Electronic Supplementary Information for

Stimuli Responsive Chiral Liquid Crystal Phases of Phenylboronic Acid Functionalized Rodlike Viruses and Their Interaction with Biologically Important Diols[**]

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Experimental

Materials

Most of the reagents used in the synthesis were purchased from J&K Scientific (Beijing, China) and used without further purification. Some organic solvents were supplied by local suppliers. Ultrapure water from a Milli-Q UltraPure system (18.2 mΩ·cm) (Millipore) was always used. The M13 virus was grown and purified following the standard biochemical protocol using the ER2738 strain of *E. coli* as the host bacteria.[1]

Instrumental

HPLC-MS was performed on the LCMS-2020 from Shimadzu. ^{1}H NMR spectra were recorded on an AVANCE III 400MHz spectrometer (Bruker).The concentration of the virus was determined with a UV-Vis 2550 spectrometer (Shimadzu). Fluorescence spectra were recorded on an F-4600 fluorescence spectrophotometer (Hitachi High-Technologies). Centrifugation and ultracentrifugation were performed on a benchtop Allegra X15R centrifuge and Optima L-90K ultracentrifuge (Beckman Coulter), respectively.

Synthesis of 2,5-Dioxopyrrolidinyl 3-[N-(3-(1,3,2-Dioxaboran-2-yl)phenyl)carbamoyl]propanoate (DDPCP, 3)

Synthesis of DDPCP was followed the method described in the reference (Scheme S1).[2] All of the organic solvents were pre-dried following standard procedures. To 30 mL of anhydrous pyridine was added 5 g of 3-aminophenylboronic acid monohydrate. The resulted mixture was cooled in an ice-water bath for 10 min. Under strong stirring, 10g succinic anhydride (SA) was added and the mixture was kept in the ice-water bath until SA dissolved completely (ca. 30 mins). The reaction mixture was kept at 25 °C and stirred for 12 h. After this, the solvent was evaporated by rotary evaporation and 40 mL water was added. Under stirring, NaOH was added slowly until all of the solids were dissolved. The pH of the mixture was then adjusted to ca. 3 by adding HCl solution under stirring, during which some precipitate appeared. The mixture was then kept at 3 °C overnight. The precipitate was collected by filtration and dried in vacuo, resulting in raw products with a reddish brown color. The raw product was dissolved in water containing NaOH and recrystallization was repeated twice and finally resulted in 3.6 g product **1** as white needle crystals.

For the subsequent reaction, the two hydroxyl groups of the boronic acid of **1** has to be protected. To 40 mL of 1,4-dioxane in a 100 mL round-bottom flask installed with a West condenser was added 3.6 g of **1** and 8.7 mL 1,3-propanediol. The suspension was kept in an oil bath at 100 °C and refluxed under stirring for 10 min until the entire solid dissolved. The solution was then cooled down to room temperature, and to which 0.84 g *N*-hydroxysuccinimide was added. The mixture was placed in an ice-water batch for 10 min and then 1.56 g dicyclohexylcarbodiimide (DCC) was added under strong stirring. The reaction was further kept in the ice-water bath for 30 min. The reaction was then kept at 25 °C and stirred under argon overnight. The white predicate was filtered away. Most of the solvent was then removed by rotary evaporation. To the vigorously stirred concentrate was slowly added diethyl ether with a volume ten times of the concentrate in the flask. The resulted white precipitate was filtered and dried under vacuo and 1.6 g white product **3**, i.e. DDPCP was obtained. The product **1** and **3** was characterized by ^{1}H NMR (DMSO-d_6) (Figure S1). The end product DDPCP (**3**) was further analyzed by HPLC-MS (Figure S2). DDPCP (1mg) was dissolved in 2 mL methanol (HPLC), the mobile phase was 50% acetonitrile+50% water. A sharp peak appeared at 2′48″, corresponding to an organic compound with a molecular weight of 335.10 which is the exact M_w of the product **3** minus 1,3-propanediol and combined with a hydrogen ion in the positive ion mode of MS. The protection groups of the DDPCP (Mw=374.15) was removed since there was trifluoroacetic acid in the mobile phase (Inset in Figure S2).

Chemical modification M13 viruses with DDPCP (3)

To prepare M13 virus coupled with phenylboronic acid (PBA), i.e. M13-PBA, M13 viruses were dispersed in phosphate buffer (pH 8.0, 100 mM) to form 2 mg mL^{-1} suspensions. To 18 mL such suspension placed in an ice-water bath, 200 μL DDPCP (**3**) in DMF (235 mg mL^{-1}) was added dropwise under strong stirring. The reaction was further kept in the ice-water bath and stirred for another 30 min. The temperate was then brought to 10 °C and kept at such temperature overnight. The reaction mixture was dialyzed in a dialysis tube (MWCO 10k Da) against 100 mM PBS buffer of which the pH was adjusted to 5.0 for two days. During dialysis, the buffer was constantly replaced. During this procedure, the protection groups of the PBA moieties can be removed to free the two hydroxyl groups of the boronic acid. After this, the M13-PBA suspension was further transferred into the targeted

buffer by dialysis. Cycles of centrifugation at 100000g for about 6h were used to concentrate the virus, if necessary.

