritin (440 kDa), Albumin (66 kDa) and Ovalbumin (45 kDa).

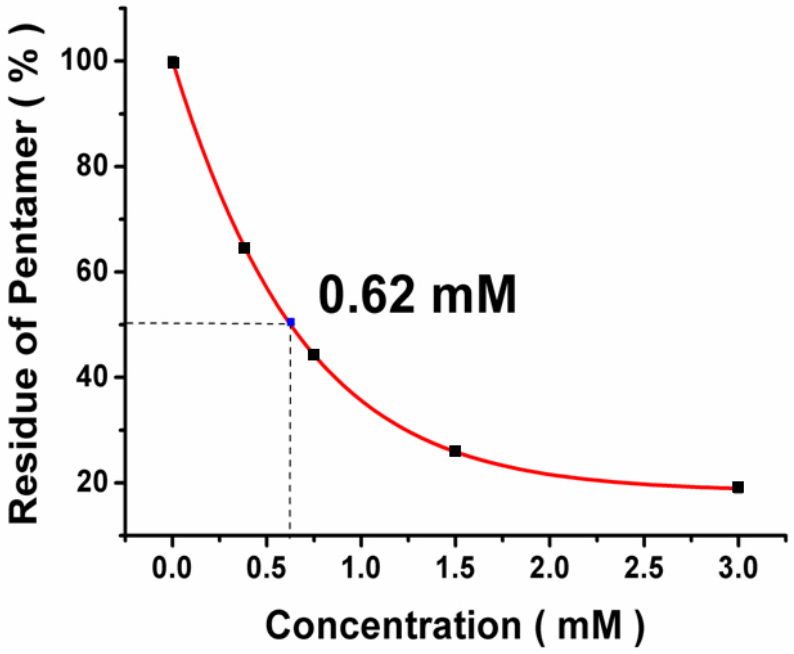


Fig. S7 The given curve illustrated the analysis of the pentamer formation under the inhibition of CP5A, which shows a gradual decrease of pentamer yield with the addition of CP5A and the IC_{50} response to the CP5A *vs* pentamer formation.

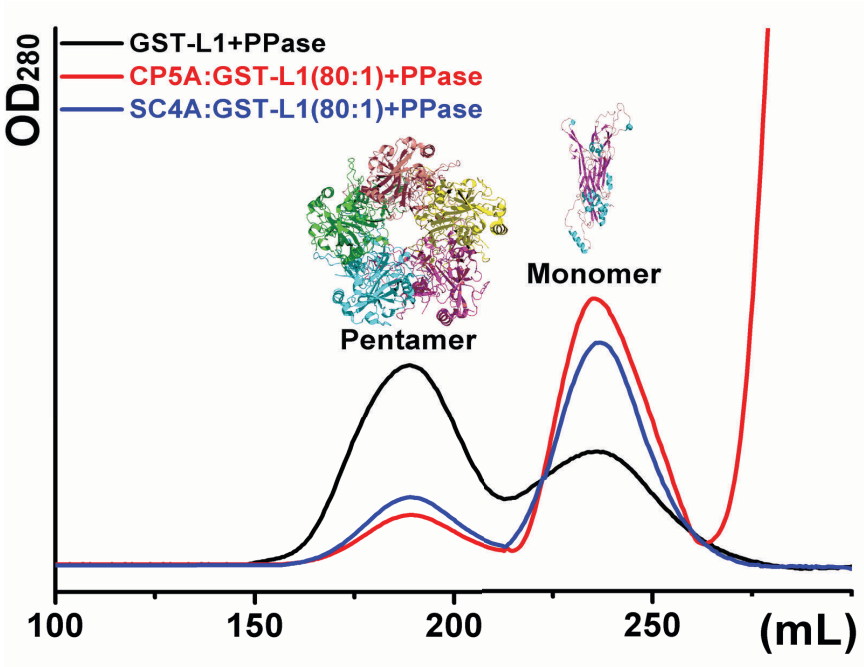


Fig. S8 The diagram shows the comparison of the inhibition efficiency of CP5A and SC4A on L1 pentamer formation by the size-exclusion chromatography. The concentration of initial GST-L1 was 0.1 mg/mL, and the molar ratio of SC4A / CP5A to GST-L1 was 80:1. HPV 16 L1 crystal structure came from Protein Data Bank (code 2R5H).

1.5. Dynamic Light Scattering (DLS) Measurements

The DLS measurement was often used to monitor the size distribution of sample particles in solution. Firstly, the dust particles in sample solution were removed through a 0.22 μm filter unit, then the particle size analyzer (Malvern Zetasizer Nano-ZS 90) with a 4 mL cuvette and the Dispersion Technology Software (DTS, V6.01) were used to collect and analysis data.

Firstly, the protein was treated at different molar ratios of CP5A (or SC4A) to GST-L1, after which it was digested by PPase overnight to remove GST-tag, then the sample was monitored by DLS measurements. After CP5A was released by further dialysis in buffer L and assembly buffer, the two kinds of L1 particle sizes were monitored by DLS again. Combined with the TEM experiments (Fig. 4), we further compared three different L1 particles in morphology and size distribution.

