Interactions between Rotavirus and Suwannee River Organic Matter: Aggregation, Deposition, and Adhesion Force Measurement

Supporting Information

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Materials and Methods

Solution chemistries and reagents

Deionized (DI) water (Millipore, Barnstead, USA) of an 18 MΩ-cm resistivity was used for preparing all the solutions for deposition,aggregation, and AFM experiments. The unadjusted pH of fresh DI water remained stable for the entire duration of the QCM, TR-DLS, and AFM experiments (3 hours, 4 hours, and 1 hour respectively). Analytical grade NaCl, $CaCl_2$, $MgCl_2$, poly-L-lysine (PLL) hydrobromide, and HEPES buffer were utilized in this research. HEPES buffer was prepared with 100 mM NaCl and 10 mM N-(2-hydroxyethyl) piperazine-N′-2-ethanesulfonic acid at a final pH of 5.9. PLL hydrobromide solution was prepared in HEPES buffer at a final concentration of 0.1 g/L. All electrolyte solutions and HEPES buffer were filtered through a 0.22 μm sterile cellulose acetate membrane and sonicated for 30 minutes before use. The polyglutamic sodium salt (PLG) with a molecular weight ranging from 50,000–100,000 g/mol (Cat# P4886, Sigma) was prepared in solution by adding 25 mg of PLG to 4.2 g of DI water.

Suwannee river natural organic matter (NOM, International Humic Substances, IHSS, St. Paul, MN) was used as a dissolved organic matter model. The procedure for NOM solution preparation was previously described.[1] Total dissolved organic carbon (DOC) concentration of the NOM stock solution was measured using a Phoenix 8000 TOC analyzer (Dohrmann, USA) at 101.4 mg C/L. The NOM stock was stored at 4^oC and covered from light by aluminum foil. All the solutions were kept at pH 5.9 with the exception of the solutions used for studying the effect of pH. For these experiments, pH was adjusted to 8.3 using NaOH. The average D_h of NOM in 1 mM NaCl solution measured every 20 seconds for 240 minutes was 2 nm and 1 nm for 2 consecutive TR-DLS experiments. However, due to the small size of NOM and its fluorescent

nature we believe that fluorescence correlation spectroscopy would be a more sensitive technique for estimating the size of NOM in solution.[2]

Rotavirus preparation and focus forming unit (FFU) infectivity assays

Purification of rotavirus was conducted by sequential centrifugation and filtration as described previously.[3] While care was taken for virus purification, it is possible that protein contamination remained in the virus stock used for this study. Due to the biological nature of virus, it is impossible to obtain pure virion without influencing virus infectivity or causing aggregation.[4-6] Enumeration of rotavirus was carried out using FFU infectivity assays.[7] The stock concentration was ~5×10^6 FFU/ml and was stored at 4^oC in a 1 mM NaCl and 0.1 mM $CaCl_2$ solution. Calcium in rotavirus stock was kept above the critical free calcium concentration to avoid solubilization of outer capsid proteins VP4 and VP7.[8] This membrane-purified rotavirus stock was also used in our previous study.[9] After preparation the virus stock was carefully aliquoted and stored for almost 2 years with no significant change in infectivity or hydrodynamic diameter. Another rotavirus stock was grown and purified using CsCl gradient method described previously[10] to a final concentration of ~10^8 FFU/mL. Standard SDS-PAGE was carried out for the rotavirus stock using 7.5% Mini-PROTEAN TGX Precast minigels stained overnight using SYPRO Ruby protein gel stain according to the manufacturer's instructions (Bio-Rad, Hercules, CA). Bands were analyzed for molecular weight using a Gel-Doc imager (Bio-Rad, Hercules, CA).

Electrophoretic mobility (EPM) measurements

A ZS90 Zetasizer instrument and clear disposable cells (Malvern, UK) were used to determine the EPM of rotavirus in solution containing 20 mg C/L and a broad range of salt concentrations (5 mM to 600 mM for NaCl, 5 mM to 200 mM for $CaCl_2$ and $MgCl_2$) at an

unadjusted pH of 5.9 at room temperature (~25^{o}C). At least three measurements were conducted for each salt concentration. Membrane-purified rotavirus was added to these solutions to a final concentration of 8×10^{5} FFU/mL, which ensured an optimal signal for EPM measurements.

QCM sensor cleaning and QCM deposition protocol

Cleaning protocol before each experiment was as follows: quartz sensors were soaked for 2 hours in a 2% Hellmanex II cleaning solution (Hellma GmbH & Co. KG, Mullheim, Germany), thoroughly rinsed with DI water, dried with ultrapure N_2, and oxidized in an Ozone/UV chamber for 30 minutes (BioForce Nano-sciences, Inc., Ames, IA). The electrolyte solutions were injected into the QCM system using a precision syringe pump (Kd Scientific Inc., Holliston, MA) operating at a withdrawal mode at a 0.1 mL/min flow rate.

After a stable baseline was established in water, the QCM sensors were sequentially coated with PLL and then NOM as described previously.[1, 11] After coating, the system was equilibrated with 2 mL of electrolyte solution (NaCl, $CaCl_2$, $MgCl_2$) at the concentration of interest (i.e., 0.1, 0.3, 0.5, 0.7, or 1 mM). Equilibrium (frequency shift with time <0.1 Hz/min) was obtained after the addition of this 2 ml of electrolyte solution. This step was followed immediately by virus adsorption experiments, which were performed by flowing 2 mL of rotavirus suspensions at a concentration of 8×10^{5} FFU/ml and in the same electrolyte concentration as the previous step. For non-repulsive conditions, rotavirus deposition rates on PLL-coated silica surface in $MgCl_2$ and $CaCl_2$ solutions from our previous research[9] were used in this investigation.

Surface preparation for AFM experiments

The quartz and silica surfaces were first cleaned by immersion in 2% Hellmanex (Hellma Analytics, USA) solution for 30 minutes and subsequently rinsed in excess with DI water. Next,

approximately 300 μL of 98% sulfuric acid with 30 g/L nochromix solution were pipetted on top of the surfaces for 24 hours and then removed. The surfaces were finally rinsed in excess with DI water. NOM-coated surfaces were prepared following the layer-by-layer protocol introduced previously.[11, 12] The PLL coating protocol of the silica surface (QCM sensor) was conducted by pipetting 300 μL of PLL hydrobromide solution prepared in HEPES buffer at a final concentration of 0.1 g/L and left undisturbed for 24 hours. Next, the PLL solution was removed and the surface was rinsed with DI water. Similarly, PLL layer was coated by pipetting 300 μL of approximately 240 mg C/L SRNOM solution or 6 g/L of PLG solution and left undisturbed for 24 hours. The SRNOM solution was then removed and the surface was rinsed with DDI water. For some selected experiments, the PLL layer was coated by pipetting 300 μL of solution containing ~1×10^{8} FFU/ml rotavirus and left undisturbed for 8 hours. The viral solution was then removed and the surface rinsed with DI water.