MALDI-TOF MS of the coat protein P8 of M13 virus and M13-PBA

To 24 μL M13 or M13-PBA suspension (1 mg mL^{-1}) was added 6 μL guanidinium chloride (6M) and incubated for 5 mins at room temperature to denaturate the virus. After this, the mixture was filtered through a Millipore $ZipTip_{\mu\text{-}C18}$ tip to remove the salts. To collect the coat proteins P8, a mixture of TFA (0.1%) and ACN (90 %) was used as the eluent. The P8 proteins was spotted onto a MALDI plate using sinapic acid as the matrix and analyzed on a MALDI-TOF AutoflexIII LRF200-CID mass spectrometer (Bruker). Several previous works have mentioned the difficulty in analyzing boronic acid containing peptides or proteins due to the dehydration and formation of anhydride (boroxine).[3] Anyhow, additional peaks appear in the mass spectrum of the coat protein of M13-PBA while only a single peak corresponding to the unmodified P8 exists in that of M13 (Figure S3). The assignment of several peaks is possible by taking into account dehydration or detachment of the phenylboronic acid (PBA) moieties.[3b] For instance, the weak peaks at 5463 m/z can be assigned to the coat protein p8 modified by one DDPCP with the PBA moieties in the trigonal form ($PhB(OH)_2$); the peak at 5445.6 m/z can be assigned to the coat protein p8 modified by one DDPCP minus one OH; the peak at 5336.7 m/z can be assigned to the coat protein p8 modified by one DDPCP from which the $PhB(OH)_2$ is subtracted. The other peaks should be due to the complicated intermediates formed in the procedure of analysis under the conditions, which are necessary for MALDI-TOF MS of the coat protein P8 of M13 in order to avoid irreversible aggregation of the denaturized coat proteins p8. The number of the PBA moieties on each virus was calculated by comparing the area of peaks of the natural P8 and these coupled with PBA.

Fluorescence measurement

For the Alizarin Red S (ARS) fluorescence,[4] M13-PBA was suspended in phosphate buffer (100 mM, pH=7.4). To 200μL 10 mg/mL such suspension, 7 μL ARS solution of various concentration in the same buffer was added. The final concentration of the ARS was in the range of 2.5 ~ 30 μM. After incubation for 10 min, the fluorescence spectra were recorded at T= 25 °C. The excitation wavelength is 469 nm and scanning range is in the 500-700nm.

For the catechol titration of M13-PBA in the presence of ARS, 200μL aqueous mixture in phosphate buffer (100 mM, pH=7.4) was prepared, containing 10 mg mL^{-1} M13-PBA and 20 μM ARS. To such mixture, 7 μL catechol in the same buffer was added and the end concentration of catechol was controlled in the range of 0 ~ 60 μM. After incubation for 10 mins, the fluorescence spectra were recorded at T= 25 °C with an excitation wavelength of 469 nm and scanning range of 500-700nm.

For the endogenous tryptophan fluorescence of the coat protein p8, 1.3 mg mL^{-1} M13 or M13-PBA in phosphate buffer (100 mM, pH = 7.4) was used. The excitation wavelength is 295 nm and the emission scanning range is 300-400 nm. The results listed in Figure S4.

Circular dichroism (CD) spectroscopy of viruses

CD spectra were obtained on a MOS-500 spectropolarimeter (Bio-Logic, France). The virus concentrations used for the CD measurements was 0.1 mg mL^{-1} in phosphate buffer (100 mM, pH=7.4). The CD spectra were registered at 25 °C in the region from 200 to 400 nm, averaging over three scans and subtracting contributions from corresponding blanks. The scan speed is 1 nm/min scan and the band width is 2.0 nm band width. The results for M13 and M13-PBA are listed in Figure S4A.

Dynamic light scattering of viruses under difference conditions

Dynamic light scattering (DLS) measurements of the viruses were carried out on a laser light scattering goniometer (BI-200SM, Brookhaven, USA) equipped with a digital correlator (BI-10000AT) and a laser source of 532 nm. The correlation functions were collected at a scattering angle of 90° and T = 25°C. All samples for DLS were filtered through a 0.45μm Millipore filter. The concentration of the virus was diluted to 0.05 mg mL^{-1}. The intensity distribution of hydrodynamic diameter was reported by using cumulant analysis. The samples at the pH 7.4 was suspended in the phosphate buffer (100 mM, pH = 7.4) while carbonate buffer (200 mM, pH 10) was used for the samples at the pH 10.

To investigate the influence of diols, M13-PBA suspensions and diol solutions in the same buffer were filtered through a 0.45μm Millipore filter respectively into a clean cuvette and gentle shaking was applied to mix the two filtered solutions. Incubation for 10 mins was carried out before any DLS measurements. The concentration of M13-PBA in the mixture was kept at 0.05 mg mL^{-1} and the concentration of diols was 3 mM. The results are listed in Figure S5.

Apparent zeta potential

The apparent electrophoretic zeta potentials of viruses were determined on the Brookhaven ZetaPALS instrument (Brookhaven, USA). M13 viruses with or without chemical modifications were dispersed in aqueous buffer to form 0.2 mg mL^{-1} suspensions. The aqueous buffers were 1.1 mM PBS (pH 7.0) or 2 mM carbonate buffer (pH 10.2). The average electrophoretic mobility from five runs consisting of 15 cycles per run was converted to the zeta potential (ξ), using the Smoluchowski formula ($\xi = 4\pi\eta\mu/\varepsilon$, where μ, η and ε are the mobility of the particle, the viscosity and the dielectric constant of the dispersion, respectively).[5] The results are reported in Table S1.

