

Identification of a β CD-Based Hyper-Branched Negatively Charged Polymer as HSV-2 and RSV Inhibitor

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Supplementary Material

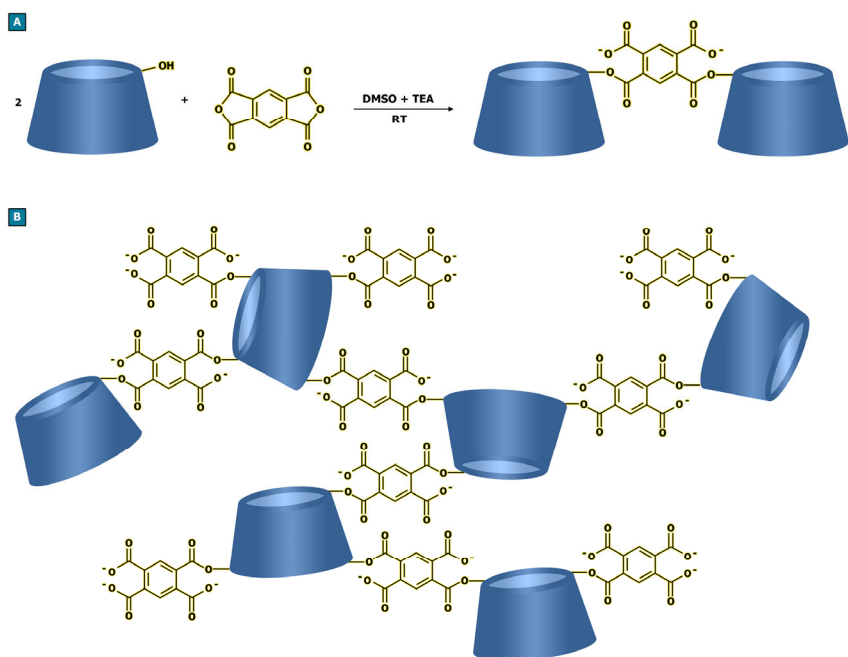


Figure S1. (A) Reaction occurring between β CD and PMDA, (B) P_{PMDA} proposed structure.

