2nd International School/Conference "Applied Biosciences and Biotechnology 2019"
Tbilisi, Georgia

Virus attachment to surfaces:
Assessing relative contributions of electrostatic, van der Waals, and acid-base interactions

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Michigan State University

April 4, 2019

Goal, objectives, premise

Overarching goal

Elucidate physicochemical mechanisms that control virus adhesion to surfaces

Specific objective

Predict virus adhesion to practically important surfaces (e.g. household paint, lipstick, human skin) from relevant solutions (saliva, respiratory fluid)

Longer term objective

Select bacteriophage surrogates for

- evaluating human adenovirus fate in water treatment systems
- designing sample concentration processes for human adenovirus detection in aqueous media

Premise

Understanding the relationship between

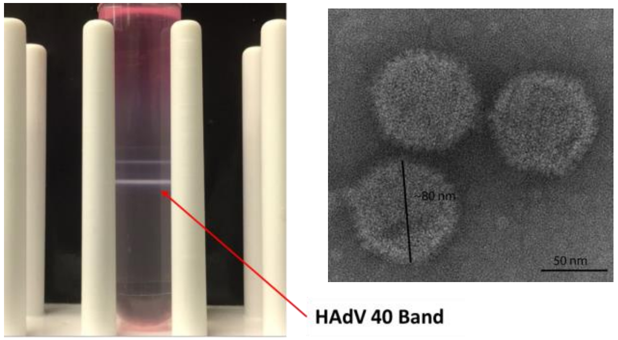
a) virus deposition conditions and properties of the collector surface

b) the deposition kinetics

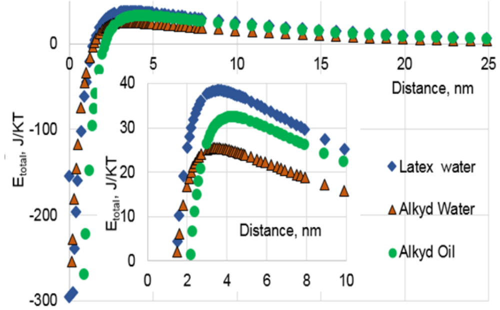
can guide the design of specialty surfaces for regulating virus adsorption.

Three-pronged approach

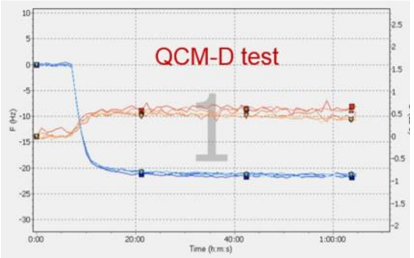
1. Detailed characterization of physicochemical properties of a virus
   - ζ potential vs pH (electrophoretic tests)
   - size and morphology (TEM, dynamic light scattering)
   - hydrophobicity and surface energy components



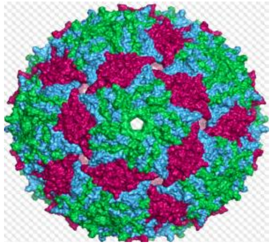
2. Prediction of virus-surface interactions using extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) model



3. Experimental quantification of virus attachment to surfaces using quartz crystal microbalance with dissipation (QCM-D)



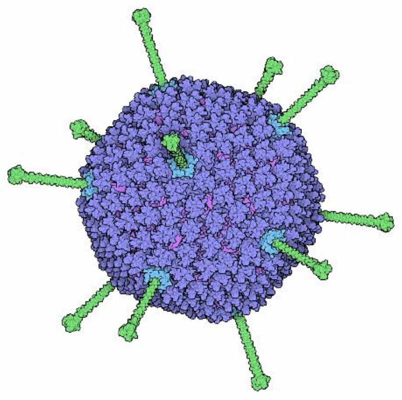
Approach: Selecting target viruses



(*Image source: Wikipedia.org*)

Bacteriophage MS2

- Diameter: ~28 nm
- Non-enveloped single stranded RNA

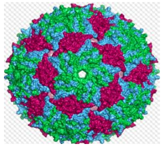


(Image source: PDB-101.ocsb.org)

Human adenovirus 40 (HAdV40)

- Diameter: ~80 nm
- Non-enveloped double-stranded DNA

Approach: Selecting target viruses



(*Image source: Wikipedia.org*)

Bacteriophage MS2
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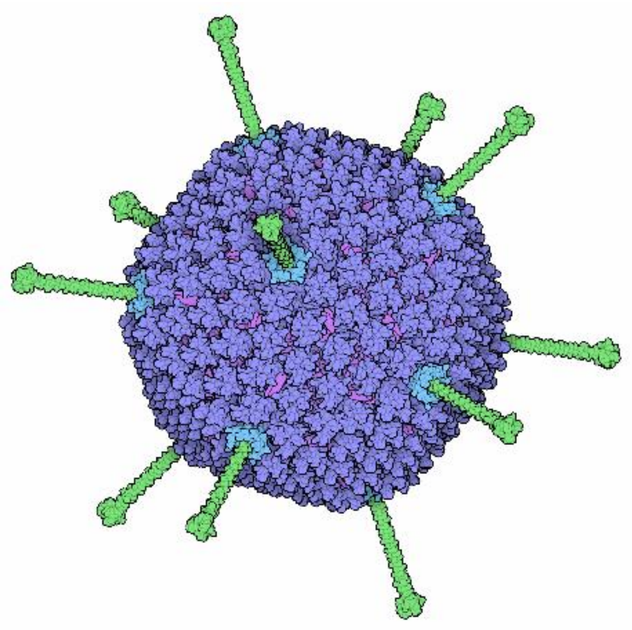

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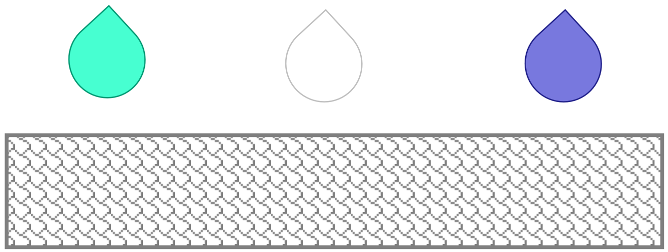
Human adenovirus 40 (HAdV40)
- Diameter: ~80 nm
- Non-enveloped double-stranded DNA

Approach: Characterization

✓ surface energy component: contact angle of 3 probe liquids

water glycerol diiodomethane



$$(1+\cos\theta)\gamma_l^{TOT} = 2\left(\sqrt{\gamma_s^{LW}\gamma_l^{LW}} + \sqrt{\gamma_s^{+}\gamma_l^{-}} + \sqrt{\gamma_s^{-}\gamma_l^{+}}\right)$$

$$(1+\cos\theta)\gamma_l^{TOT} = 2\left(\sqrt{\gamma_s^{LW}\gamma_l^{LW}} + \sqrt{\gamma_s^{+}\gamma_l^{-}} + \sqrt{\gamma_s^{-}\gamma_l^{+}}\right)$$

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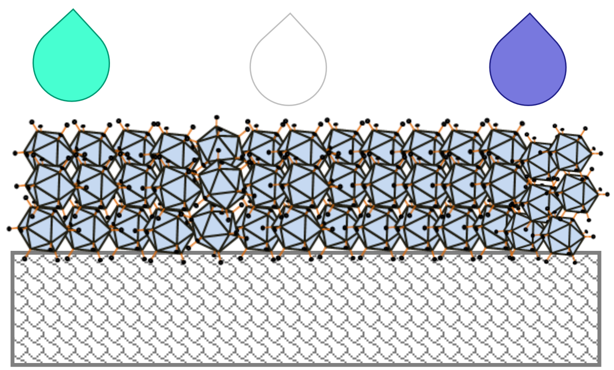
where $\gamma_l^{TOT} = \gamma_l^{LW} + 2\sqrt{\gamma_l^{+}\gamma_l^{-}}$

Goniometer / Tensiometer

(*image source: ramehart.com*)

Approach: Characterization

✓ surface energy component: contact angle of 3 probe liquids



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where $\gamma_l^{TOT} = \gamma_l^{LW} + 2\sqrt{\gamma_l^{+}\gamma_l^{-}}$

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ΔG as a measure of hydrophobicity

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The free energy of interfacial interaction of two virions when immersed in water:

$$\Delta G_{wvw} = -2\left(\sqrt{\gamma_v^{LW}} - \sqrt{\gamma_w^{LW}}\right)^2 - 4\left(\sqrt{\gamma_v^{+}\gamma_v^{-}} + \sqrt{\gamma_w^{+}\gamma_w^{-}} - \sqrt{\gamma_v^{+}\gamma_w^{-}} - \sqrt{\gamma_v^{-}\gamma_w^{+}}\right)$$

TABLE 3 Contact angles, calculated surface energy parameters, and free energy of interfacial virion-virion interactions in water for HAdV 40[a]

Parameter	Value
Contact angle (°) with indicated probe liquid	
H_2O	68 ± 2
Glycerol	64 ± 1
Diiodomethane	36 ± 2
Surface energy parameter (mJ/m^2)	
γ^{LW}	41.6
γ^{+}	0.01
γ^{-}	14.7
γ^{AB}	0.8
γ^{TOT}	42.4
Free energy of interfacial virion-virion interactions in water (ΔG_{vwv} [mJ/m^2])	−30.4

So HAdV 40 is hydrophilic?

