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

# Bacteriophages as surrogates for human viruses

## Fundamentals and applications to water quality control

Vlad Tarabara, Professor

Department of Civil and Environmental Engineering
Michigan State University

April 3, 2019

Image source: http://en.wikipedia.org/wiki/Michigan

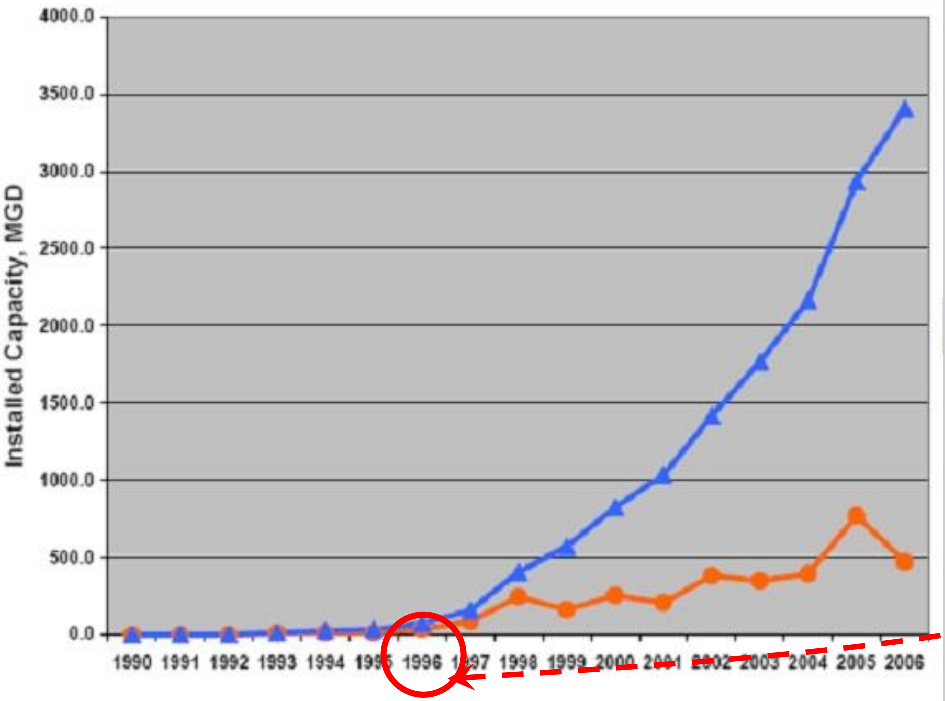
Image source: michigan.gov

# From Victorian to 21st century technologies

## Victorian technologies dominated water treatment just 30 years ago

[D. Furukawa, 2004]

global low pressure membrane installed capacity:
**annual** and **cumulative**

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**Milwaukee learned its water lesson, but many other cities haven't**

September 2, 1996
Web posted at: 9:30 p.m. EDT

*Editor's Note: This is the first in a three-part series on water quality in the United States.*

From Correspondent Dan Rutz

(CNN)

MILWAUKEE (CNN) -- Until 1993, most Americans took the cleanliness of public drinking water for granted.

The United States has a reputation for high standards in its water systems; it wasn't until a parasite slipped through the cracks in Milwaukee and killed more than 100 people that water systems managers started to take a closer look at how they monitored their

Dialectic nature of water management: "Each new success gives rise to new challenges. … State of water management is always provisional" (J. Brisco)

# Water quality control technologies

Needed but presently lacking



**Integrity monitoring for water treatment systems**

- Real time of near real-time sentinel detection is desirable

**Fast and reliable detection of viruses in complex water matrices**

- Sample concentration as a bottleneck

**Cyber security of water infrastructure**

- Protection against the other kind of viruses

# Viruses listed on the EPA's CCL

and diseases they cause

| Virus | Associated Disease | CCL 1 | CCL 2 | CCL 3 | CCL 4 |
|---|---|---|---|---|---|
| Adenoviruses | conjunctivitis, ocular infections, gastroenteritis, respiratory disease, encephalitis, pneumonia, genitourinary infections, pharyngoconjunctival fever | Yes | Yes | Yes | Yes |
| Enteroviruses * | hand- foot-and-mouth-disease, gastroenteritis, heart anomalies, myocarditis, pericarditis, meningitis, pancreatitis, paralysis | | | Yes | Yes |
| Coxsackieviruses | | Yes | Yes | | |
| Echoviruses | | Yes | Yes | | |
| Hepatitis A | hepatitis | | | Yes | Yes |
| Caliciviruses | gastroenteritis | Yes | Yes | Yes | Yes |

*Polioviruses, coxsackieviruses, and echoviruses are generally referred to enteroviruses. Enteroviruses are listed in the CCL3 and CCL4.

# Why to be concerned about viruses

## in recreational water and their removal in WWTPs



- Many of infectious disease outbreaks due to unidentified sources are likely caused by enteric viruses (USEPA, 2006)
  - Viruses were identified as etiological agents for 54% of hospitalized patients (Children's Infectious Diseases Hospital, Tbilisi, Georgia, 2004-2008 period)
- Wastewater is one of the major sources of human viruses
  - More than 100 types of enteric viruses are excreted in human feces and present in contaminated waters
- Human enteric viruses are detected in the effluents of state-of-the art wastewater treatment plants worldwide
  - Even large viruses (e.g. human adenovirus) are found in conventional WWTP and MBR plant effluents
- … and yet, virus removal is not a design criterion or treatment goal for wastewater treatment plants

COLLEGE OF ENGINEERING

# Waterborne disease outbreaks

## in the U.S. (1971-2012)

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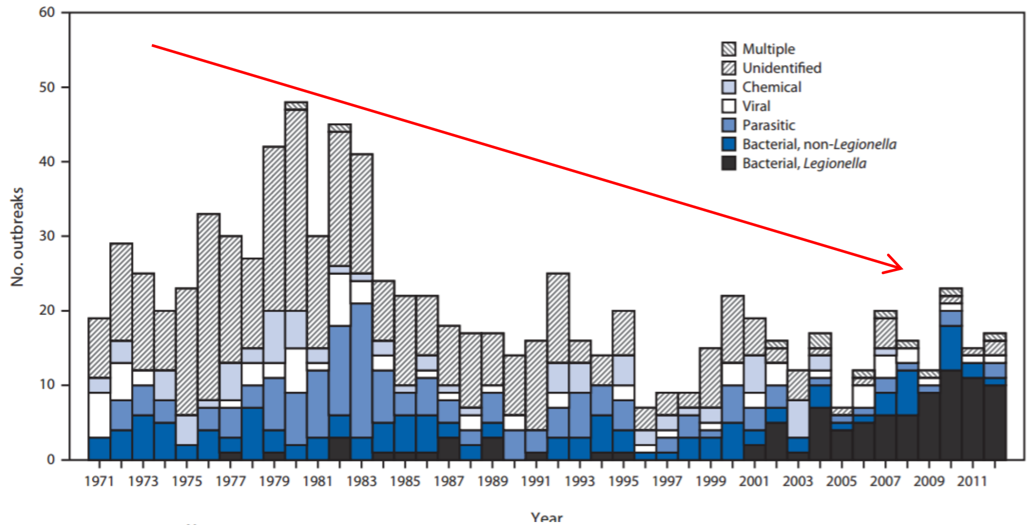
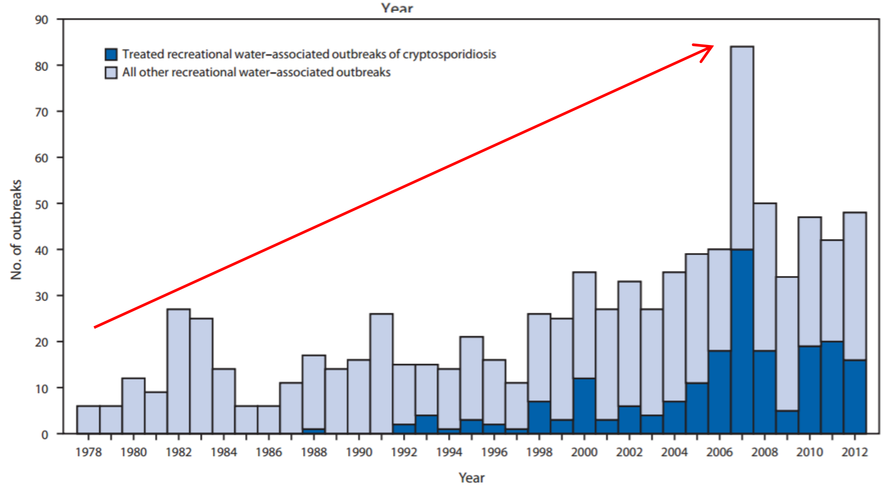
Source: "Surveillance for Waterborne Disease Outbreaks Associated with Drinking Water and Other Non-Recreational Water — United States, 2011-2012" by Centers for Disease Control and Prevention



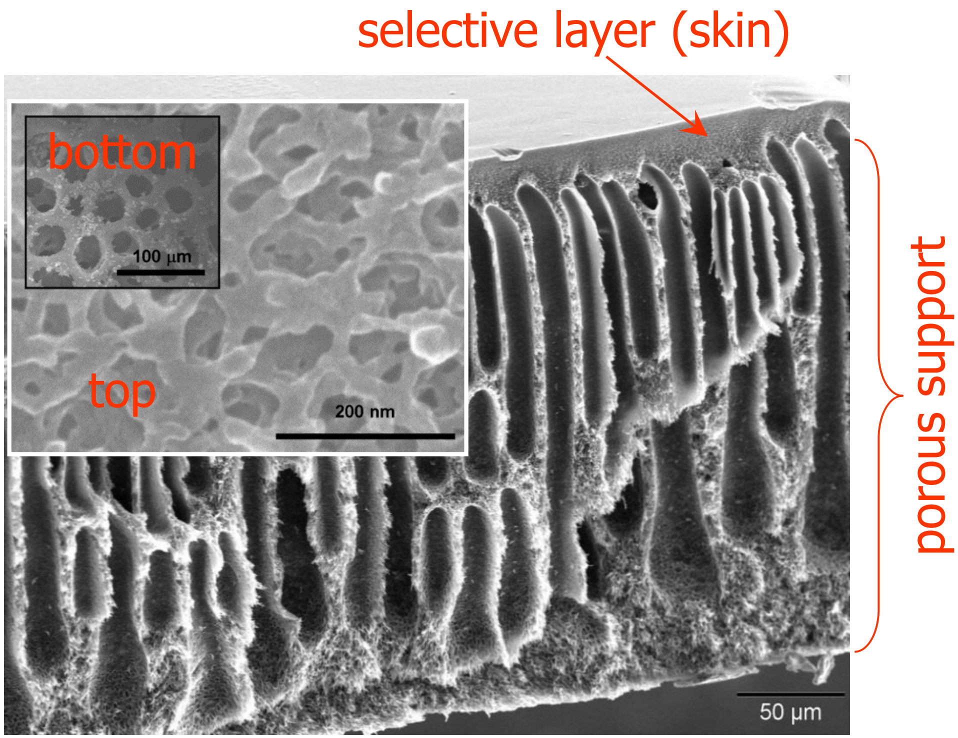

Source: "Recreational water-associated disease outbreaks, United States, 2011-2012" by Centers for Disease Control and Prevention



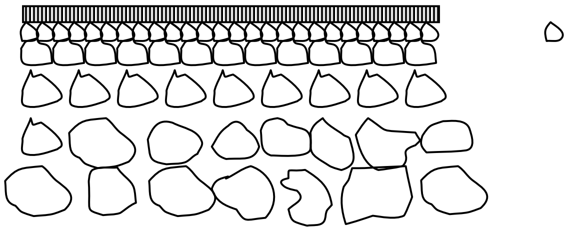

* Total n = 879.