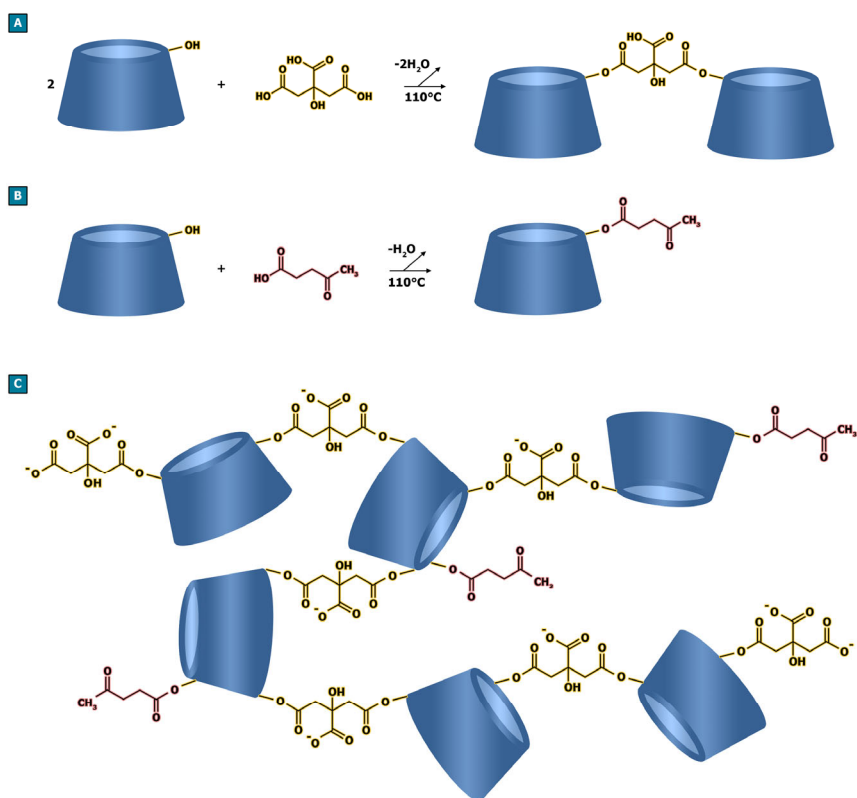


Figure S2. (A) Reaction occurring between β CD and CA, (B) Reaction occurring between β CD and LA, (C) P_{CA_LA} proposed structure.

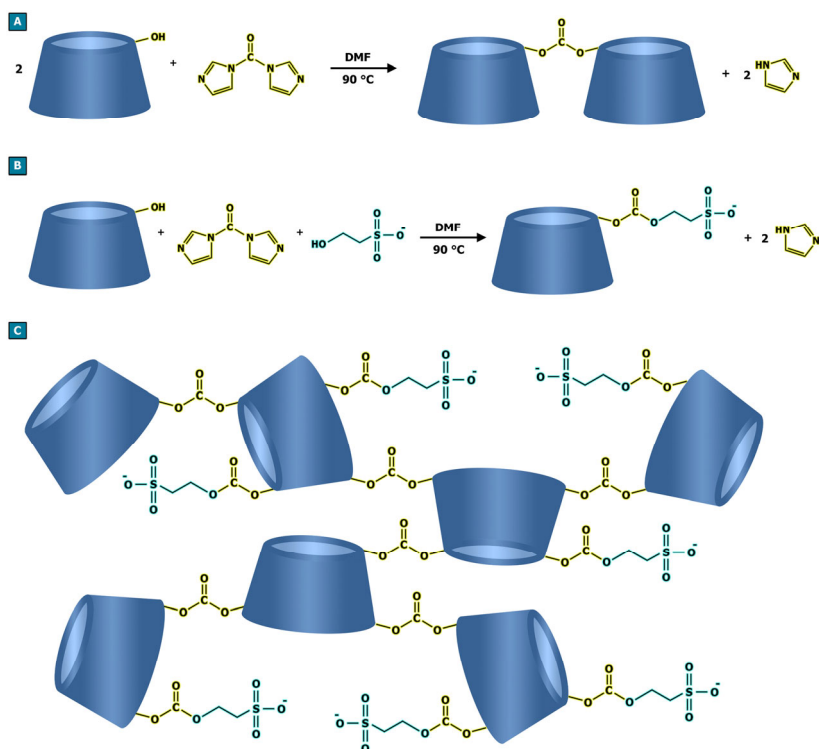


Figure S3. (A) Reaction occurring between β CD and CDI, (B) Reaction occurring between β CD, CDI, and ISE, (C) P_CDI_ISE proposed structure.