Transmission electron microscopy (TEM) of viruses

For the TEM, diluted M13-PBA viruses was dropped onto a carbon-coated copper electron microscopy (EM) grid and negatively stained by standard 2% uranyl acetate solution. In the case of M13-PBA in the presence of glucose, the sample for the dynamic light scattering measurement was used. Transmission electron microscopy (TEM) was performed on a Philips CM200 microscope.

Atomic force microscopy of modified viruses.

To the smooth surface of a freshly cut mica was added 20 μL M13-PBA suspension with a concentration of 10^{-4} mg mL^{-1}. One of the edge of the mica was attached vertically to a filter paper to blow away some of the buffer during which the virus suspension formed a homogeneous film on the mica surface. This step turned out to align the virus particles and was extremely useful for the visualization of the viruses by AFM. The mica was dried for two days under vacuum and at room temperature. AFM images were obtained with a Nanoscope IV atomic force microscopy (Veeco) operating in the tapping mode under ambient conditions using etched silicon cantilever tips.

Isotropic-nematic phase transition of the (modified) M13 virus

Home made crosspolarizors was used to check the birefringence of the virus suspensions. Concentrated M13 or M13-PBA suspensions in Tris-HCl-NaCl buffer (pH 8.2, ionic strength = 110 mM) were prepared and then diluted with the same buffer to the coexistence region between the isotropic (*I*) and nematic (*N*) phase (*I*-*N* transation). The vials containing the suspensions were left alone until macroscopic phase separation occurred, with the lower birefringent part the nematic phase and the the upper part the isotropic phase. Aliquots of samples from the two phases were taken for the concentration determination (Table S1).

Observation of the nematic LC phase and measurement of the pitch of the cholesteric LC phases

Concentrated M13 or modified virus suspensions in various buffers and with a concentration in the range for the formation of the nematic LC phase were prepared and filled into freshly cleaned glass capillaries with a OD 1.5 mm. Both ends of the glass capillary were then sealed with flame to avoid solvent evaporation. The samples in the capillaries were equilibrated for at least three days before any observation. The structure of the (chiral) nematic LC phase of the samples in the capillaries was then monitored on a polarizing optical microscopy (Olympus BX41). The "fingerprint" texture, if exists, was recorded with a CCD camera (Qimaging micropublisher 3.3 RTV) and analyzed with the software ImageJ. The cholesteric pitch, *P*, was determined. For each sample, the reported *P* value is an average of about fifty measurements sampling the different areas along the whole capillary. Three capillaries were measured for each case.

General protocol for investigating the influence of diols on the cholesteric LC phase of M13-PBA or M13.

To 3 mL M13 or M13-PBA suspension in the carbonate buffer (pH 10.2, 200 mM) was added 20 μL catechol and derivatives or fructose. The mixture was bubbled with argon for 30 mins in dark to remove oxygen in order to avoid oxidation driven self-polymerization of the catechol and derivatives.[6] Binding of the diols to the surface phenylboronic acid moieties was performed in dark for 1 hrs. The mixture was then concentrated by ultrafiltration centrifugation in the Amicon Ultra-4 Centrifugal Filter Devices (MWCO 100KDa, Milipore). The final concentration of the virus was 30 mg mL^{-1} which are deep in the range of the nematic phase. The final concentration of the diols ranged from 2 ~ 40μM. To investigate the nature of the nematic LC phase, the final mixture was filled into a glass capillary which was flame sealed. The capillary was observed in the following three months (See the section titled "Observation of the Nematic LC phase and measurement of the pitch of the cholesteric LC phases").

Rheology measurement of concentrated virus suspensions

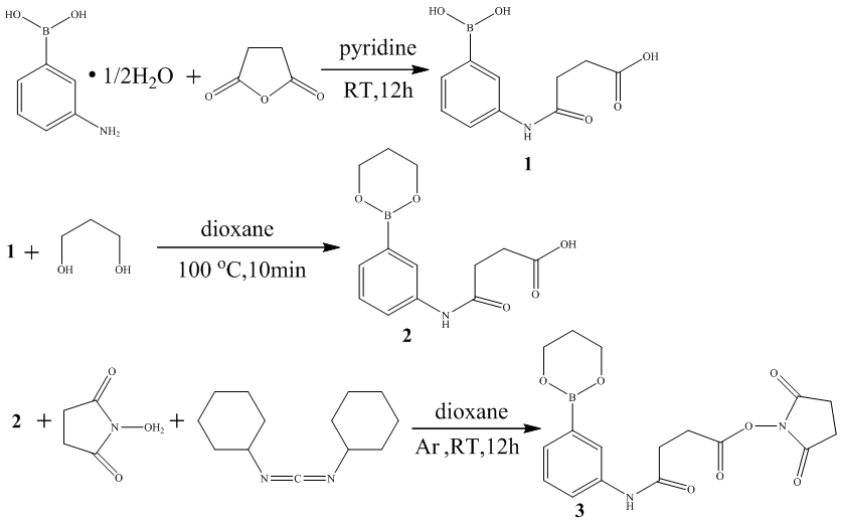
The steady state viscosity versus shear rate of the concentrated virus suspension was measured on a AR-G2 rheometer (TA Instruments). A double wall Couette geometry was used due to the low visicosity of the samples. M13, M13-PBA or M13-PBA in the presence of dopamine were measured. The concentration of M13-PBA is 30 mg mL^{-1} dispersed in carbonate buffer (200 mM carbonate buffer, pH =10.2). The results are listed in Figure S8.