No. Hydrophobic, actually!

4984 aem.asm.org Applied and Environmental Microbiology August 2016 Volume 82 Number 16

Elution Is a Critical Step for Recovering Human Adenovirus 40 from Tap Water and Surface Water by Cross-Flow Ultrafiltration

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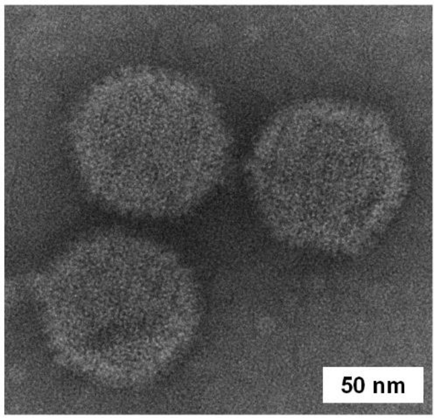
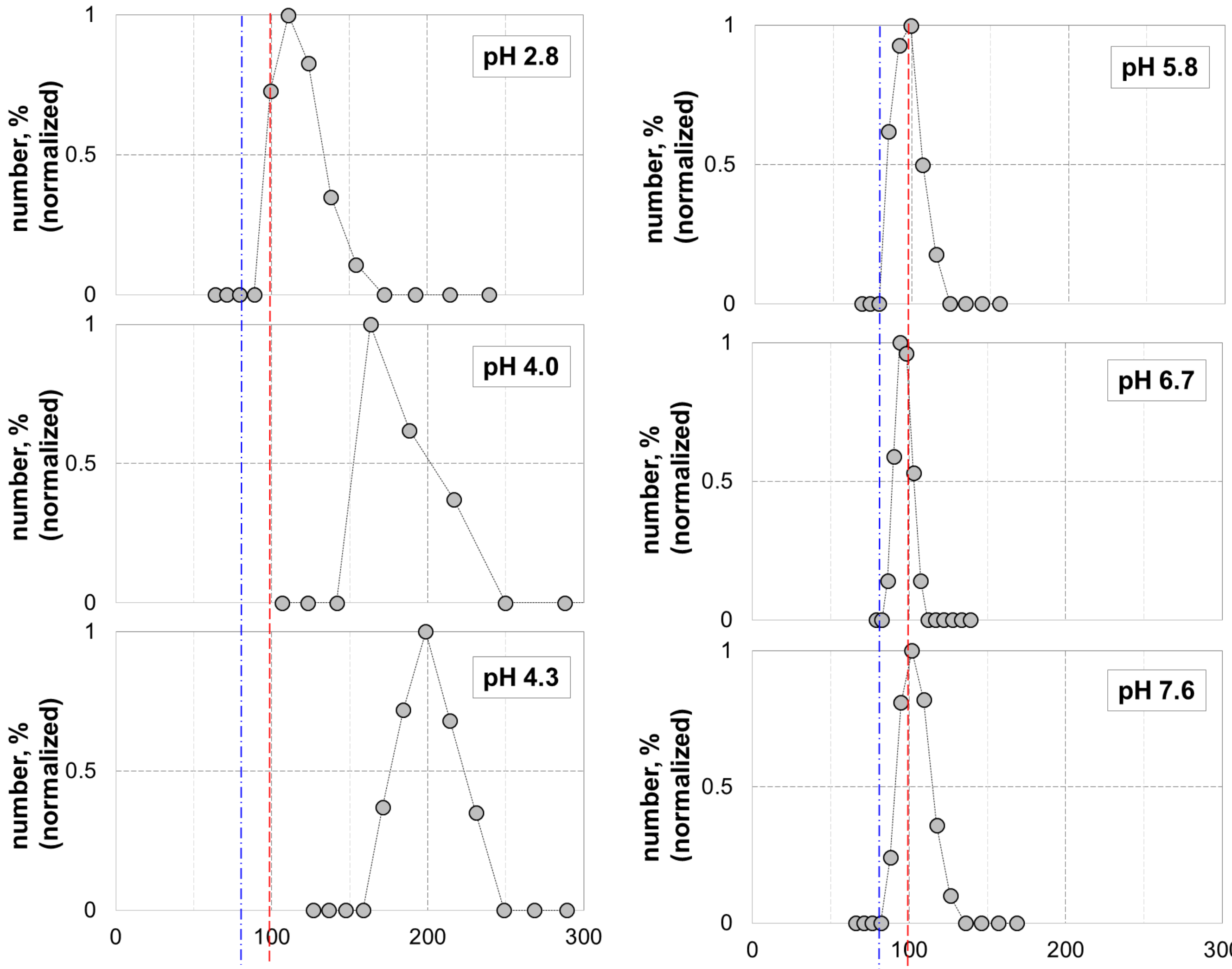
Department of Civil and Environmental Engineering, Michigan State University, East Lansing, Michigan, USA[a]; Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan, USA[b]; Department of Chemistry, Michigan State University, East Lansing, Michigan, USA[c]