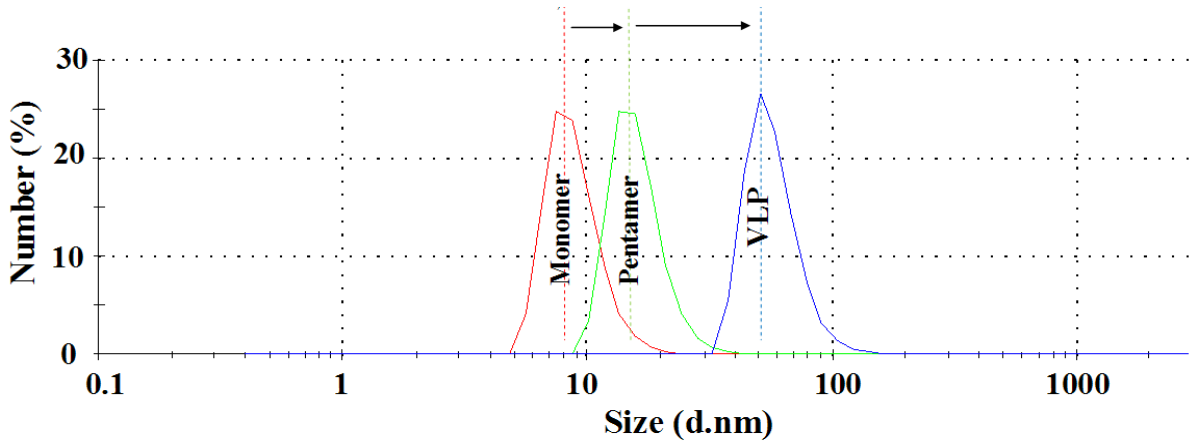


Fig. S9 Dynamic light scattering (DLS) profiles showing the particles size of HPV L1 monomer (L1-m, after incubated with CP5A at the molar ratio of 1:80 and GST cleavage); pentamer (after removal of CP5A by dialysis in buffer L) and VLPs (after further assembly from L1-p in an assembly buffer G), which are consistent with the results of TEM below well. The initial concentration of GST-L1 used here was 0.1 mg/mL.

1.6. Transmission Electron Microscopy (TEM) Measurements

For the TEM measurements, the corresponding sample in above **1.5** was spotted on carbon and formvar-coated copper grids for 2 min and dried in air. Then it was negatively stained with phosphotungstic acid for 2 min and air-dried. The samples were examined using a H-7650 transmission electron microscope (Hitachi Japan), with an accelerating voltage at 80 kV.

For the samples used for TEM measurements, the monomer (L1-m) was prepared by using 0.1 mg/mL of GST-L1 in buffer L, after incubation with CP5A or SC4A at a molar ratio of 1:80 for 30 min and further PPase cleavage on GST; the pentamer (L1-p) was prepared by using the above L1-m after removal of CP5A or SC4A by dialysis in buffer L; and the VLPs were prepared by using the above L1-p after further assembly in an assembly buffer solution G (1 M NaCl, 50 mM $H_2PO_4^-/HPO_4^{2-}$, pH = 5.3).

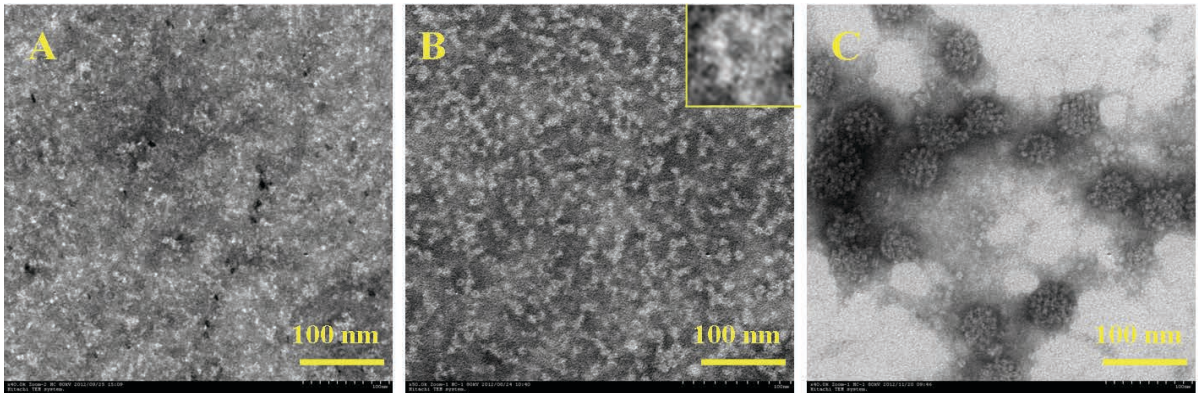


Fig. S10 Comparison of the electronic micrograms (EM) of HPV L1 in (A) monomer (L1-m, after incubated with SC4A at the molar ratios of 1:80 and GST cleavage); (B) pentamer (L1-p, after removal of SC4A by dialysis in buffer L) and (C) VLPs (after further assembly from L1-p in an assembly buffer G). The initial concentration of GST-L1 used here was 0.1 mg/mL.

1.7. The Analysis on Crystal Structure of HPV 16 L1

Crystal structure of neighbour monomers from a HPV L1 pentamer was displayed by application of PyMOL Molecular Graphics System, Version 1.4.1 and by using the crystal structure of HPV16 L1 (Protein Data Bank code2R5H) as a template. The Polar Contacts can be displayed with the menu A > find > polar contacts and selecting from one of the many submenus and the distances can be measured between two atoms and expressed in the same unit as the XYZ coordinates within the PDB file by PyMOL.