Supplymental results

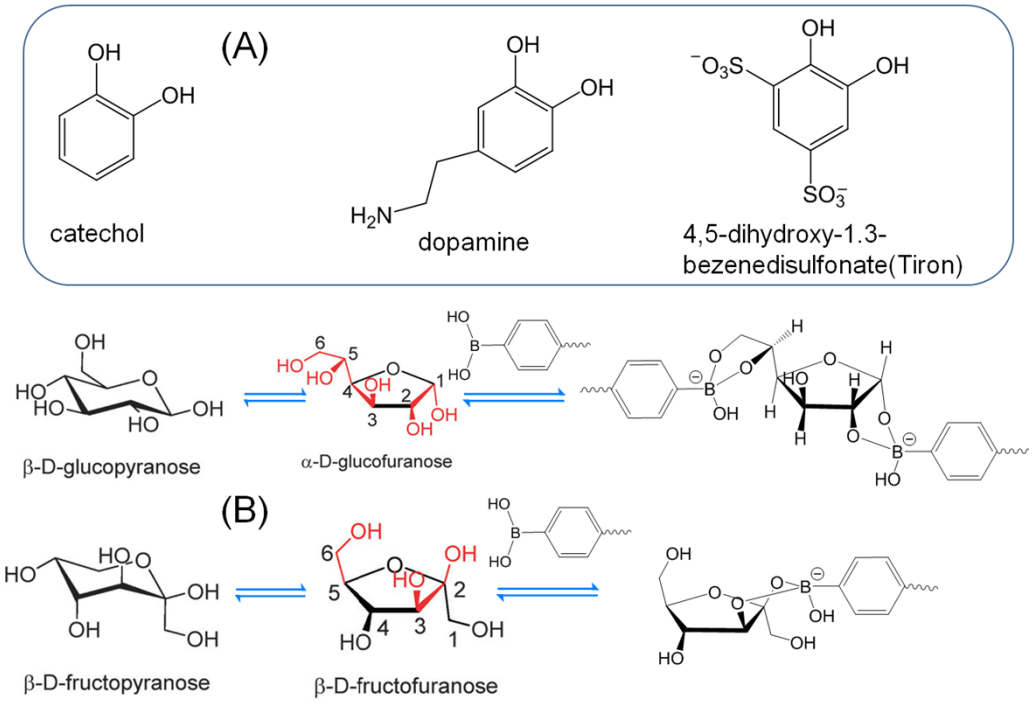
Table S1. Some general properties of the (Modified) M13 viruses

Sample	ζ (mV)[a]		*I/N* (mg mL^{-1})[b]	$K_{b,app}$ (M^{-1})[c]			
	pH = 7.0	pH = 10.2		ARS	Catechol	Fructose	Glucose
M13	-40.49	-45.34	20.8/25.9	--	--	--	--
M13-PBA	-32.25	-52.08	21.3/26.2	6800±200	4650± 330	230±55	85±39

[a] Apparent zeta potentials. The aqueous buffers for pH 7.0 and 10.2 were 1.1 mM PBS and 2 mM carbonate buffer, respectively. [b] Virus concentration in the isotropic (*I*) and nematic phase (*N*) at the *I-N* phase transition equilibrium. The buffer for the the *I-N* transition is Tris-HCl-NaCl buffer (pH 8.2, I =110 mM). [c] The apparent binding constant of diols to the PBA moieties on the virus surface was determined by the fluorogentic method based on Alizarin red S (ARS).[4] $K_{b,app}$ is the average value of triplicate measurements.



Scheme S1. Synthesis of 2,5-Dioxopyrrolidinyl 3-[N-(3-(1,3,2-Dioxaboran-2-yl)phenyl)carbamoyl]propanoate (DDPCP)



Scheme S2. (A) Molecular structure of catechol and derivatives; (B) Molecular structure of the pyranose and furanose form of glucose and fructose. Complexes of the monosaccharide with a phenylboronic acid derivatives are also shown.