Human Adenovirus 40

pH dependence of the aggregation state

TEM: ~ 80 nm
DLS: ~ 99 nm



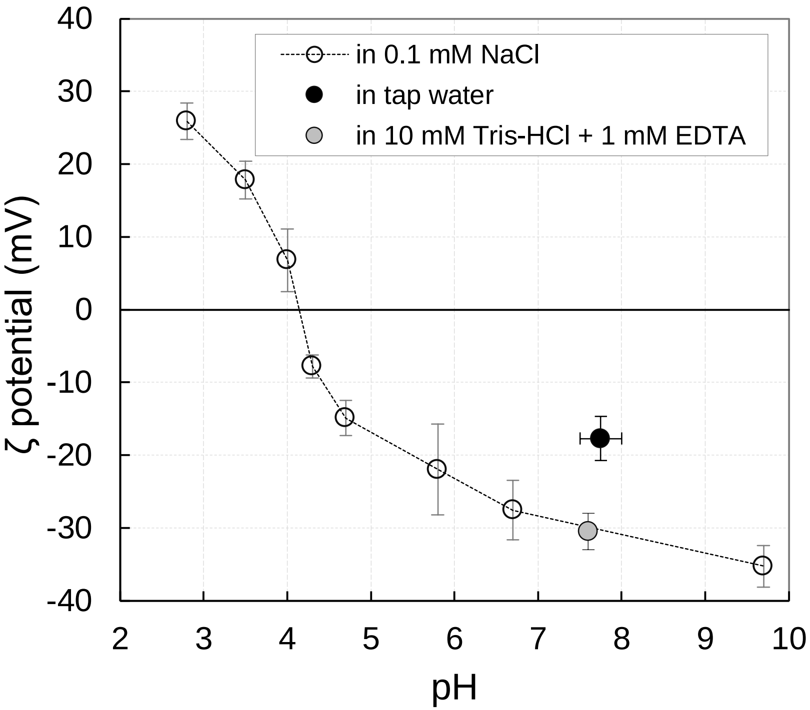


Shi, H. et al. Appl. Environ. Microbiol. 2016

Human Adenovirus 40

Hydrophobicity, surface charge and XDLVO virus-virus interaction

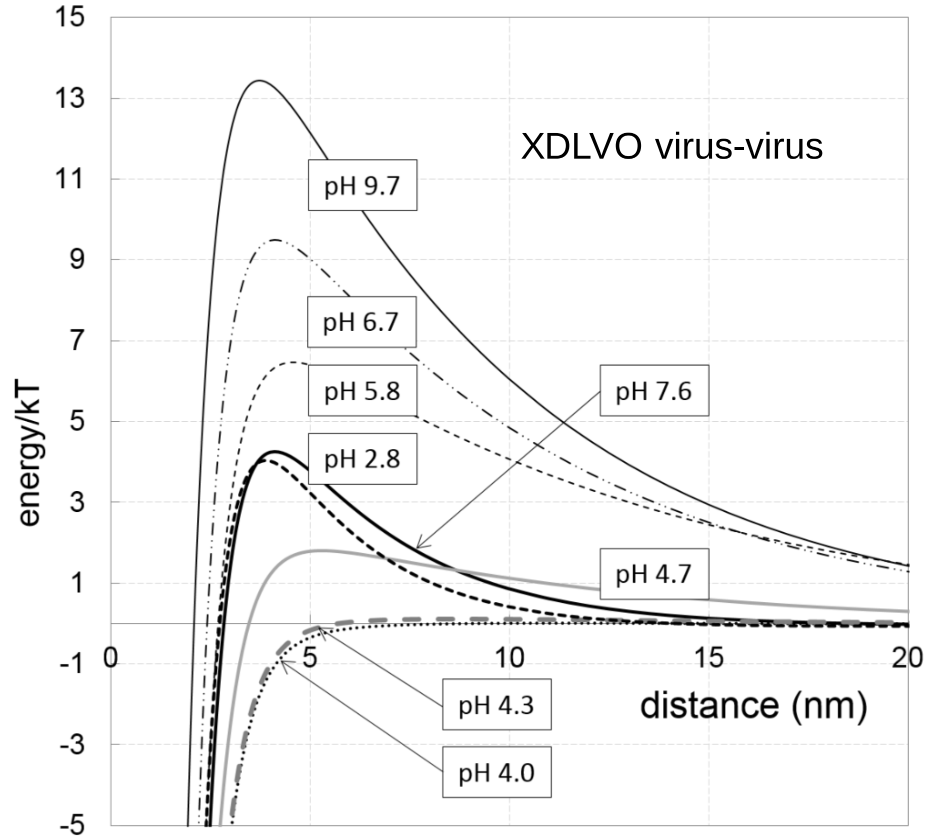
ζ–potential of individual (non-aggregated) HAdV virions



Shi, H. et al. Appl. Environ. Microbiol. 2016

$$\sigma = \frac{2\varepsilon_r \varepsilon_0 \kappa kT}{ze} sinh\left(\frac{ze\zeta}{2kT}\right)\sqrt{1 + \frac{1}{\kappa\frac{d_p}{2}}\frac{2}{cosh^2\left(\frac{ze\zeta}{4kT}\right)} + \frac{1}{\left(\kappa\frac{d_p}{2}\right)^2}\frac{8\, ln\left[cosh\left(\frac{ze\zeta}{4kT}\right)\right]}{sinh^2\left(\frac{ze\zeta}{2kT}\right)}}$$

Makina and Ohshima, 2010

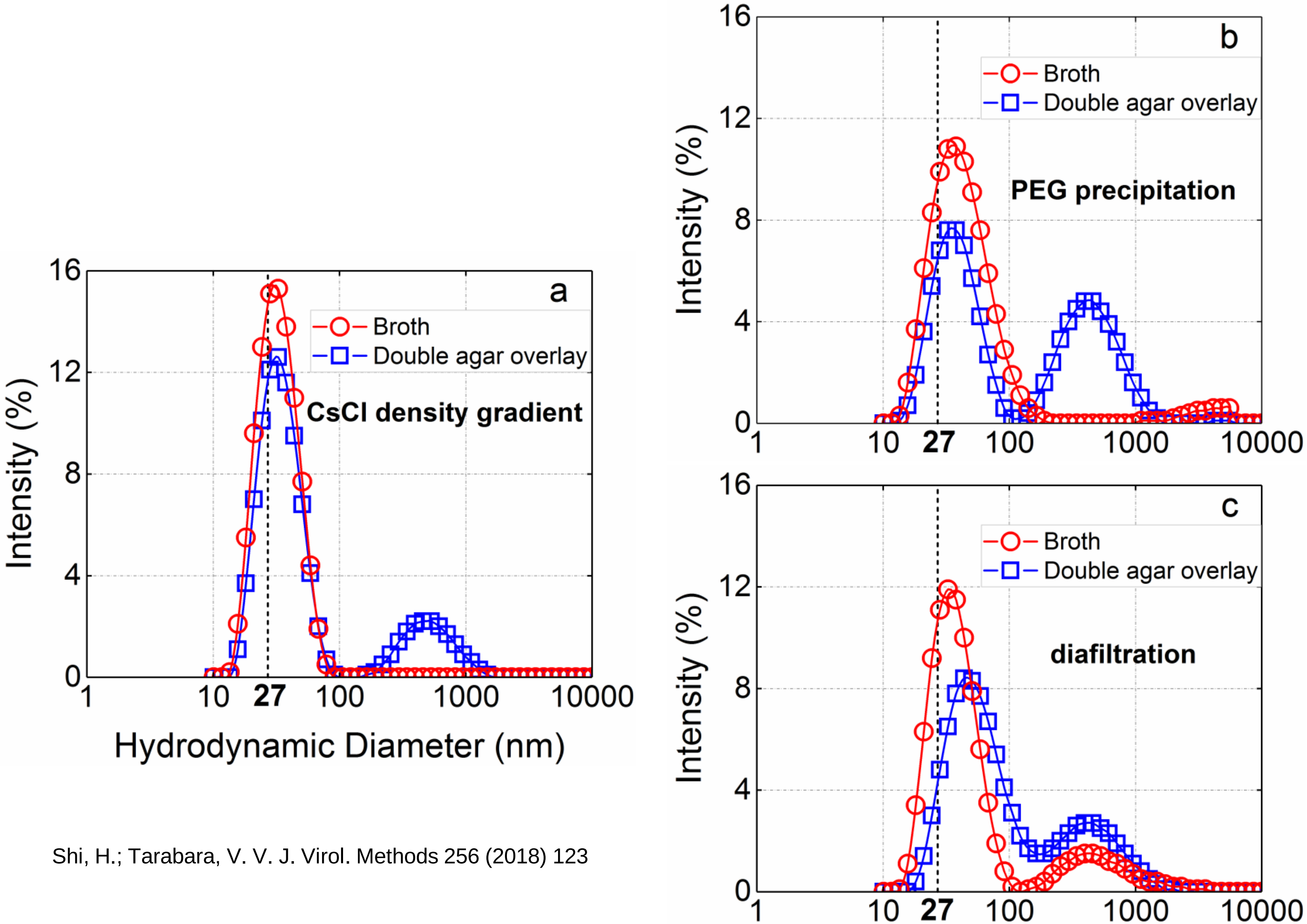


In ultrapure water (pH 5.8−6.0):

θ_w = 68 ^{o}

ΔG_{vwv} = - 30.4 mJ/ m^2

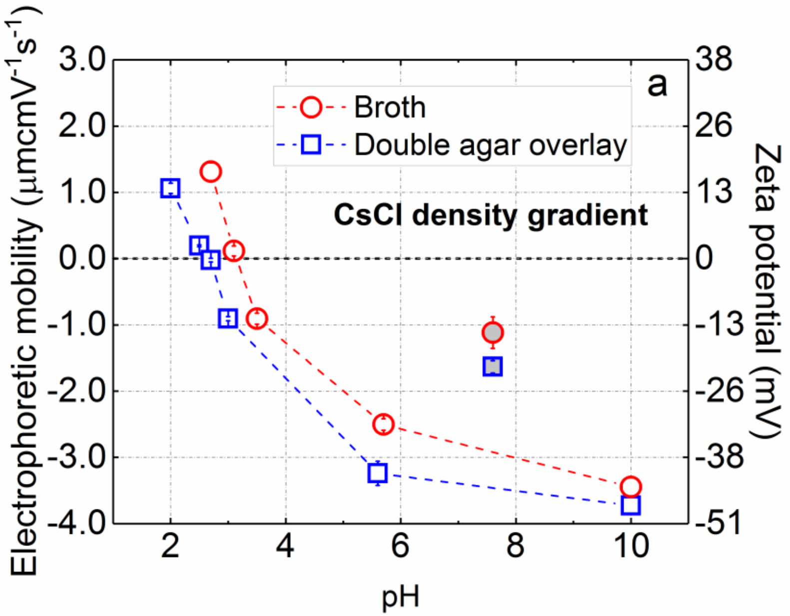
Effect of sample preparation
on virus (here – MS2 phage) size measurements