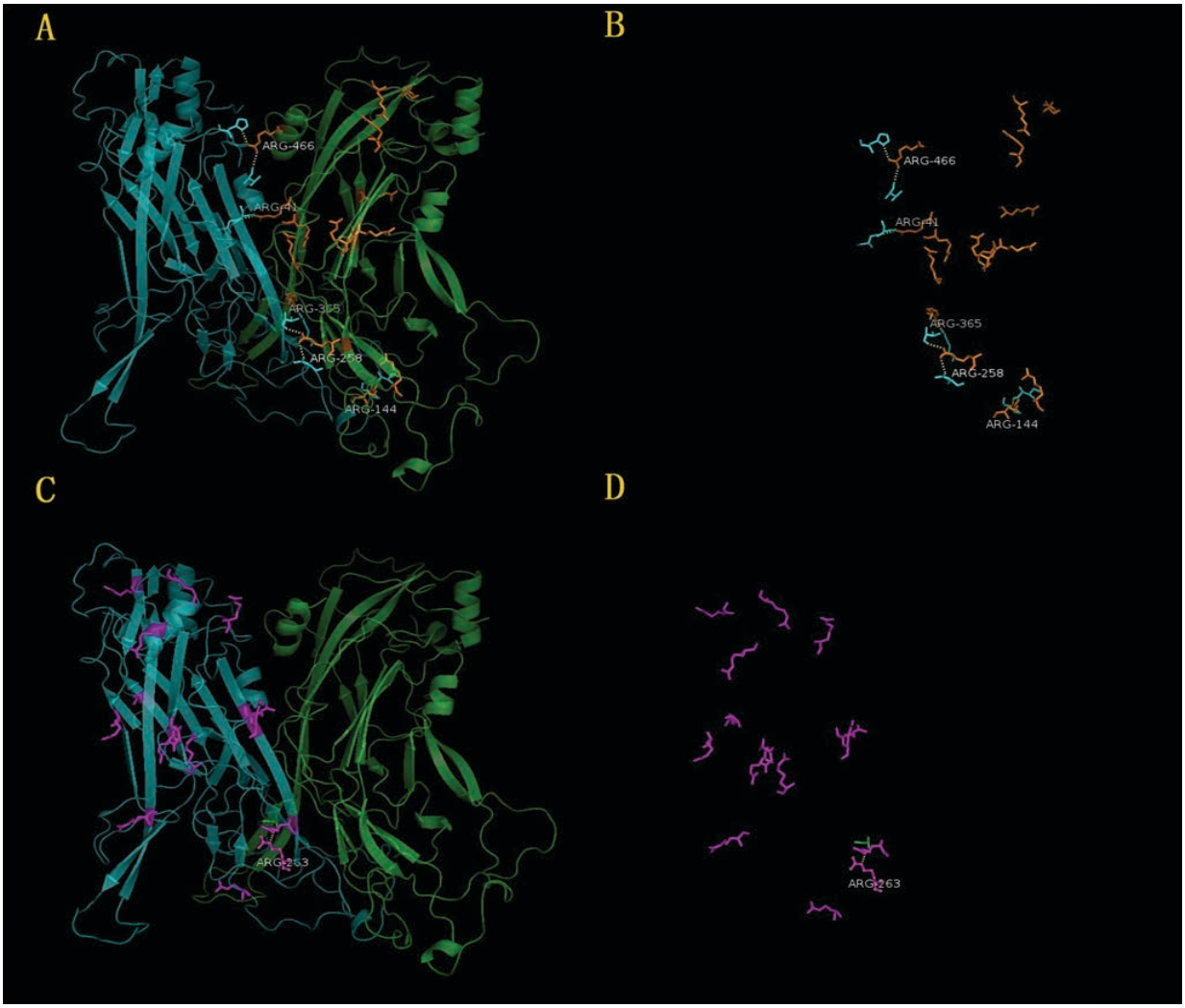


Fig. S11 (A), (C) show the crystal structure of neighbour monomers from a L1 pentamer (Protein Data Bank code 2R5H), where each monomer is shown in different colours to show the intertwining of the surface loops and the binding sites; (B), (D) show the lateral **arginines** from one monomer interact with the specific site of the neighbour monomer by forming hydrogen bonds or others.

