Detecting Infectious Viral Pathogens Toward Developing Smart Water Reuse Disinfection Systems

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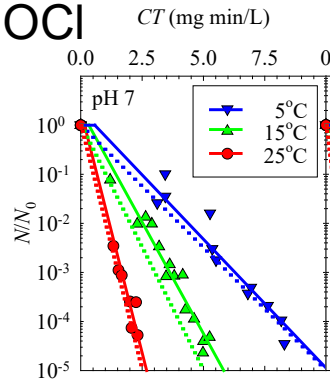
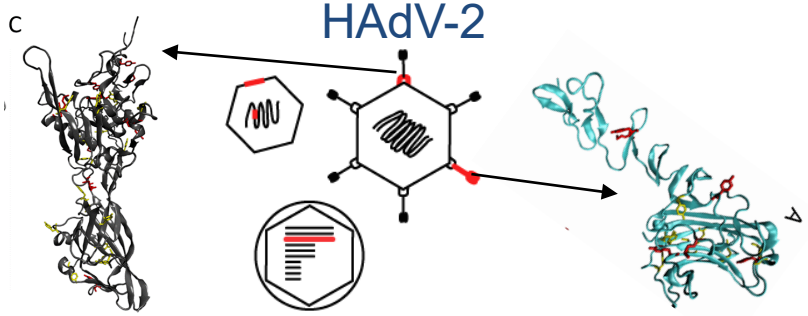
Inter/Transdisciplinary Research Approach

Kwanrawee Sirikanchana, Martin Page, Aimee Gall, Bernardo Vazquez, Dana Al-Qadi, Kelley Gonçalves, Shiliang Tian, Wen Cong, Ana S. Peinetti, Anisa Hardin
Joanna L. Shisler, Yi Lu

HOCl



HAdV-2



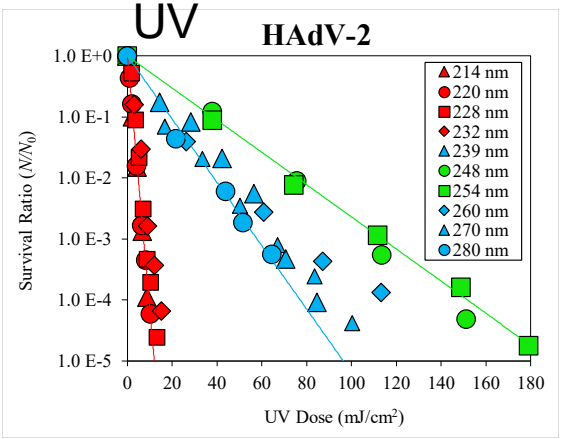
Protein/Genome transformation

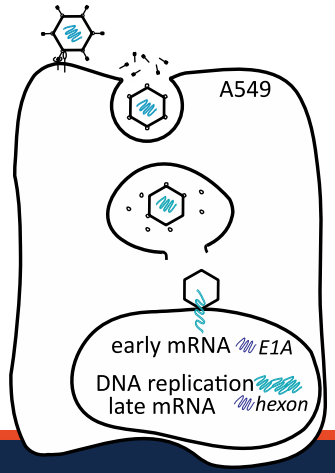
Inactivation kinetics

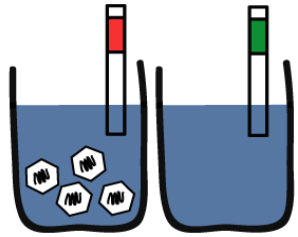
Replication cycle disruption

Sensor development

UV







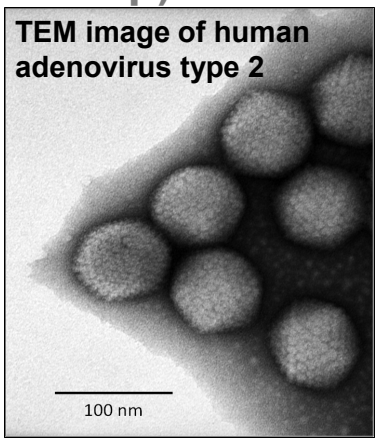
Gall et al., *PLoS Pathog* **2015,** *11* (6), e1004867

Viral Pathogens, Disinfectants & Objectives

Martin Page, Kwanrawee Sirikanchana, Aimee Gall, Bernardo Vazquez, Kelley Gonçalves, Wen Cong, Anisa Hardin
Joanna L. Shisler

Pathogens:

- **Human Adenovirus HAdV-2 (HAdV species C; non-enveloped capsid ~90 nm; dsDNA ~36 kbp)**



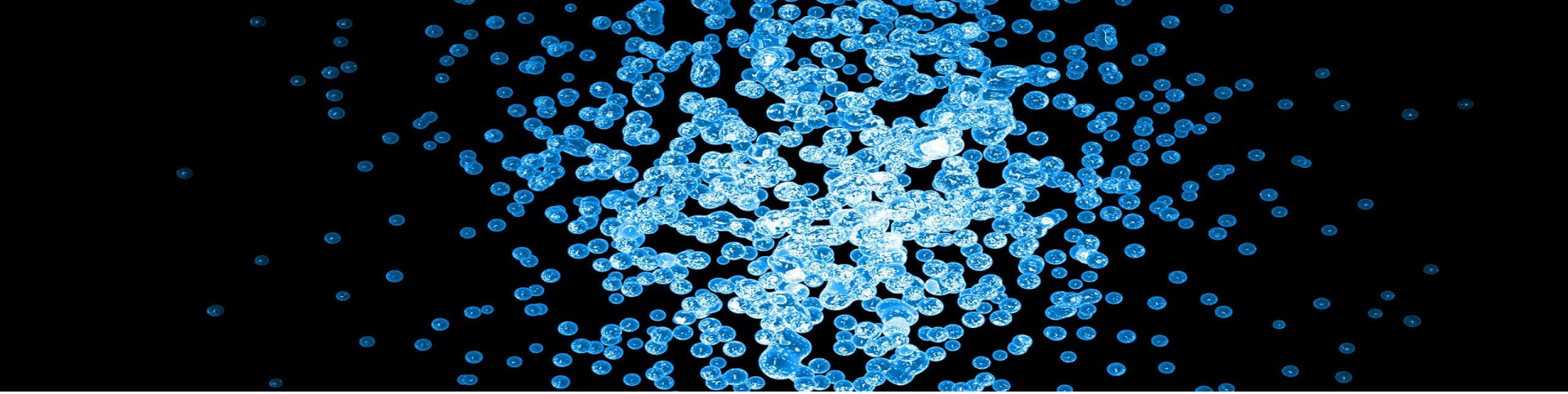
- Coxsackievirus B5 (enterovirus species B; non-enveloped capsid ~30 nm; +ssRNA ~7.4 kb)

Disinfectants:

- **Free chlorine**
- Monochloramine
- UV light
  - Experiments performed with synthetic buffered solutions in batch reactors
  - Viability assessment by plaque assay using human lung A549 carcinoma cells (HAdV-2), or Buffalo Green Monkey Kidney (BGMK) cells (CoxV-B5)