# Membranes provide an “absolute barrier” to pollutants

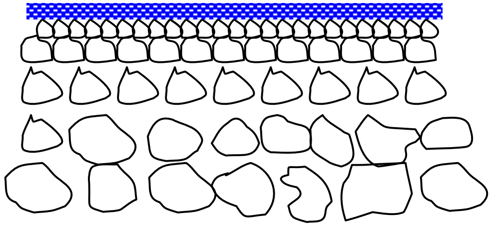
Membranes are made asymmetric for higher permeability



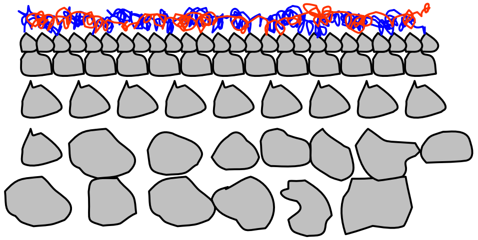

Integrally-skinned



Thin-film composite

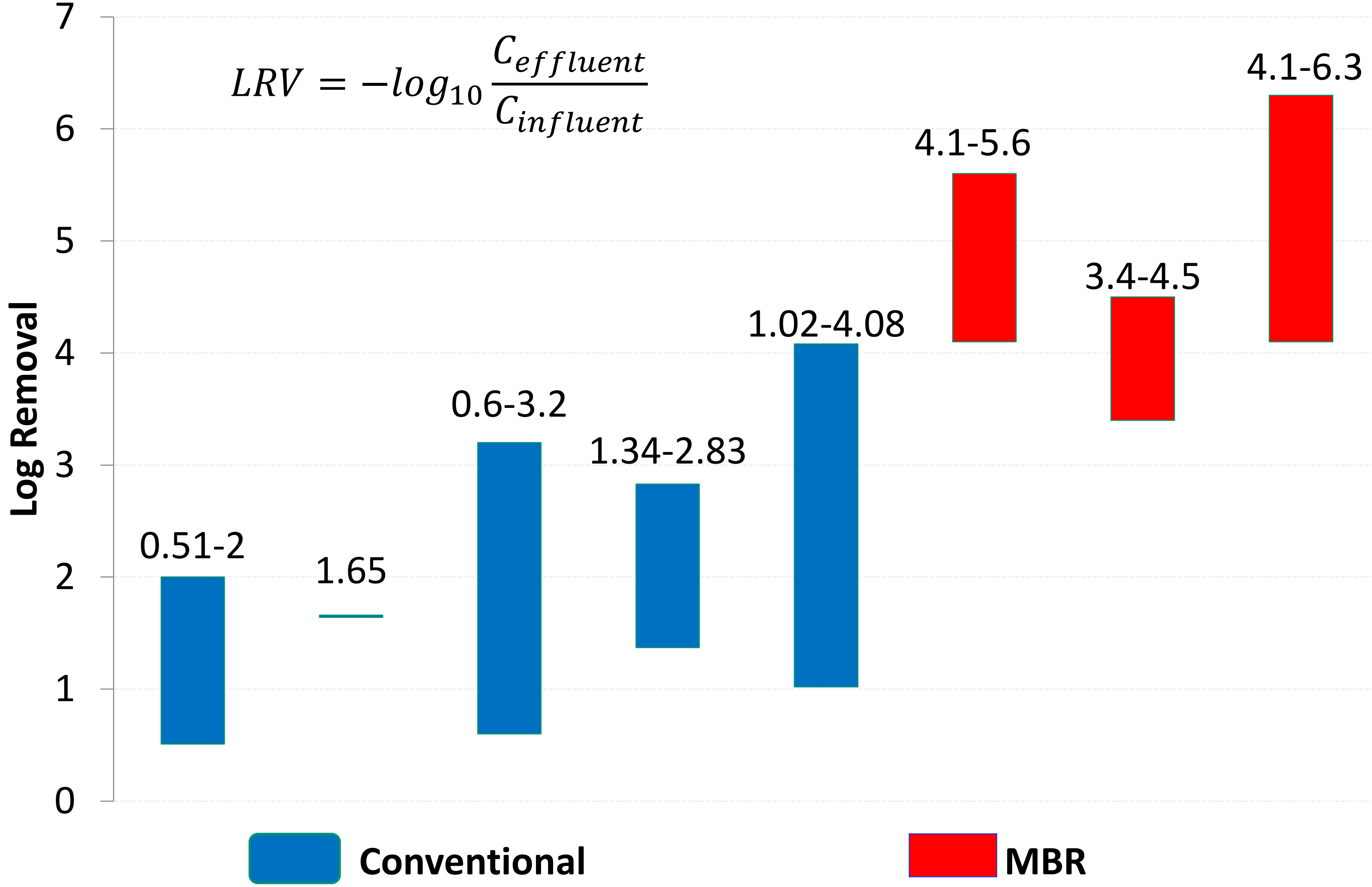


Polyelectrolyte multilayer skin



# Removal of human adenovirus

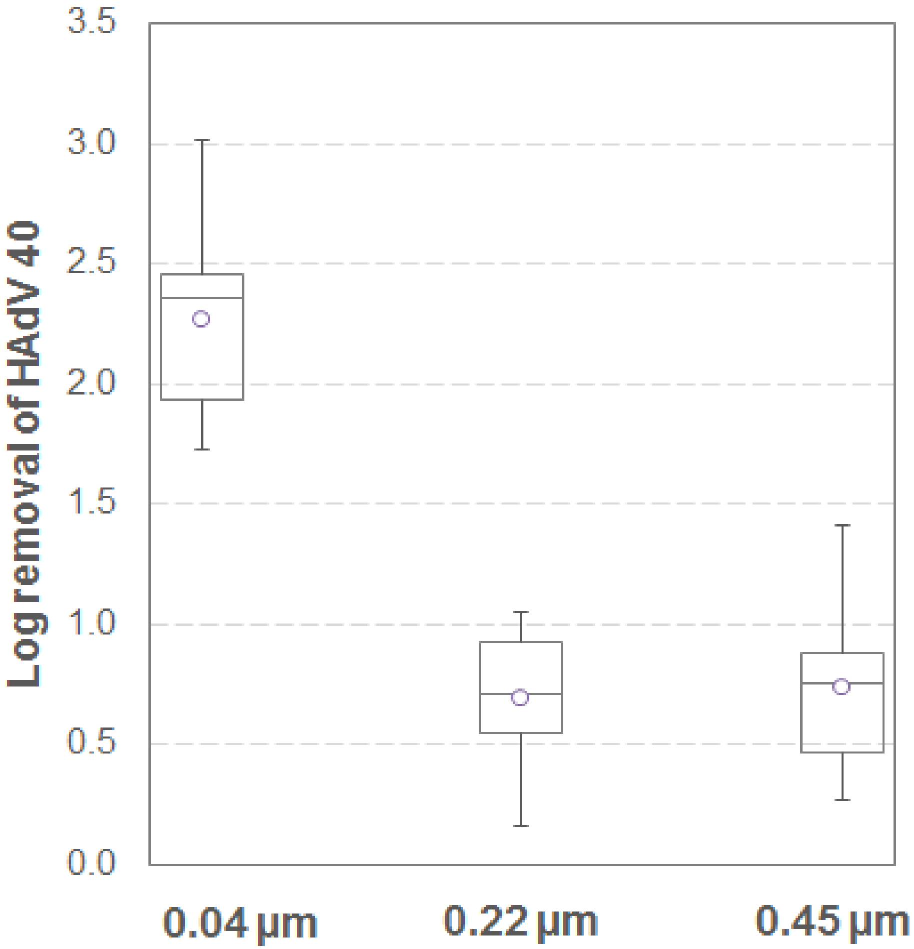
in MBR and conventional WWTPs (literature data)



# Removal of HAdV 40 from DI water

by three types of hollow fiber membranes






virus size
& membrane pore sizes:

UF: $d_{pore}$ = 40 nm

**HAdV40: $d_h$ = 80 - 90 nm**

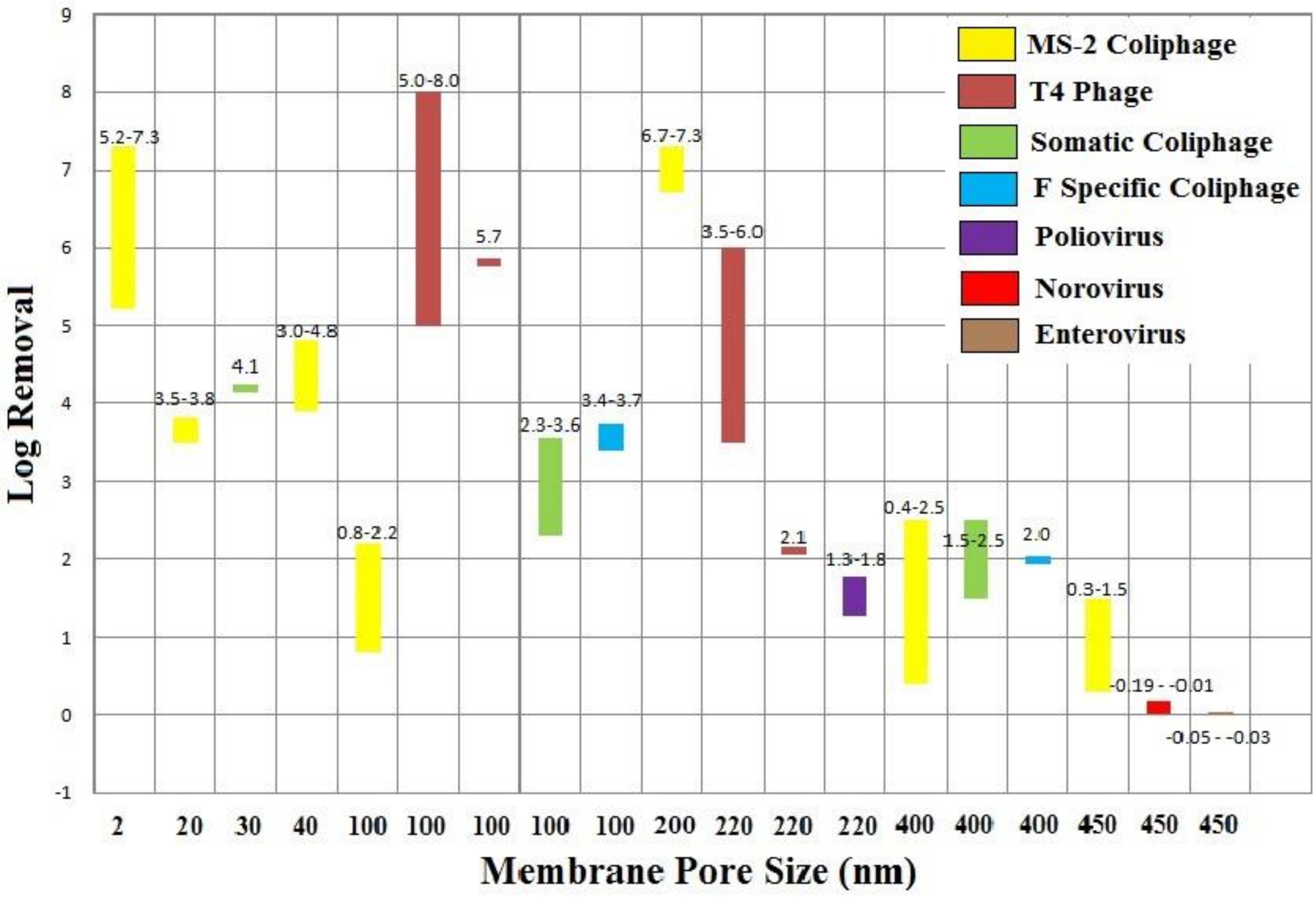
MF1: $d_{pore}$ = 220 nm

MF2: $d_{pore}$ = 450 nm

Yin, Z.; Tarabara, V. V.; Xagoraraki, I. Human adenovirus removal by hollow fiber membranes: Effect of membrane fouling by suspended and dissolved matter. J. Membr. Sci. 482 (2015) 120-127.

# Virus removal in membrane bioreactors
(literature data)

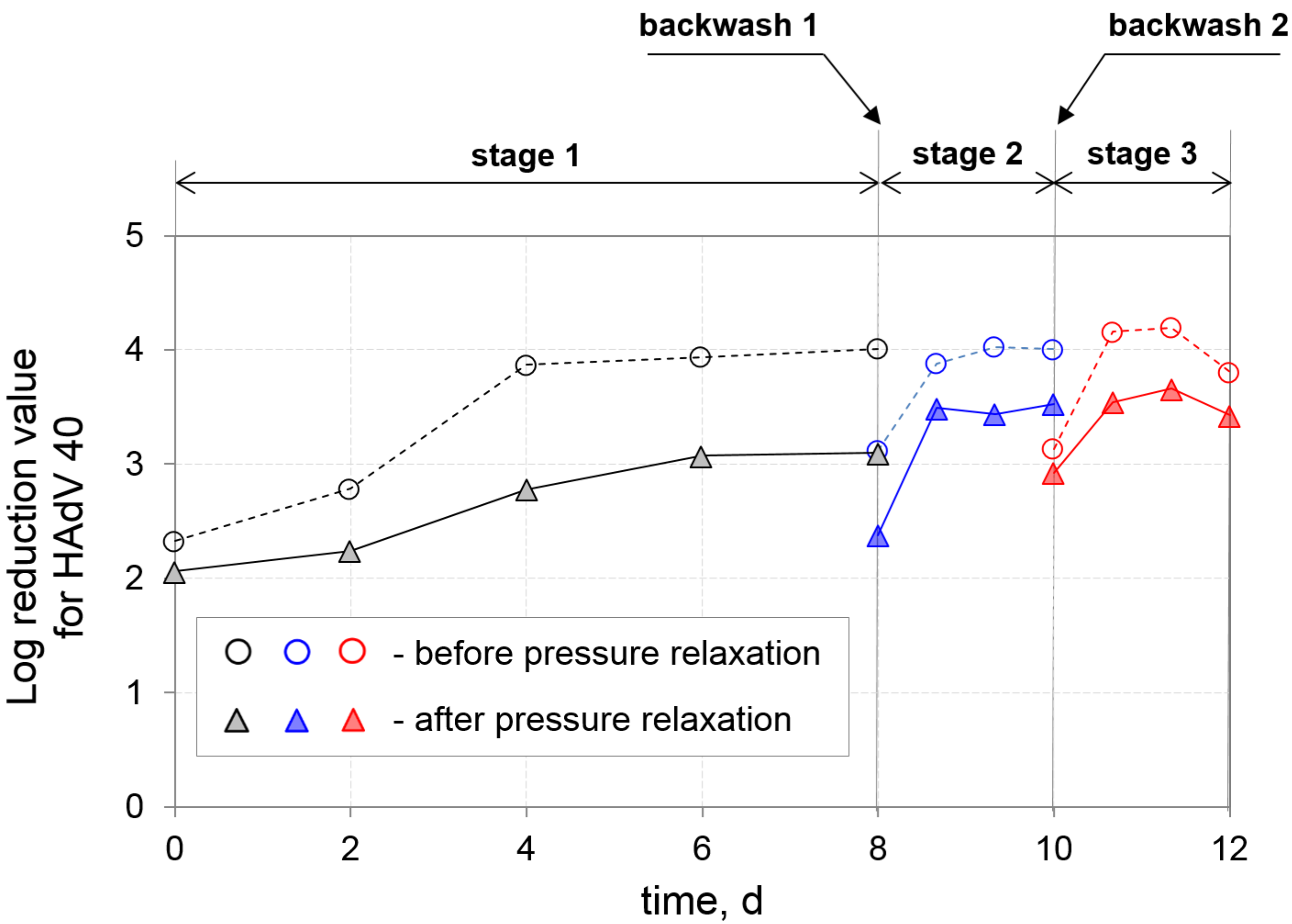





Credit: Dr. Ziqian Yin (MSU)

# Virus removal in a bench-scale MBR

Probing effects of membrane cleaning




Yin, Z.; Tarabara, V. V.; Xagoraraki, I. Effect of pressure relaxation and membrane backwash on adenovirus removal in a membrane bioreactor. Water Res. 88 (2016) 750-757

# Potential for viruses in drinking water

due to failure if high pressure membranes



**In-service failures**

- damage to the membrane layer as a result of chemical or biological degradation and particulate abrasion
- delamination of the membrane supporting layers
- failures of O-rings, gaskets, connectors and other fittings

**Manufacturing defects** (e.g. incomplete glue-lines)

*membranes* MDPI

*Review*

**Current and Emerging Techniques for High-Pressure Membrane Integrity Testing**

**Eddy R. Ostarcevic [1], Joseph Jacangelo [2], Stephen R. Gray [1] and Marlene J. Cran [1,*]**

1 Institute for Sustainable Industries and Liveable Cities, Victoria University, Werribee 3030, Australia; eddy.ostarcevic@vu.edu.au (E.R.O.); stephen.gray@vu.edu.au (S.R.G.)

2 Stantec, Washington, DC 20005-3957, USA; joseph.jacangelo@stantec.com

* Correspondence: marlene.cran@vu.edu.au; Tel.: +61-3-9919-7642

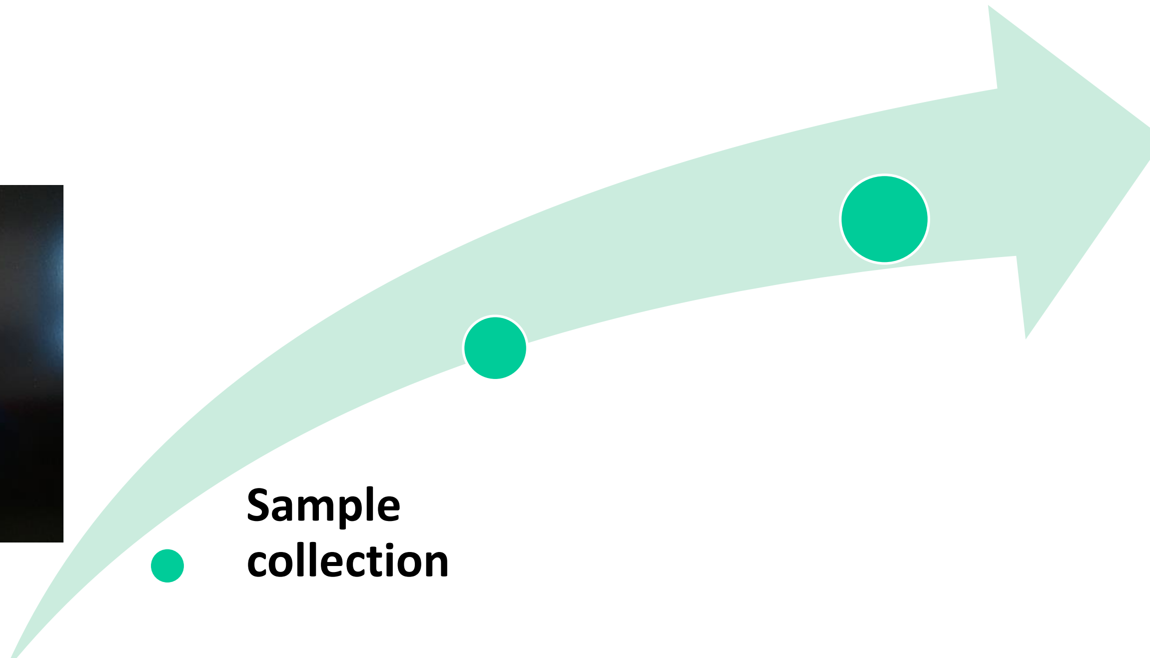
# Waterborne pathogen detection

## The challenge of sample concentration



# Waterborne pathogen detection

## The challenge of sample concentration




# Waterborne pathogen detection

## The challenge of sample concentration

**Sample concentration**

**Sample collection**

# Waterborne pathogen detection

## The challenge of sample concentration



- **Sample collection**
- **Sample concentration**
- **Sample detection**

# Waterborne pathogen detection

## The challenge of sample concentration

Sample detection

Sample concentration

Sample collection

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Cite this: *Environ. Sci.: Water Res. Technol.*, 2017, 3, 778

## Membrane-based methods of virus concentration from water: a review of process parameters and their effects on virus recovery†

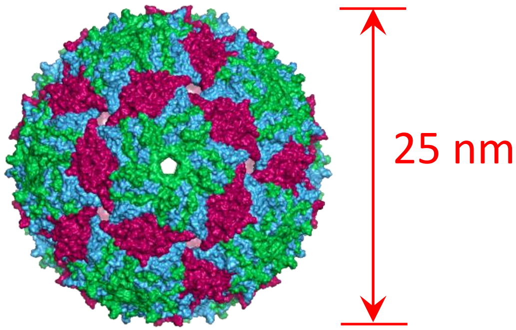
Hang Shi, Elodie V. Pasco and Volodymyr V. Tarabara *