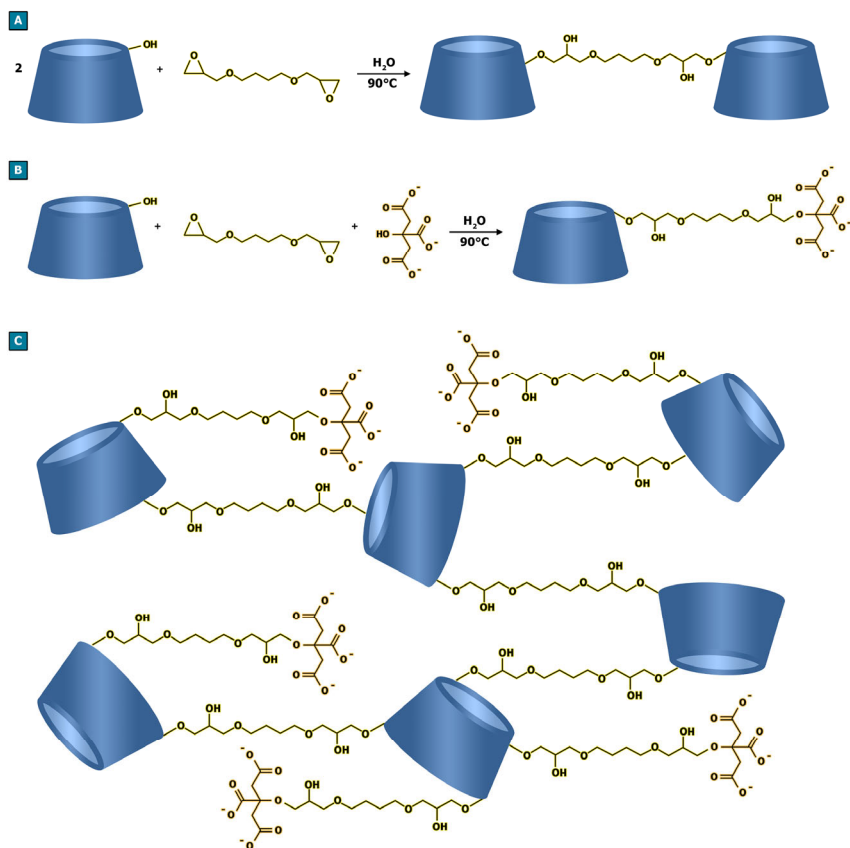


Figure S4. (A) Reaction occurring between β CD and BDE, (B) Reaction occurring between β CD, BDE, and CIT, (C) P_BDE_CIT proposed structure.

