Supplemental Information

Thermostabilization of Viruses via Complex Coacervation

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Table S1. Table of counter-ions, molecular weights and polydispersity index (PDI) of polypeptides.

	Counter Ion	M_w (g/mol)	PDI[c]
K_{400}	TFA[a]	97,000	1.08
E_{400}[b]	Na^+	60,000	1.01

[a] TFA is defined as trifluoroacetate.
[b] E_n was purchased from Alamanda Polymers and is racemic, but without sequence control.
[c] PDI was determined using gel permeation chromatography, as reported by Alamanda Polymers.

PPV Coacervate Formation Recipes:

Coacervate samples containing PPV were prepared by adding the components listed in Table S2 in order from right to left into 1.5 mL Eppendorf tubes.

Table S2. Table of volumes for preparing PPV-containing complex coacervates.

Sample #	Charge Fraction K(+) (mol/mol)	Volume E400(-) (µL)	Volume K400(+) (µL)	Volume Stock Virus (µL)	Volume Buffer (µL)	Volume Water (µL)
1	0.100	151.2	16.8	4.4	6.00	61.6
2	0.300	117.6	50.4	4.4	6.00	61.6
3	0.425	96.6	71.4	4.4	6.00	61.6
4	0.475	88.2	79.8	4.4	6.00	61.6
5	0.500	84.0	84.0	4.4	6.00	61.6
6	0.525	79.8	88.2	4.4	6.00	61.6
7	0.550	75.6	92.4	4.4	6.00	61.6
8	0.600	67.2	100.8	4.4	6.00	61.6
9	0.700	50.4	117.6	4.4	6.00	61.6
10	0.900	16.8	151.2	4.4	6.00	61.6

Total sample volume (µL)	240
Concentration Stock PPV (log_{10} (MTT_{50}/mL))	7.74 for 6 log PPV coacervates 6.74 for 5 log PPV coacervates 5.74 for 4 log PPV coacervates
Concentration Stock HEPES Buffer (M)	0.40
Concentration Stock K_{400} (+) (mM)	10.0
Concentration Stock E_{400} (-) (mM)	10.0
Final Polymer Concentration (mM)	7.00

BVDV Coacervate Formation Recipes:

Coacervate samples containing BVDV were prepared by adding the components listed in Table S3 in order from right to left into 1.5 mL Eppendorf tubes.

Table S3. Table of volumes for preparing BVDV-containing complex coacervates.

Sample #	Charge Fraction K(+) (mol/mol)	Volume E400(-) (µL)	Volume K400(+) (µL)	Volume Stock Virus (µL)	Volume Buffer (µL)	Volume Water (µL)
1	0.100	151.2	16.8	25.7	6.00	40.3
2	0.300	117.6	50.4	25.7	6.00	40.3
3	0.425	96.6	71.4	25.7	6.00	40.3
4	0.475	88.2	79.8	25.7	6.00	40.3
5	0.500	84.0	84.0	25.7	6.00	40.3
6	0.525	79.8	88.2	25.7	6.00	40.3
7	0.550	75.6	92.4	25.7	6.00	40.3
8	0.600	67.2	100.8	25.7	6.00	40.3
9	0.700	50.4	117.6	25.7	6.00	40.3
10	0.900	16.8	151.2	25.7	6.00	40.3

Total sample volume (µL)	240
Concentration Stock BVDV (log_{10} (MTT_{50}/mL))	5.97 for 5 log BVDV coacervates 4.97 for 4 log BVDV coacervates
Concentration Stock HEPES Buffer (M)	0.40
Concentration Stock K_{400} (+) (mM)	10.0
Concentration Stock E_{400} (-) (mM)	10.0
Final Polymer Concentration (mM)	7.00

The recovery of the virus was calculated as:

$$Recovery\ (\%) = \frac{C_c \times V_c}{C_i \times V_i} \times 100 \qquad \text{(S1)}$$

where, C_c is the virus concentration in the coacervate phase, V_c is the virus volume in the coacervate phase, C_i is the initial virus concentration, and V_i is the initial virus volume in the overall system.