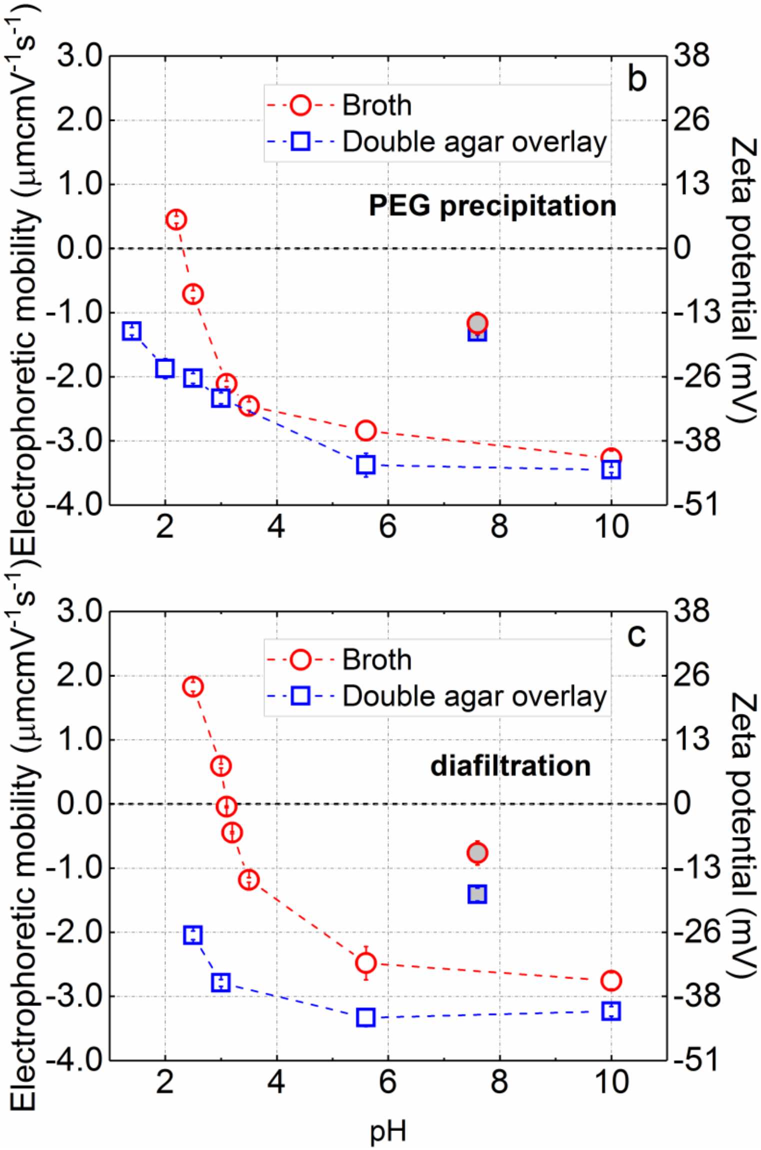


Shi, H.; Tarabara, V. V. J. Virol. Methods 256 (2018) 123

Effect of sample preparation

on virus (here – MS2 phage) charge measurements





Shi, H.; Tarabara, V. V. J. Virol. Methods 256 (2018) 123

Approach: Modeling

Extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) model

$$E^{XDLVO} = E^{LW} + E^{EL} + E^{AB}$$

E^{LW}: *Lifshitz - van der Waals (LW) interaction energy*

E^{EL}: *Electrical double layer (EL) interaction energy*

E^{AB}: *Lewis acid-base (AB) interaction energy*

Approach: Modeling

Extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) model predicts energy of virus-surface interaction, E_{slv}:

$$E_{slv}^{TOT} = E_{slv}^{LW} + E_{slv}^{EL} + E_{slv}^{AB}$$

where the following characteristics of the virus and the surface are taken as inputs:

For virus:
- d_v (hydrodynamic diameter)

For virus and surface:
- ζ (zeta potential)
- γ^{LW} (apolar component of surface tension)
- γ^{+} (electron-acceptor component of surface tension)
- γ^{-} (electron-donor component of surface tension)

Approach: Modeling

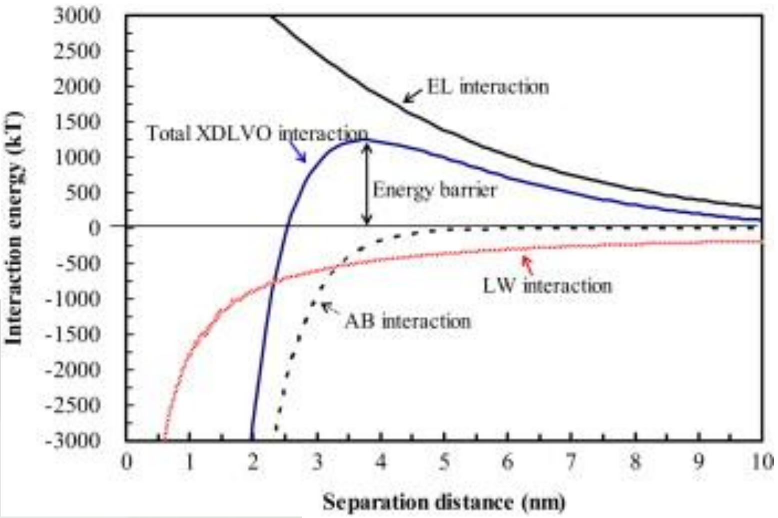
XDLVO theory

$$E_{slv}^{XDLVO} = E_{slv}^{LW} + E_{slv}^{EL} + E_{slv}^{AB} \quad (1)$$

$$E_{slv}^{LW} = -\frac{Aa}{6d} \quad (2)$$

$$E_{slv}^{EL} = \pi\varepsilon_r\varepsilon_0 a\left[2\psi_c\psi_s \ln\left(\frac{1+e^{-k_D d}}{1-e^{-k_D d}}\right) + (\psi_c^2+\psi_b^2)\ln(1-e^{-2k_D d})\right] \quad (3)$$

$$E_{slv}^{AB} = 2\pi a\lambda\Delta G_{d_0}^{AB} \exp\left(\frac{d_0-d}{\lambda}\right) \quad (4)$$



(*source: Lin et al. [47]*)

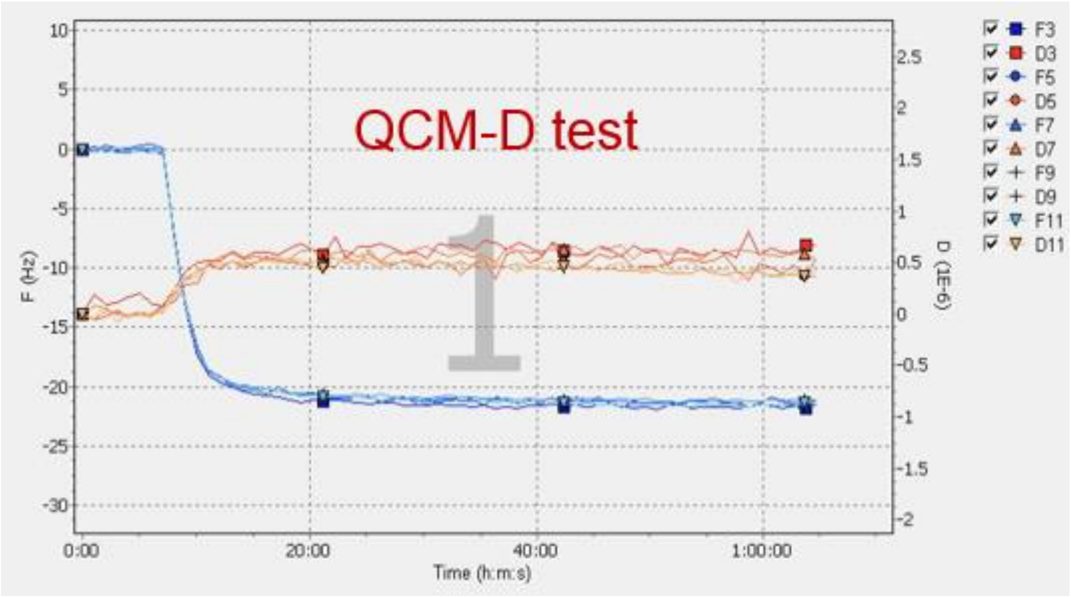
- ε_r: dielectric constant of water (ε_r = 79)
- ε_0 : relative permittivity in vacuum (ε_0 = 8.854·10^{12} CV^{-1}m^{-1})
- ψ_c and ψ_s: surface potentials
- k_D: reverse Debye length
- λ: characteristic delay length of the AB interaction (λ = 0.6 nm)
- d_0: minimum separation distance (d_0 = 0.158 nm)