Rotavirus-coated membranes were prepared following a modified procedure previously used for oocysts.[13] Briefly, 10 mL of ~5×10^{6} FFU/ml rotavirus solution were vacuum-filtered onto a 13 mm in diameter piece of 0.05 μm polycarbonate track-etched membrane (Whatman Nucleopore, USA) and afterwards carefully rinsed with DI water. A layer of water was maintained to prevent rotavirus exposure to air. After this filtration step, the membrane was immediately glued by the edges to a glass slide. Rotavirus coating of the membrane was checked by tapping mode imaging at a scan rate of 0.5 Hz with a chromium-gold-coated silicon nitride probe with a spring constant of ~0.27 N/m (Budget Sensors, Bulgaria).

Samples of rotavirus-coated silica probe were similarly prepared by the layer-by-layer method described above.[11, 12] Twenty μL of PLL hydrobromide solution prepared in HEPES buffer at a final concentration of 0.1 g/L were added as a drop on top of the glass slide. Using a

DMI5000M Leica inverted microscope (Leica, Germany) and a 10× lens, only the probe was carefully introduced to the PLL drop and allowed to coat for 6 hours. The probe was then removed from the PLL drop and rinsed with DI water. Following the same procedure, the probe was carefully introduced in a 20 μL ~5×10^6 FFU/ml rotavirus drop and allowed to coat for 6 hours. The probe was then removed from the virus solution drop and rinsed with DI water. The preparation for the rotavirus-coated silica surface was described above.

Interaction force measurement protocols

For the first set of control experiments, the coating completeness of surfaces was tested using a silicon nitride (SiNi) tip with 20 nm tip radius (0.24 N/m, NP series, Bruker, USA). The approaching curves were separately measured in 1 mM $NaHCO_3$ solution at a buffered pH of 8.3 with the following surfaces: a) ultra-pure quartz; b) QCM silica sensors (i.e., silica surface); c) silica surface coated with PLL; d) silica surface coated sequentially with PLL and then NOM or PLG or rotavirus. Ultrapure quartz surface (Cat # 26016, 19×19×0.5 mm thick, Ted Pella, USA) was used as a reference for negatively charged surface. Note that zeta potential of this surface has been measured in our previous work (-2.53 to -0.18 $\mu m cm V^{-1} s^{-1}$ at 1 and 200 mM NaCl, respectively).[12] QCM silica sensor surface (QSX 303 silica sensor, Q-Sense, Sweden) was used in most AFM experiments because this surface was also used in deposition experiments.

For the second set of control experiments, a 1 μm silica sphere mounted on a silicon nitride tip-less cantilever with a spring constant of ~0.06 N/m (Novascan Technologies, USA) was used. For the first subset of this control set, we obtained approaching force curves for the silica sphere probe with quartz surface in solution with and without 20 mg C/L NOM. The solution also contained either 1 or 10 or 100 mM NaCl at unadjusted pH 5.9. For the second subset of this control, we obtained approaching force curves for the following cases: 1) silica

probe and rotavirus-coated membrane; 2) silica probe and rotavirus-coated silica surface; 3) rotavirus-coated silica probe and silica surface. This 1 μm silica sphere was used for AFM force measurement as recommended in previous studies reviewed by Butt et al.,[14] so that nanometer scale roughness of the substrate did not influence AFM force measurement.

For the third set of experiments, approaching and retracting force was obtained for rotavirus-coated silica probe with one of the following surfaces: 1) rotavirus-coated membrane; 2) NOM-coated silica surfaces; and 3) PLG-coated silica surfaces. We used unadjusted pH 5.9 solution containing 0 or 20 mg C/L NOM and 1 or 10 or 100 mM NaCl, or 33.3 mM $MgCl_2$, or 33.3 mM $CaCl_2$ to study interactions between rotavirus and rotavirus. The interaction between rotavirus and NOM was studied using solution composition similar to the ones used for QCM experiments, i.e., solution containing 3 mM NaCl or 1 mM $MgCl_2$ or 1 mM $CaCl_2$. For the experiment using 1 mM $CaCl_2$, we used two pH conditions: unadjusted pH 5.9 or 1 mM bicarbonate buffered solution at pH 8.3. The interaction between rotavirus and PLG was studied using solution containing 1 mM $CaCl_2$ at unadjusted pH 5.9 or 1 mM bicarbonate buffered solution at pH 8.3.

Results and Discussion

Control experiments for PLL and NOM coating completeness on silica surface

Representative force curves are shown in Figure 4S. As shown in Figure 4aS, electrostatic repulsion was observed in 1 mM bicarbonate buffer solution at pH 8.3 when the SiNi tip was approaching the negatively charged quartz surface. This control experiment was conducted first to ensure that the SiNi tip was negatively charged in 1 mM bicarbonate solution at pH 8.3. This solution condition and the SiNi tip was further used to determine the surface charge of silica sensor surface and the polycarbonate membrane. The negative charge of the

159 silica surface and the polycarbonate (PC) membrane was evidenced because repulsive
160 electrostatic forces were recorded (Fig. 4aS).