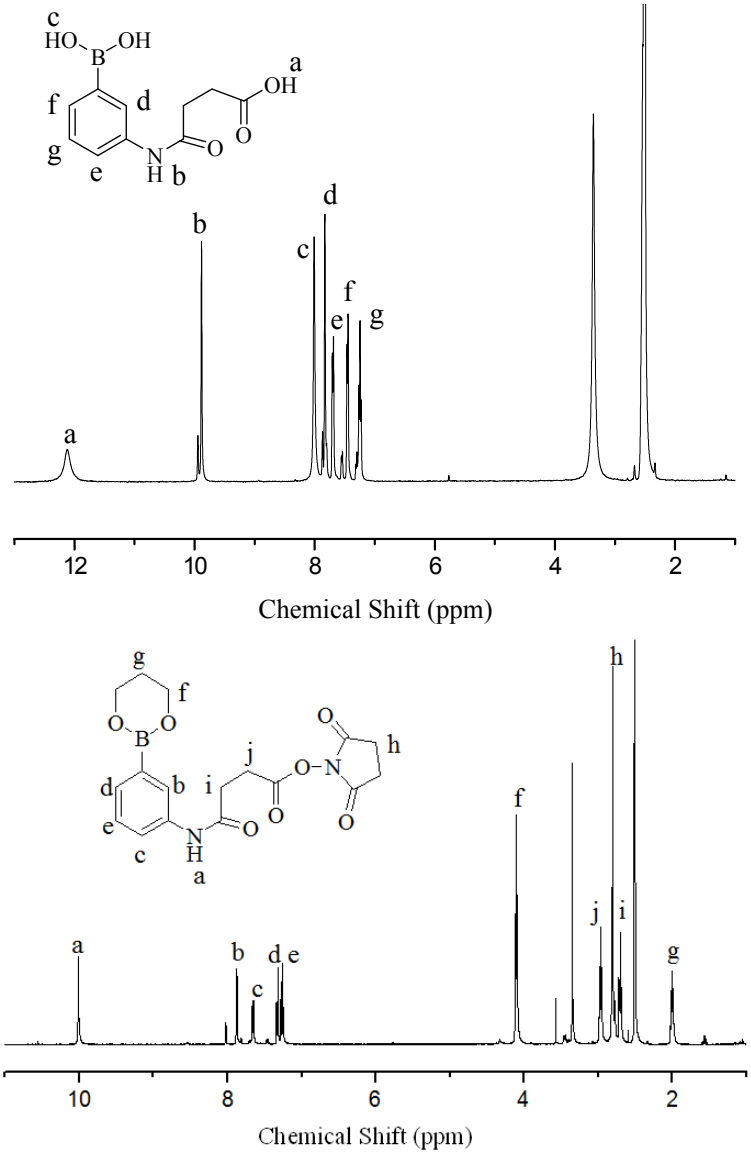


Figure S1. ^{1}H NMR spectra (DMSO-d_6) of product **1** and **3**.

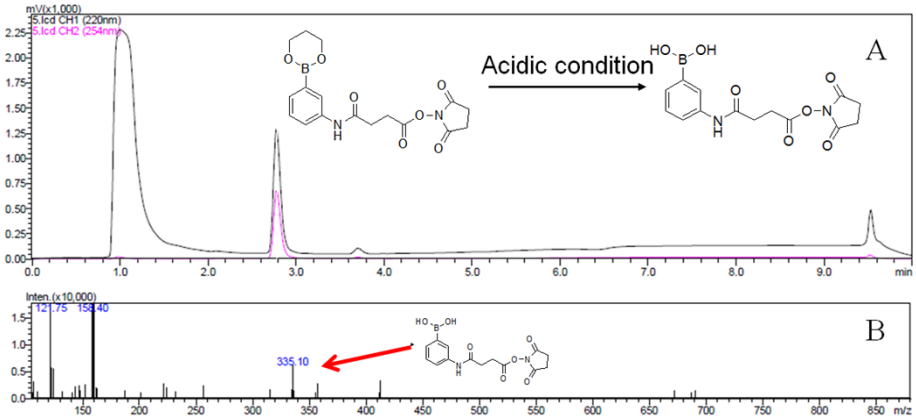


Figure S2. Analysis of DDPCP (3) by HPLC-MS. DDPCP (**3**)(1mg) was dissolved in 2 mL methanol for the HPLC and the mobile phase was 50% acetonitrile+50% water with some trifluoroacetic acid. (A) A sharp peak appeared at 2′48″ in the HPLC. (B) The molecular weight of the organic compound corresponding to the sharp peak in (A) is 335.10 which is the exact Mw of the product 3 minus 1,3-propanediol and combined with a hydrogen ion in the positive ion mode of MS.