Approach

Measuring virus attachment to surfaces using QCM-D

Quartz crystal microbalance with dissipation (QCM-D) measurements

(*image source: q-sense.com*)



Sauerbrey equation

$$\Delta m = -\frac{C\Delta f}{n}$$

- Δm: mass per unit surface area, $ng.cm^{-2}$
- C: mass sensitivity constant ($C=-17.7\ ng.Hz^{-1}.cm^{-2}$)
- Overtone number (n=3)
- Δf : frequency shift, Hz

Approach: Measuring virus adhesion

Virus: bacteriophage MS2

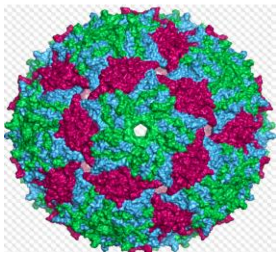


image source: Wikipedia.org

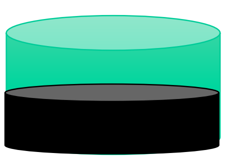
Virus purification steps:

- Add PEG 6000
- Centrifuge 10,000 rpm for 30 min
- Filter through 0.22 µm
- Dialyze through 100 kDa

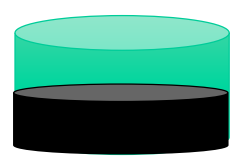
Deposition surface: Polyelectrolyte multilayer

Polyanion: PSS
poly(allylamine hydrochloride)

Polycation: PDADMAC
poly(diallyldimethyl ammonium chloride)

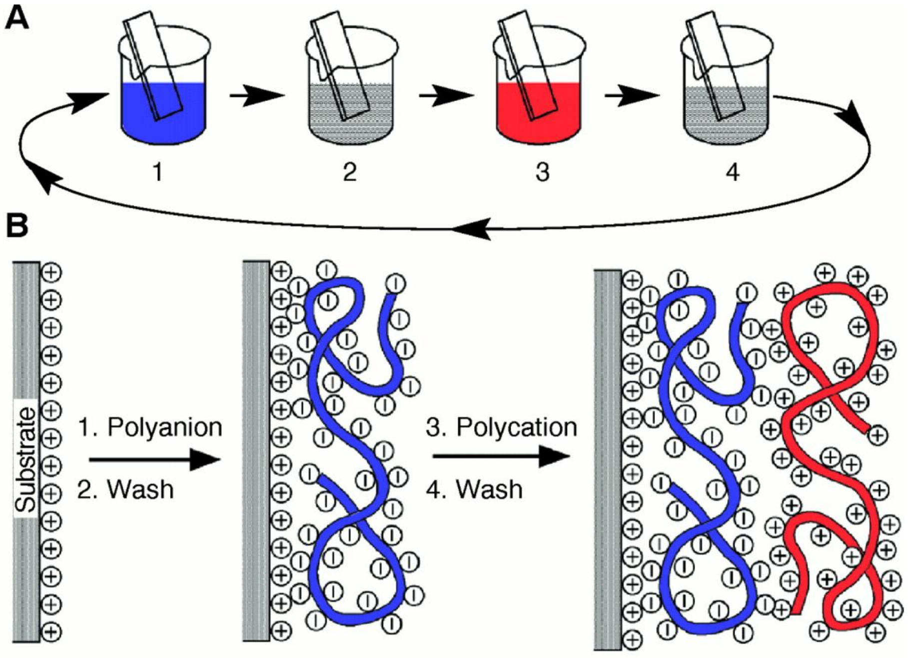


PSS
0.02 mM / pH 4.6



PDADMAC
0.02 mM / pH 6.4

PEM film preparation:
Electrostatic layer-by-layer self-assembly



[Decher, G. *Science* 277 (1997)]

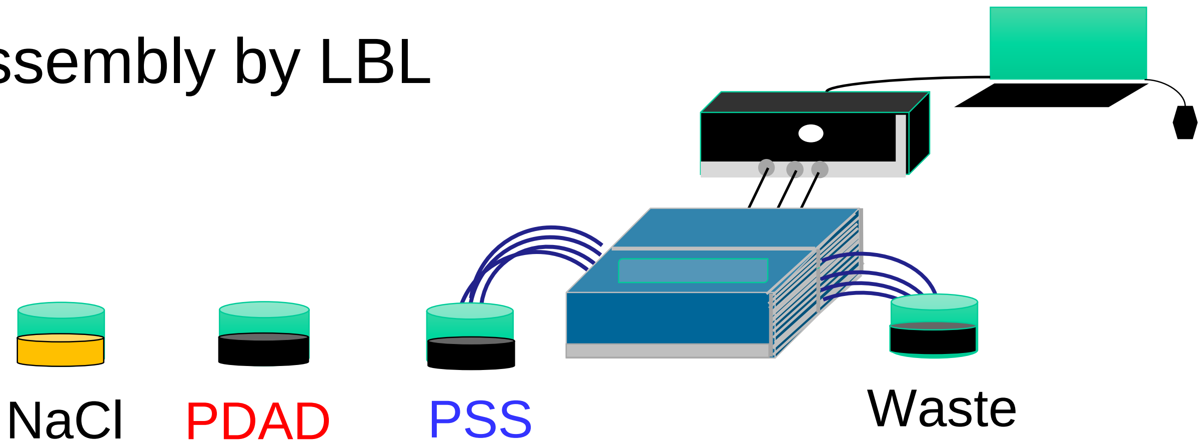
Two-step polyion adsorption process:
1) Anchoring
2) Relaxation into a denser film

- ❑ Easy control of film thickness, surface chemistry, swelling
- ❑ Wide range of materials applicable for LbL adsorption
- ❑ Possibility of tuning the surface properties by adjusting design parameters (pH, ionic strength, PE M_w, etc)
- ❑ Possibility of post assembly modification (grafting, cross-linking, annealing, etc)
- ❑ Easy disassembly to remove adhered materials (particles, viruses) or for regeneration

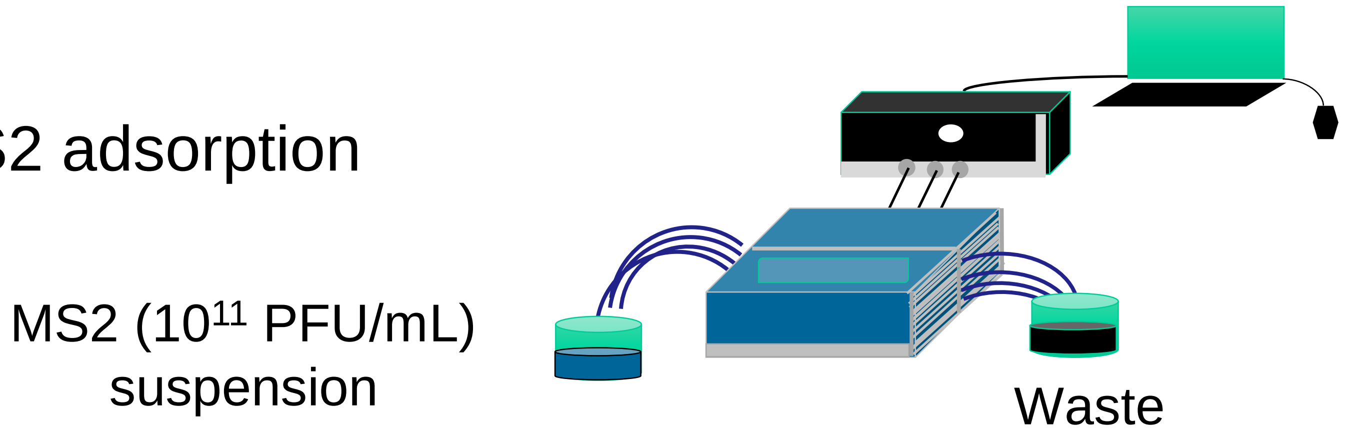
Approach

Surface design and virus attachment measurements

Step 1: PEM assembly by LBL



Step 2: MS2 adsorption



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Properties of the model surfaces

