**SUPPLEMENTARY INFORMATION**

**Single-particle chemical force microscopy to characterize virus surface chemistry**

**Additional Materials & Methods**

**Materials.** Potassium phosphate monobasic (molecular biology grade, ≥99.0%), sodium phosphate monobasic monohydrate (ACS grade, 98.0-102.0%), and sodium chloride (ACS grade, ≥99.0%) were a gift from Millipore Sigma (MA, USA). Sodium phosphate dibasic heptahydrate (ACS grade, 98.0-102.0%), 12-mercaptododecanoic acid (HS (CH2)11COOH, 96%), 1-dodecanethiol (HS(CH2)11CH3, ≥ 98%), (11-mercaptoundecyl)-N,N,N-trimethylammonium bromide (HS(CH2)11N(CH3)3Br), and primary amine functionalized silica nanoparticles (NH2-NPs <30 nm (DLS)) were purchased from Sigma-Aldrich (MO, USA). N-hydroxysulfosuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Thermo Fisher Scientific (MA, USA).

All aqueous solutions or buffers were prepared using purified water with a resistivity of ≥18 MΩ·cm from a Nanopure filtration system (Thermo Scientific) and filtered with a 0.2 µm bottle top filter (VWR, Radnor, PA) or a 0.2 µm syringe filter (VWR) prior to use. A standard phosphate buffered saline (PBS) (pH 7.2) was prepared by dissolving 0.21 g potassium phosphate monobasic, 0.726 g sodium phosphate dibasic heptahydrate, and 9 g sodium chloride into 1000 mL Nanopure water. A 20 mM phosphate buffer (PB) (pH 7.0) was prepared by dissolving 1.0763 g sodium phosphate monobasic monohydrate and 3.2705 g sodium phosphate dibasic heptahydrate into 1000 mL of Nanopure water.

Porcine parvovirus (PPV) strain NADL-2 was a gift from Dr. Ruben Carbonell (North Carolina State University, NC, USA). Bovine viral diarrhea virus (BVDV) strain NADL was purchased from USDA APHIS.

**Table S1** Model virus properties.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Virus** | **Capsid** | **Family** | **Nucleic acid** | **Size (nm)** | **pI** | **Related human viruses** | **References** |
| **Porcine parvovirus (PPV)** | Non-enveloped | Parvoviridae | ssDNA | 18-26 | 4.8-5.1 | B-19 human parvovirus | [1, 2] |
| **Bovine viral diarrhea virus (BVDV)** | Enveloped | Flaviviridae | ssRNA | 40-60 | 4.3-4.5 | Hepatitis C | [1-3] |

**Table S2** AFM probes summary.

|  |  |  |  |
| --- | --- | --- | --- |
| Application | AFM probe name | Tip Radius (nm) | Spring constant (N/m) |
| Topographic image | Bruker ScanAsyst-Fluid+ silicon nitride | 2 | 0.7 |
| NT-MDT CSG30 | 6 | 0.6 |
| Force measurement | Bruker AC-40 | 10 | 0.1 |
| NT-MDT CSG10/Au | 35 | 0.11 |

**References**

1. Mi X, Bromley EK, Joshi PU, Long F, Heldt CL. Virus isoelectric point determination using single-particle chemical force microscopy*.* *Langmuir* 36(1), 370-378 (2020).

2. Norkin LC. *Virology: molecular biology and pathogenesis*. ASM press, (2010).

3. Kim IS, Choi YW, Lee SR. Optimization and validation of a virus filtration process for efficient removal of viruses from urokinase solution prepared from human urine*.* *J. Microbiol. Biotechnol.* 14(1), 140-147 (2004).