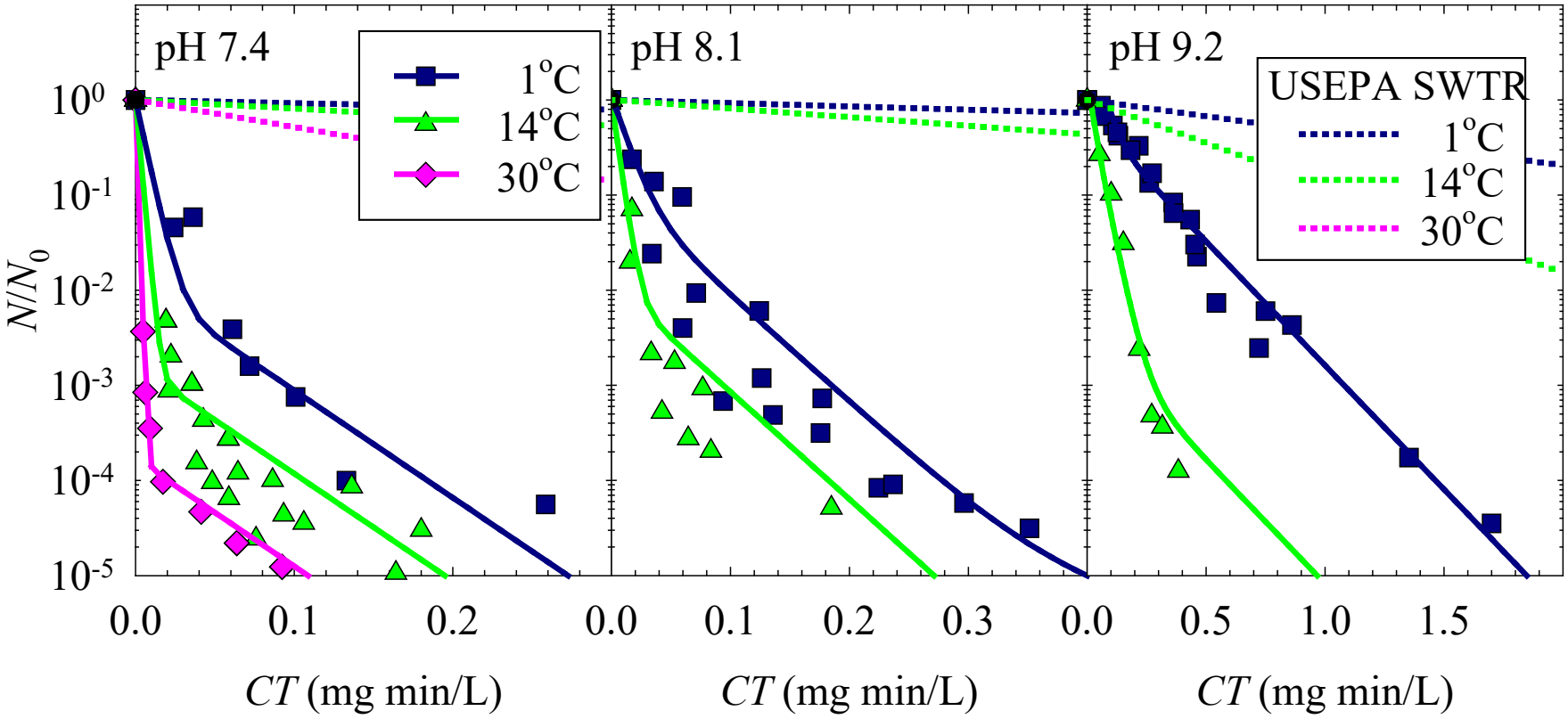
Broad Objective:

- **Achieve better control of viruses in water reuse**
  - Determination of inactivation kinetics
  - Elucidation of inactivation mechanisms
  - **Simple, rapid, robust sensors to detect infectious viruses** (ultimately including those for which cell culture is not available, e.g., human norovirus)



INACTIVATION KINETICS

Inactivation of Human Adenovirus 2 with Free Chlorine



M.A. Page, J.L. Shisler, B.J. Marinas, *Wat. Res.*, 2009, 43, 2916-2926

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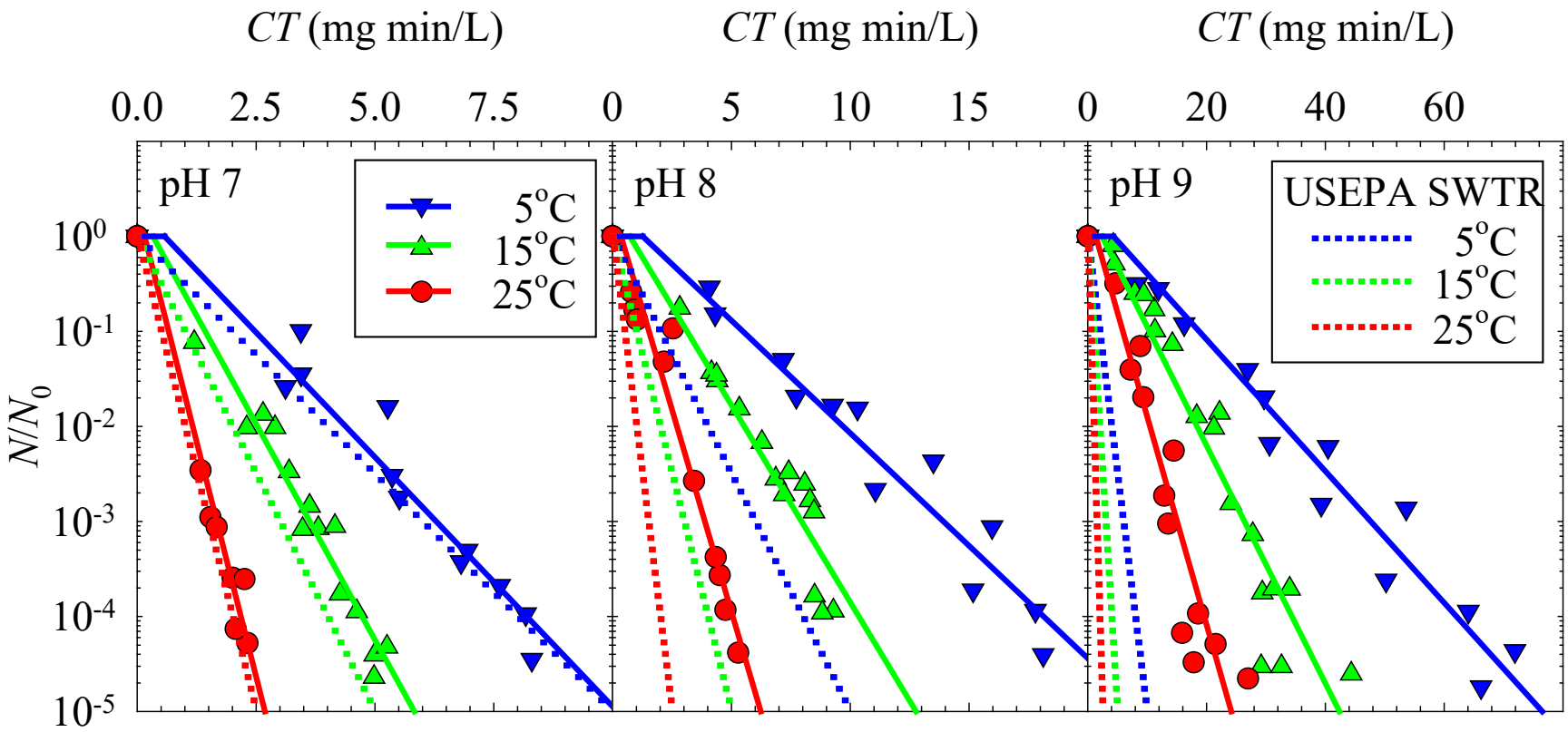

W. Cong, A. Hardin, B.J. Marinas, *in preparation*

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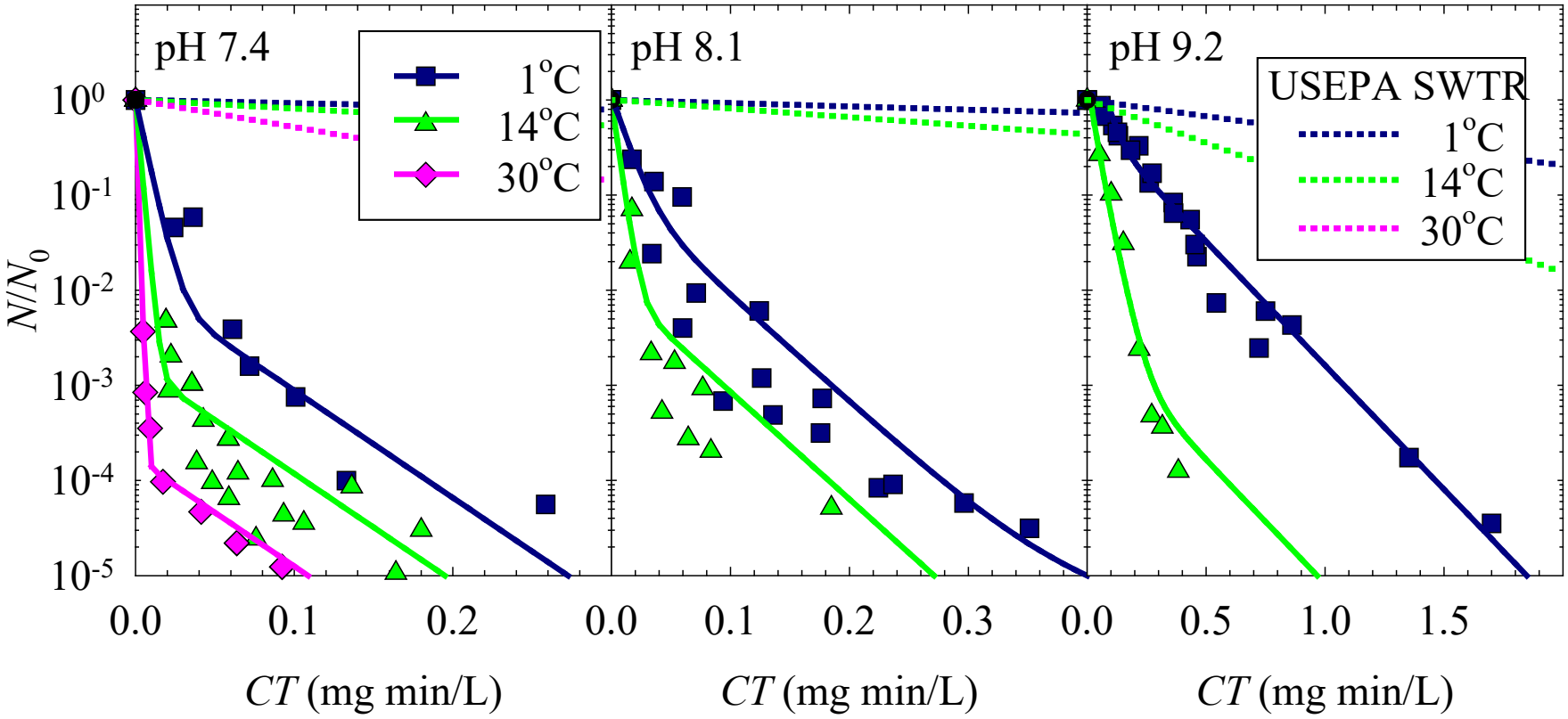