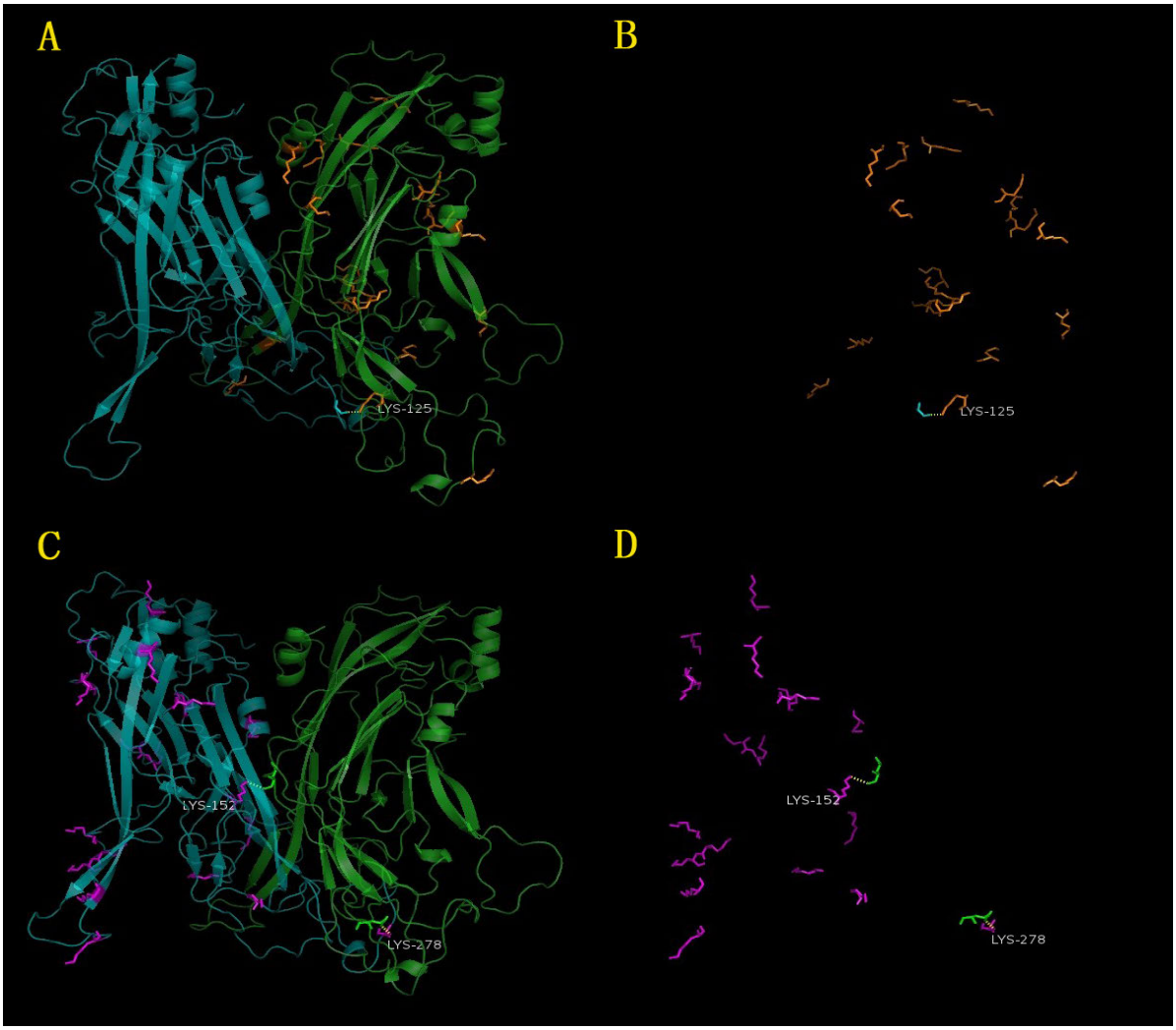
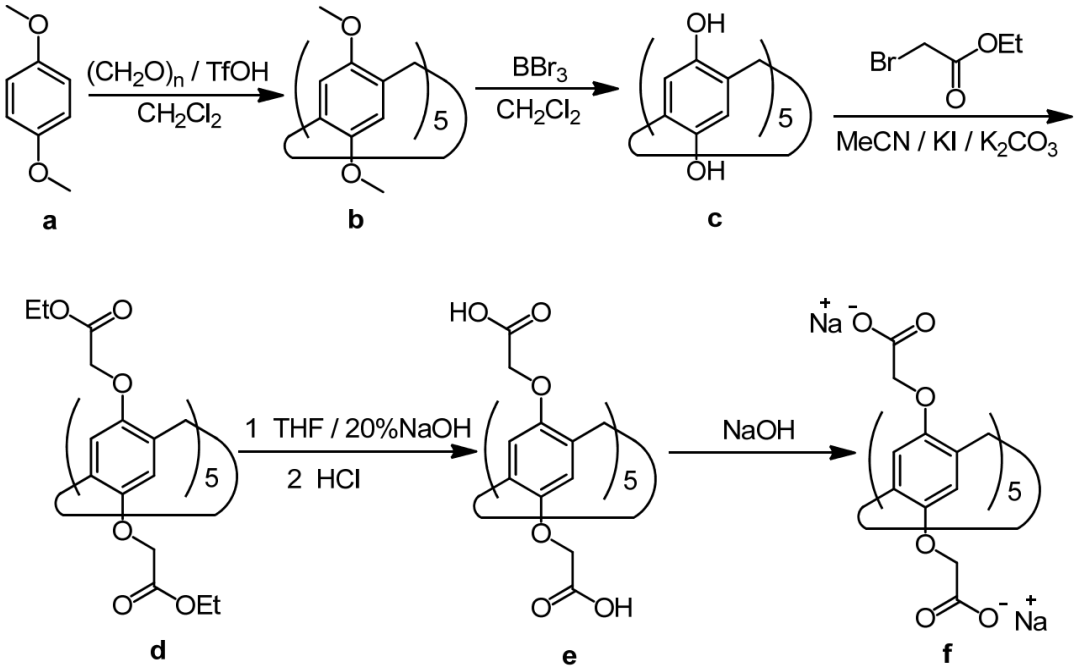


Fig. S12 (A) and (C) show the crystal structure of two neighbour monomers from a L1 pentamer (Protein Data Bank code 2R5H). Each monomer is shown in different color to show the intertwining of the surface loops and the binding surfaces; (B), (D) show the lateral **lysines** from right and left monomer including the specific site of the neighbour monomer by forming hydrogen bonds or others.