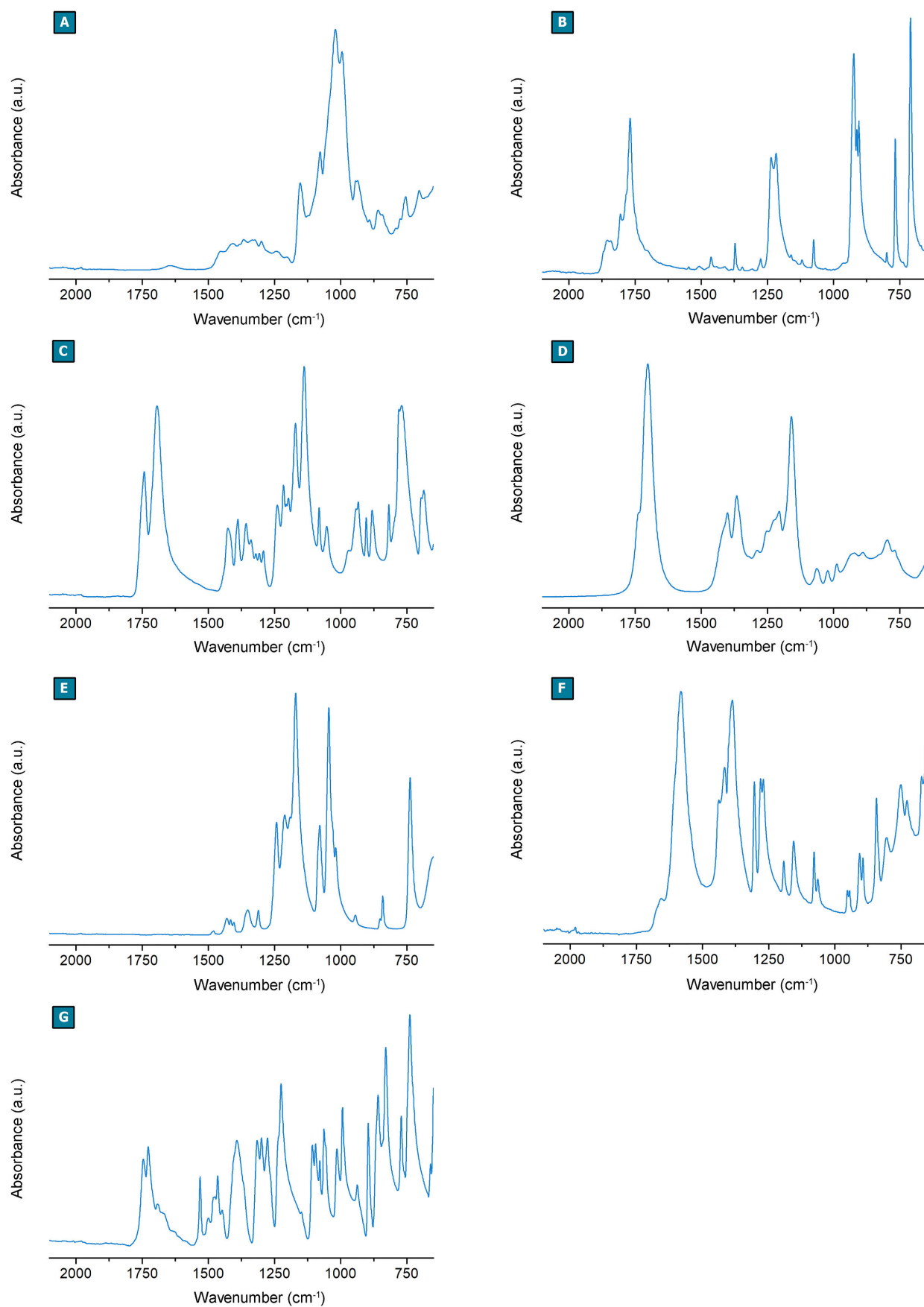


Figure S5. FTIR-ATR spectra of (A) β CD, (B) PMDA, (C) CA, (D) LA, (E) ISE, (F) CIT, (G) CDI.

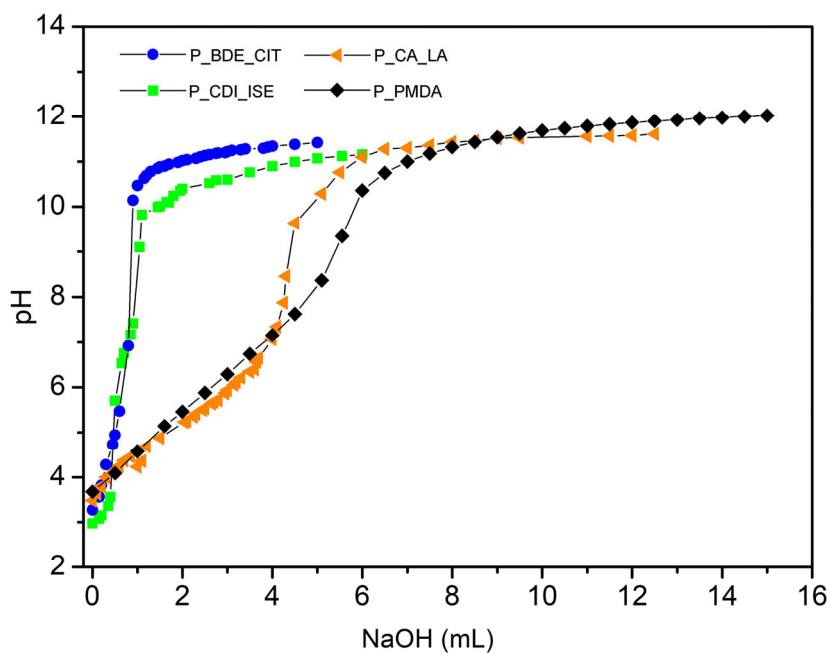


Figure S6. Potentiometric titration curves.