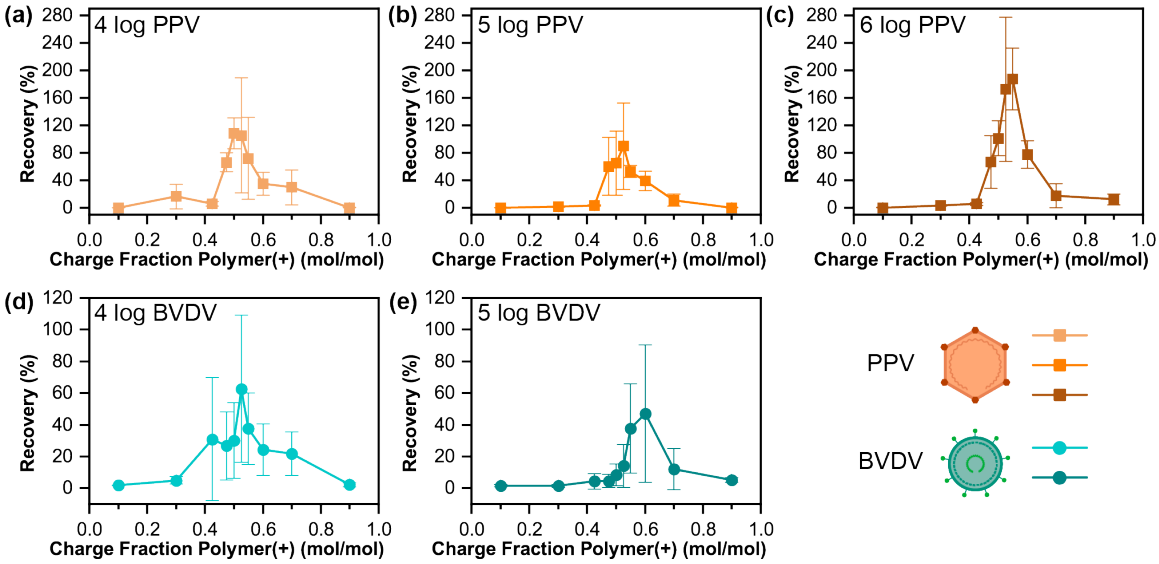

Figure S1. Recovery of live **(a-c)** PPV and **(d-e)** BVDV in the coacervate phase at different total virus loadings. All data points are the average of three separate tests and error bars represent the standard deviation.

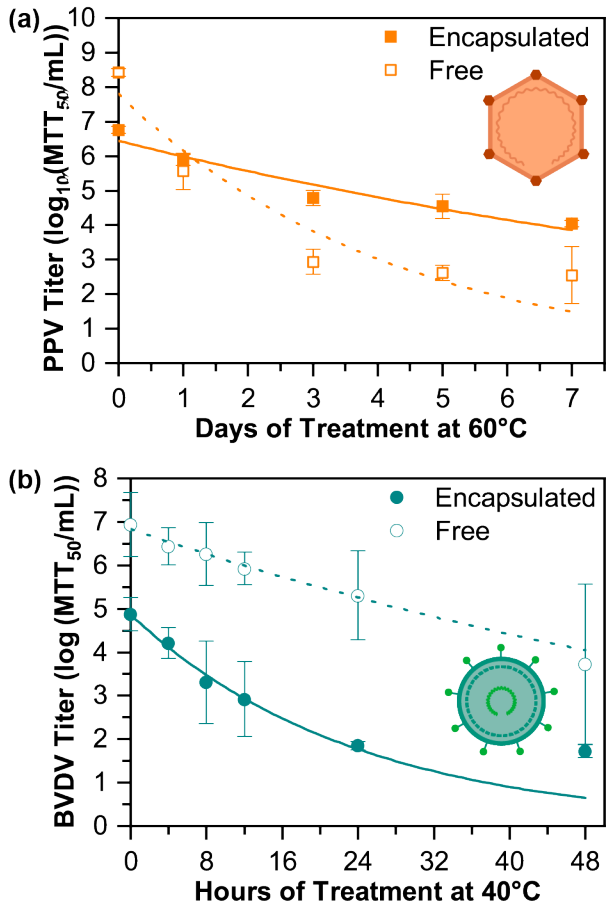

Figure S2. The infectivity loss of free and encapsulated **(a)** PPV and **(b)** BVDV. All data points are the average of three separate tests and error bars represent the standard deviation. The lifetime of encapsulated and free PPV at 60°C is 14 and 4 days, respectively. The lifetime of encapsulated and free BVDV at 40°C is 24 and 92 hrs, respectively. Encapsulated PPV is prepared at charge fraction 0.5, while encapsulated BVDV is prepared at charge fraction 0.6.