Table S4. The weak interactions and bond lengths related to Arg and Lys at the interface of two monomers, which are obtained from the crystal structure of a HPV16 L1 pentamer (Protein Data Bank code 2R5H).

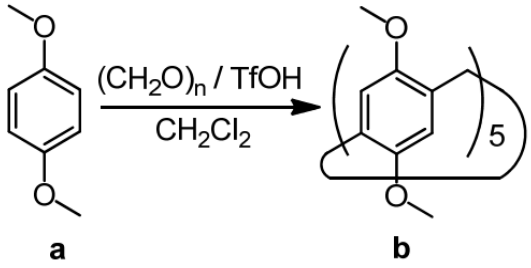
Two neighbour L1, L1(α)-L1(β)	Bond distance (Å)	
	H…Y	X-H⋯Y
41R ——233D	2.0	2.7
——192N	2.3	3.2
144R——277I	1.7	2.7
——278K	2.5	2.9
258R——130E	3.0	3.5
——257V	2.0	2.9
365R——269E	1.7	2.3
466R——238V	2.6	3.3
——319H	2.7	3.4
343S ——263R	2.7	3.4
125K ——132A	1.9	2.4
144R——276K	2.5	2.9
113L——152K	2.6	3.4

1.8. Synthesis of Carboxylatopillar[5]arene (CP5A)[7]



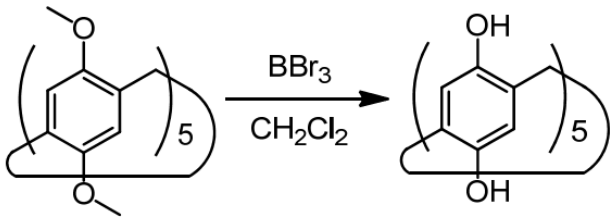
Scheme S1. Synthetic route to CP5A

1.8.1. Synthesis of Compound b



Dimethoxypillar[5]arene (DMP5A) was prepared by the reaction of 1,4-dimethoxybenzene (a) and paraformaldehyde in CH_2Cl_2, in the presence of 5% mmol of trifluoromethanesulfonate (TfOH) as catalyst. The ratio of paraformaldehyde to 1,4-dimethoxybenzene was 3:1. The resulting reaction mixture was stirred for 5 h at RT, and then quenched by the addition of H_2O. The mixture was washed with H_2O, the organic phase was collected, concentrated and then subjected to column chromatography (SiO_2: light petroleum / EtOAc, 10:1 to 6:1) to give the final product (b) as white powder. Yield: 50%. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C) δ (ppm): 6.76 (s, 10H), 3.77 (s, 10H), 3.65 (s, 30H).

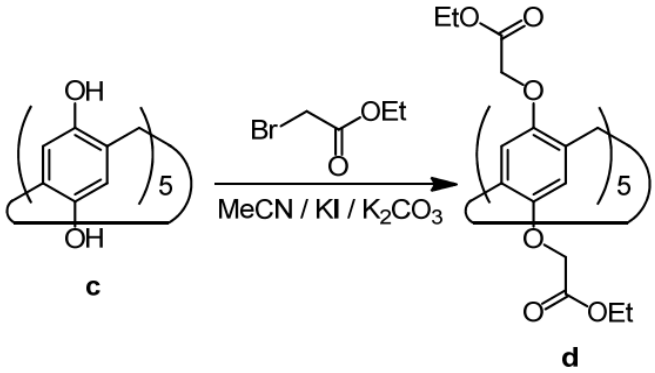
1.8.2. Synthesis of Compound c



b c

To a solution of b (1.5 g, 2 mmol) in dry CH_2Cl_2 (60 mL), boron tribromide (24.3 g, 97.3 mmol) was added in the condition of ice bath, and the mixture was stirred at RT for 2 days. Then, H_2O (60 mL) was added into the mixture, which was stirred for another 72 h at RT. The precipitate formed was filtered and washed with water to result in pillar[5]arene (P5A, c) as white powder. Yield: 1.2 g, 96% ^{1}H NMR (300 MHz, CD_3COCD_3, 25 °C); δ (ppm): 6.66 (s, 10H), 3.59 (s, 10H), 7.97 (s, 10H).

