Supplementary material

for the manuscript

Deposition of bacteriophage MS2 onto polyelectrolyte-coated surfaces

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Hien T. T. Dang, Volodymyr V. Tarabara

Department of Civil and Environmental Engineering, Michigan State University,
East Lansing, MI 48824, USA
S1. Propagation, purification and quantification of bacteriophage MS2

MS2 propagation

The bacteriophage MS2 was purchased from American Type Culture Collection (ATCC). To propagate MS2, an actively growing broth culture was prepared by incubating few drops of overnight *E. coli* in soy broth for 6 h at 37 °C. The broth culture was then spiked with 0.1 mL of the diluted phage suspension (~10^6 PFU/mL) and incubated overnight at 37 °C. The MS2 suspension was centrifuged at ~5000 rpm for 15 min to remove debris [1]. The supernatant was filtered through a 0.22 μm filter (Millex-GS, Merck Millipore) and then stored at 4°C as a stock for future use.

MS2 purification

To purify MS2, polyethylene glycol 6000 (Sigma-Aldrich) and NaCl were added into the MS2 stock to a final concentration of 10 % and 0.5 M, respectively [1, 2]. The mixture was stirred gently overnight to settle down MS2 and then followed by centrifuging at 10,000 rpm for 30 min. After centrifugation, the supernatant was discarded and the pellets were suspended with 1 mM NaCl by vibrating the sample on a shaker over 24 h. After the pellets were completely dissolved, the MS2 suspension was filtered through 0.22 μm filter and then dialyzed through 100 kDa dialysis tubes in 1 mM NaCl for 24 hours. The NaCl solution was exchanged after the first 12 h. The entire purification process was performed in dark at 4 °C. The PEG precipitation was chosen over centrifugal diafiltration because the latter gives a broader size distribution in the product MS2 suspension [3].
MS2 quantification using double-layer plaque assay

MS2 concentration was measured using a double-agar layer assay. To quantify MS2, two agar media were prepared. The bottom layer contained 10 g tryptone, 1 g yeast extract, 8 g NaCl and 15 g agar, 1 g glucose, 0.294 g CaCl$_2$ and 10 mg thiamine in 1L of DI water. The top layer contained the same mixture of the components except for having 5 g of agar [4]. These media were boiled and cooled down to 50 °C. The bottom layer was then poured into 100 x 5 mm Petri dishes (15 ml in each), followed by drying until the agar layer solidified. While still hot (~50 °C), the top layer agar was poured into 20 mL culture tubes at a volume of 2.5 mL and kept in a water bath at 43-45 °C. As for MS2 stock, it was serially diluted by pipetting 1 mL of the phage in 9 mL of broth until its final concentration was ~ 10$^3$ PFU/mL. After incubating the E. coli suspension for 6 h, one or two drops of the host stock was pipetted into every culture tube, followed by 100 μm of diluted MS2 suspensions. The culture tube containing melted soft agar, E. coli and phage was gently mixed before pouring over a hard agar plate. After the top agar layer dried, the petri dish was placed upside down in the incubator for overnight until the lysis became visible. Every MS2 dilution was analyzed for MS concentration at least three times. The petri dishes with a number of countable lysis spots in the 50-300 range were chosen to determine MS2 concentration of MS2 stock.
S2. DLVO and XDLVO theories

The XDLVO theory developed by Van Oss [5] defines the total energy of interaction $E_{stlv}^{XDLVO}$ between a particle (such as virus ($v$)) and a planar surface ($s$) in liquid medium ($l$) as a sum of the Lifshitz van der Waals $E_{stlv}^{LW}$, electrostatic double layer $E_{stlv}^{EL}$, and acid-base $E_{stlv}^{AB}$ energies:

$$E_{stlv}^{XDLVO} = E_{stlv}^{LW} + E_{stlv}^{EL} + E_{stlv}^{AB}$$

where

$$E_{stlv}^{LW} = -A \frac{a}{6d} = -12\pi \gamma_0^2 \Delta G_{y_0}^{LW} \frac{a}{6d}$$

$$E_{stlv}^{EL} = \pi \varepsilon_r \varepsilon_0 a \left[ 2\psi_c \psi_s \ln \left( \frac{1 + e^{-\kappa d}}{1 - e^{-\kappa d}} \right) + (\psi_c^2 + \psi_s^2) \ln(1 - e^{-2\kappa d}) \right]$$

$$E_{stlv}^{AB} = 2\pi a \lambda \Delta G_{d_0}^{AB} \exp \left( \frac{d_0 - d_0}{\lambda} \right)$$