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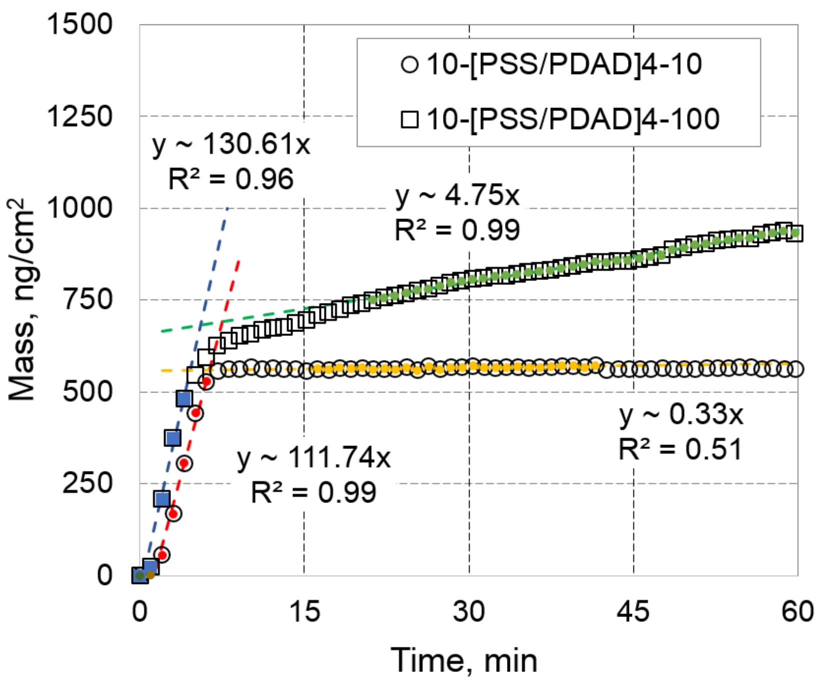
IS of PEs	10 mM PEM		100 mM PEM	
IS of MS2	10 mM	100 mM	10 mM	100 mM
	$[PSS/PDAD]_4$			
Zeta potential (mV)	6.7 ± 2		27.5 ± 1	
Free energy (mJ/m^2)	-16.3 ± 2.1		6.9 ± 14.4	
Roughness of PEMs (nm)	0.3	3.3	2.3	6.3
	$[PSS/PDAD]_4[PSS]$			
Zeta potential (mV)	-5.7 ± 2		-17.8 ± 0	
Free energy (mJ/m^2)	45.2 ± 0.6		44.3 ± 10.2	
Roughness of PEMs (nm)	0.2	1.3	2.5	6.5

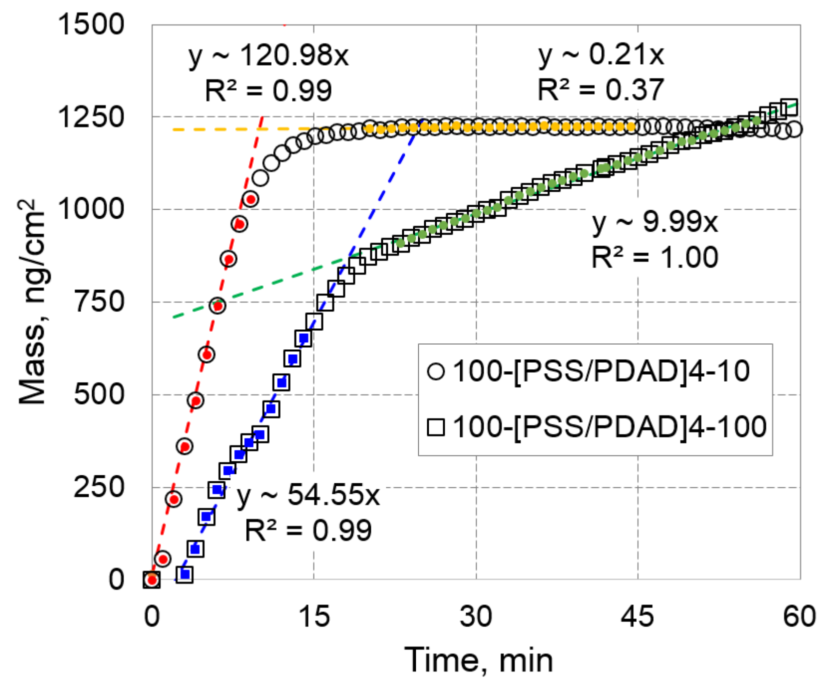
Note: the negatively-charged PEMs are more hydrophilic than the positively-charged PEMs

MS2 adsorption onto [PSS/PDAD]$_4$

a positively-charged and relatively hydrophobic surface

Deposition phase 1: red and blue symbols and trend lines
Deposition phase 2: yellow and green symbols and trend lines

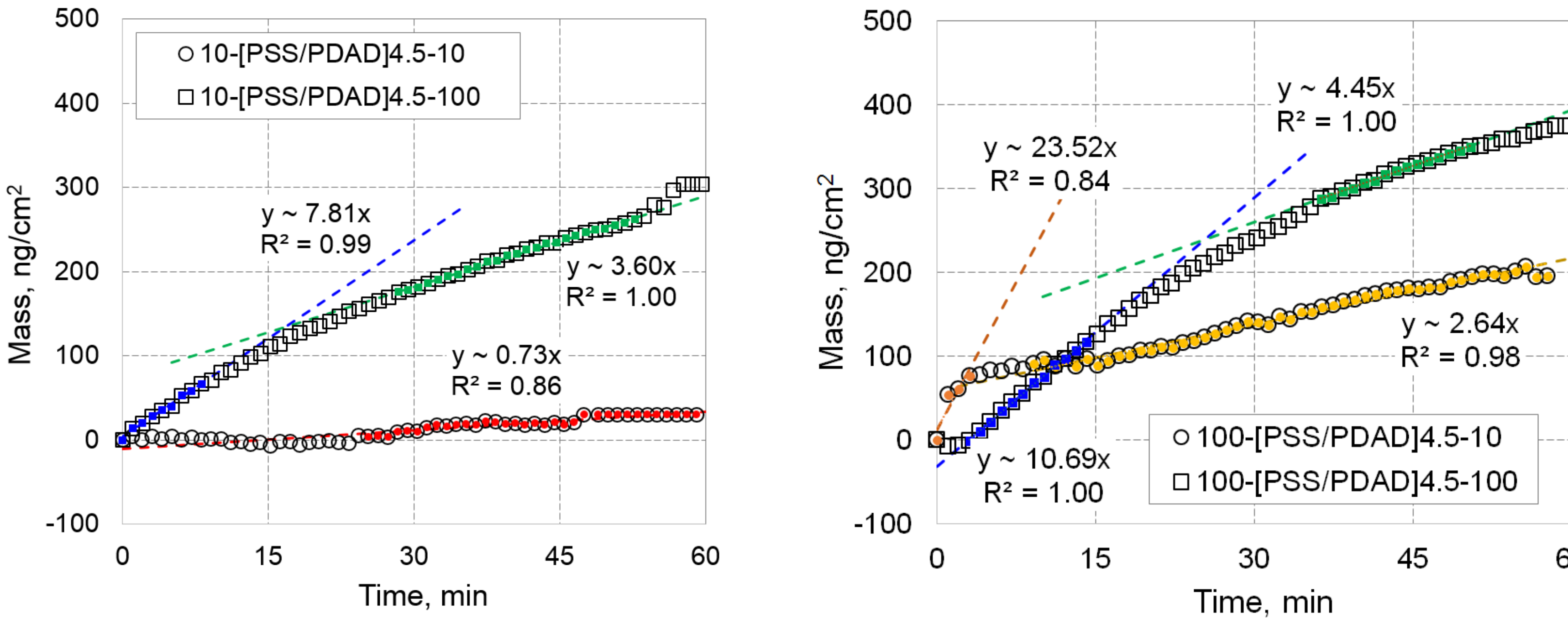




Phase 1: the surface is relatively virus-free
Phase 2: the surface charge is affected by the virus accumulation on the surface.

MS2 adsorption onto $[PSS/PDAD]_4[PSS]$
a negatively-charged and hydrophilic surface

Deposition phase 1: red and blue symbols and trend lines
Deposition phase 2: yellow and green symbols and trend lines



Phase 1: the surface is relatively virus-free
Phase 2: the surface charge is affected by the virus accumulation on the surface.