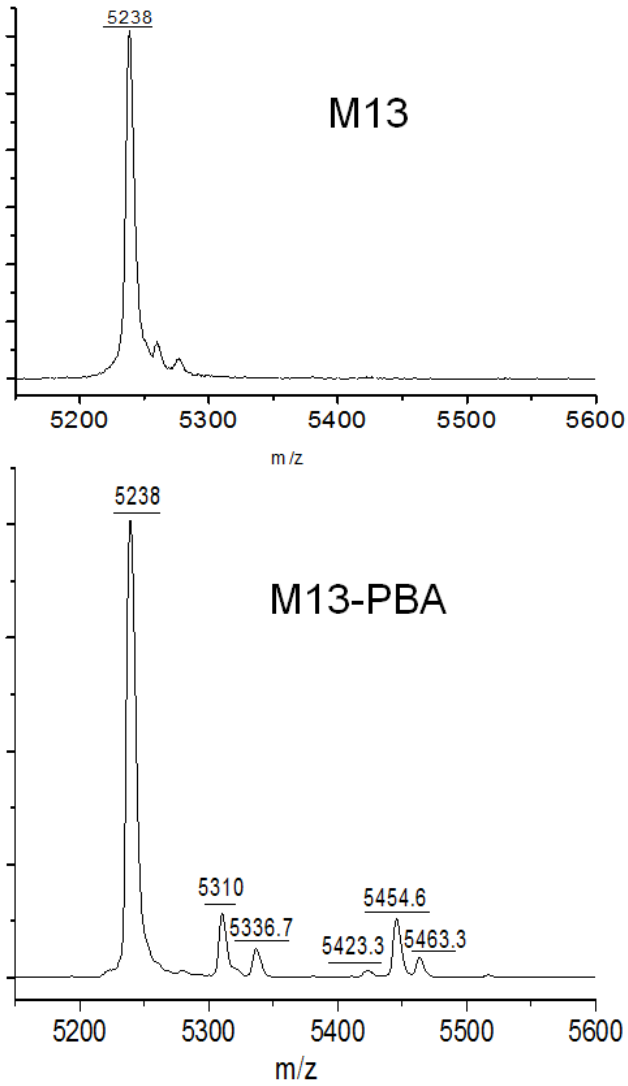


Figure S3. MALDI-TOF MS of the coat protein P8 of M13 and modified M13 (M13-PBA). The numbers nearby each peak are the corresponding m/z values.

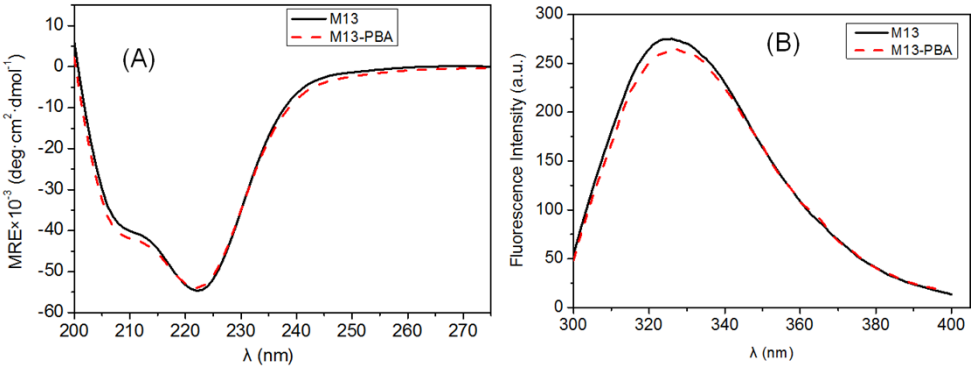


Figure S4. Circular dichroism (CD) spectroscopy (A) and the endogenous tryptophan fluorescence spectra of the coat protein p8 (B) of M13 and M13-PBA.

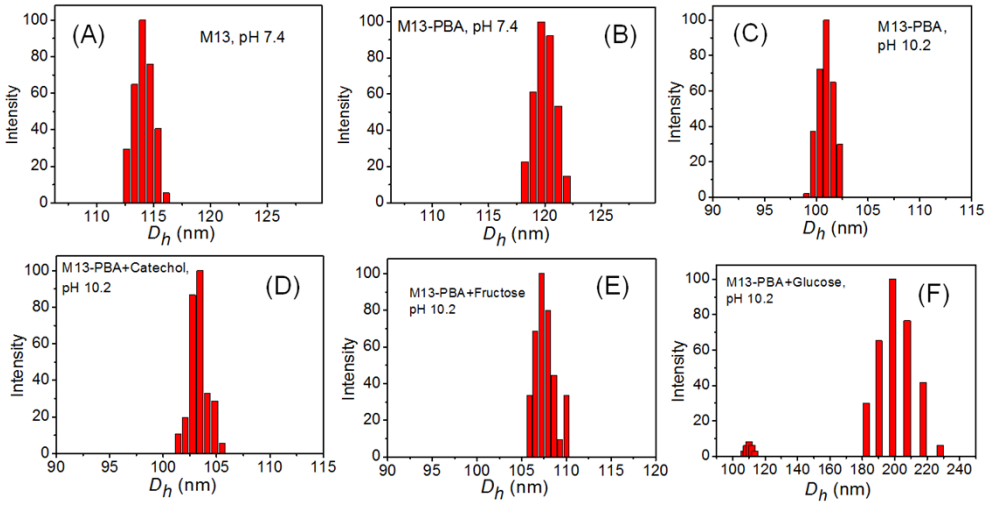


Figure S5. Distribution of the apparent hydrodynamic diameter (D_h) of M13 or M13-PBA under different conditions obtained from DLS. M13-PBA+Catechol (D), +Fructose (E), +Glucose (F) specify measurements were performed in the presence of catechol, fructose and glucose, respectively, after incubating for 10 mins. The concentration of M13-PBA in the case of virus + diols was kept at 0.05 mg mL^{-1} and the concentration of diols was 3 mM.