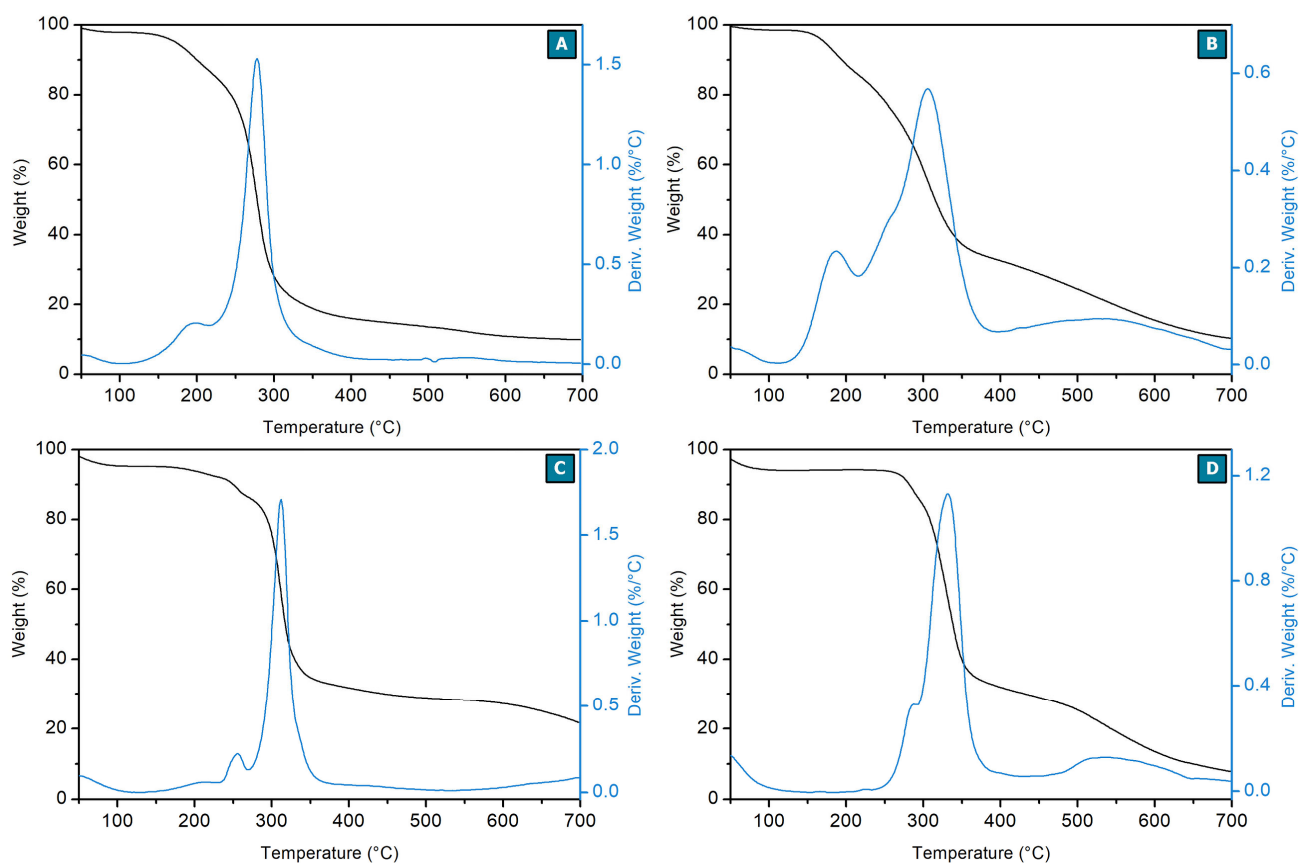


Figure S7. TGA and DTGA of (A) P_PMDA, (B) P_CA_LA (C) P_CDI_ISE, and (D) P_BDE_CIT.