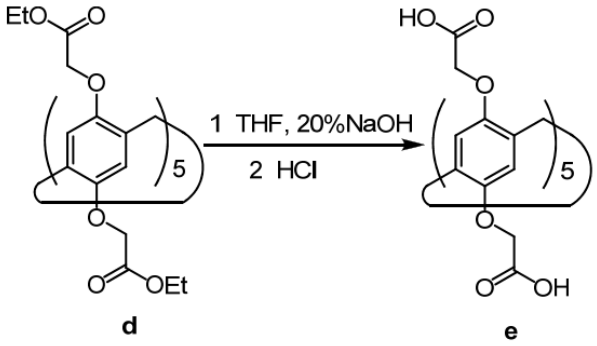
1.8.3. Synthesis of Compound d



c d

P5A (c) (1.22 g, 2 mmol) was dispersed in MeCN (60 mL) and K_2CO_3 (3.5 g) was added. The mixture was stirred for 40 min at RT, then a small amount of KI and excess of ethyl bromoacetate (5 mL, 45 mmol) were added. The mixture was heated under reflux under a N_2 atmosphere for 18 h, and then filtered and washed with $CHCl_3$ after cooling down. The filtrate was concentrated, and the residue was subjected to column chromatography (SiO_2: CH_2Cl_2 / Me_2CO, 100:0 to 30:1). The crude product was crystallized by slow diffusion of n-hexane into a $CHCl_3$ solution to result in ethoxycarbonyl-substituted P5A (d) after filtration and dryness. Yield: 2.35 g, 80%. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C) δ (ppm): 7.05 (s, 10H), 4.54 (q, J = 15 Hz, 20H), 4.09 (m, J = 6 Hz, 20H), 3.86 (s, 10H), 0.98 (t, J = 6 Hz, 30H).

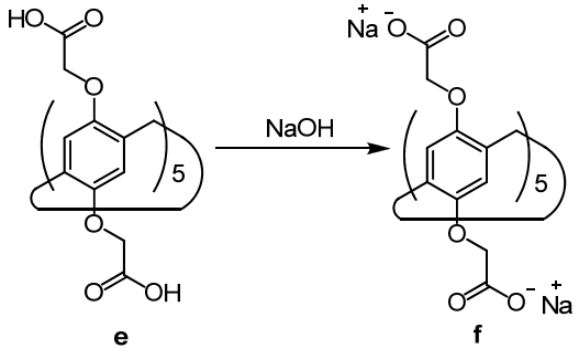
1.8.4. Synthesis of Compound e



d e

Aqueous NaOH solution (30 mL, 20%) was added to a solution of d (1.47 g, 1 mmol) in THF (60 mL). The mixture was heated under reflux for 15 h. After cooling to RT, the mixture was concentrated under reduced pressure. The residue was diluted into 100 mL of H_2O, and then acidified with HCl. The resulting precipitate was collected by filtration, washed with H_2O, and dried under vacuum to give CP5A (e). Yield: 1.1 g, 92.4%. ^{1}H NMR (300 MHz, CD_3SOCD_3, 25 °C) δ (ppm): 12.93 (s, 10H), 7.11 (s, 10H), 4.69 (d, *J* = 15 Hz, 10H), 4.41 (d, J=15 Hz, 10H), 3.74 (s, 10H).

1.8.5. Synthesis of Compound f



e f

Compound e (0.24 g) was dispersed in deionized H_2O (3 mL). Then, NaOH solution (80 mg NaOH in 1 mL of deionized H_2O) was added dropwise into the mixture until the reaction mixture was clear. Final product (f) of CP5A was obtained by drying under vacuum. Yield: 0.25 g, 95%. ^{1}H NMR (500 MHz, D_2O, 25 °C) δ (ppm): 6.80 (s, 10H), 4.51 (d, *J* = 20 Hz, 10H), 4.29 (d, *J* = 15 Hz, 10H), 3.86 (s, 10H); ^{13}C NMR (500 MHz, D_2O, 25 °C) δ (ppm): 177.496, 149.239, 128.593, 114.588, 67.722, 29.086; HR-MS (ESI): m/z594.1177 $[M-10Na+8H]^{2-}$, 605.6116 $[M-9Na+7H]^{2-}$, 616.0996 $[M-8Na+6H]^{2-}$, 627.0900 $[M-7Na+5H]^{2-}$, 638.0882 $[M-6Na+4H]^{2-}$.