161 Coating completeness of positively charged PLL on silica surface was confirmed for 25
162 approaching force curves along a 0.7 cm^2 area when attractive electrostatic forces were detected
163 using a sharp SiNi tip of 20 nm-curvature-radius; adhesion was also always detected during
164 retraction (Fig. 4bS). Finally, negatively charged NOM coverage on PLL-coated silica surface
165 was confirmed when repulsive forces were recorded during approaching force curves between
166 the SiNi probe and NOM layer (Fig. 4cS). Similar to NOM -coated surfaces, the PLG-coated
167 and the rotavirus-coated silica surfaces also showed electrostatic repulsion (Fig. 4cS and 4dS,
168 respectively). These results suggest that the coating protocol completely covered the positively
169 charged PLL layer relative to the 20-nm tip radius of the SiNi probe used over 0.7 cm^2 probing
170 area of the studied surface. Note that the SiNi probe is 2 times smaller than the rotavirus
171 particles. Thus, the surface coating is considered complete on the length scale of the SiNi probe.
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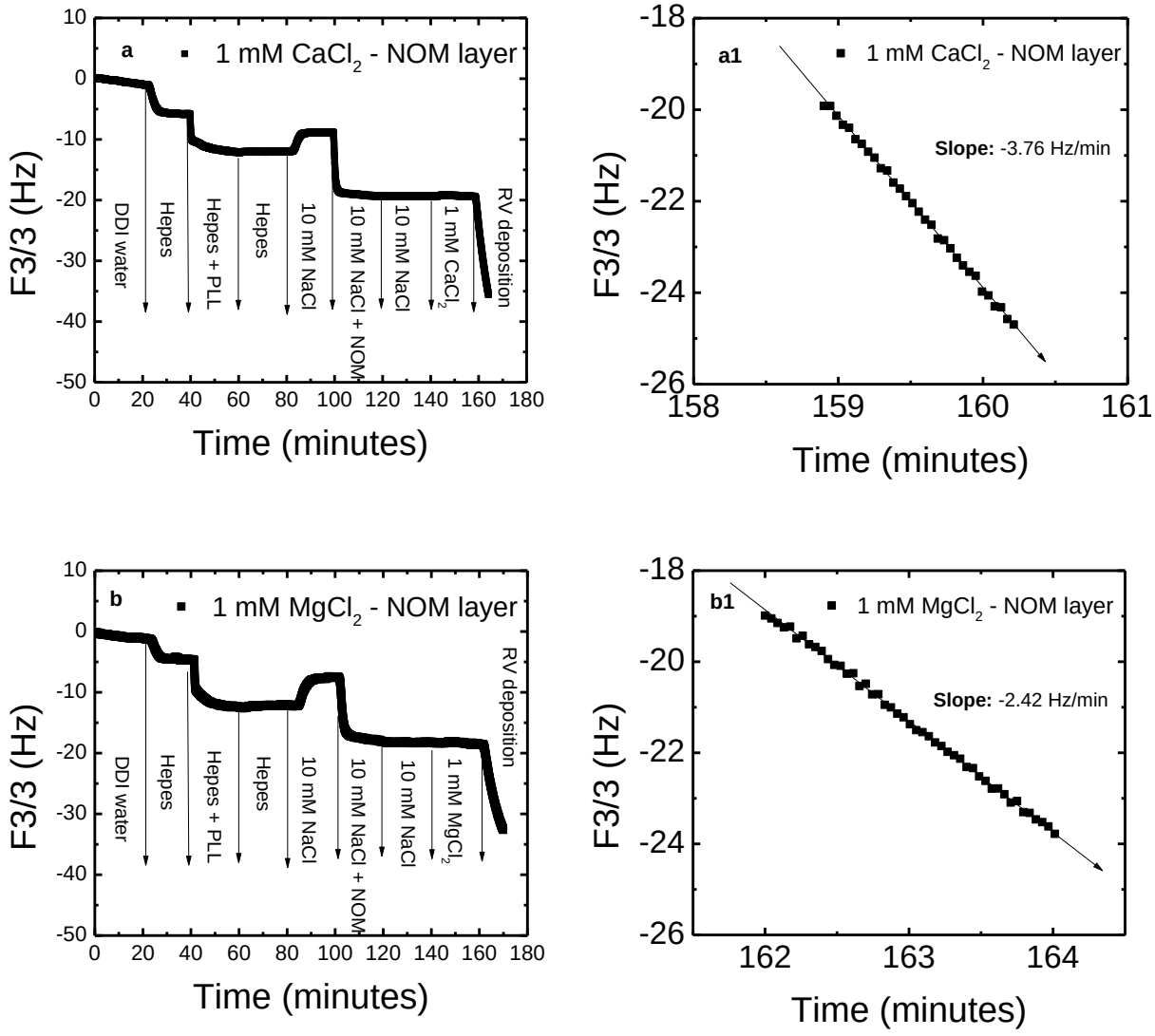
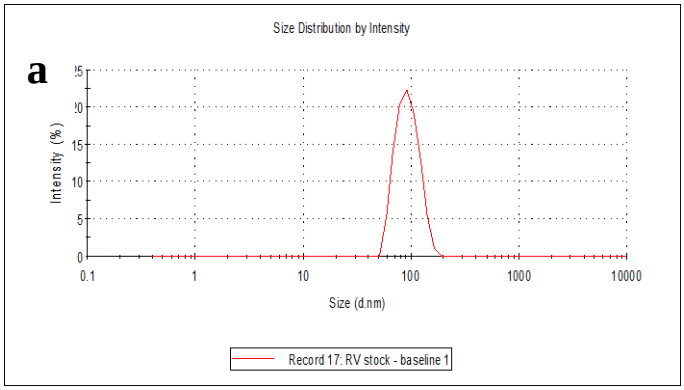
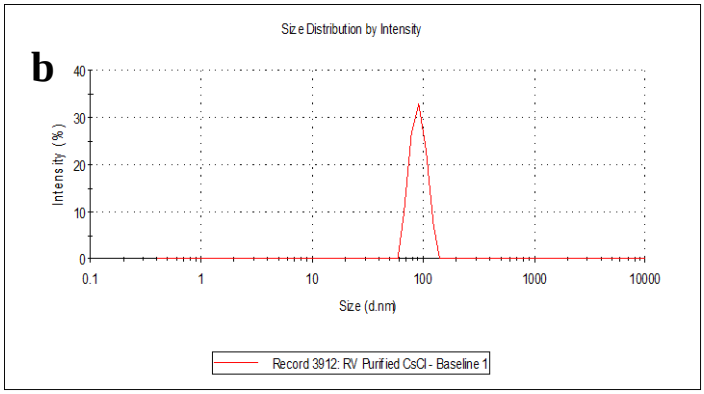


Figure 1S. Measurement of deposition of RV on SRNOM layer in solution containing **a)** 1 mM $CaCl_2$ or **b)** 1 mM $MgCl_2$ with their corresponding deposition rate calculation (**a1** and **b1**). Rotavirus concentration was ~8×10^5 FFU/mL in solution at 25°C.





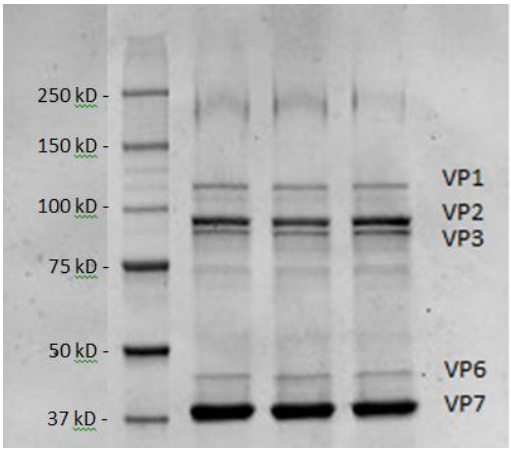


Figure 2S. Measurement of hydrodynamic diameter of rotavirus purified following **a)** dialysis-concentration using Amicon ultrafiltration membrane cell, **b)** CsCl gradient method, and **c)** SDS-PAGE gel for CsCl-purified rotavirus. The mixture of rotavirus, Laemmli sample buffer, and 2-mercaptoethanol was heated at 100°C for 5 min. Then 5-µL of the mixture was loaded and the gel was run at 200 V constant voltage until the dye front reached the line near the bottom edge of the gel cassette for approximately 34 min. Each sample was loaded in triplicate, i.e., three lines on the right. A 5-uL sample of Biorad unstained protein standard solution was loaded with each gel, i.e., left band. SYPRO Ruby protein gel stain was used. Small amount of other proteins, which may be broken pieces of rotavirus protein produced during purification, is also present.