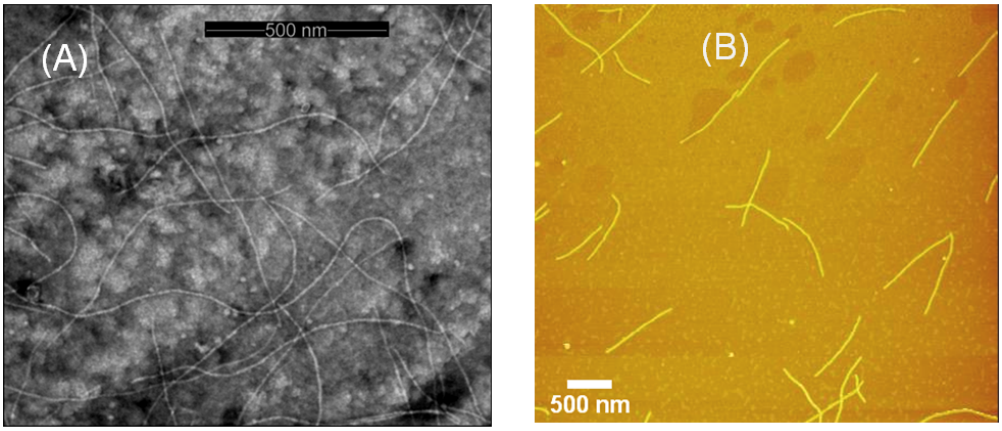


Figure S6. Transmission electron microscopy (A) and atomic force microscopy (B) of of M13-PBA.

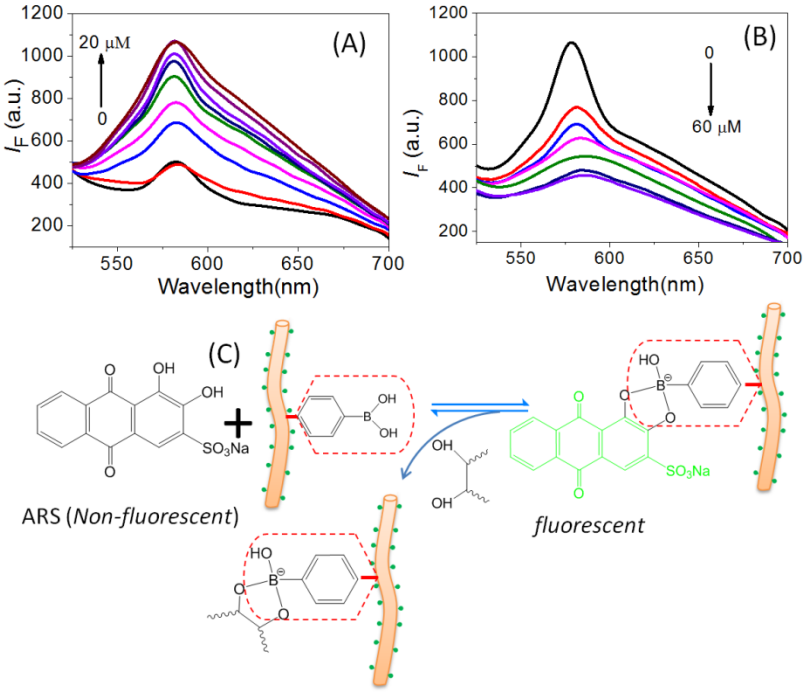


Figure S7. (A) Fluorescent behaviour of Alizarin red S (ARS) in the presence of M13-PBA. Varied amount of ARS was added to the M13-PBA suspension in PBS buffer (100mM, pH 7.4). The fluorescent intensity (I_F) increased with increasing amount of ARS and saturated above certain concentration. Such phenomena clearly highlight the solvent exposure and chemical accessibility of the PBA moieties on the virus surface. (B) Fluorescent titration by catechol. Varied amount of catechol was added to a mixture of M13-PBA and ARS in PBS buffer (100mM, pH 7.4). When diols such as catechol was added to the mixture of M13-PBA and ARS which showed fluorescent emission, the fluorescent intensity decreased with increasing amount of diols, due to the replacement of the ARS by the competitive diols. This result confirms the binding capability of the viral surface PBA moieties to diols. (C) Schematic illustration of the reversible fluorescent emission behavior of Alizarin Red S via binding with boronic acid moieties to form a boronate complex or being replaced by diols.

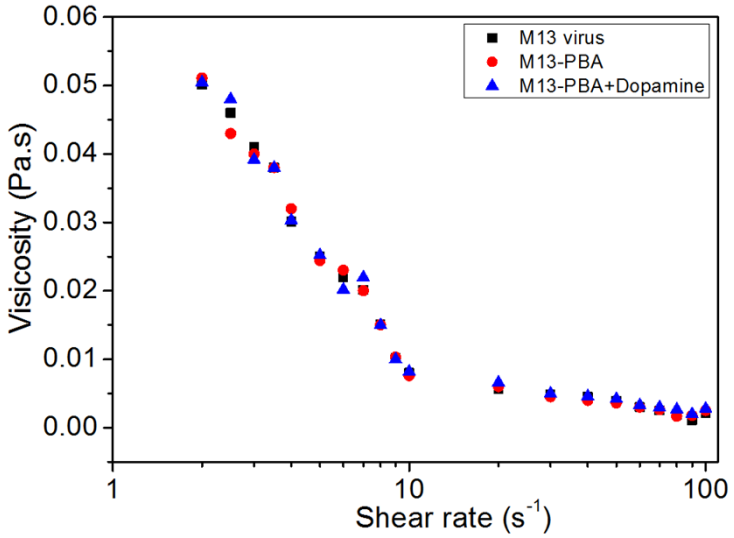


Figure S8. Shear viscosity as a function of shear rate for M13 virus and virus modified with the derivatives of PBA in the presence or absence of dopamine.