# Bacteriophages as surrogates

For human viruses



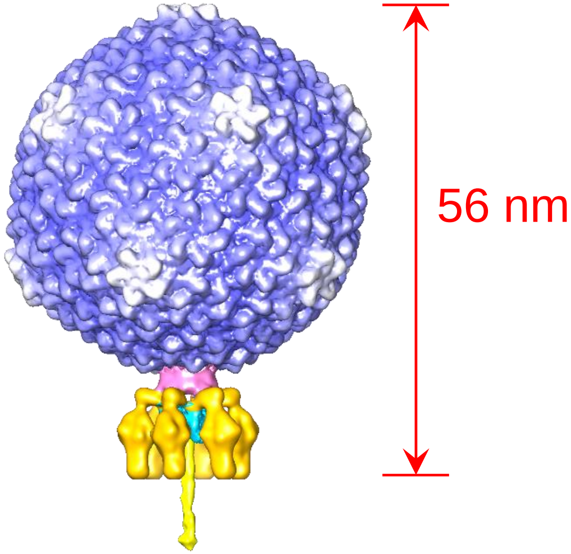
**MS2**




ssRNA virus

surrogate for norovirus

**P22**




dsDNA virus

surrogate for adenovirus

MS2 image source:
https://en.wikipedia.org/wiki/Bacteriophage_MS2

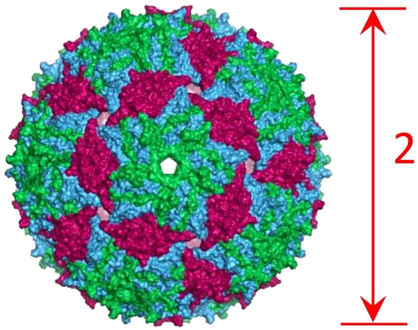
P22: image source:
Dr. Kristin Parent, Michigan State University

# Bacteriophages as surrogates

For human viruses



**MS2**

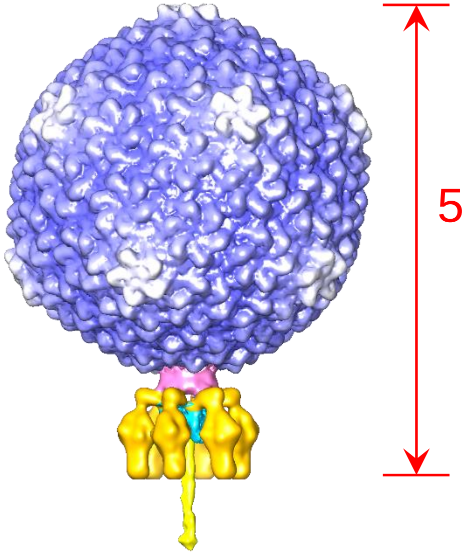



ssRNA virus

surrogate for norovirus

MS2 image source:
https://en.wikipedia.org/wiki/Bacteriophage_MS2

**P22**




dsDNA virus

surrogate for adenovirus

P22: image source:
Dr. Kristin Parent, Michigan State University

Odilon Redon. 1883 Frontispiece for “Les Origines”, lithograph

# How to select suitable surrogates?

Three steps to identifying the "right phage"

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

2. Model virus-surface interaction
   - For example, using extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) model

3. Experimentally validate the choice of the surrogate in the target application
   - Example 1: measure phage removal by a sand filter or a membrane
   - Example 2: measure phage adhesion to a fomite

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MICHIGAN STATE UNIVERSITY

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# Human Adenovirus 40 (HAdV 40)

## Regulated waterborne pathogen



### Contaminant Candidate List 3 – CCL

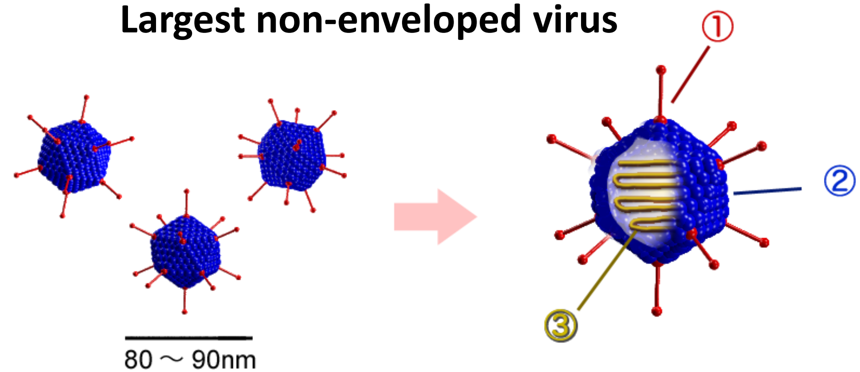
CCL & Regulatory Determinations Home | Basic Information | CCL 1 | CCL 2 | CCL 3 | CCL 4

**Microbial Contaminants**

| Microbial Contaminant Name | Information |
|---|---|
| Adenovirus | Virus most commonly causing respiratory illness, and occasionally gastrointestinal illness |

- **Longer survival time**
  in tap water and sea water:
  1) inactivation log around **1-2** for **HAdV 40/41** after 60 days;
  2) inactivation log around **2-4** for **poliovirus 1** after 60days.

**Largest non-enveloped virus**



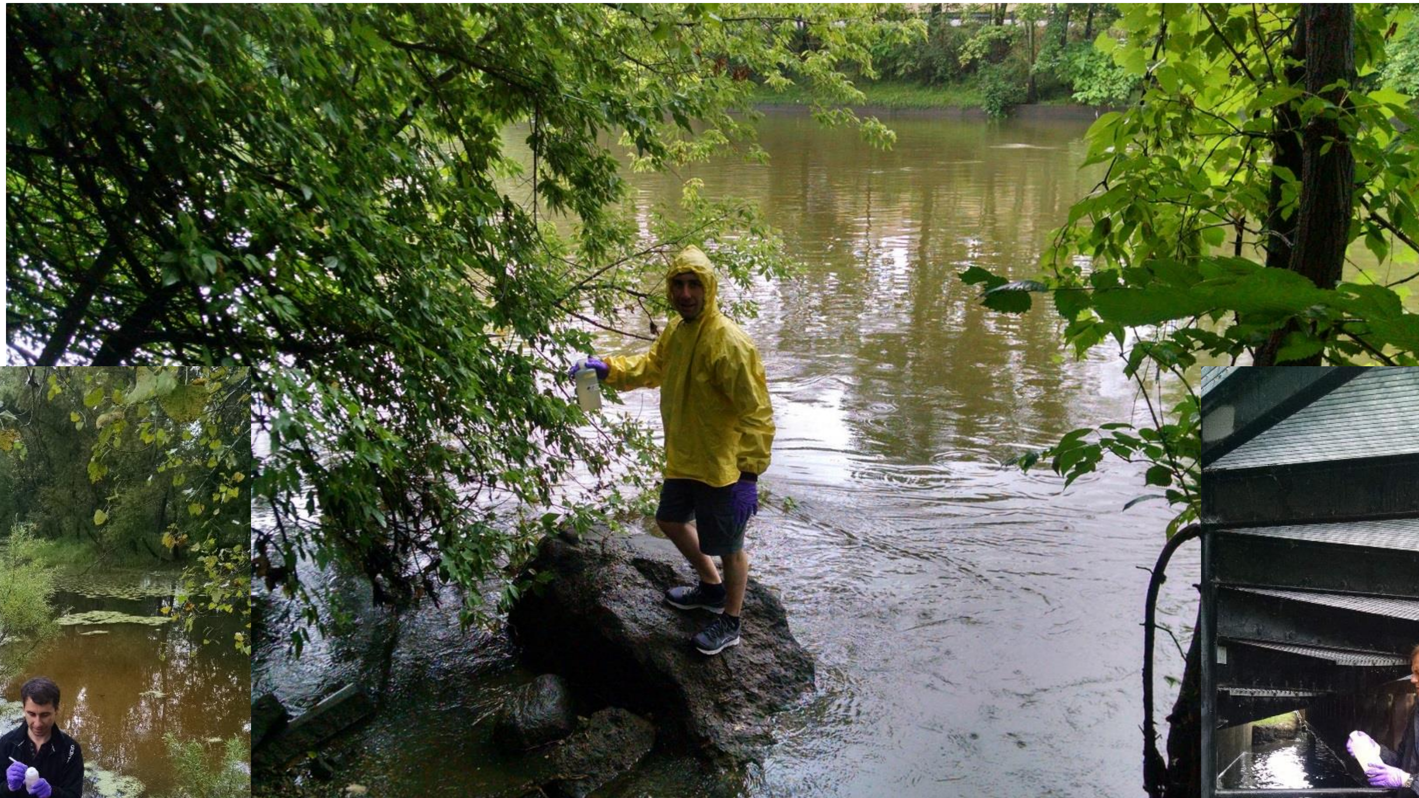

**1= penton capsomeres**
**2=hexon capsomeres**
**3= linear ds DNA**

- **Resistant to UV**
  UV dose for 99% inactivation is 109 mJ/cm$^2$ (only 55 mJ/cm$^2$ for MS2)

- **Recognized as emerging human health threat**
  serotypes 40/41 in subgroup F responsible for gastroenteritis in children

# Isolating bacteriophages

## to identify optimal HAdV40 surrogates



# 40 phages isolated → 12 candidates

Identified based on morphology and size

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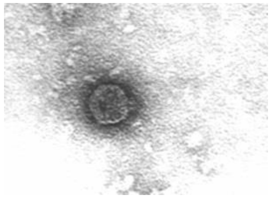
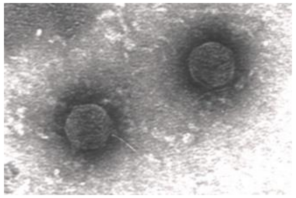
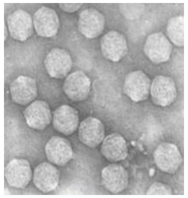


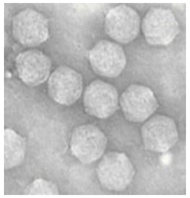
BL-1



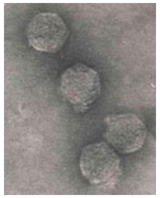
BL-2



Black Sea

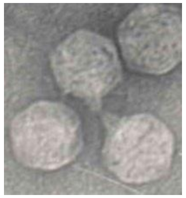


GBC

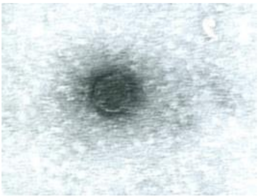


Cass

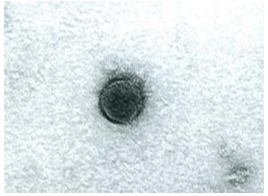
Ec-09



Ec-56D

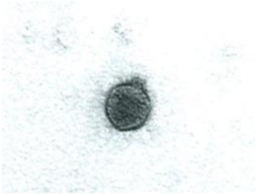


Ec-C



Lake Lansing

Ps.a.