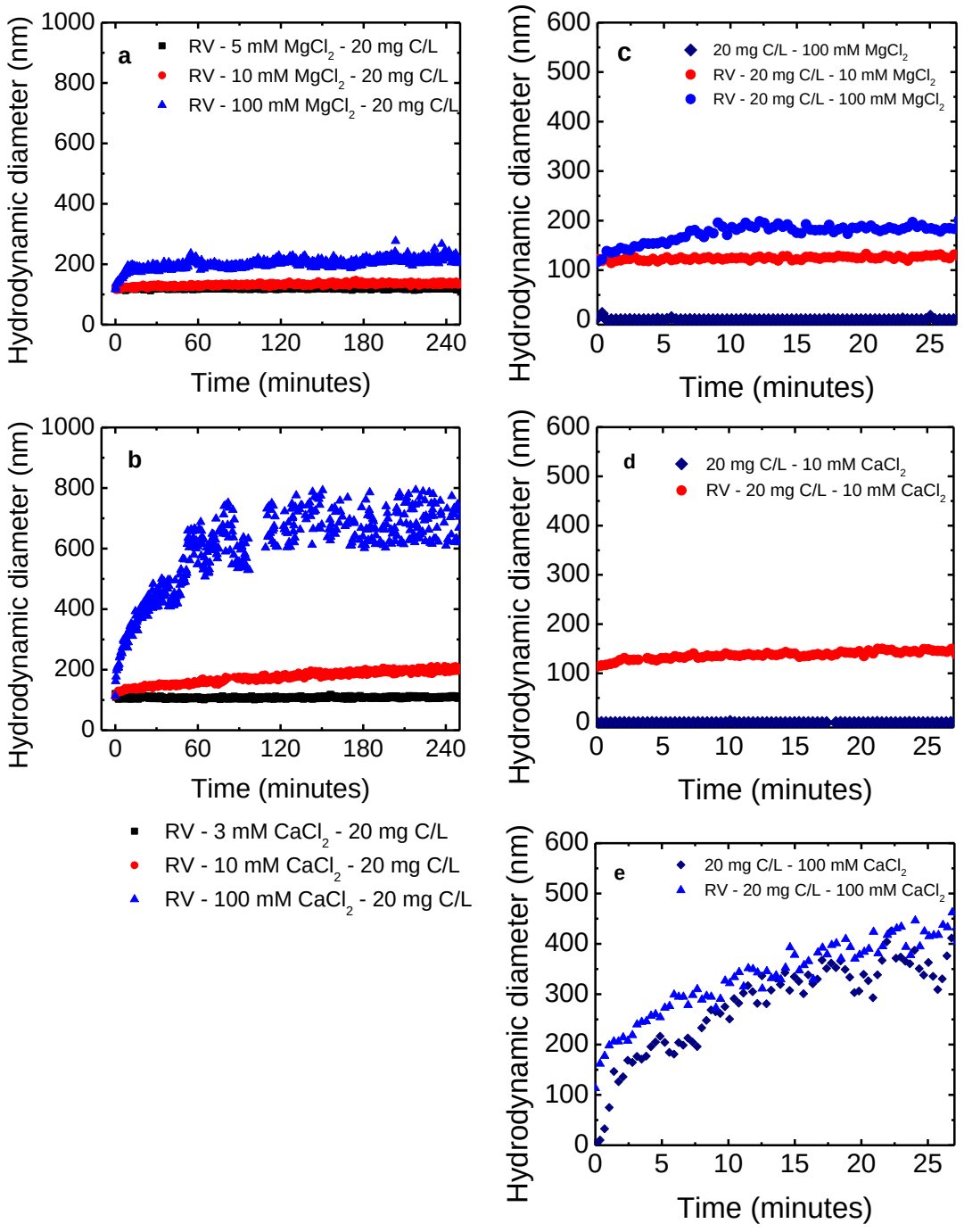


Figure 3S. Aggregation kinetics of rotavirus in solutions containing 20 mg C/L SRNOM and **(a)** $MgCl_2$ or **(b)** $CaCl_2$ recorded for 250 minutes. Rotavirus concentration was ~8×10^5 FFU/mL in solution at 25°C. (c) Aggregation kinetics of rotavirus in solutions containing 20 mg C/L SRNOM and 10 or 100 mM $MgCl_2$,and aggregation kinetics of 20 mg C/L SRNOM and 100 mM $MgCl_2$. (d) Aggregation kinetics of rotavirus in solutions containing 20 mg C/L SRNOM and 10 mM $CaCl_2$,and aggregation kinetics of 20 mg C/L SRNOM and 10 mM $MgCl_2$. (e) Aggregation kinetics of rotavirus in solutions containing 20 mg C/L SRNOM and 100 mM $CaCl_2$,and aggregation kinetics of 20 mg C/L SRNOM and 100 mM $MgCl_2$.