Table S1. Raw data of P_PMDA and P_PMDA₅₀ virucidal activities

	Sample	Titer (PFU/mL)	*R
RSV	Untreated	257178	
	P_PMDA (500 µg/mL)	18471	1.1
	P_PMDA (300 µg/mL)	43170	0.8
	P_PMDA (100 µg/mL)	107325	0.4
	P_PMDA ₅₀ (500 µg/mL)	30101	0.9
	P_PMDA ₅₀ (300 µg/mL)	43486	0.8
	P_PMDA ₅₀ (100 µg/mL)	200138	0.1
HSV-2	Untreated	897075	
	P_PMDA (1.5 µg/mL)	599075	0.2
	P_PMDA (15 µg/mL)	315150	0.5
	P_PMDA (100 µg/mL)	330480	0.4
	P_PMDA ₅₀ (1.5 µg/mL)	493514	0.3
	P_PMDA ₅₀ (15 µg/mL)	203016	0.6

*Logarithm of inactivation R = Log (Untreated sample) – Log (Treated sample)

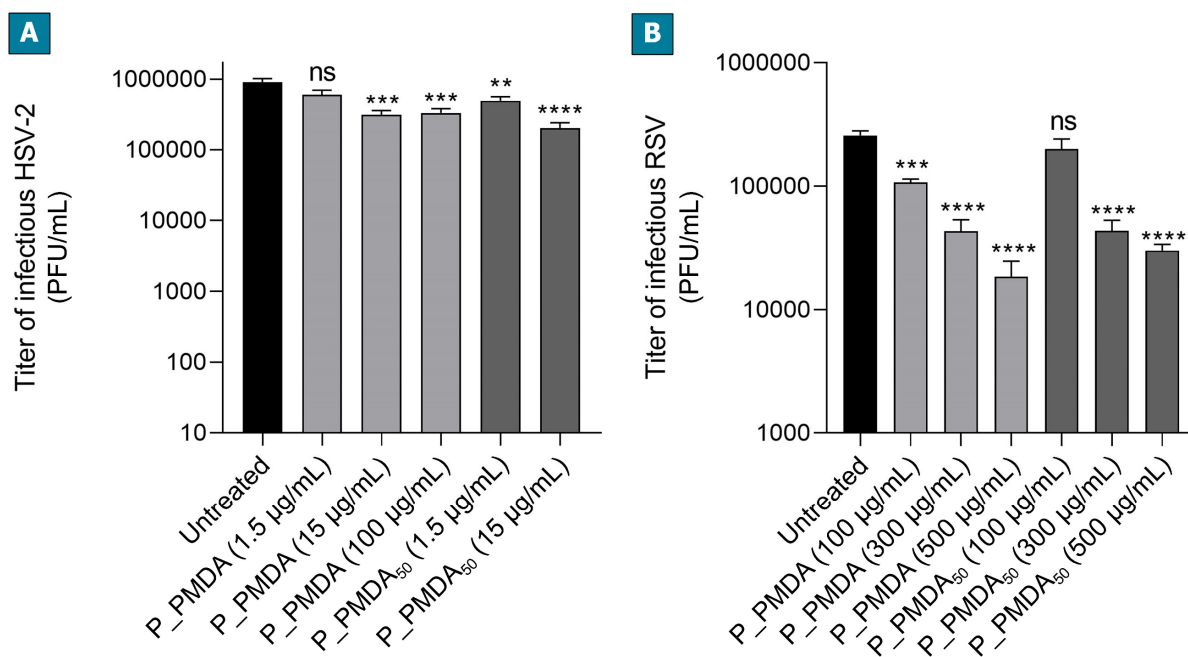


Figure S8. HSV-2 and RSV inactivation assays with additional concentrations of compounds. HSV-2 (A) and RSV (B) inactivation assays. HSV-2 or RSV infectious particles were additionally incubated with 100 µg/mL of P_PMDA or P_PMDA₅₀ for 2 hours at 37°C and the residual viral infectivity was then evaluated by titration to the non-inhibitory concentration of the polymers. Graphs including all tested doses are reported. On the y-axis, the infectious titers are expressed as plaque-forming units per mL (PFU/mL). Error bars represent standard error of the mean (SEM) of three independent experiments (ANOVA and Bonferroni Post hoc test; ns $p > 0.05$, ** $p < 0.005$, *** $p < 0.0005$, **** $p < 0.0001$)