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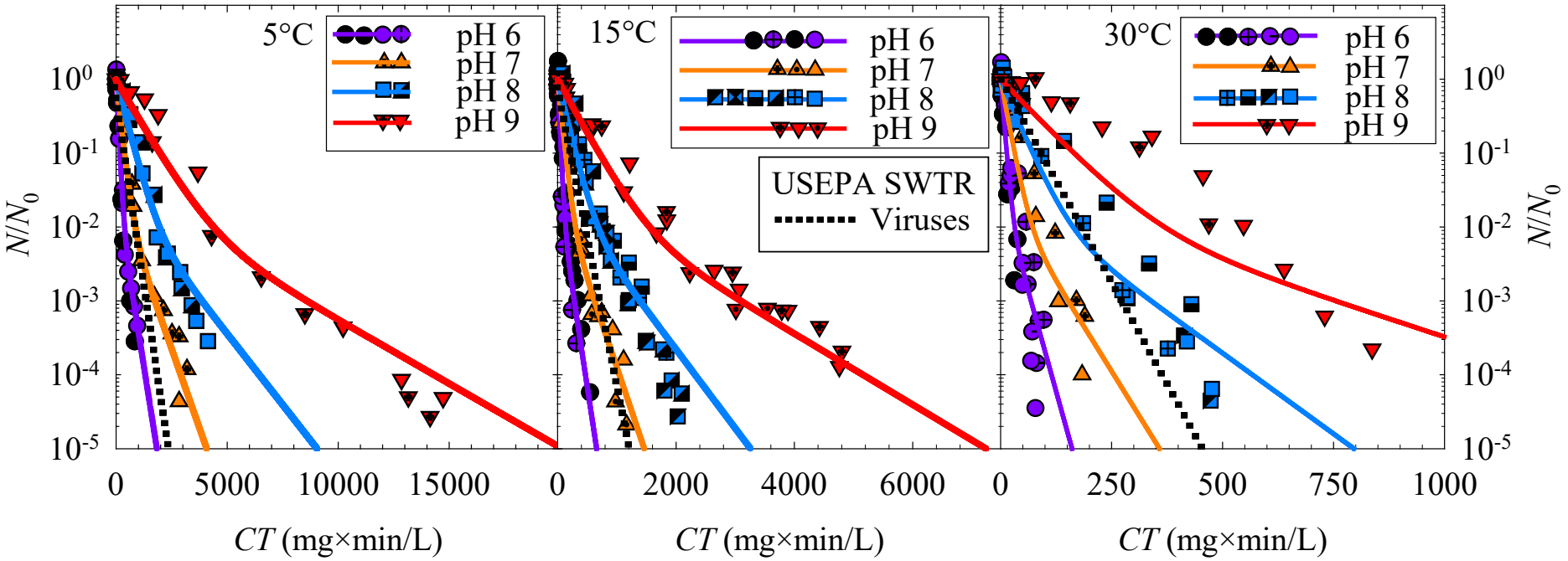
Inactivation of Human Adenovirus 2 with Monochloramine



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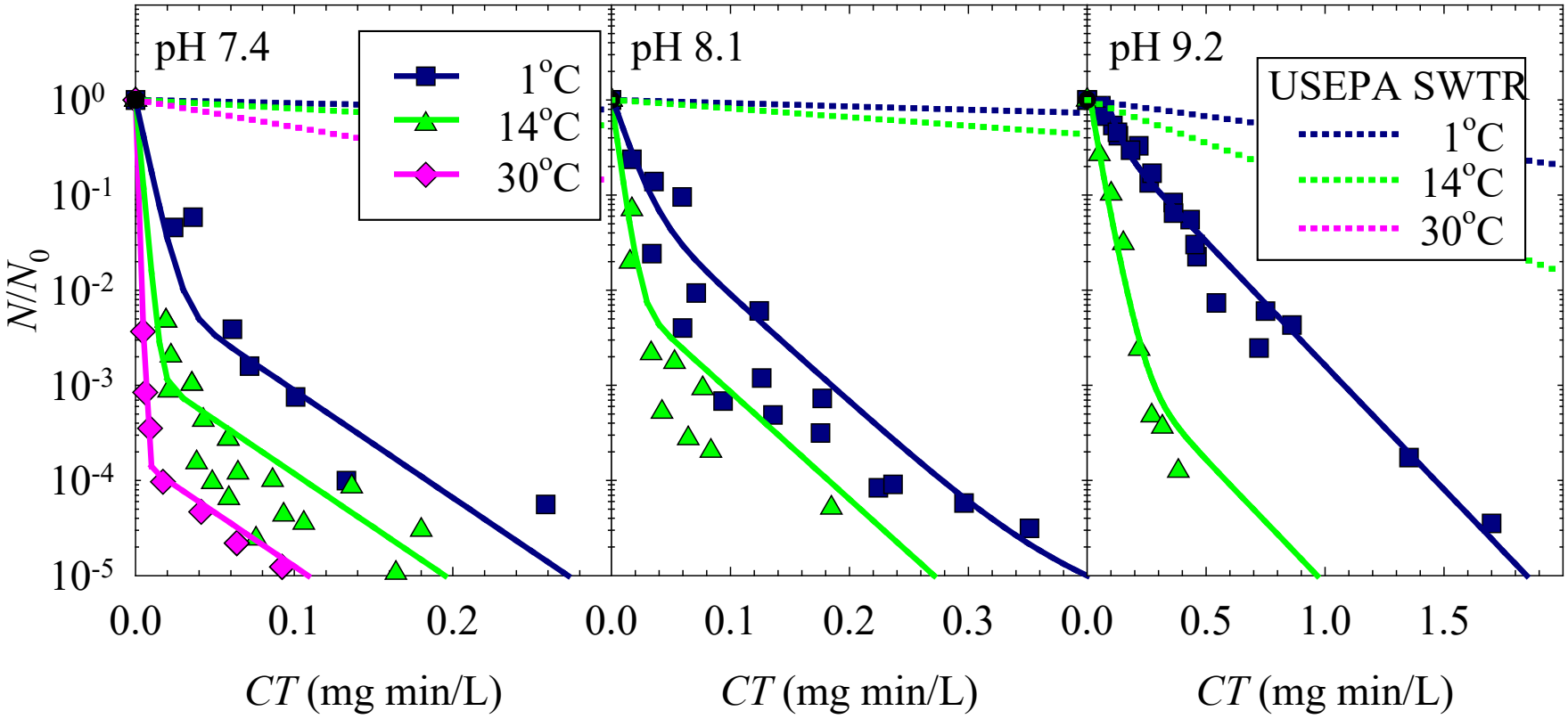