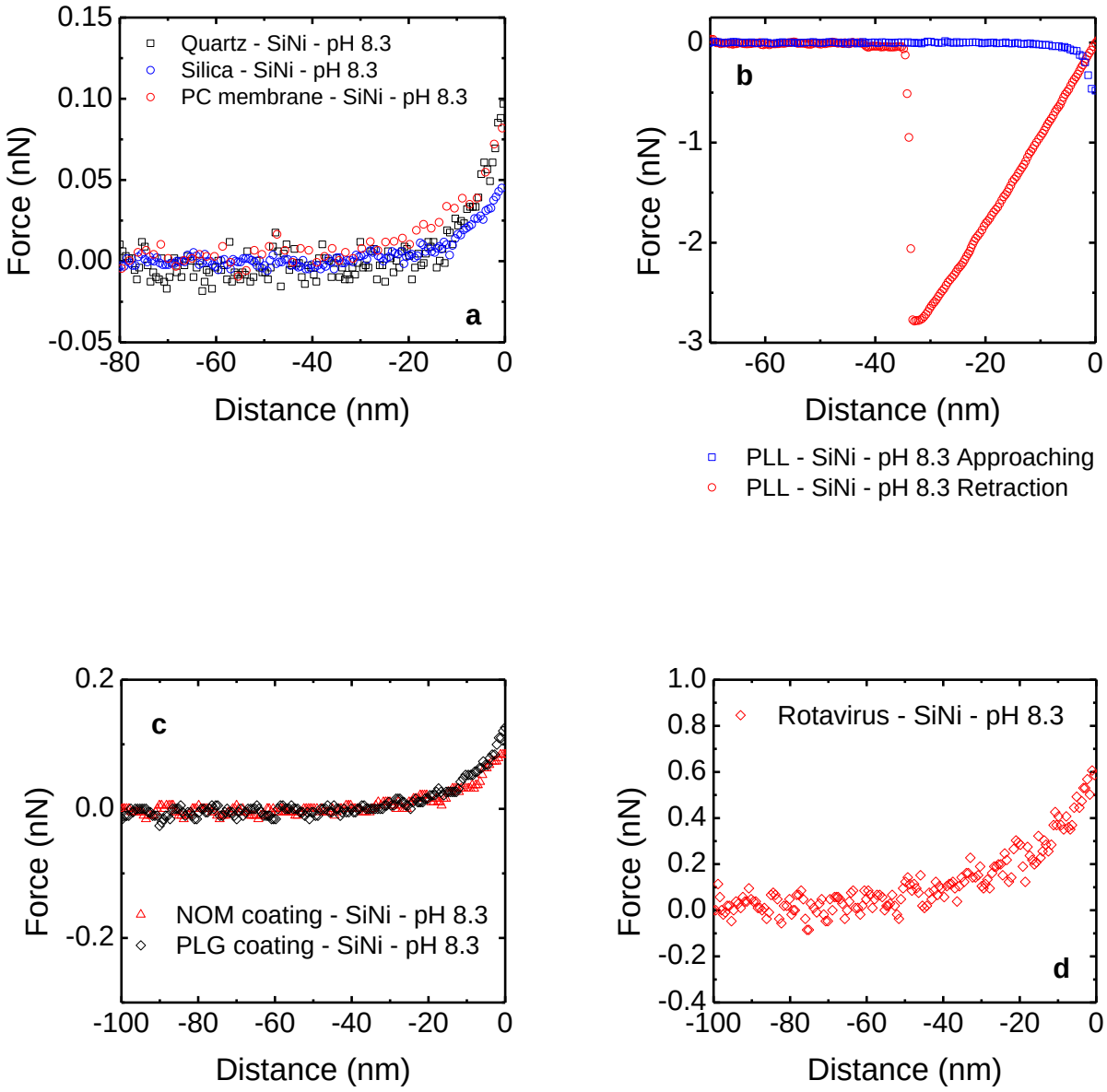


Figure 4S. Control experiments showing **a)** electrostatic repulsion between quartz surface and silicon nitride probe, silica surface and silicon nitride probe, and polycarbonate surface and silicon nitride probe, **b)** electrostatic attraction between PLL layer and silicon nitride probe during approaching and adhesion during retraction, **c)** repulsion forces between NOM layer and silicon nitride probe, PLG layer and silicon nitride probe, and **d)** repulsion force between silicon nitride probe and rotavirus layer adsorbed on a PLL layer. All the control experiments were conducted at 1 mM $NaHCO_3$ (buffered pH of 8.3).

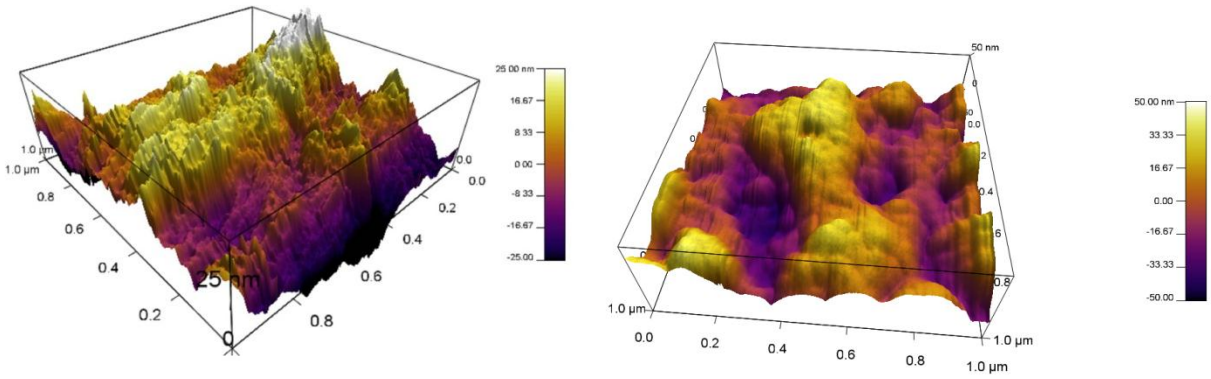


Figure 5aS. Left image: AFM Z-retrace image of polycarbonate membrane. **5bS.** Right image: AFM Z-retrace image of a lawn of rotavirus particles on a polycarbonate membrane.

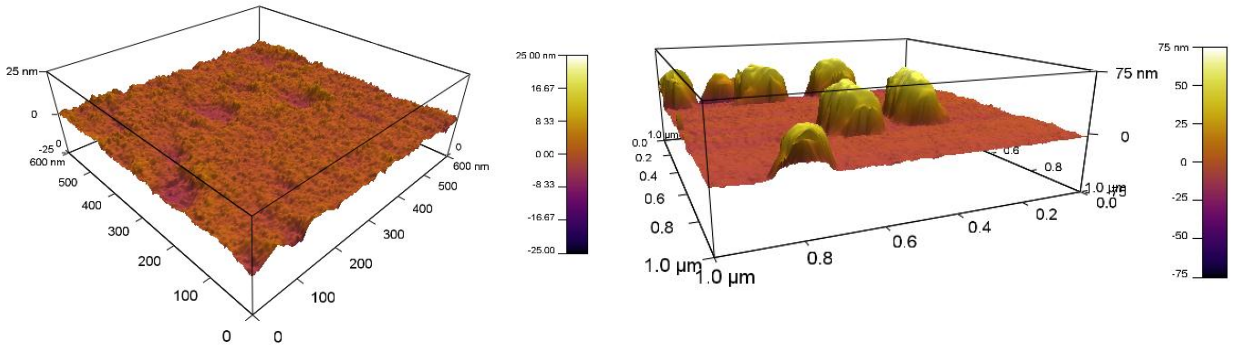
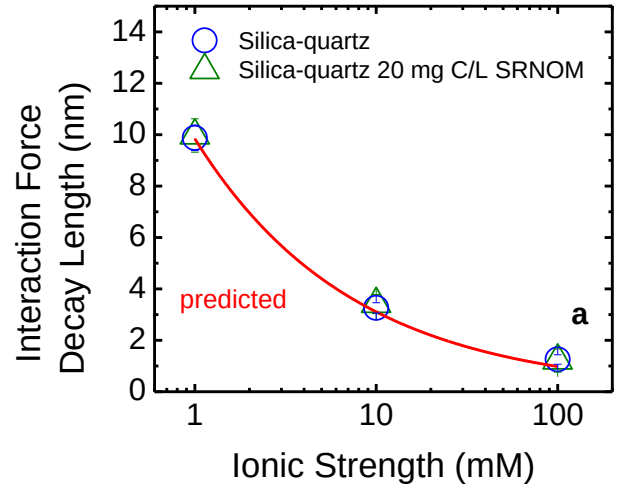


Figure 5cS. Left image: AFM Z-retrace image of PLL-coated silica surface. **5dS.** Right image: AFM Z-retrace image of rotavirus particles on a PLL-coated silica surface. Using the AR-MFP-3D software, shapes and dimensions of monodispersed rotavirus particles were differentiated from the surrounding substrate by height and Z-retrace imaging and 3D analysis. Rotavirus stock was significantly diluted (2 orders of magnitude) for imaging monodispersed virus particles adsorbed on substrate. Specifically, 300µL of 5×10^4 FFU/mL was used for Figure 5dS and 10 mL of ~5×10^6 FFU/ml was filtered through a polycarbonate membrane (Fig. 5aS) to prepare for image shown in Figure 5bS. Images were obtained by tapping mode AFM in 1 mM NaCl with a scan rate of 1 Hz over 1 µm×1 µm area.