In the expressions above, $a$ is the virus radius, $d$ is the separation distance, $\varepsilon_r$ is the dielectric constant of water ($\varepsilon_r = 79$), $\varepsilon_0$ is the relative permittivity in vacuum ($\varepsilon_r = 8.854 \cdot 10^{-12} \text{ CV}^{-1} \text{m}^{-1}$), $\psi_c$ and $\psi_s$ are the surface potentials of the colloid and surface, respectively, $\kappa$ is the reverse Debye length, $\lambda$ is the characteristic delay length of the AB interaction in water ($\lambda = 0.6 \text{ nm}$), $d_0$ is the minimum separation distance ($d_0 = 0.158 \text{ nm}$).

The AB and LW free energies of adhesion per unit area (J/m$^2$) at the separation distance $d_0$ are given by equations (S5) and (S6), respectively:

$$\Delta G_{d_0}^{AB} = 2 \sqrt{\gamma_i^+} (\sqrt{\gamma_s^-} + \sqrt{\gamma_v^-} - \sqrt{\gamma_i^-}) + 2 \sqrt{\gamma_i^-} (\sqrt{\gamma_s^+} + \sqrt{\gamma_v^+} - \sqrt{\gamma_i^+}) - 2(\sqrt{\gamma_s^+} \gamma_v^- + \sqrt{\gamma_s^-} \gamma_v^+)$$

$$\Delta G_{d_0}^{LW} = 2(\sqrt{\gamma_i^{LW}} - \sqrt{\gamma_s^{LW}})(\sqrt{\gamma_v^{LW}} - \sqrt{\gamma_i^{LW}})$$

(S5)  (S6)
where, $\gamma^+$ is the electron acceptor parameter, $\gamma^-$ is the electron donor parameter, $\gamma_{LW}^+$ is the apolar surface tension component, and indices $l$, $s$, and $v$ refer to liquid, surface and virus. The surface tension components ($\gamma_s^{LW}$, $\gamma_l^+$ and $\gamma_s^-$) are calculated using eq. (S7) based on the measured contact angles, $\theta$, of three probe liquids (DI, glycerol and diiodomethane) and the known surface tension components of the probe liquids ($\gamma_l^{LW}$, $\gamma_l^+$ and $\gamma_l^-$). The square root of the electron donor parameter ($\sqrt{\gamma^+}$) was calculated to be negative (from -1.1 to -2.1) so the value of $\gamma^+$ was assumed to be zero. Negative values of $\sqrt{\gamma^+}$ or $\sqrt{\gamma^-}$ are often reported for microbial cell surfaces due to hydration [6, 7]; this is more likely to occur for surfaces that have higher $\gamma_{LW}^+$ and lower water contact angle values.

\[
(1 + \cos \theta) \gamma_i^{TOT} = 2 \left( \sqrt{\gamma_s^{LW} \gamma_l^{LW}} + \sqrt{\gamma_s^+ \gamma_l^-} + \sqrt{\gamma_s^- \gamma_l^+} \right) \tag{S7}
\]

When both $\gamma_s^+$ and $\gamma_v^+$ are zero, eq. (S5) simplifies to:

\[
\Delta G_{d0}^{A|B} = 2 \sqrt{\gamma_l^+} \left( \sqrt{\gamma_s^-} + \sqrt{\gamma_v^-} - 2 \sqrt{\gamma_l^-} \right) \tag{S8}
\]

**S.2.1 Quantifying hydrophobicity/hydrophilicity of a macroscopic surface**

The hydrophobicity of a surface can be evaluated based on its free energy of cohesion when immersed in water ($\Delta G_{sws}$). $\Delta G_{sws}$ is twice the interfacial tension $\gamma_{sw}$ between the surface and water:

\[
\Delta G_{sws} = -2 \gamma_{sw} = 2 \left( \sqrt{\gamma_s^{LW}} - \sqrt{\gamma_w^{LW}} \right)^2 - 4 (\sqrt{\gamma_s^+ \gamma_s^-} + \sqrt{\gamma_v^+ \gamma_v^-} - \sqrt{\gamma_s^+ \gamma_w^-} - \sqrt{\gamma_v^+ \gamma_s^-}) \tag{S9}
\]

A positive value of $\Delta G_{sws}$ indicates a hydrophilic surface while a negative value indicates that the surface is hydrophobic.