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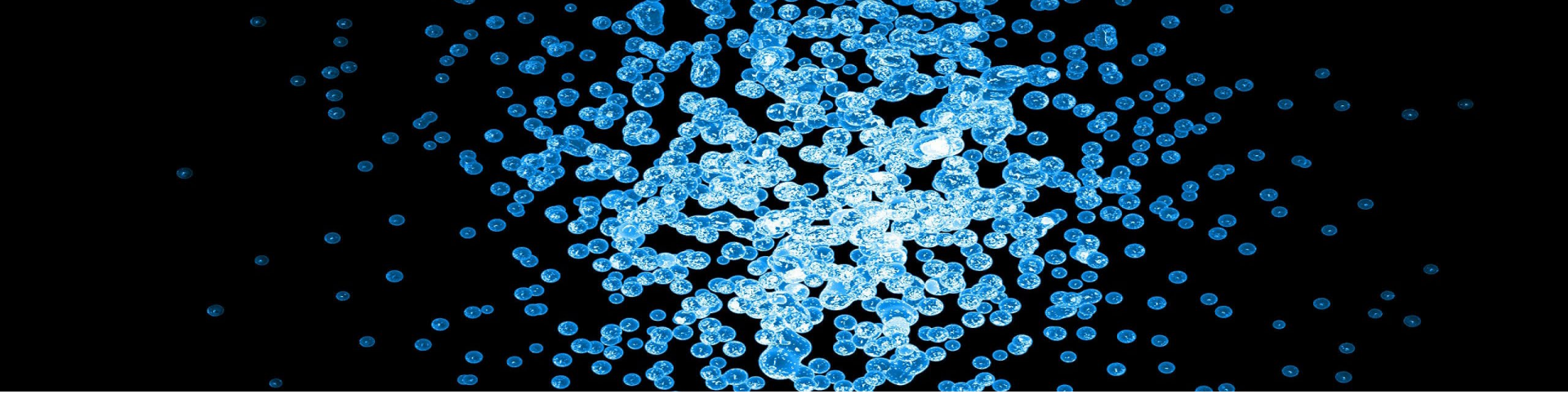
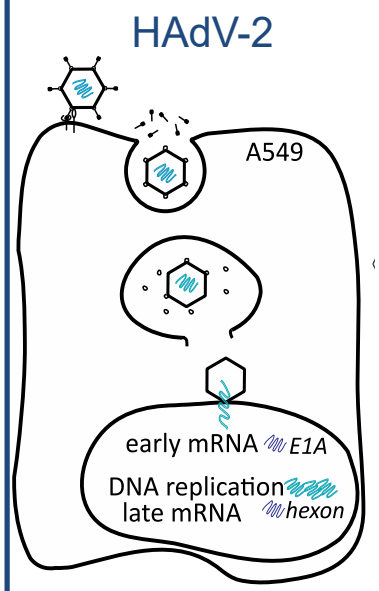


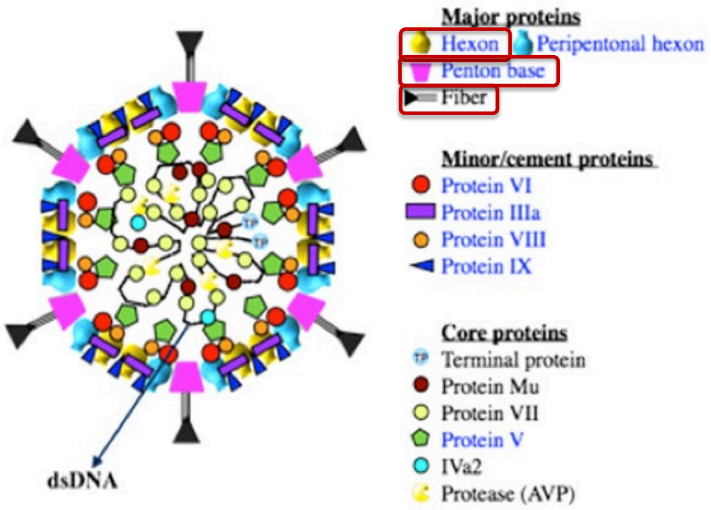
INACTIVATION MECHANISMS: REPLICATION CYCLE STEP INHIBITION

HAdV-2 Replication Cycle

Selected HAdV-2 (90 nm, ~36,000 bp) Replication cycle events:

- Attachment (fiber to CAR) – 0 h p.i.
- Internalization with endosome (penton base to integrin) – 15 min p.i.
- Virion uncoating and endosome rupture
- DNA release into nucleus
- Early mRNA (E1A) synthesis – 1-2 h p.i.
- DNA replication and late mRNA (hexon) synthesis – 5-8 h p.i.
- Cell lysis – 48 h p.i.





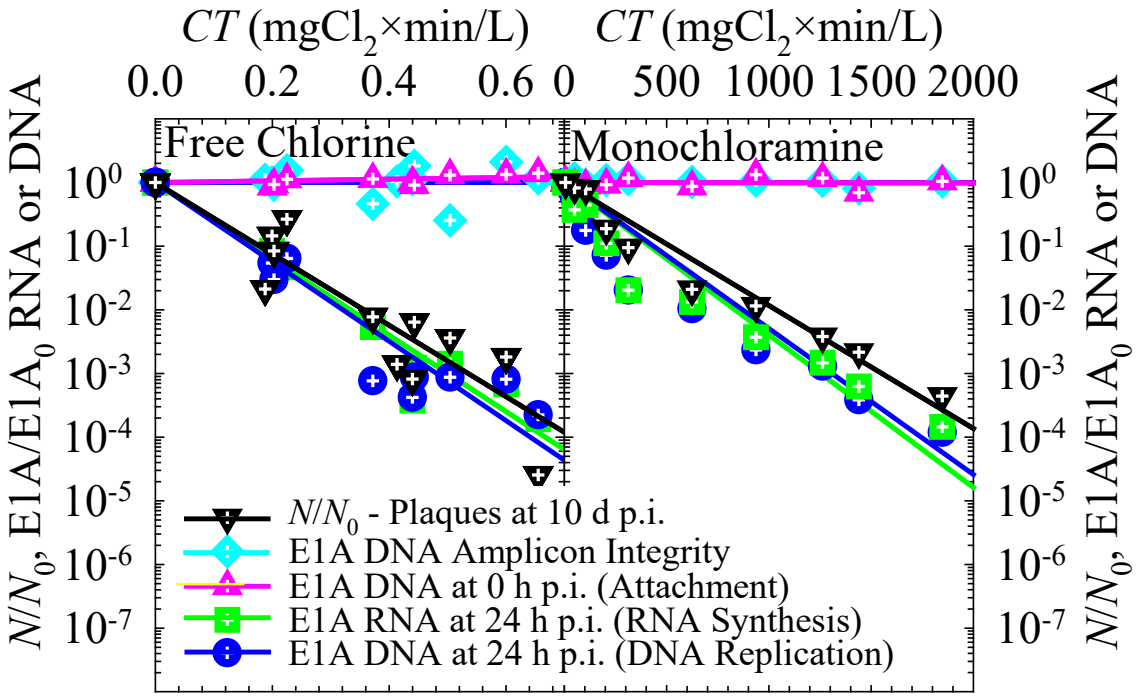
(Reddy and Nemerow, 2014)

Location of selected HAdV-2 DNA amplicons

DNA: Quantitative Polymerase Chain Reaction (qPCR)
mRNA: Reverse Transcriptase qPCR (RT-qPCR)

Inactivation Human Adenovirus 2 with free chlorine (pH 9.2, 15°C) and monochloramine (pH 8, 15°C) compared to relative quantity of early (E1A) and late (hexon) DNA and RNA

- Adenovirus inactivation at levels up to 99.99% did not result in loss of amplicon integrity or ability to attach
- However, synthesis of viral DNA and early E1A and late hexon gene transcription were inhibited



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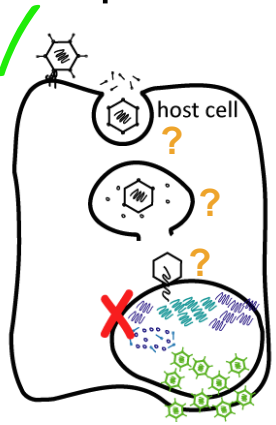