WP

Credit for TEM images: Dr. Besarion Lasareishvili (AgrUni)

# Optimal sample preparation procedure?

Propagation and purification procedures should be selected together as an appropriate sample preparation method

Double agar overlay → Host bacteria cultivated on agar plate

Agar-propagated phage

Broth → Host bacteria suspended in broth

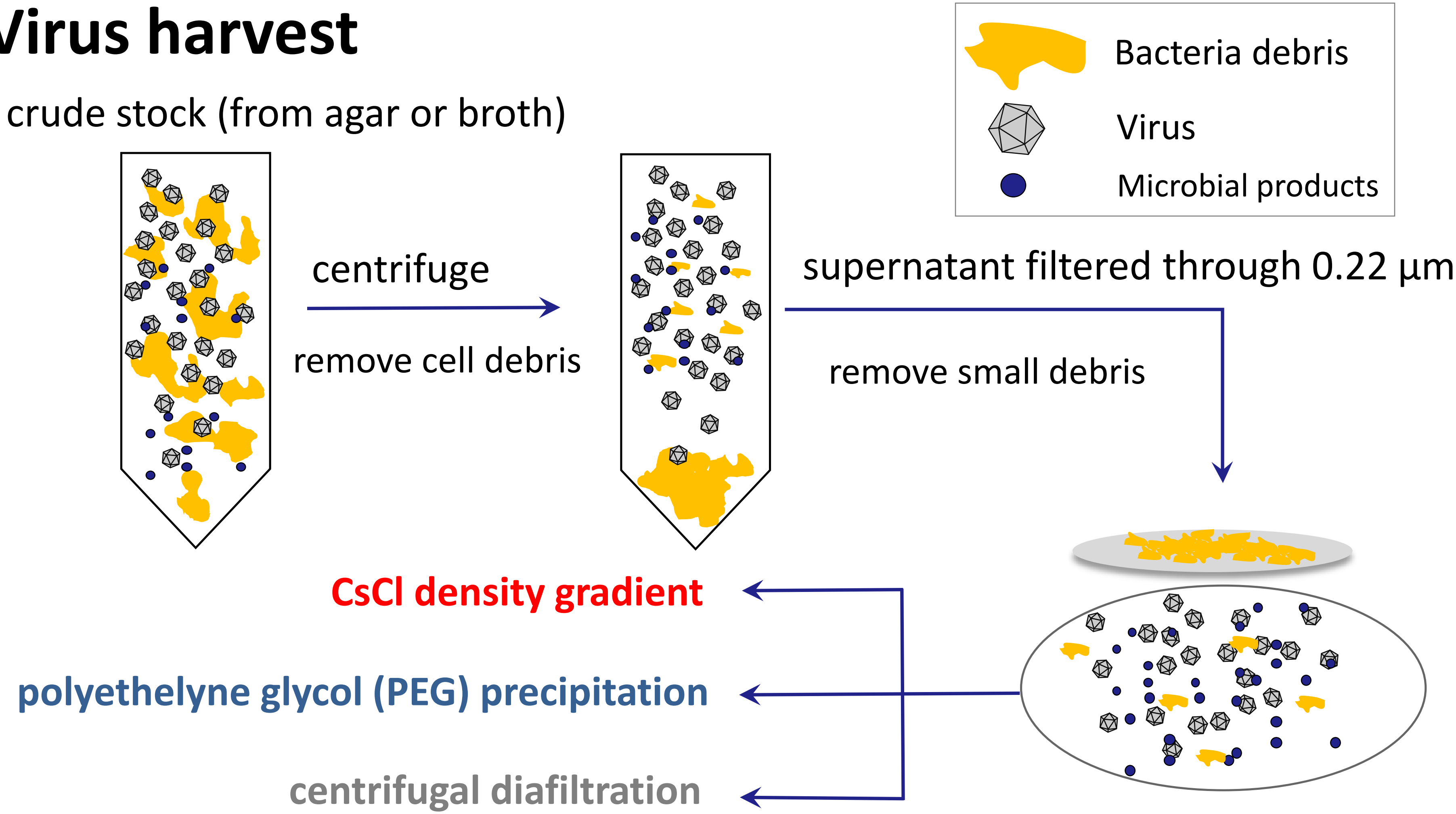
Broth-propagated phage

Slide from the PhD defense presentation by Dr. Hang Shi (2017 PhD, MSU)

# Propagation method evaluated



## Virus harvest

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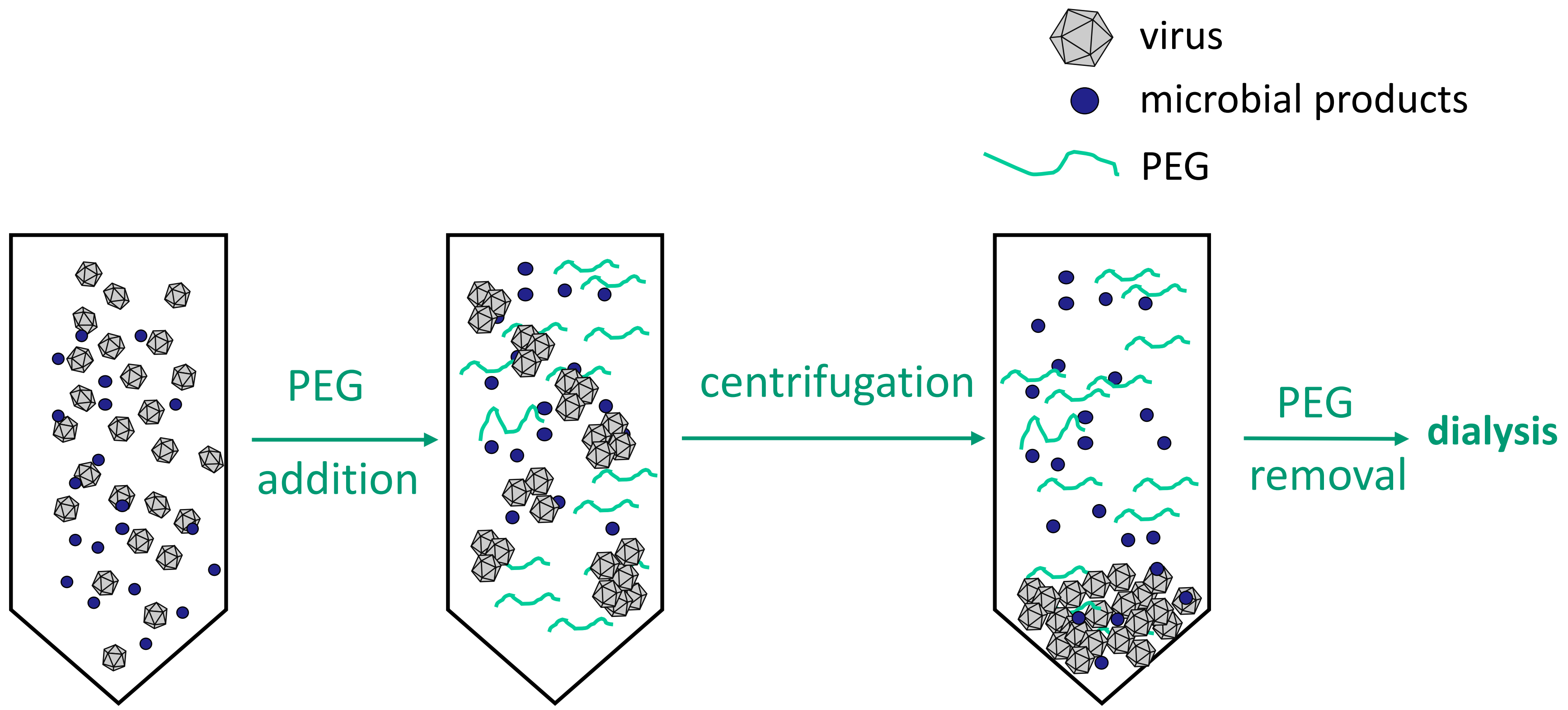
# Purification method evaluated



## Method 1: PEG precipitation

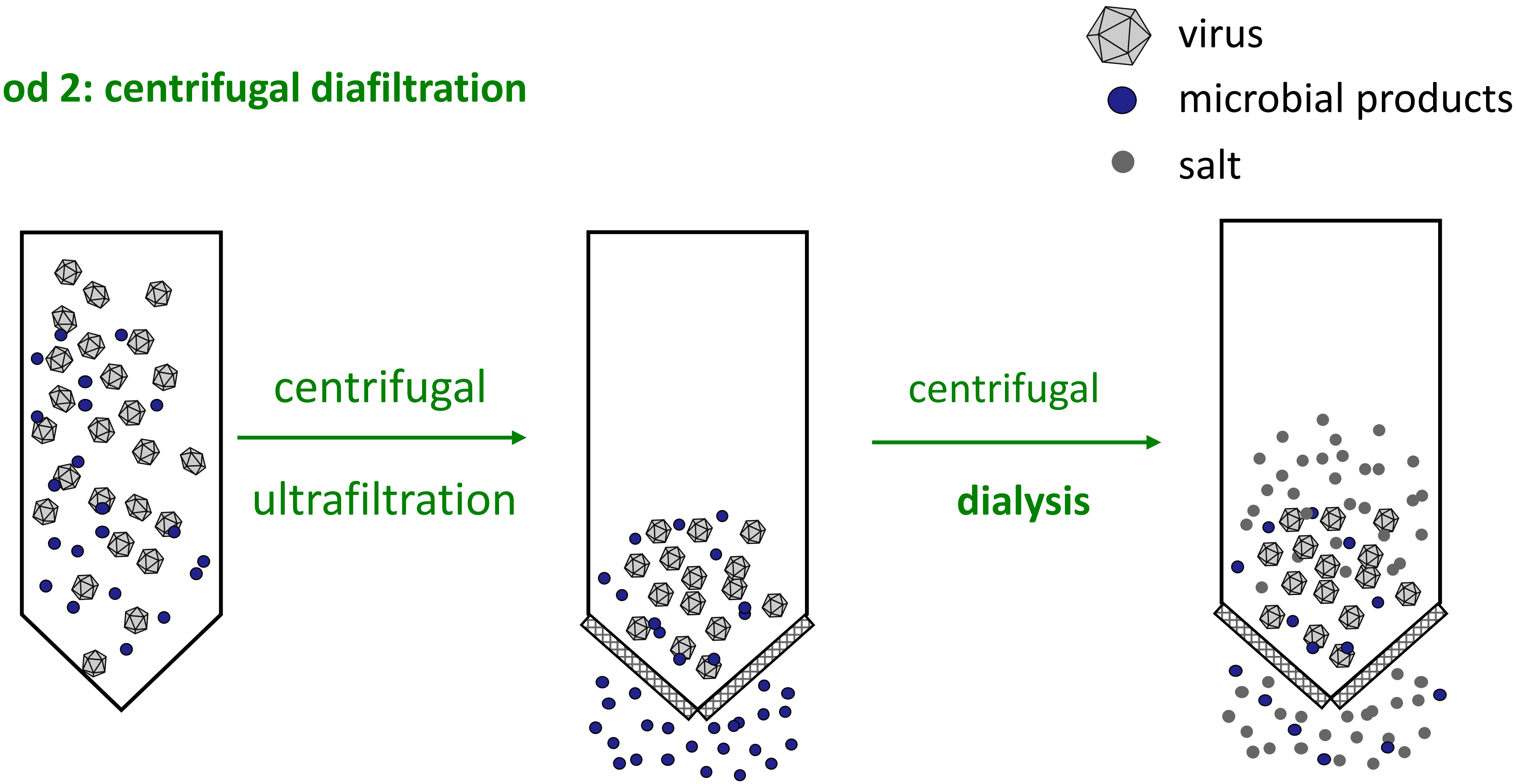



Slide from the PhD defense presentation by Dr. Hang Shi (2017 PhD, MSU)