Cytotoxicity experiments were performed using the same approach as described in the experimental section to test the cytotoxicity of the various individual peptides and the resulting coacervate. Cells were seeded in a 96-well plate in 100 μL of media well. Stock solutions of 35 mM K_{400} and E_{400} on a monomer basis were made in 50 mM HEPES at pH 8.0 ± 0.03. A similar stock coacervate solution of a 1:1 mixture of K_{400}/E_{400} was also prepared. After one day of incubation, 25 μL of the relevant stock solution was added to the first well, and mixed (a 1:5 dilution). Subsequent samples were prepared via serial dilution, transferring 25 μL from one well to the next for a total of 8 conditions for each of the individual peptides and the coacervate. A control sample of cells with HEPES buffer, and a blank consisting of media and buffer were also run. The MTT assay was then run after 5 days of incubation. All samples were run in triplicate with each biological replicate having three technical replicates, each of which were measured three times at 550 nm.

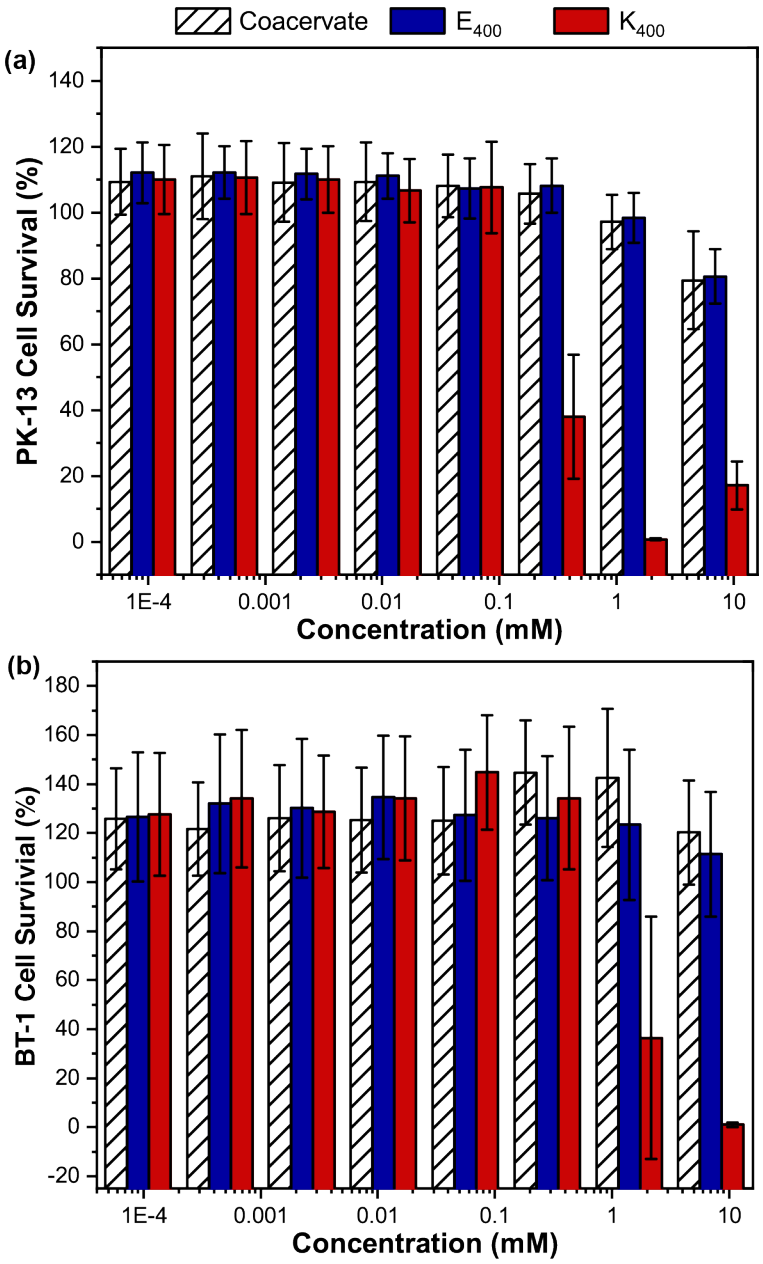

Figure S3. Cytotoxicity of polypeptides measured using an MTT assay in the **(a)** PK-13 cells used to study PPV and **(b)** BT-1 cells used for BVDV. Polypeptide concentrations indicated are on a monomer basis.