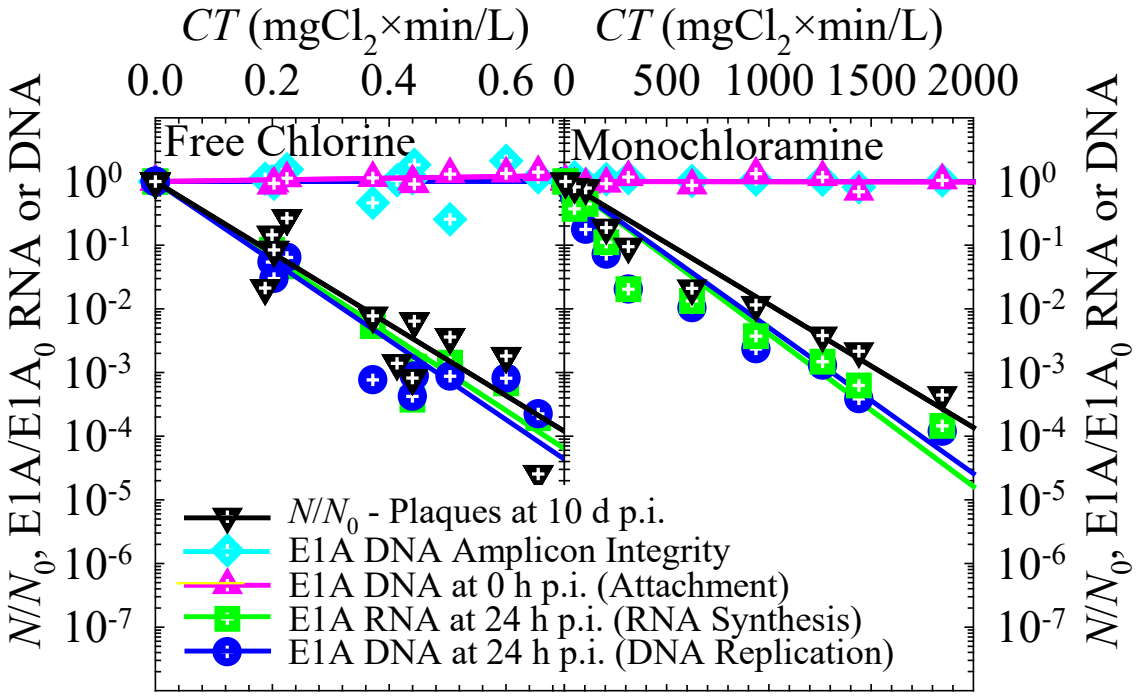

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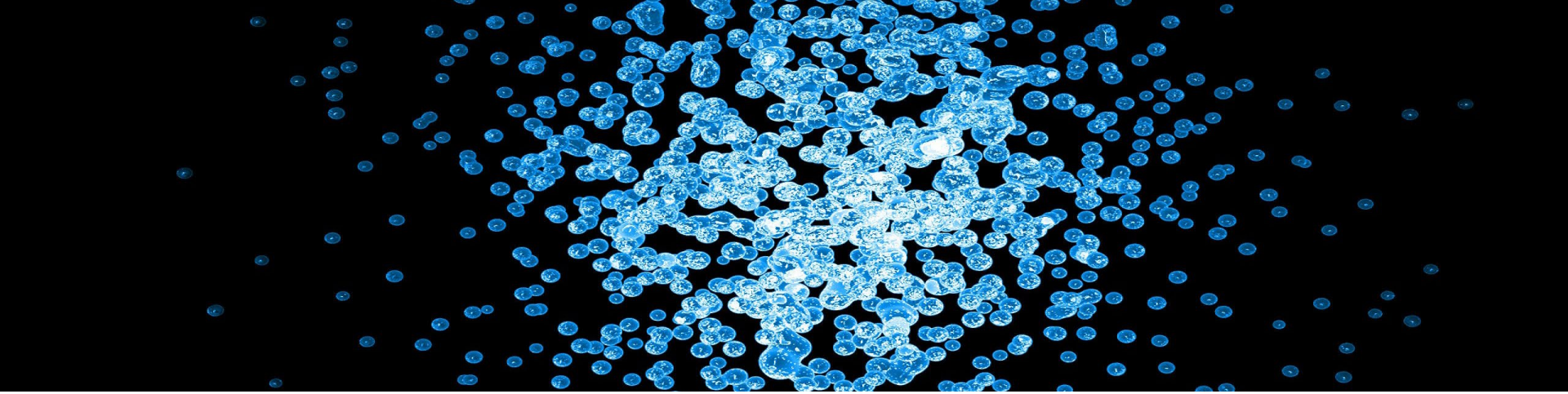
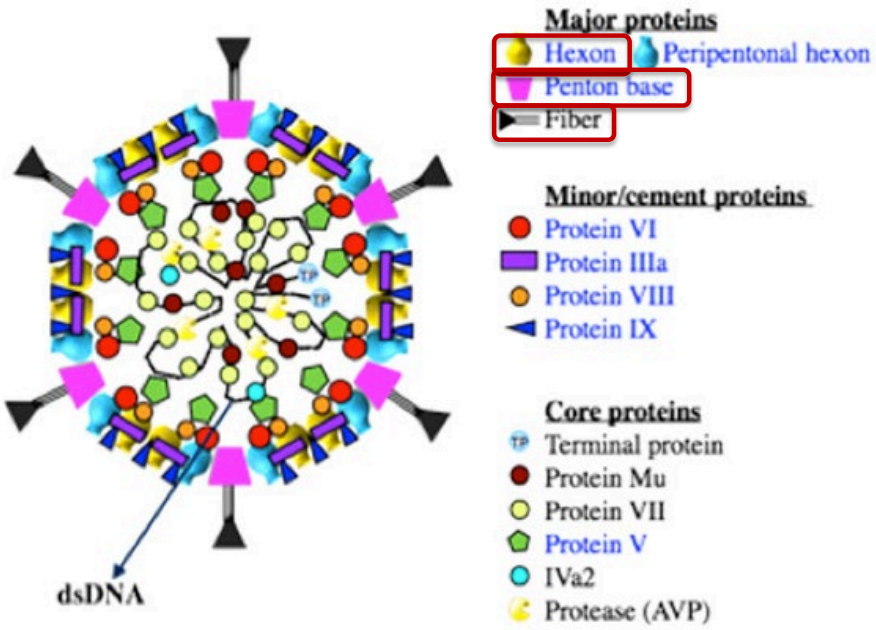


INACTIVATION MECHANISMS: CAPSID PROTEIN TRANSFORMATION

Modifications of Human Adenovirus 2 Capsid Proteins with Free Chlorine

- Proteins targeted were: fiber, penton base, hexon
- The genes of the adenovirus fiber, penton base, and hexon were expressed in gene-modified *E. coli*
- 1 uM of each protein monomer (with 571, 582, 967 residues) was reacted with 100μM free chlorine for 5 min (*CT*≈3.6 mg min/L, pH 8, 22°C) before quenching with excess sodium thiosulfate
- Proteins digested (Trypsin) and residues analyzed by LC-MS/MS



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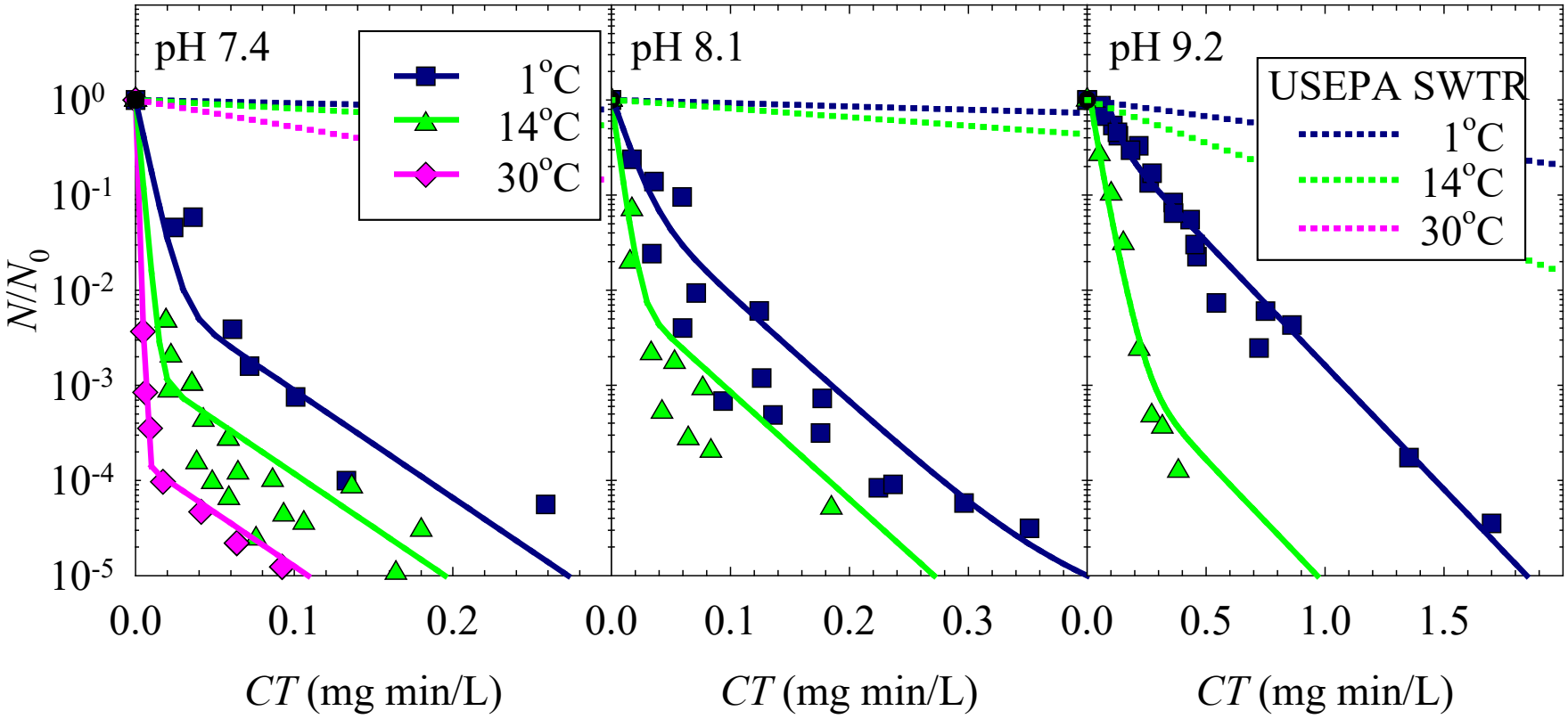