# Purification method evaluated



**Method 2: centrifugal diafiltration**

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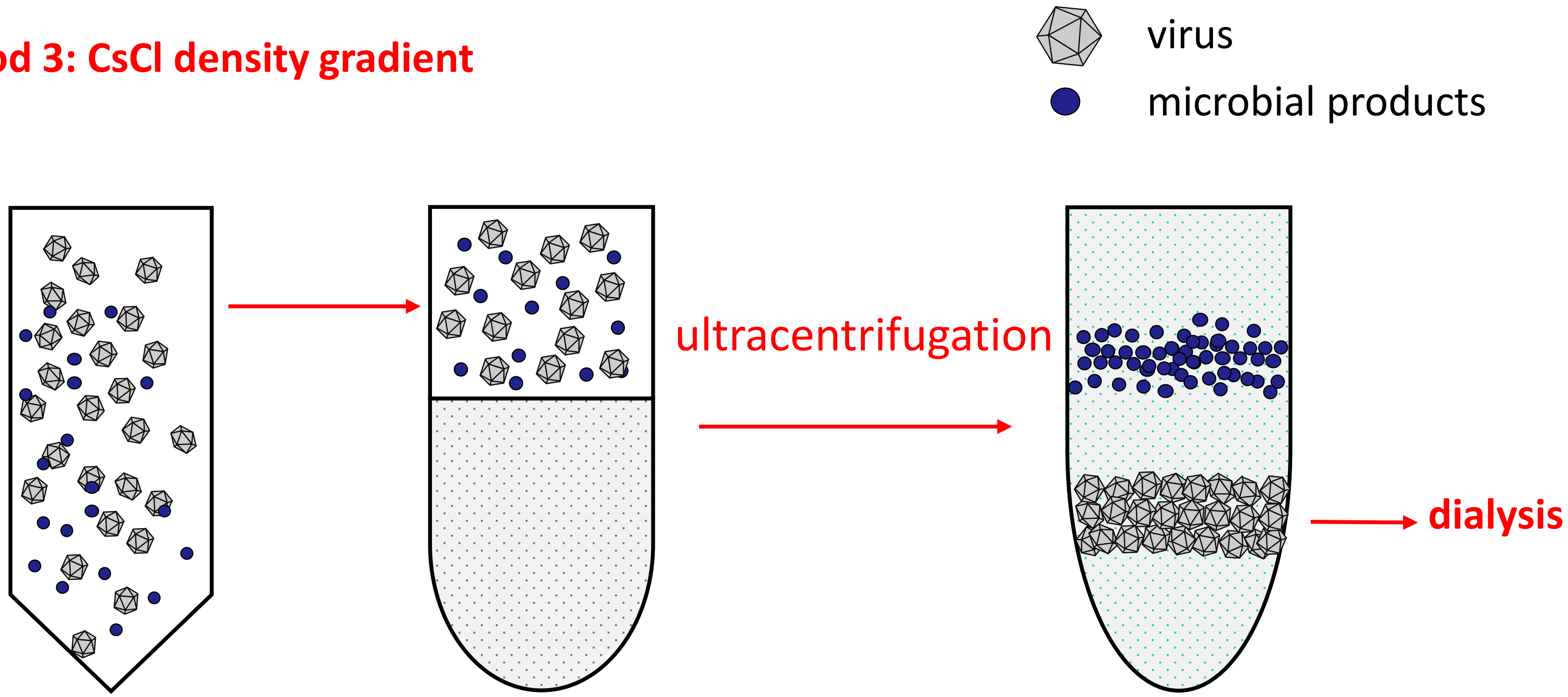
# Purification method evaluated



**Method 3: CsCl density gradient**

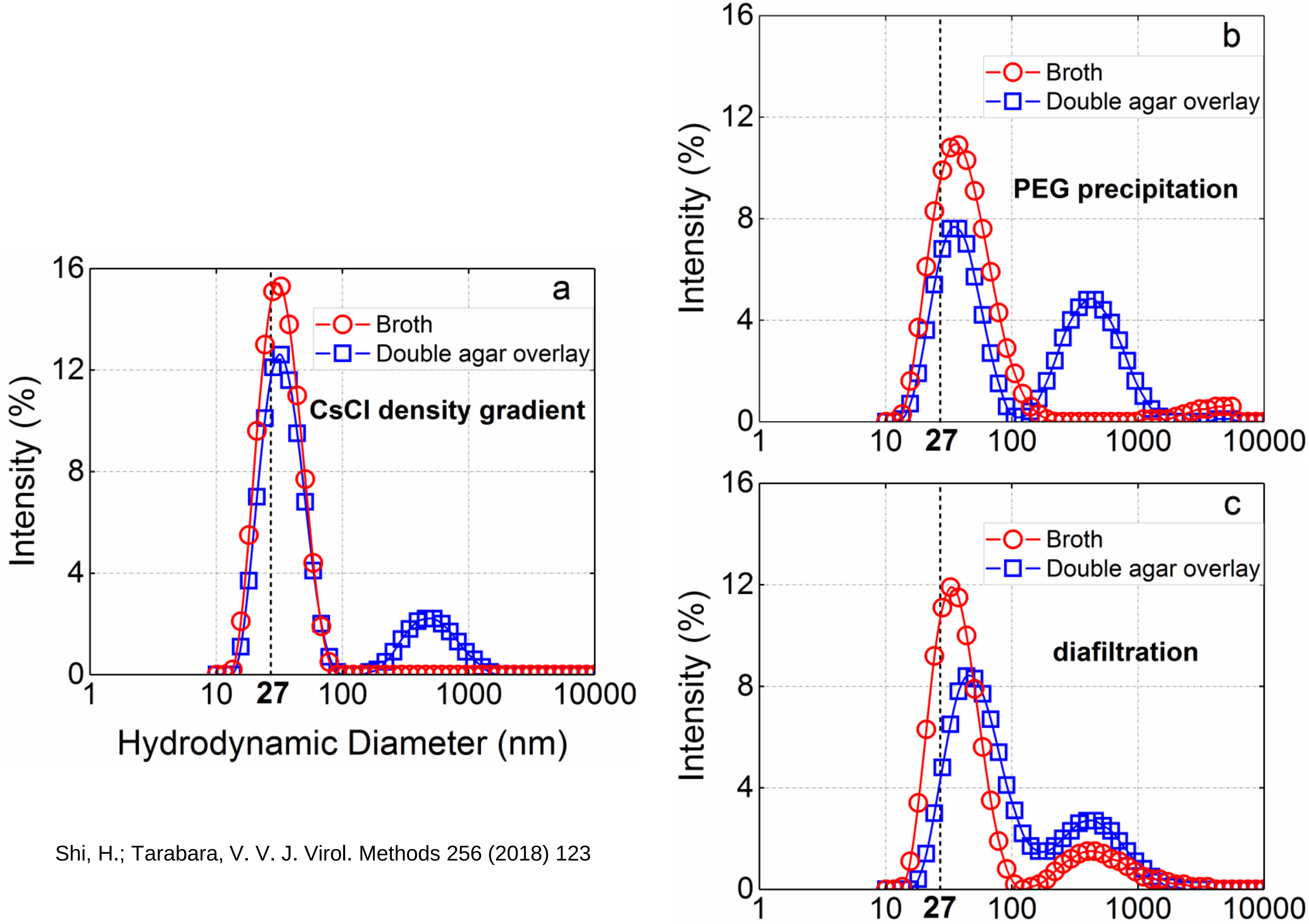



Slide from the PhD defense presentation by Dr. Hang Shi (2017 PhD, MSU)