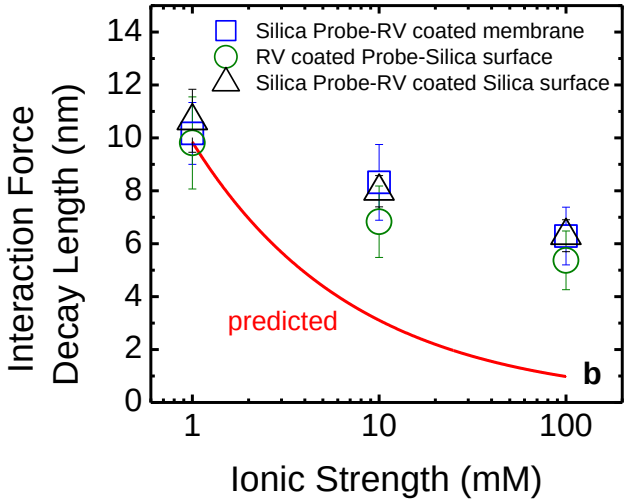
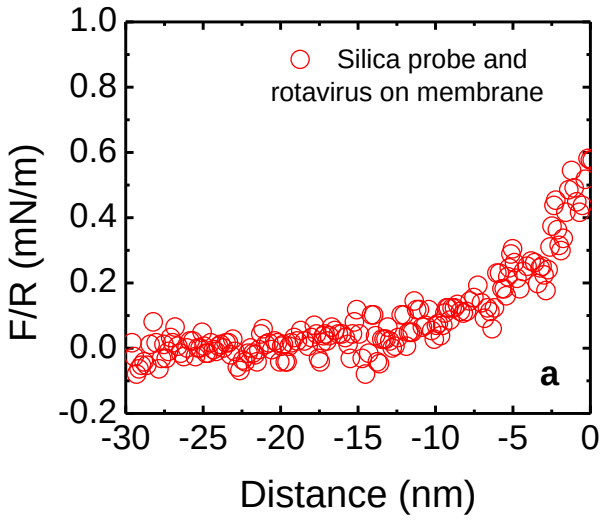


Figure 6S. Interaction force decay length determined for **a)** silica probe with quartz cover slip, and silica probe with quartz cover slip in 20 mg C/L NOM and **b)** silica probe with rotavirus-coated membrane, and rotavirus-coated probe with silica surface, and silica probe with rotavirus-coated silica surface. Predicted Debye length is plotted for comparison purposes.



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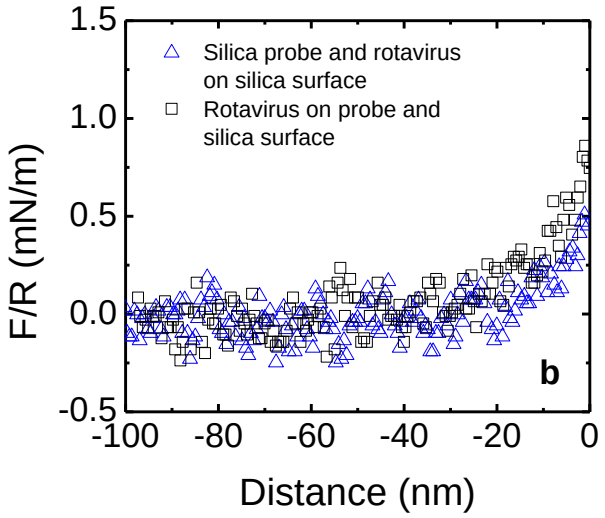

319
320 **Figure 7S.** Approaching force curves for **a)** silica probe with rotavirus layer on polycarbonate
321 membrane and **b)** silica probe with rotavirus layer on silica surface, and rotavirus on probe with
322 silica surface.
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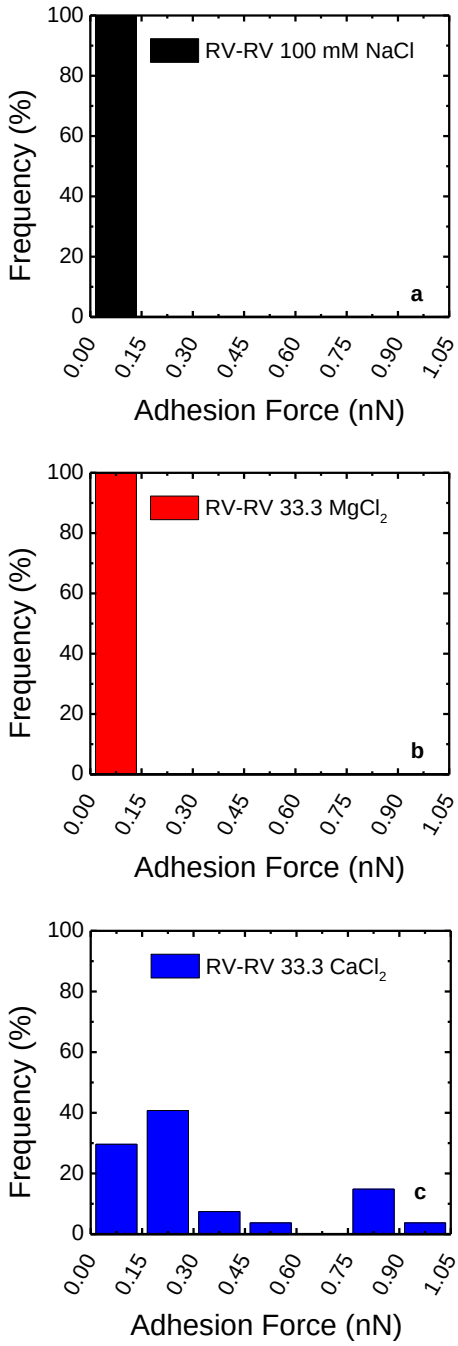


Figure 8S. Adhesion forces of RV with RV layer in solution containing **a)** 100 mM NaCl or **b)** 33.3 mM $MgCl_2$ or **c)** 33.3 mM $CaCl_2$.