Table S2. Raw data of P_PMDA and P_PMDA₅₀ binding inhibition activities

	Sample	Titer (PFU/mL)	*R
RSV	Untreated	7119	
	P_PMDA (500 µg/mL)	2655	0.4
	P_PMDA (300 µg/mL)	1508	0.7
	P_PMDA ₅₀ (500 µg/mL)	1917	0.6
	P_PMDA ₅₀ (300 µg/mL)	1755	0.6
HSV-2	Untreated	251809	
	P_PMDA (1.5 µg/mL)	7721	1.5
	P_PMDA (15 µg/mL)	2817	2.0
	P_PMDA (100 µg/mL)	5428	1.7
	P_PMDA ₅₀ (1.5 µg/mL)	68876	0.6
	P_PMDA ₅₀ (15 µg/mL)	13355	1.3
	P_PMDA ₅₀ (100 µg/mL)	25158	1.0
	P_PMDA ₅₀ (300 µg/mL)	5802	1.8
	P_PMDA ₅₀ (500 µg/mL)	6056	1.8

*Logarithm of inhibition R = Log (Untreated sample) – Log (Treated sample)

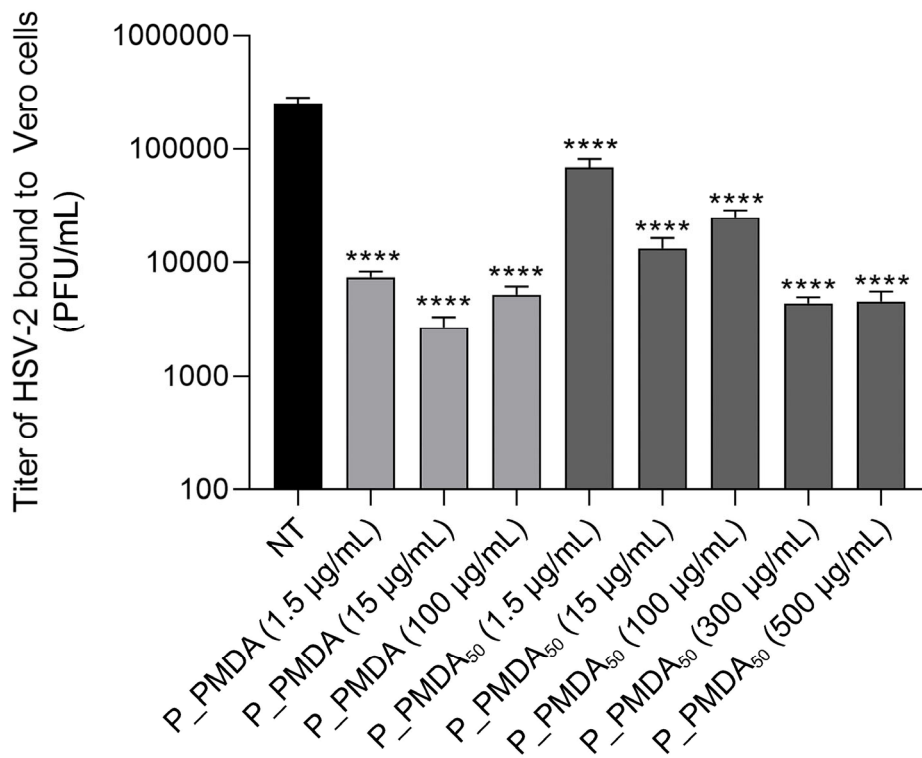


Figure S9. HSV-2 and RSV binding assays with additional concentrations of compounds. HSV-2 (MOIs = 3) was allowed to attach to cells in presence of additional 100 µg/mL of P_PMDA, or 100, 300 and 500 µg/mL of P_PMDA. The cell-bound virus titers were determined by means of titration on confluent cells. A graph including all tested doses is reported. On the y-axis, the infectious titers are expressed as plaque-forming units per mL (PFU/mL). Error bars represent standard error of the mean (SEM) of three independent experiments (ANOVA and Bonferroni Post hoc test; ****p<0.0001).