# Effect of sample preparation

on virus (here – MS phage) charge measurements






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

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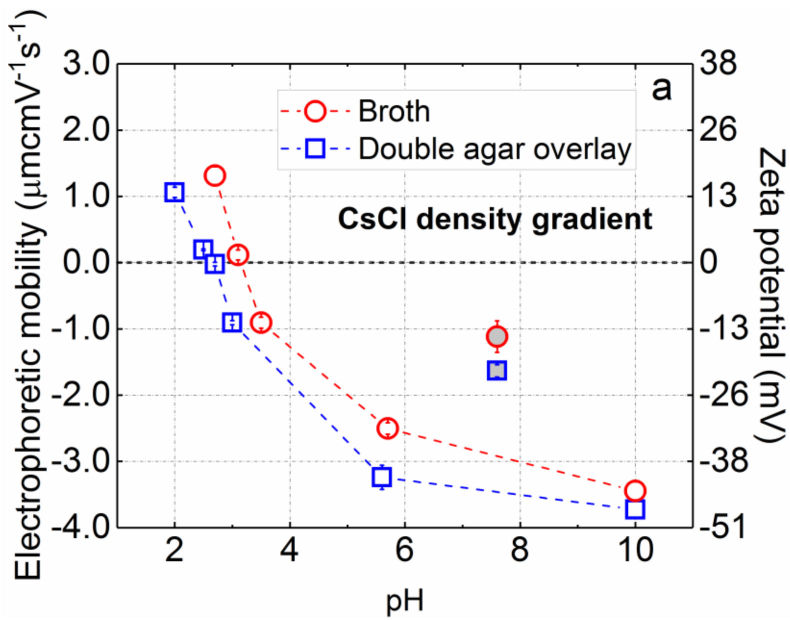
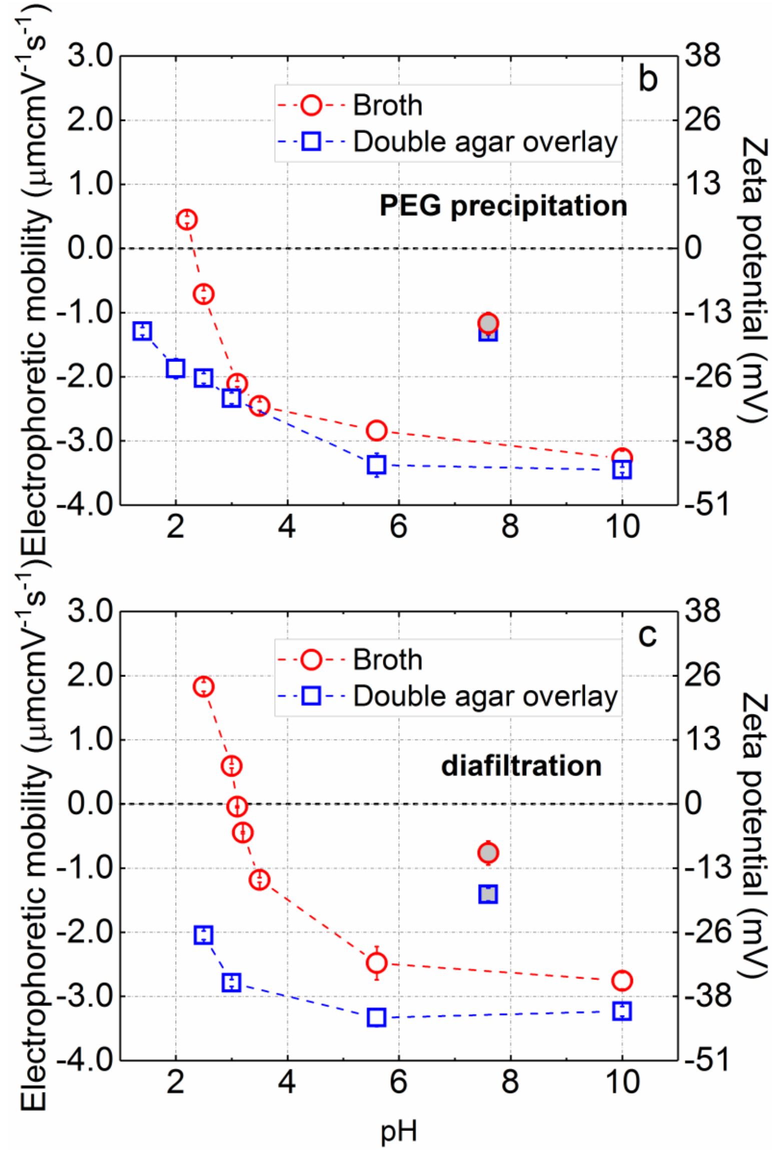


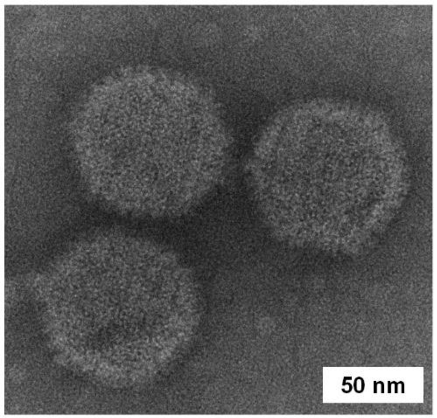

Shi, H.; Tarabara, V. V. J. Virol. Methods 256 (2018) 123

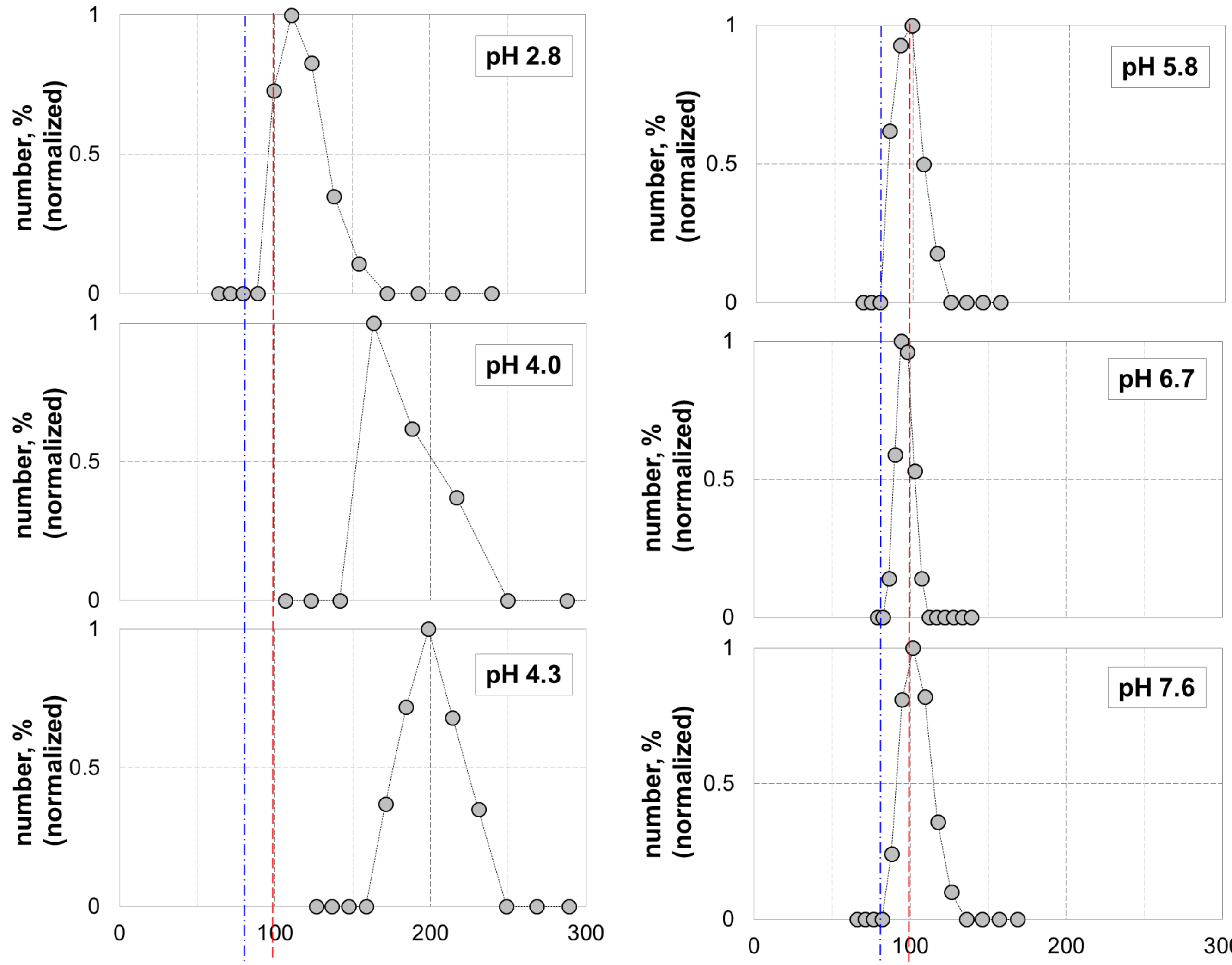
# Human Adenovirus 40

## pH dependence of the aggregation state



TEM: ~ 80 nm
DLS: ~ 99 nm



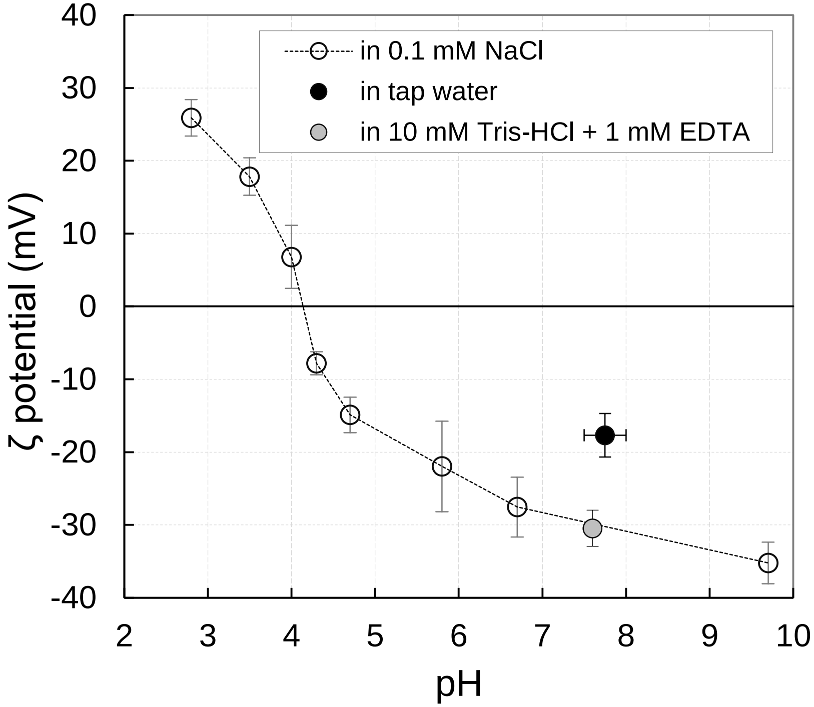

Shi, H. et al. Appl. Environ. Microbiol. 2016

# Human Adenovirus 40

## Hydrophobicity, surface charge and XDLVO virus-virus interaction



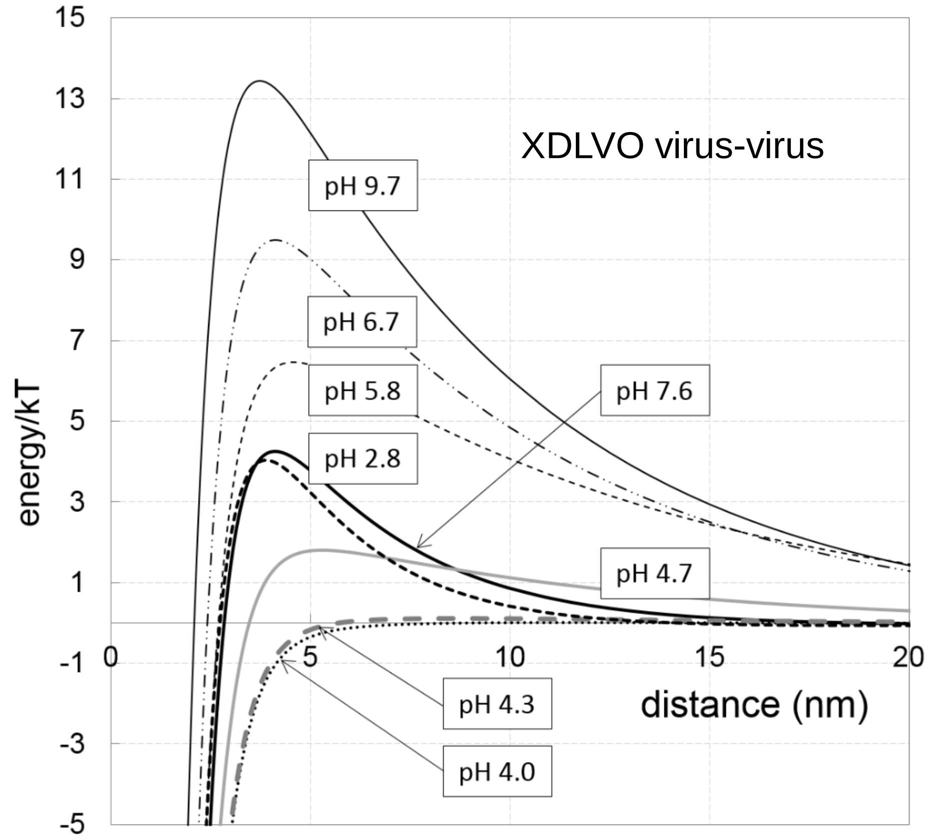
$\zeta$–potential of individual (non-aggregated) HAdV virions