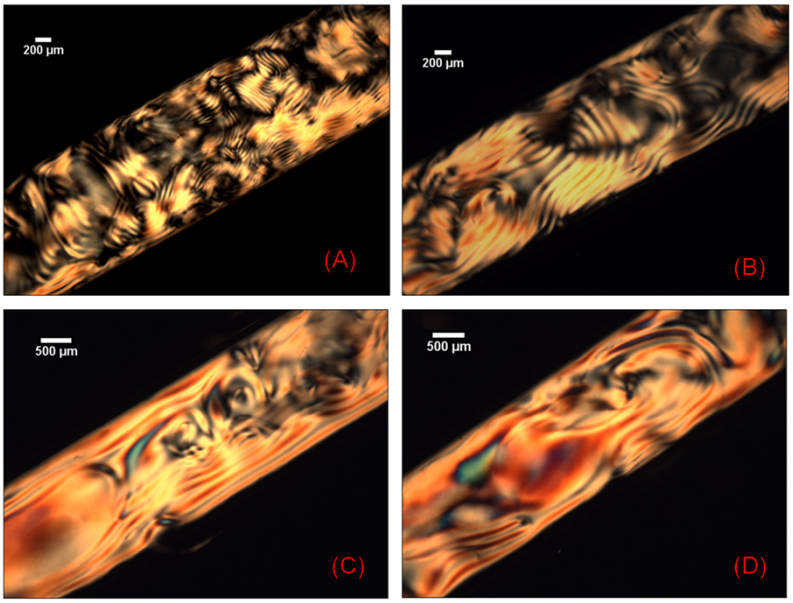


Figure S9. Influence of diols on the cholesteric LC phase of M13-PBA. The M13-PBA suspension has a concentration of 30 mg mL^{-1} in carbonate buffer (pH 10.2, 200 mM), which contains catechol with the following concentration: 5 (A), 10 (B),15 (C), 20 μM (D).

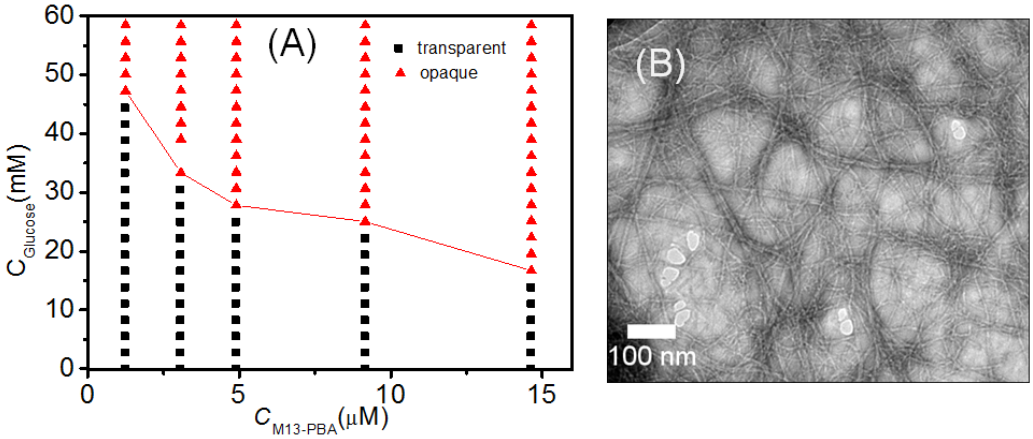


Figure S10. Solution behavior of M13-PBA in the presence of glucose. (A) Apparent phase diagraph determined by visual observation of the state of M13-PBA at varied ratio of glucose to M13-PBA. (B) TEM photograph reveals bundles of virus in the presence of glucose.

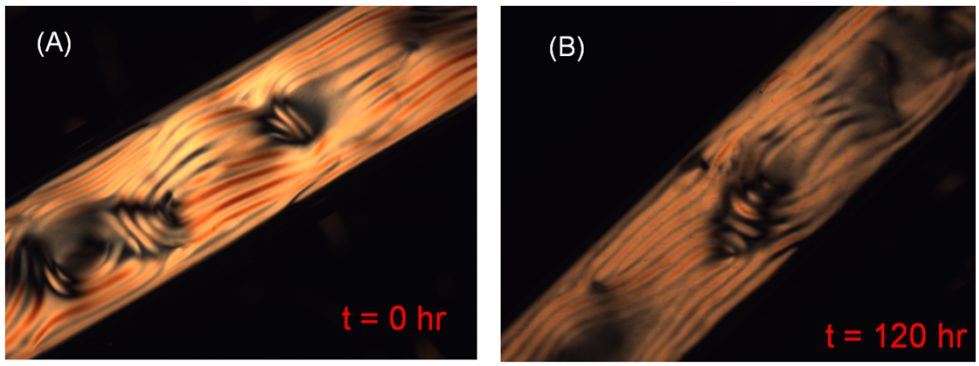


Figure S11. Results of the control experiment of Figure 3 in the main text. The M13-PBA was filled in a glass capillary which was immerged into a carbonate buffer containing no any catechol. The typical texture of the chiral nematic LC phase of M13-PBA still exists after long-term storage in the carbonate buffer containing no any catechol. The slight difference of the texture between (A) and (B) can be attributed to the annealing effect of the storage which normally improves the fingerprint texture of the chiral nematic LC phase.

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