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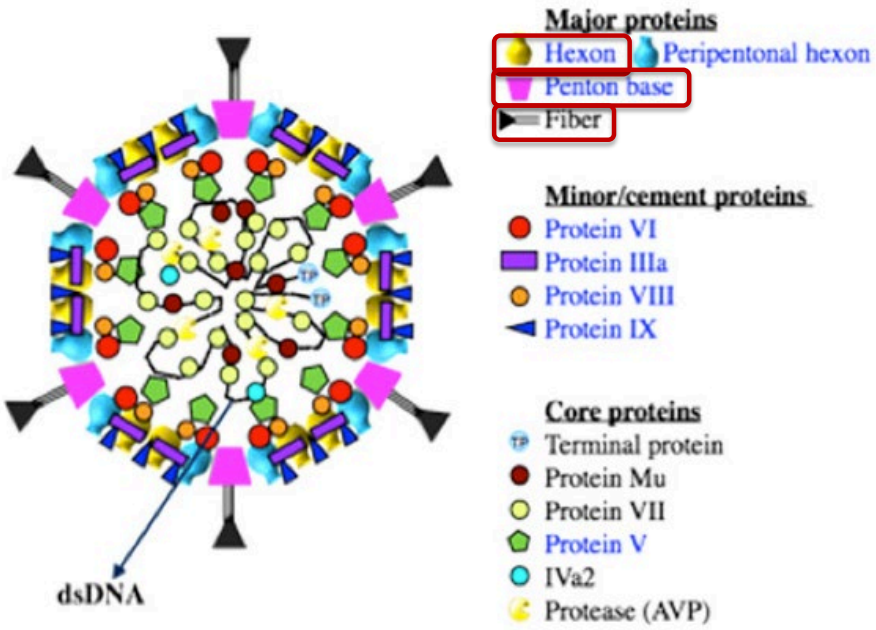
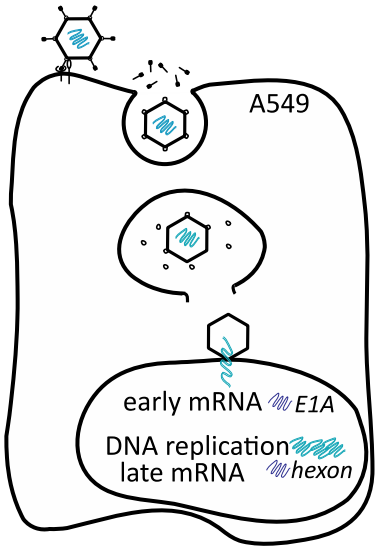


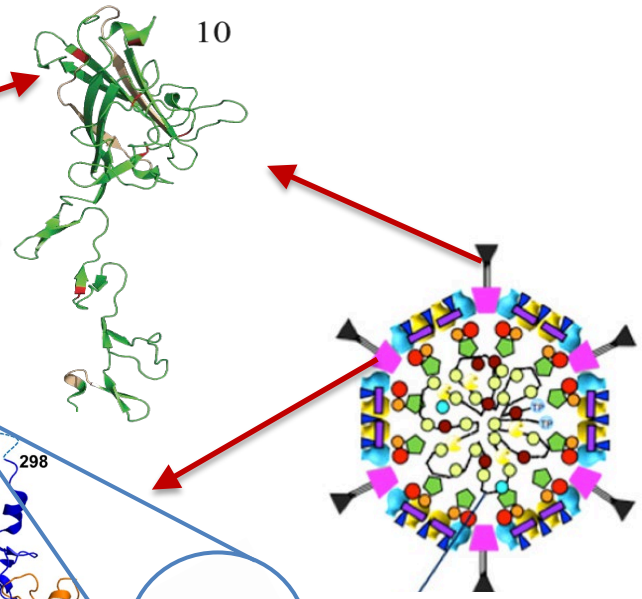
(Reddy and Nemerow, 2014)

Graphical depictions of the HAdV-2 fiber, penton based, and hexon (bottom) fiber (expressed in *E. coli*) modifications by free chlorine exposure

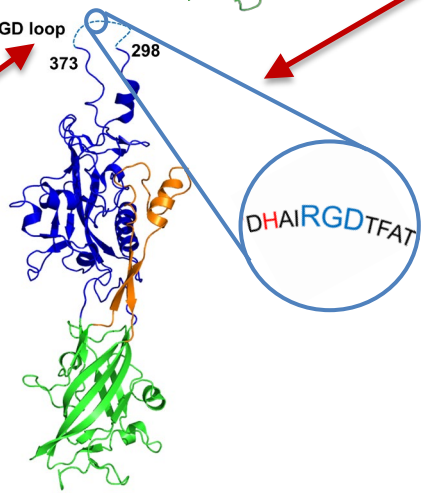
Met≈Cys>His>Trp>Tyr>Asn≈Gln



- Ribbon diagram of fiber tail, shaft, and head domains: CAR D1 binding & flexible shaft regions **preserved** at *CT*=3.6 mg min/L;



- Ribbon diagram of penton base: RGD loop, RGD integrin binding regions **transformed** (His337) at *CT*=3.6 mg min/L;



S. Tian, A.M. Gall, W. Cong, J.L. Shisler
B. Marinas, Y. Lu, *in preparation*

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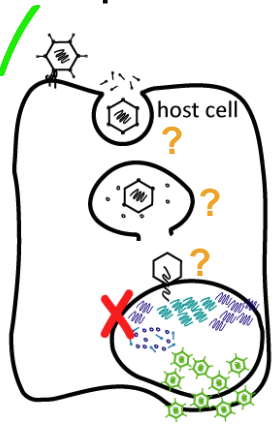