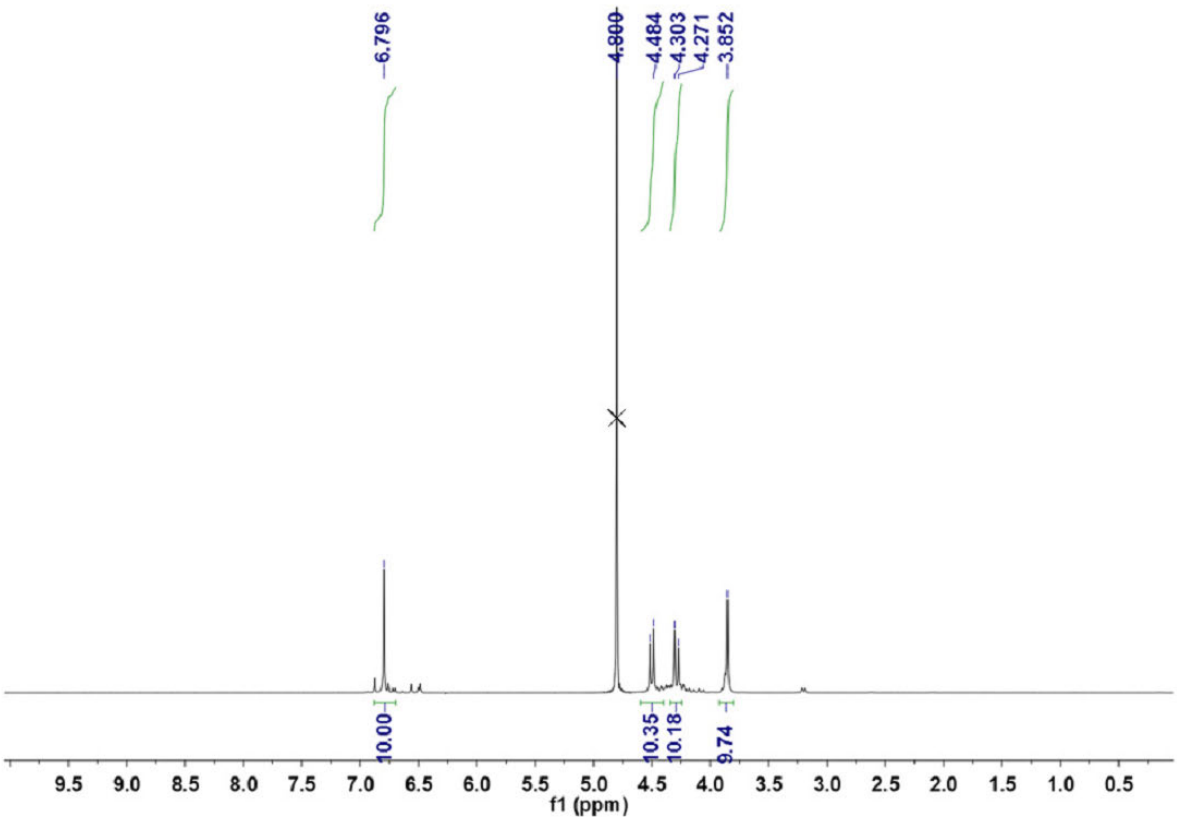


Fig. S14. ^{1}H NMR spectrum of CP5A

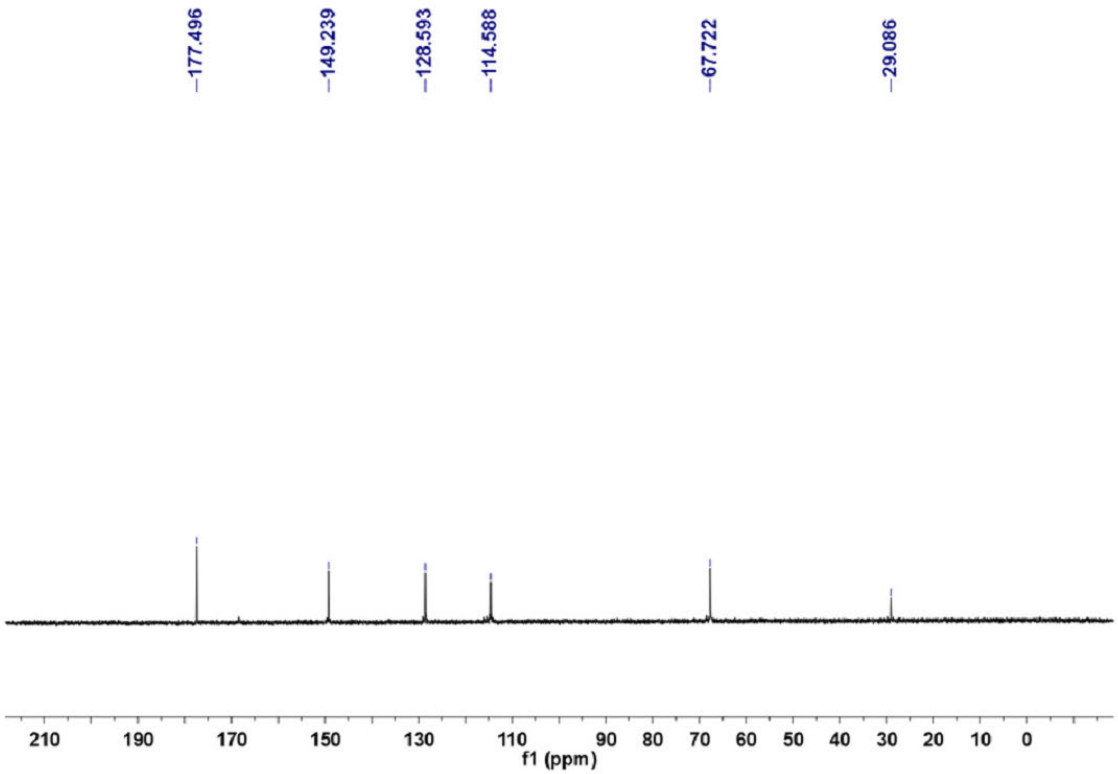


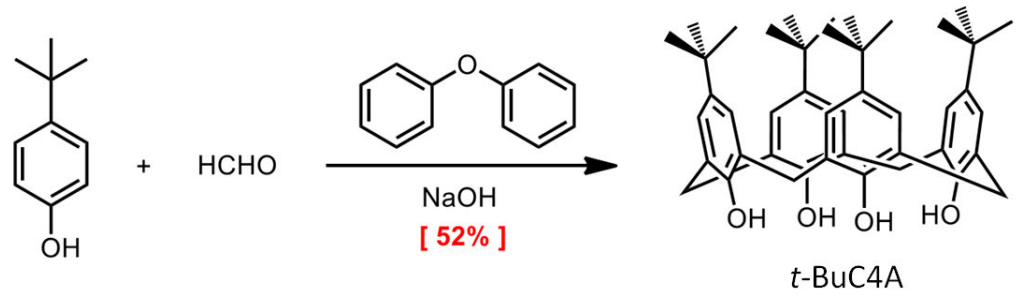
Fig. S15 ^{13}C NMR spectrum of CP5A

1.9. Synthesis of Sulfonatocalix[4]arene (SC4A)[8]

Sulfonatocalix[4]arene (SC4A) was synthesized according to a modified literature reported procedure. The synthesis route was displayed in Schemes S2, S3 and S4.