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Predicted virus-surface interactions

with poyanion- and polycation-terminated surfaces

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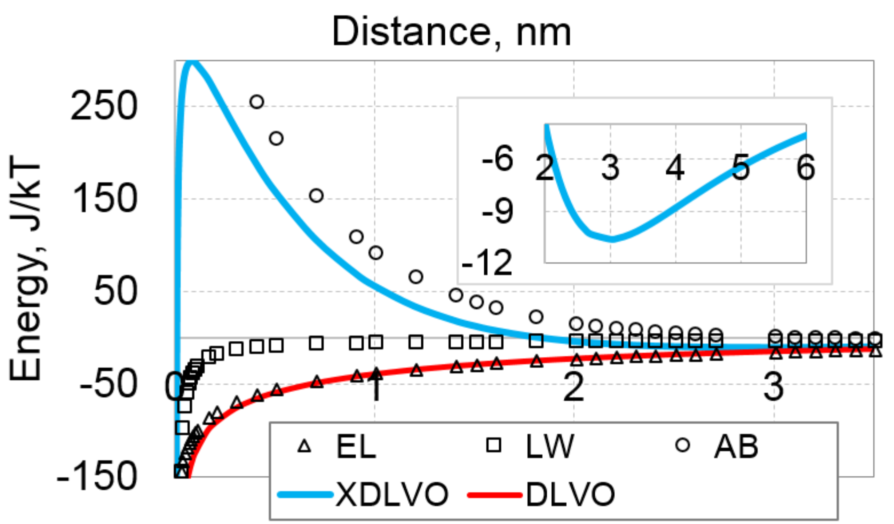
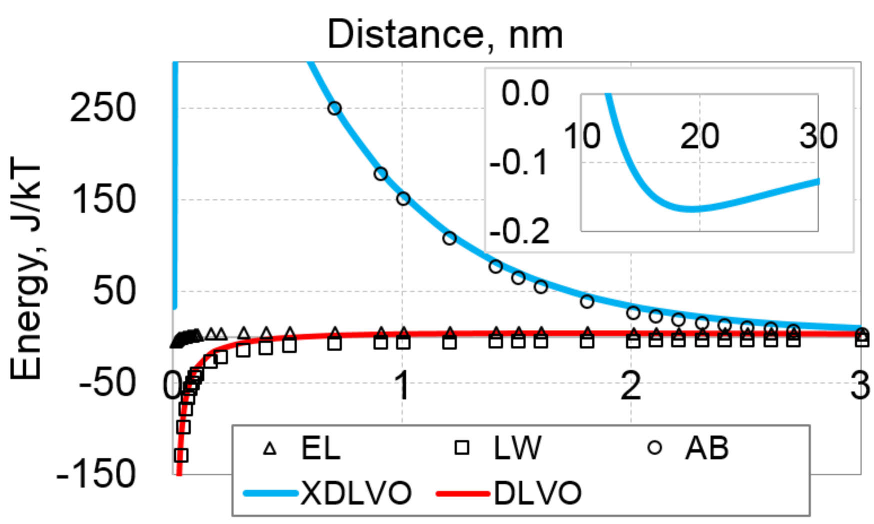
100-[PSS/PDAD]$_4$-10



100-[PSS/PDAD]$_{4.5}$-10

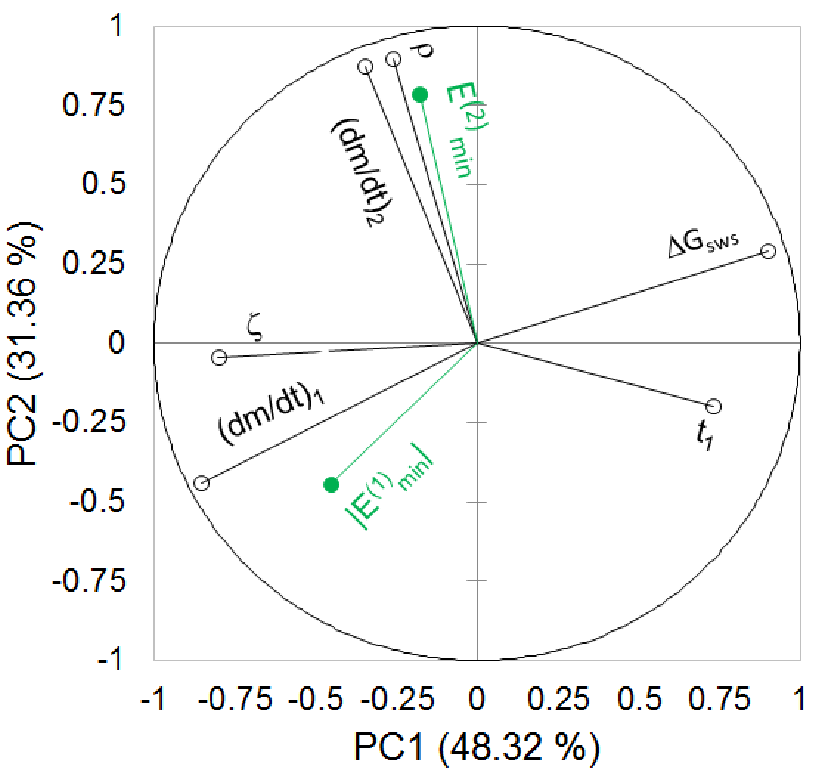


The profiles correspond to early stages of phase 1 of MS2 deposition

EL = electrostatic interactions
LW = van der Waals interactions
AB = acid-base interactions.

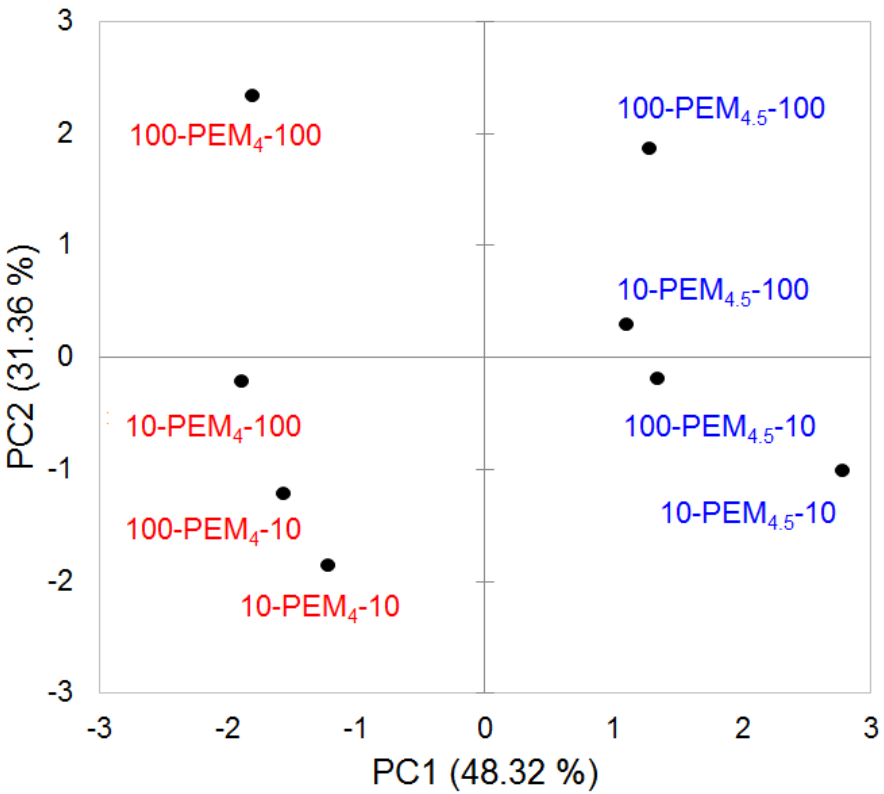
Principal component analysis
for MS2 deposition on the model surfaces