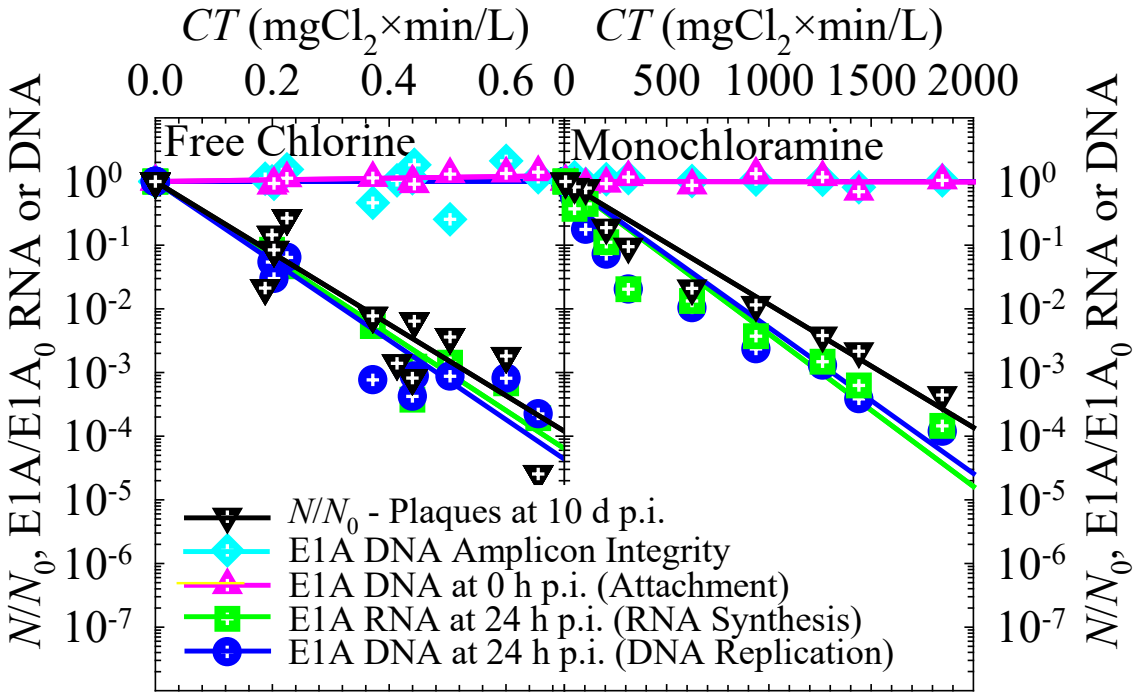

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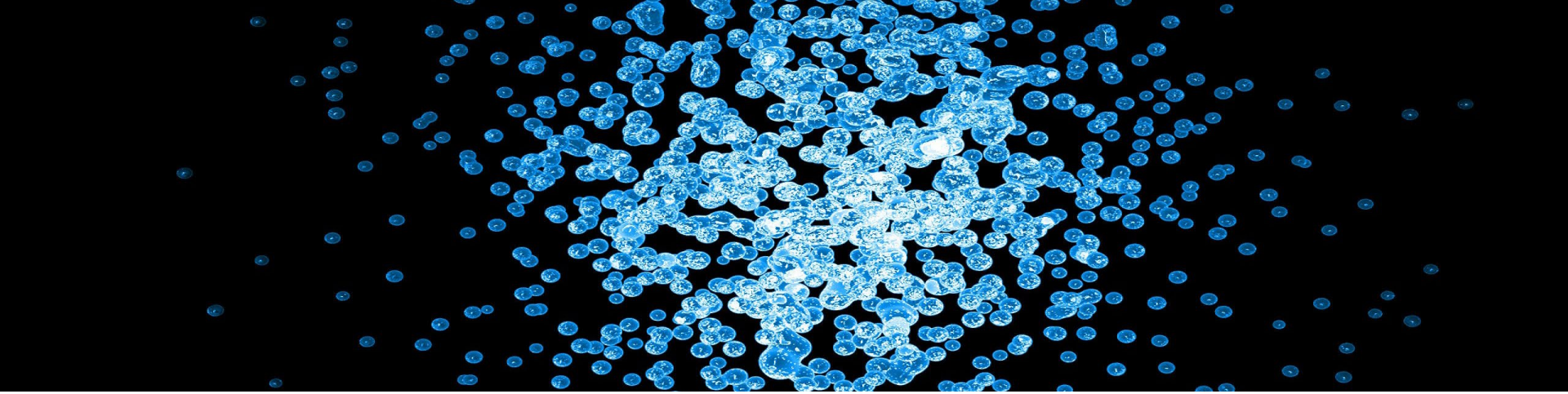
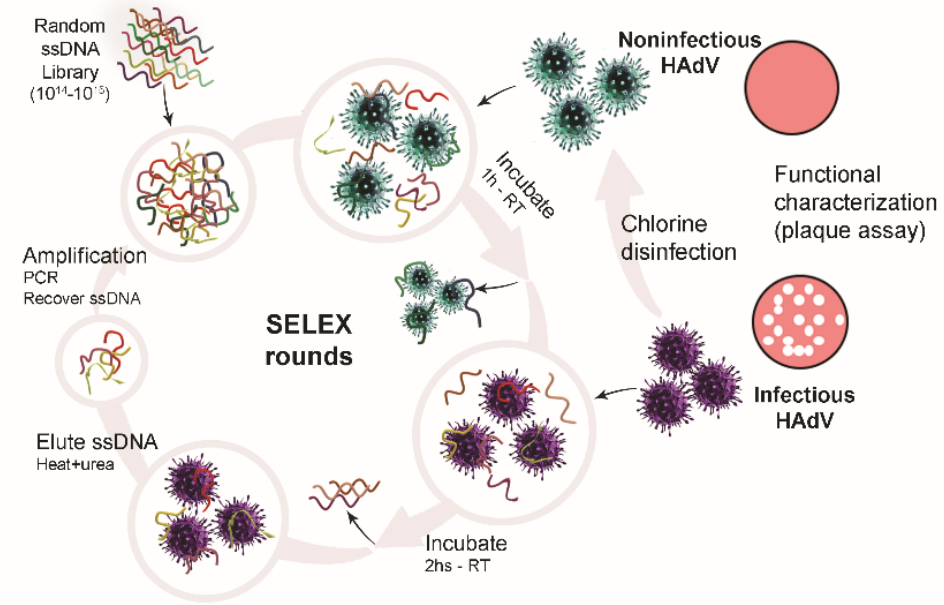


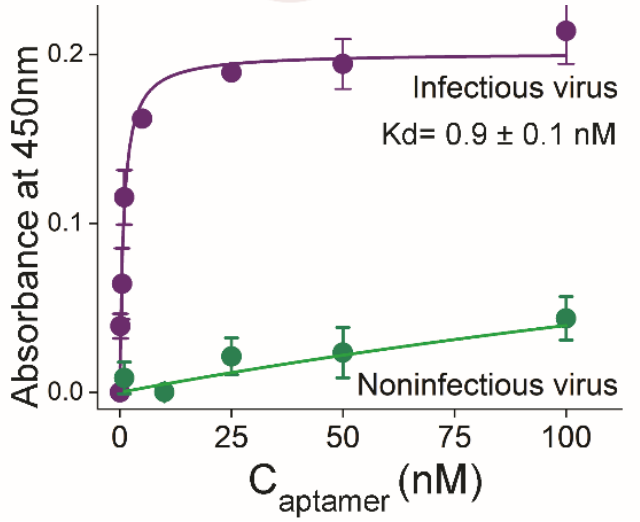
INFECTIOUS VIRUS DETECTION: APTAMER-BASED SOLID STATE NANOPORE SENSOR DEVELOPMENT

In vitro selection of infectious HAdV-2-specific aptamer

- *in vitro* selection process for infectious HAdV-2 resulting in HAdV-Seq4 aptamer: positive and counter selections steps (the latter using non-infectious viruses) added in each round to reach high specificity toward infectious virus.

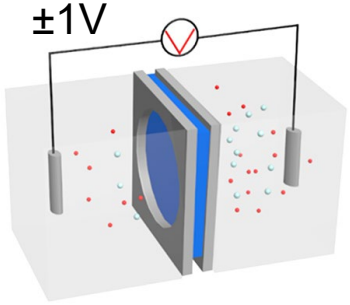


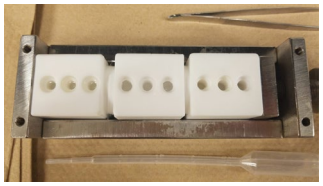
- Binding curves from affinity ELONA assays: dissociation constant (K_d=0.9 nM) of the HAdV-Seq4 aptamer for infectious HAdV-2 was more than 100 times higher than that for noninfectious HAdV-2.

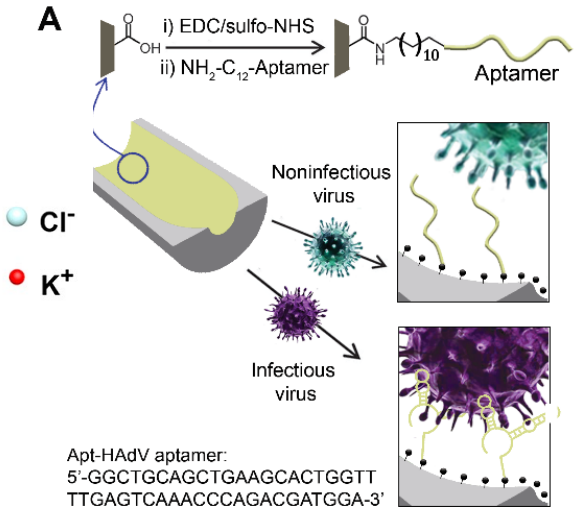


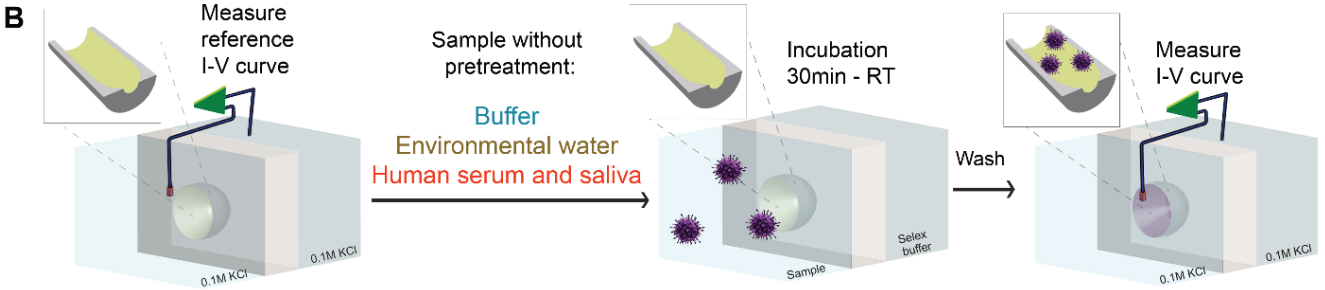
Solid State Nanopore Sensor

- electrochemical cell: 0.1 M KCl, ±1V









- **The aptamer showed great selectivity for the infectious (active) HAdV**
- **The nanopore (900±100 nm → 55±5 nm) amplified the signal several orders of magnitude**

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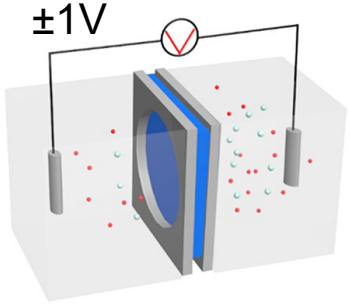