1.9.1. Synthesis of t-BuC4A

p-tert-butylphenol (40.83 g, 0.27 mol) was suspended into HCHO (24.80 mL, 37 %) in a 1000 mL three-neck round-bottomed flask under mechanical stirring. NaOH (0.50 g) was added to the reaction for 1.5 h at 110 °C, then the target compound was obtained as a brown solid after cooling to RT. Diphenyl ether (400 mL) was dispersed into the reaction glass with magnetic stirring under an atmosphere of N_2 and heated to 260 °C for 2 h. The reaction mixture was cooled to RT then was put into a 1000 mL beaker followed by the addition of 600 mL ethyl acetate under stirring to result in a white solid precipitate. The crude product was filtered and washed with water to give the final white solid after drying in vacuo (19.8 g, 52%). Further purification was done by washing with ethyl acetate (40 mL × 3), deionized water (40 mL × 3) to remove any excess impurity. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C) δ (ppm): 10.34 (s, 4H), 7.05 (s, 8H), 4.26 (d, J = 14.4 Hz, 4H), 3.50 (d, J = 13.8 Hz, 4H), 1.21 (s, 36H).

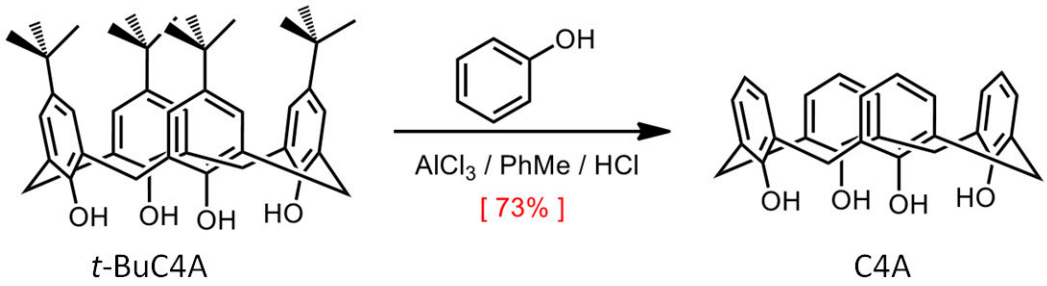


Scheme S2. Synthetic route to *t*-BuC4A

1.9.2. Synthesis of C4A

t-BuC4A (13.3 g), anhydrous aluminium trichlo (14.0 g), phenyl hydroxide (9.0 g), was suspended in anhydrous toluene (125 mL) in a 250 mL round bottom flask at RT under N_2 atmosphere for 1 h. The reaction mixture was dispersed to hydrochloric acid (250 mL, 0.2 mM) under stirring. The organic layer was collected and further washed with distilled water (100 mL × 4), which was subsequently dried over anhydrous sodium sulfate. After filtration, the solvent was removed by rotary evaporation to give a white solid product. The target

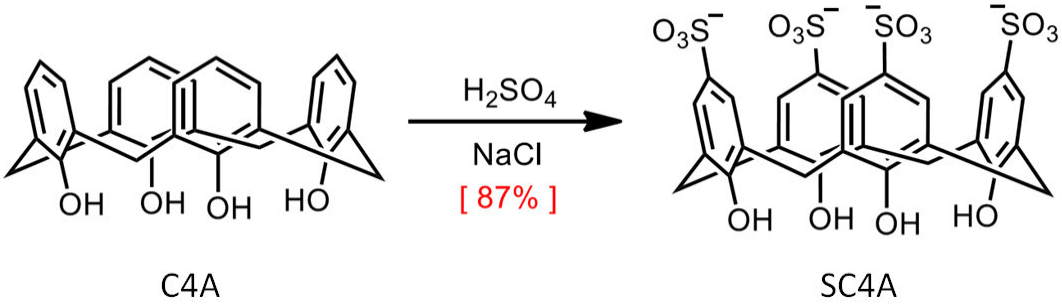
molecule was obtained in 73% yield (6.34 g) after drying under vacuum. ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C) δ (ppm): 10.19 (s, 4H), 7.05 (d, J = 7.6 Hz, 8H), 6.72 (t, J = 7.5 Hz, 4H), 4.24 (s, 4H), 3.55 (s, 4H).



Scheme S3. Synthetic route to SC4A

1.9.3. Synthesis of SC4A

A solution of C4A (5 g) in concentrated sulfuric acid (50 mL) in a round bottom flask (100 mL) was heated to 80 °C under N_2 atmosphere for 4 h. Sodium chloride (100 mL) was added dropwise into the mixed solvent and subsequently the reaction was further continued to reflux for 5 minutes. After cooling to room temperature, the crude product was collected and recrystallized from distilled water and the product was obtained as a white solid power (9.1 g, 87 %). ^{1}H NMR (300 MHz, D_2O, 25 °C) δ (ppm): 7.61 (s, 8H), 4.06 (s, 8H).



Scheme S4. Synthetic route to SC4A

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