Factor loadings



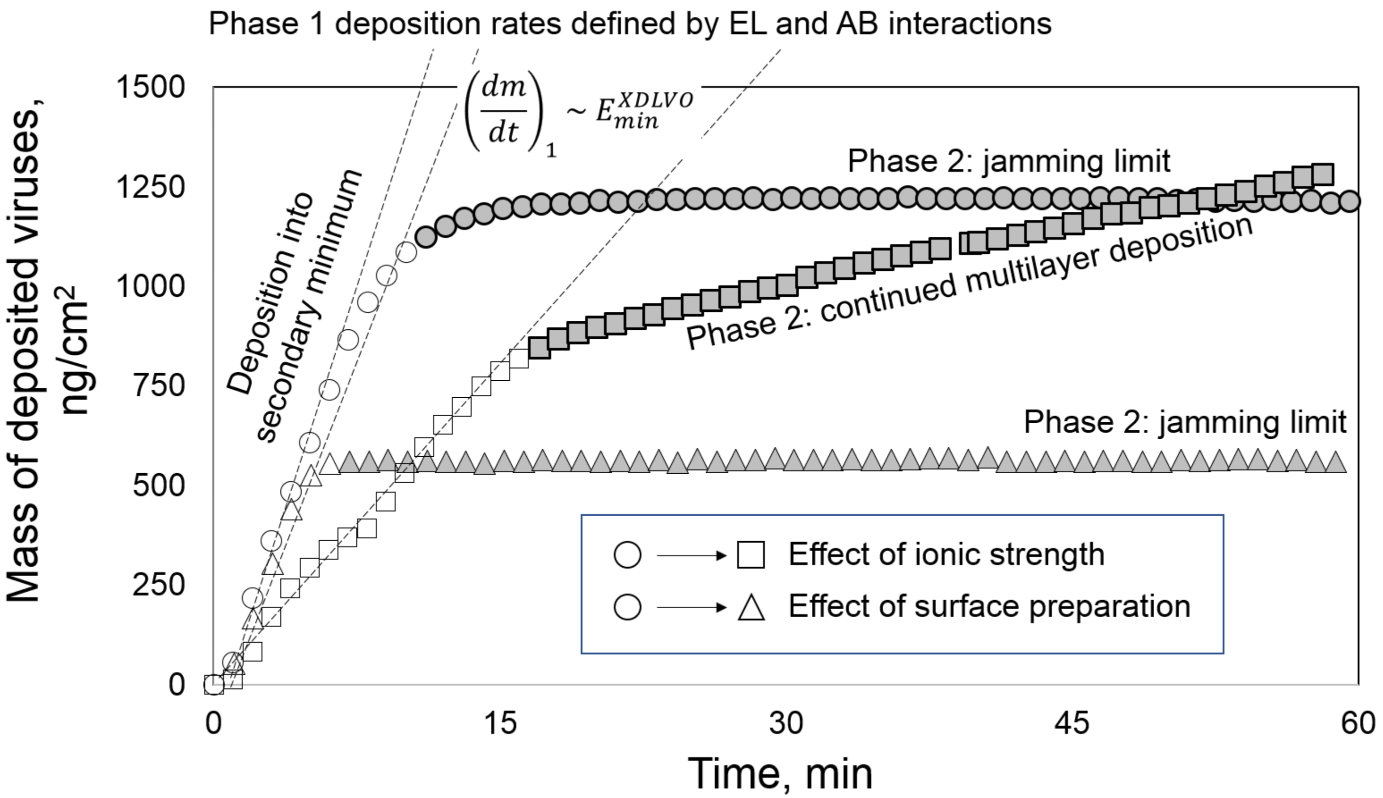
○ Surface properties and MS2 deposition kinetics data
● Supplementary variables: XDLVO model predictions

Factor scores



MS2 adsorption onto model surfaces

Summary



The **overarching observation** is that differently prepared surfaces show distinct kinetics of and capacities for virus deposition.

The findings support the **hypothesis** that surfaces can be designed to have tailored adhesive properties with respect to viruses.

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Conclusions

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Comparison of QCM-D observations with XDLVO predictions shows that the deposition rate correlates with the depth of the secondary minimum in the XDLVO energy profile.

The depth of the minima is defined primarily by the acid-base and electrostatic interactions supporting the hypothesis that charge and hydrophilicity of the polyelectrolyte multilayers control virus adhesion.

(When the deposition occurs from high ionic strengths) screening of electrostatic interactions makes the role of acid-base interactions more prominent emphasizing the importance of hydrophilicity as a surface design criterion.

Acknowledgements

Funding

U.S. NSF PIRE project "Water and Commerce - Technologies to enable environmental sustainability in global markets" (IIA-1243433)

U.S. NSF project "Virus removal in membrane bioreactors: Role of virus aggregation and adhesion" (CBET-1236393)

U.S. NSF PIRE project "New generation synthetic membranes - Nanotechnology for drinking water safety" (OISE-0530174)

U.S. Environmental Protection Agency "Science to Achieve Results" (R833010)

CRDF/SRNSF/GRDF Georgia Early Career Scholars program grant "Integrating bacteriophage and membrane separation knowledge bases to ensure microbiological safety of water supply"

U.S. Fulbright Scholar fellowship "Ensuring microbiological safety of water: Bacteriophages as human virus surrogates"

International partnership on membrane processes for research and educational excellence (MEMPREX)

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Acknowledgements

Former and current graduate students – the "virus" team

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- Elodie Pasco (PhD 2014)
- Bin Guo (PhD 2016)
- Brian Starr (MS 2016)
- Alex Casabuena (MS 2016)
- Hang Shi (PhD 2018)
- Hien Dang (PhD 2018)
- Xunhao Wang (MS in progress)
- Kyle Hillstead (MS in progress)







Acknowledgements: Colleagues from Tbilisi

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International collaborations

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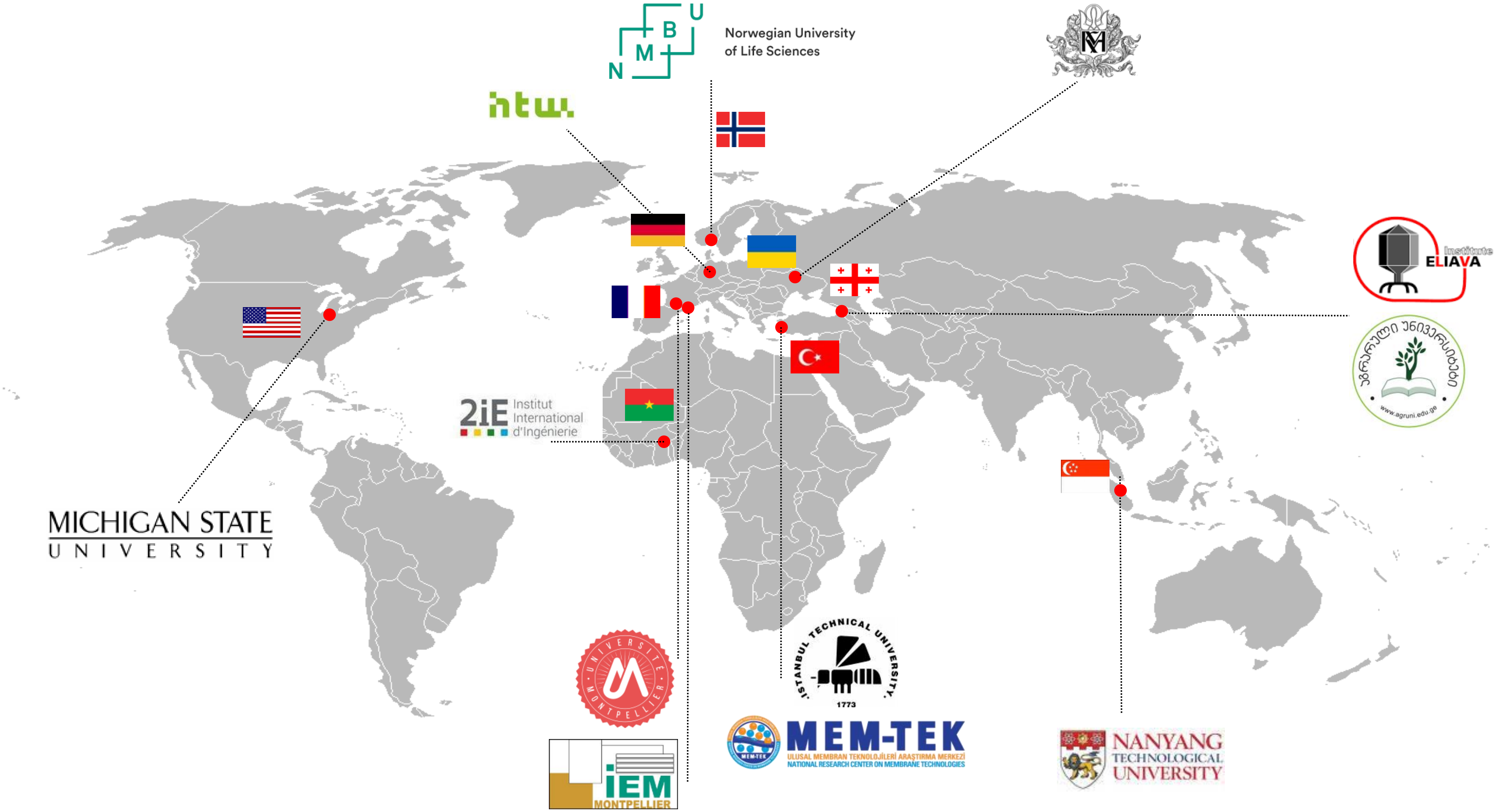

Thank you
გმადლობთ

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