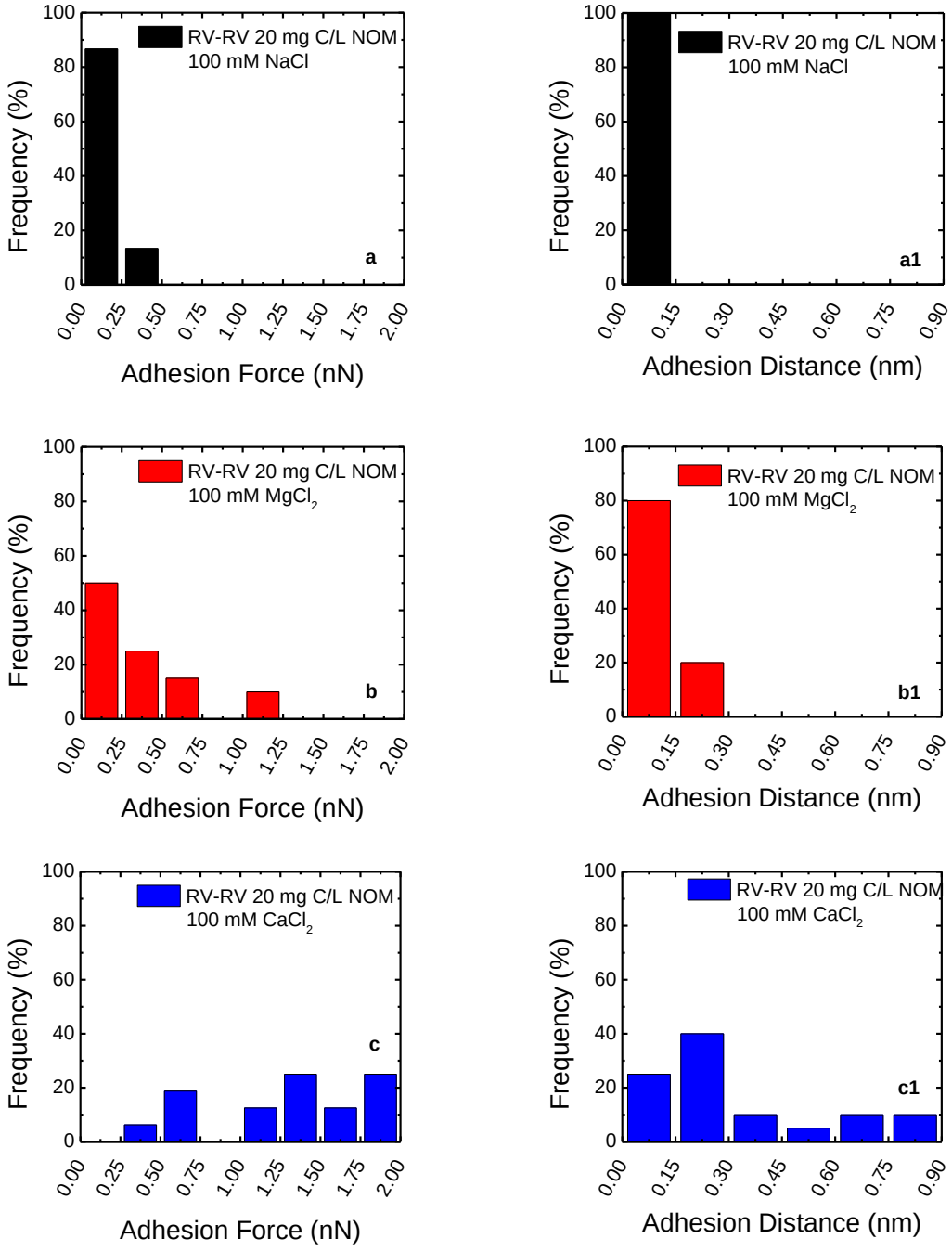
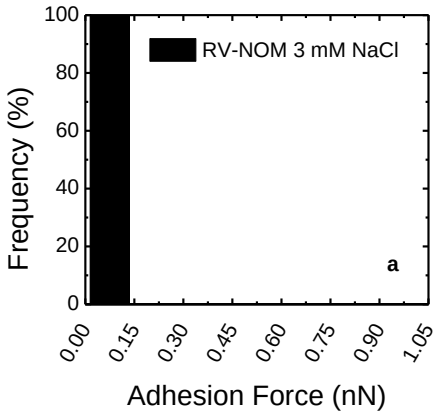


Figure 9S. Adhesion forces and adhesion distances of RV with RV layer in solution containing 20 mg C/L SRNOM and **a)** 100 mM NaCl or **b)** 33.3 mM $MgCl_2$ or **c)** 33.3 mM $CaCl_2$.



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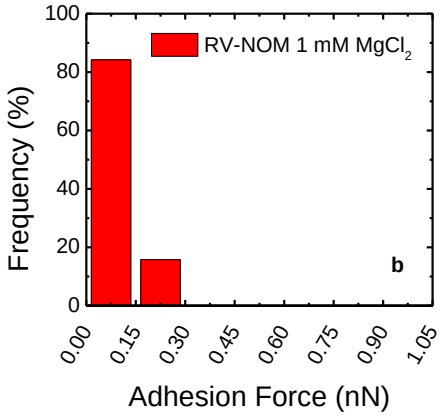

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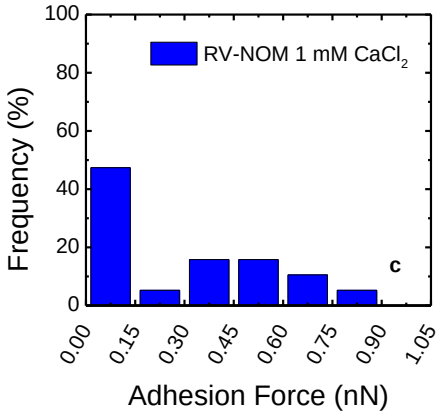

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404 **Figure 10S.** Adhesion forces of RV with SRNOM layer in solution containing **a)** 3 mM NaCl or
405 **b)** 1 mM $MgCl_2$ or **c)** 1 mM $CaCl_2$.
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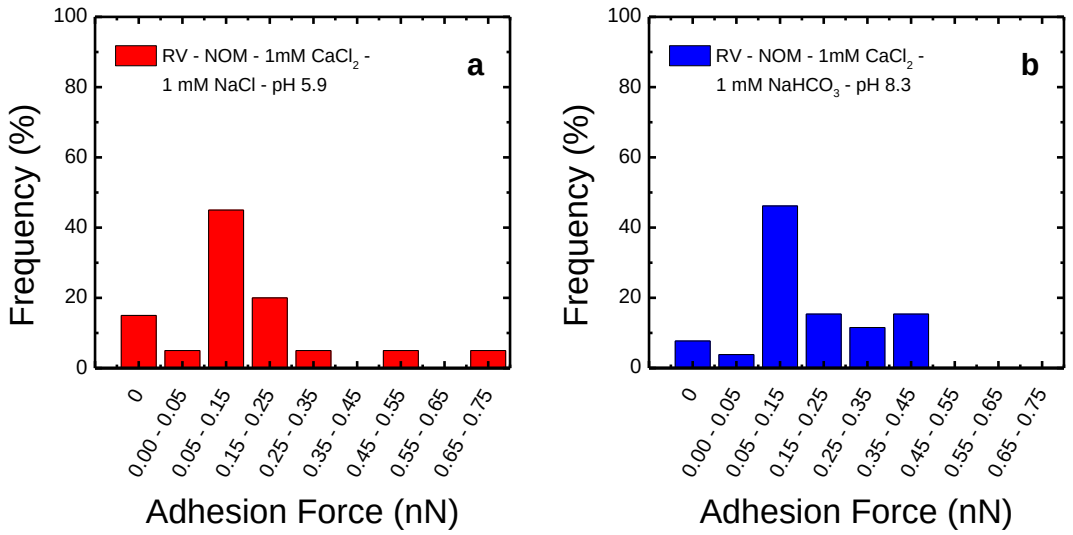