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



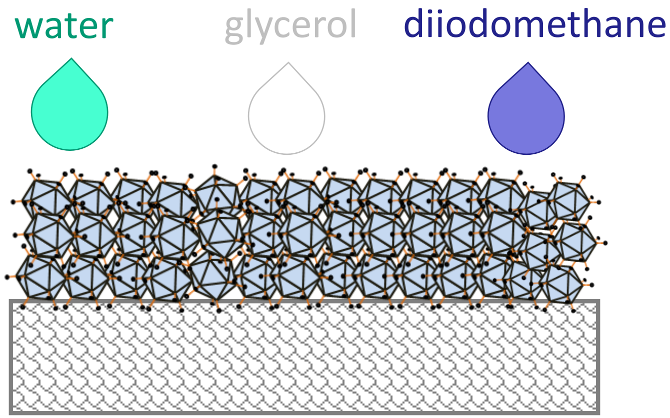

In ultrapure water (pH 5.8−6.0):

$\theta_w$ = 68 º

$\Delta G_{vwv}$ = - 30.4 mJ/ m$^2$

# How to measure hydrophobicity?






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

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

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

# How to measure hydrophobicity?

**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 ($mJ/m^2$) | |
| $\gamma^{LW}$ | 41.6 |
| $\gamma^{+}$ | 0.01 |
| $\gamma^{-}$ | 14.7 |
| $\gamma^{AB}$ | 0.8 |
| $\gamma^{TOT}$ | 42.4 |
| Free energy of interfacial virion-virion interactions in water ($\Delta G_{vwv}$ [$mJ/m^2$]) | −30.4 |

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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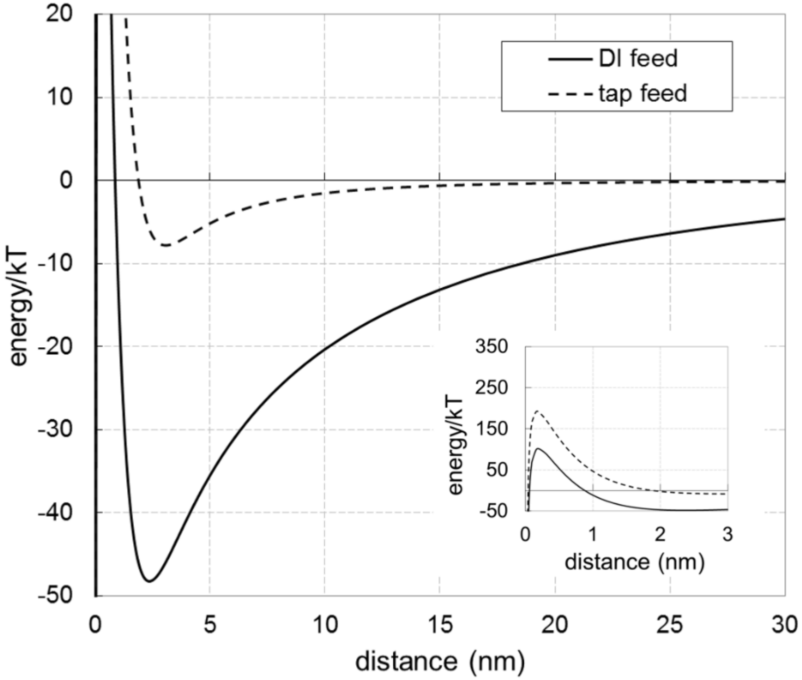
# XDLVO energy of interfacial interaction

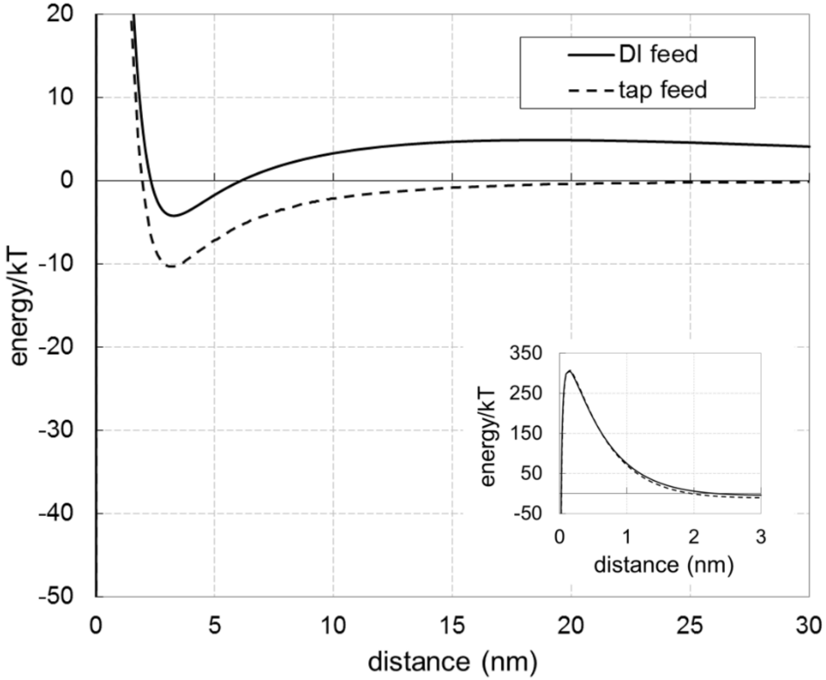
of HAdV 40 in aqueous media



with a membrane of type 1

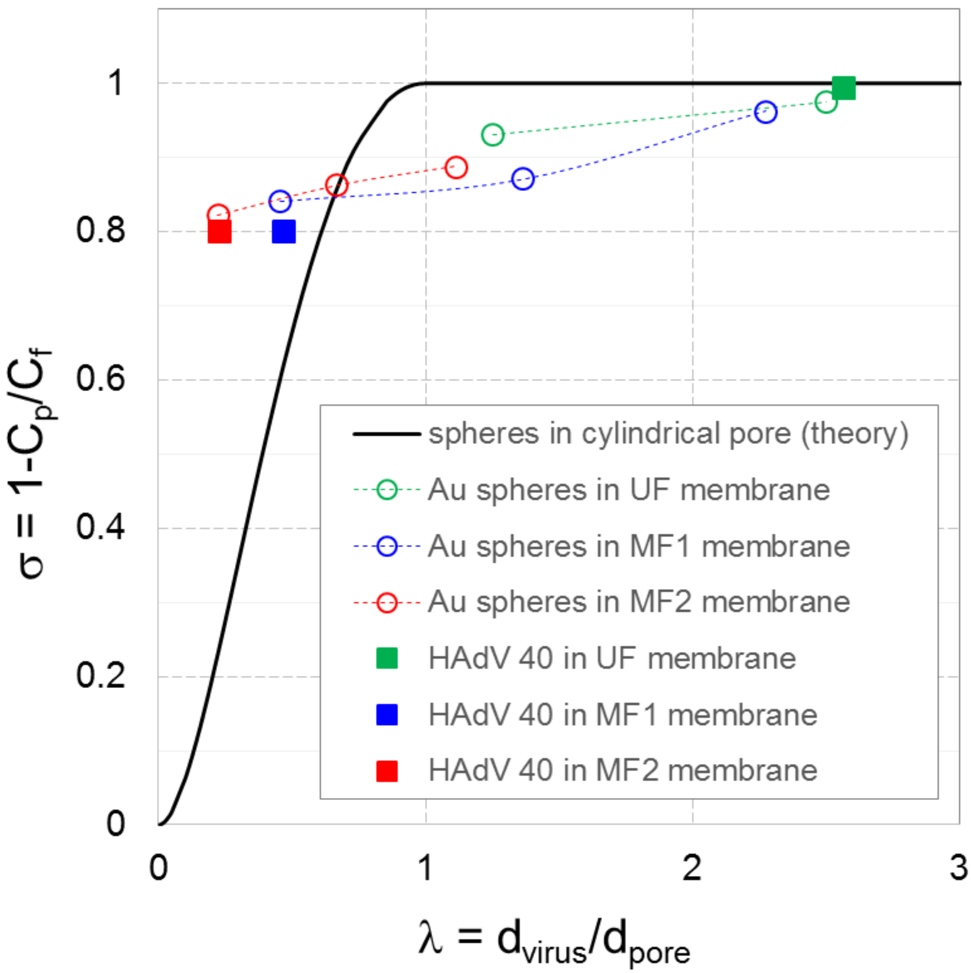



with a membrane of type 2




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

# Evaluating surrogates vs human viruses in clearance tests




Ennis et al. J. Membr. Sci. 1996, 119, 47–48
Baltus et al. Ind. Eng. Chem. Res. 2009, 48, 2404–2413

$$\sigma = 1 - [1-\lambda]^2 \left[\frac{1 + 3.867\lambda - 1.907\lambda^2 - 0.834\lambda^3}{1 + 1.867\lambda - 0.741\lambda^2}\right]$$

$$\lambda = d_{virus}/d_{pore}$$

For spherical colloids/viruses:

- Hindrance model provides good fit for membranes with straight-through cylindrical pores
- "Smearing" of the removal profile for phase inversion (polymeric) and sintered (ceramic) membranes

Data from: Yin, Z.; Tarabara, V. V.; Xagoraraki, I. Human adenovirus removal by hollow fiber membranes: Effect of membrane fouling by suspended and dissolved matter. J. Membr. Sci. 482 (2015) 120-127.

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# Summary

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- Bacteriophages can be convenient non-pathogenic surrogates of human viruses

- Surrogate selection is dictated by applications-specific demands

  Example 1: A highly charged, hydrophilic phage (e.g. MS2) can serve as a conservative tracer in testing integrity of water treatment systems

  Example 2: A surrogate that matches several key physicochemical characteristics of the target human virus can be used to evaluate transport and fate of the virus

  Example 3: A surrogate that exhibits very similar adhesive behavior as the target virus can be used to evaluate virus recovery technologies vis-à-vis range of water matrices

- Size, morphology, hydrophobicity and charge together govern virus interactions with surfaces. The surrounding matrix may have a profound effect on these interactions

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