**S3. Estimation of the mass of virus deposited on the QCM-D sensor**
The maximum possible mass of viable MS2 \( (\Delta m_{max} \approx 456 \text{ ng/cm}^2) \) deposited per unit area of a QCM-D sensor was estimated by dividing the total mass \( (M) \) of the viable virus in the feed by the active area \(^1 (A \approx 0.97 \text{ cm}^2) \) of the QCM-D sensor:

\[
\Delta m_{max} = \frac{M}{A} = \frac{C_v V m_v}{A} = \frac{C_v V \rho_v \pi d_v^3}{A} \times \frac{6}{A}
\]

where \( C_v \) is the viable virus concentration in the feed \( (C_v = 1.24 \pm 0.07 \times 10^{10} \text{ PFU/mL}, \text{see section 2.4}) \), \( m_v \) is the weight of one MS2 bacteriophage, \( \rho_v \) is the density of the virus \( (\text{for MS2, } \rho_v = 1.38 \text{ g/cm}^3 [8]) \), \( d_v \) is the diameter of the virus, and \( V \) is the volume of virus suspension passed through one QCM-D chamber that houses the sensor over the duration of the QCM-D tests. The value of \( d_v \) was taken to be the MS2 size determined by TEM imaging \( (d_v \approx 27 \text{ nm} [3]) \). The flow rate into one QCM-D chamber was 2.5 mL/h and the total duration of the test 1 h so that the total volume \( V \) was 2.5 mL.

Because the concentration of MS2 was measured using the plaque assay method, only viable virus was accounted. Thus, the true upper bound on the total (i.e. viable and non-viable) virus mass, which is what was measured in QCM-D tests, is expected to be much higher than the above estimate of 456 ng/cm\(^2\). Indeed, values > 1250 ng/cm\(^2\) in QCM-D tests with positively charged surfaces (Figure 3).

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\(^1\) Personal communication with Dr. Archana Jaiswal, Principal Application Scientist (Biolin Scientific Inc.). April 13, 2018.
Table S1: Contact angles, calculated surface energy parameters, and free energy of the PEMs and MS2 bacteriophage. Values of $\Delta G_{sws}$ are also provided in the main manuscript and are included here for convenience only.

Notes:
a: 10-[PSS/PDAD]$_4$; b: 100-[PSS/PDAD]$_4$; c: 10-[PSS/PDAD]$_{4.5}$; d: 100-[PSS/PDAD]$_{4.5}$
e: For all membranes, negative values for $\sqrt{\gamma^+}$ were obtained (see section S1).

<table>
<thead>
<tr>
<th>Property</th>
<th>[PSS/PDAD]$_4$</th>
<th>[PSS/PDAD]$_{4.5}$</th>
<th>MS2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$I_{LbL}=10^a$</td>
<td>$I_{LbL}=100^b$</td>
<td></td>
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<tr>
<td>Contact angle ($^\circ$) with the probe liquid:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H$_2$O</td>
<td>64.4 ± 2.8</td>
<td>29.6 ± 1.5</td>
<td>38 ± 6</td>
</tr>
<tr>
<td>Glycerol</td>
<td>54.1 ± 5.9</td>
<td>54.4 ± 3.0</td>
<td>63 ± 1</td>
</tr>
<tr>
<td>Diiodomethane</td>
<td>37.5 ± 3.0</td>
<td>23.2 ± 3.1</td>
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</tr>
<tr>
<td>Surface energy parameters (mJ/m$^2$)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$\gamma^LW$</td>
<td>40.8</td>
<td>46.8</td>
<td>42.5</td>
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<td>$\gamma^+e$</td>
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<tr>
<td>$\gamma^-$</td>
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<tr>
<td>$\gamma^{AB}$</td>
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<tr>
<td>$\gamma^{tot}$</td>
<td>40.8</td>
<td>46.8</td>
<td>42.5</td>
</tr>
<tr>
<td>Free energy of interfacial surface-surface interaction in water $\Delta G_{sws}$ (mJ/m$^2$)</td>
<td>-16.3</td>
<td>45.2</td>
<td>48.0</td>
</tr>
</tbody>
</table>