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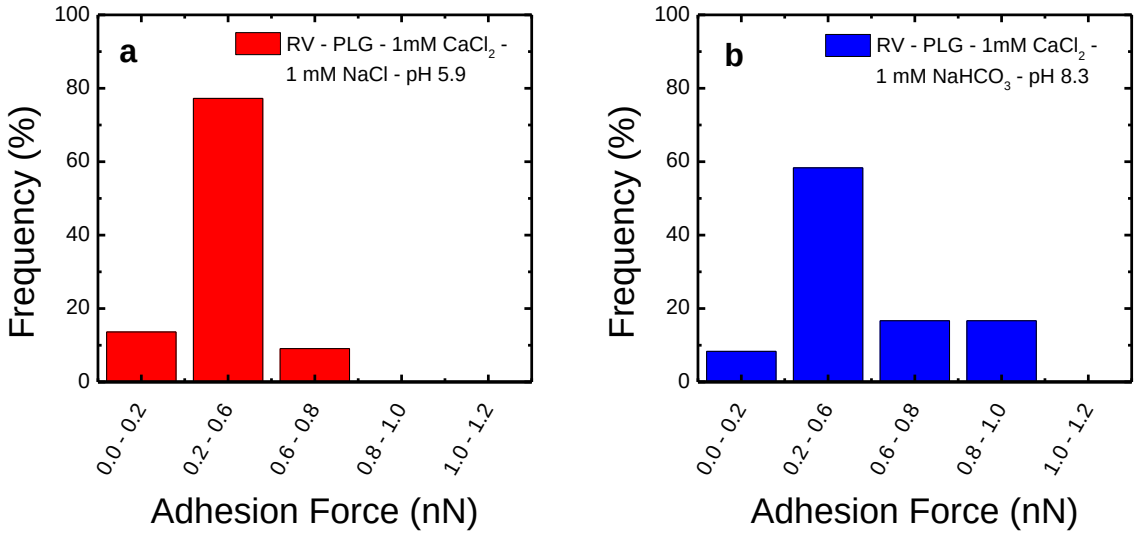
408 **Figure 11S.** Adhesion forces of RV with SRNOM layer in solution containing 1 mM $CaCl_2$ at **a)**
409 pH 5.9 (1 mM NaCl) or **b)** pH 8.3 (1 mM $NaHCO_3$).



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411 **Figure 12S.** Adhesion forces of RV with PLG layer in solution containing 1 mM $CaCl_2$ at **a)** pH

412 5.9 (1 mM NaCl) or **b)** pH 8.3 (1 mM $NaHCO_3$).

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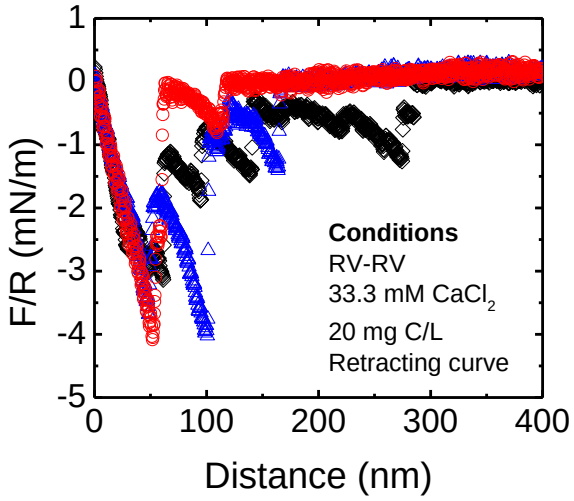

Figure 13S. Retracting force curves for rotavirus-coated probe with rotavirus-coated membrane in solution containing 20 mg C/L and 33.3 mM $CaCl_2$.

Table 1S. Predicted Debye length and measured interaction force decay length in NaCl containing solutions for:

a) Control experiments with silica probe and hard surfaces with and without 20 mg C/L NOM.

Ionic Strength (mM)	Debye Length predicted	Silica probe PC membrane	Silica probe quartz surface	Silica probe quartz surface 20 mg C/L NOM
1	10	10±1.3	10±0.5	10±0.7
10	3	4±1.6	3±0.2	3±0.4
100	1	1±0.4	1±0.2	1±0.5

b) Rotavirus interaction with silica or rotavirus with and without 20 mg C/L NOM.

Ionic Strength (mM)	Silica Probe rotavirus-coated PC membrane	Rotavirus-coated probe silica surface	Silica probe rotavirus-coated silica surface	Rotavirus-coated probe rotavirus-coated membrane 20 mg C/l NOM
1	10±1.2	10±1.7	11±1.2	12±1.5
10	8±1.4	7±1.4	8±0.6	11±1.2
100	6±1.1	5±1.1	6±0.6	11±2.6

Interaction force decay lengths were calculated from approaching force curves using AFM in contact mode.

Table 2S. Electrophoretic mobility of silica beads, NOM or PLG-coated silica beads, and rotavirus. Three measurements were conducted for each condition.

	pH 5.9		pH 8.3	
	Mob (µmcm/Vs)	StDev	Mob (µmcm/Vs)	StDev
Silica - 1 mM $CaCl_2$	-1.7	0.2	-3.0	0.2
NOM - 1 mM $CaCl_2$	-1.6	0.2	-2.2	0.1
PLG - 1 mM $CaCl_2$	-1.8	0.4	-2.1	0.4
Rotavirus - 1 mM NaCl	-1.6	0.2	-1.8	0.2
Rotavirus - 1 mM $CaCl_2$	-0.7	0.1	-0.8	0.1
Rotavirus - 1 mM $CaCl_2$ - 20 mg C/L	-1.0	0.1	-1.1	0.2

449 **Table 3S.** Hydrodynamic diameter of RV-NOM aggregates in divalent cation solutions after
450 EPM measurements by intensity analysis

Concentration	D_h (nm)	
mM	$CaCl_2$	$MgCl_2$
1	115	
2	116	
3	114	
5	121	118
7.5	120	
10	120	119
20	127	122
30	147	124
50	167	129
100	177	138
200	202	146

451

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