Notes:
a: 10-[PSS/PDAD]$_4$; b: 100-[PSS/PDAD]$_4$; c: 10-[PSS/PDAD]$_{4.5}$; d: 100-[PSS/PDAD]$_{4.5}$
e: For all membranes, negative values for $\sqrt{\gamma^+}$ were obtained (see section S1).
Table S2. Results of XDLVO modeling (values of energy barriers, \(E_{\text{max}}\), and secondary energy minima, \(E_{\text{min}}\), in XDLVO energy profiles (Figures 3, 4, S7, S8), and of QCM-D experiments (mass deposition rates for phase 1, \(\frac{dm}{dt}_1\), and phase 2, \(\frac{dm}{dt}_2\), as well as deposition lag time, \(t_1\) (Figures 1, 2)).

Notes:
* Values used as active variables in the PCA (Figure 5a and 5b)
** Values used as supplementary variables in the PCA (Figure 5a)
† The lag time estimated using linear regression was ~14 min (Figure 2a). This time was selected as the start of deposition phase1. Due to the limited duration of the QCM-D test, Phase 2 was not observed. For completeness of PCA calculations (Figure 5), \(\frac{dm}{dt}_2\) in this test was assumed to be equal to \(\frac{dm}{dt}_1\).

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Predictions and observations</th>
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<tbody>
<tr>
<td></td>
<td>Ionic strength</td>
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<tr>
<td></td>
<td>PEM formation</td>
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<tr>
<td>10-[PSS/PDAD]_{4-10}</td>
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</tr>
<tr>
<td>100-[PSS/PDAD]_{4-10}</td>
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<tr>
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<tr>
<td>100-[PSS/PDAD]_{4.5-100}</td>
<td>100</td>
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</table>
Figure S1: Effect of surface roughness on the XDLVO energy of interaction between MS2 bacteriophage and PEM surface: $^{10\text{mM}}[\text{PSS/PDAD}]_4$ (a, b) and $^{100\text{mM}}[\text{PSS/PDAD}]_4$ (c, d) during phase 1 of the deposition process.
Figure S2: Effect of surface roughness on the of XDLVO energy of interaction between MS2 bacteriophage and PEM surface: \(10\text{mM}[\text{PSS/PDAD}]_{4.5}\) (a, b) and \(100\text{mM}[\text{PSS/PDAD}]_{4.5}\) (c, d) during phase 1 of the deposition process.
Figure S3: Profiles of XDLVO energy of interaction between MS2 bacteriophage and [PSS/PDAD]₄ surface in a) 10 mM NaCl and b) 100 mM NaCl solutions during phase 2 of the deposition tests. Because this XDLVO calculation is for the case of the interaction between a sphere and smooth flat surface and because the MS2-coated surface is assumed to have the electrical charge and hydrophobicity of MS2, XDLVO profiles are identical for 10-[PSS/PDAD]₄ and 100-[PSS/PDAD]₄ surfaces.
**Figure S4:** Profiles of XDLVO energy of interaction between MS2 bacteriophage and [PSS/PDAD]_{4.5} surface in a) 10 mM NaCl and b) 100 mM NaCl solutions during phase 2 of the deposition tests.
Figure S5: QCM dissipation during MS2 deposition onto (a) 10-[PSS/PDAD]$_4$, and (b) 100-[PSS/PDAD]$_4$ surfaces from solutions of different ionic strengths (10 mM or 100 mM NaCl). The dissipation data correspond to mass data show in Figure 1.
Figure S6: QCM dissipation during MS2 deposition onto (a) 10-[PSS/PDAD]$_{4.5}$ and (b) 100-[PSS/PDAD]$_{4.5}$ surfaces from solutions of different ionic strengths (10 mM or 100 mM NaCl). The dissipation data correspond to mass data show in Figure 1.
Figure S7: Comparison of the root mean square roughness of the deposition surface with the distance corresponding to 3kT energy barrier in the XDLVO total energy profile. Surfaces within the shaded area have relatively high roughness and may potentially capture particles by physical entrapment.
References