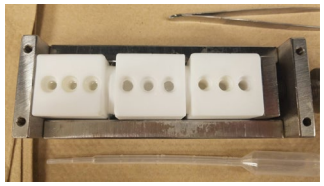

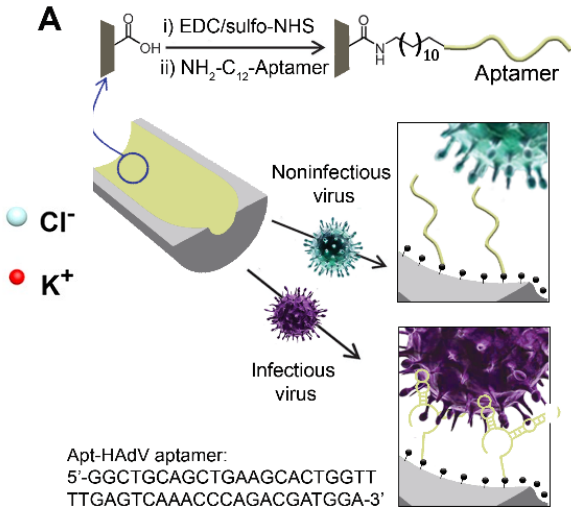

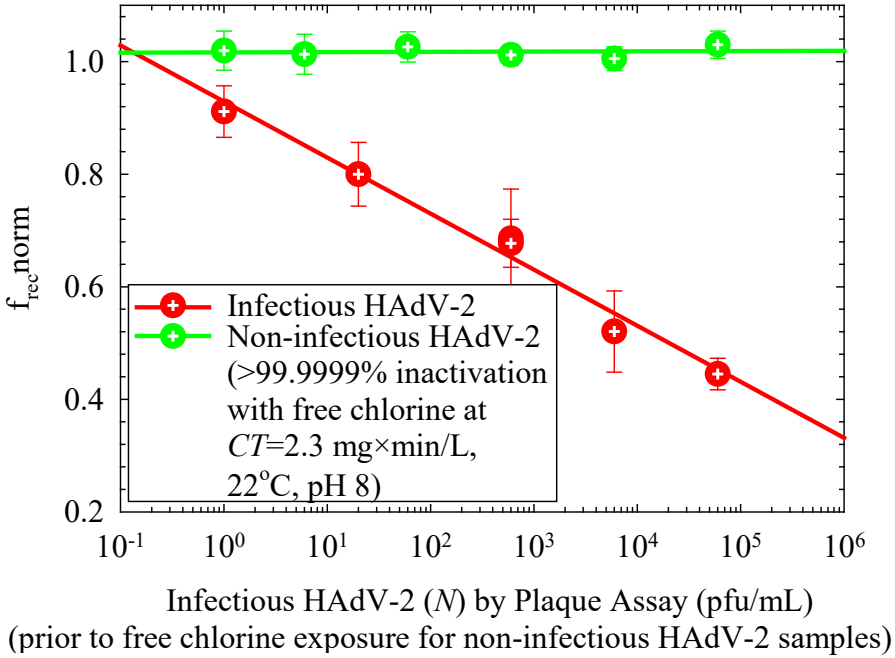

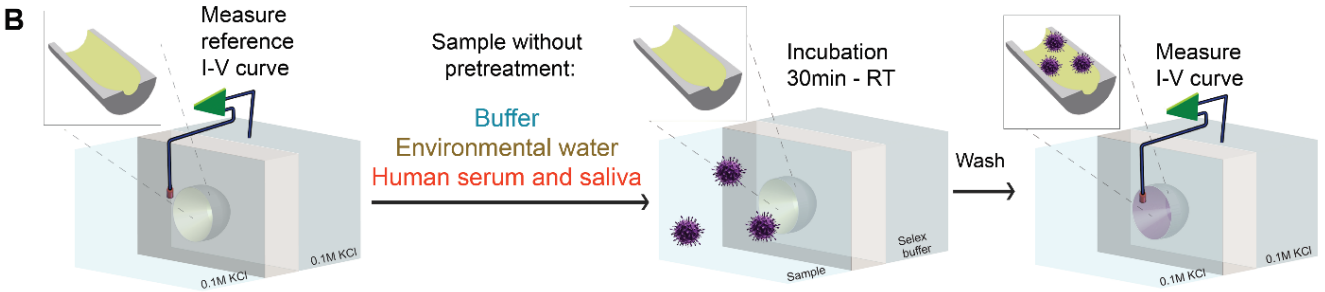

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Solid State Nanopore Sensor

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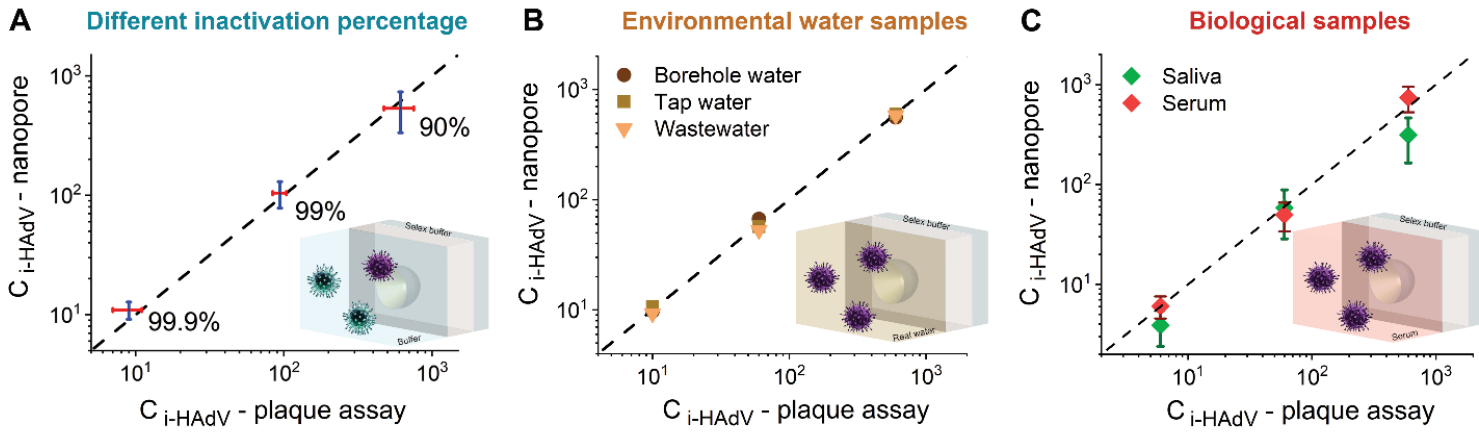
- No major background interference in buffer, tap water, WW effluent, saliva, blood serum



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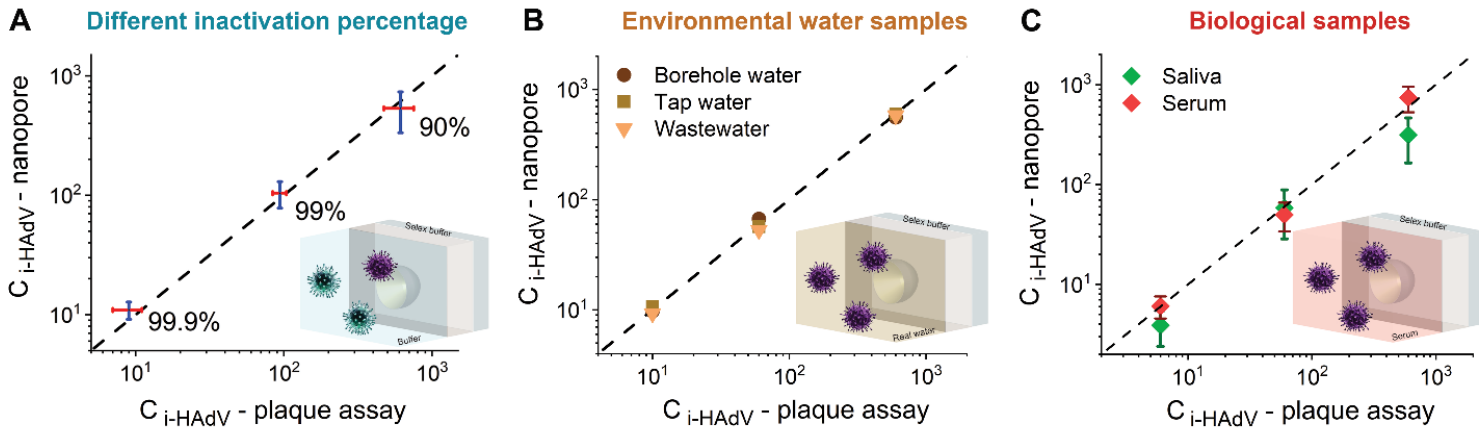
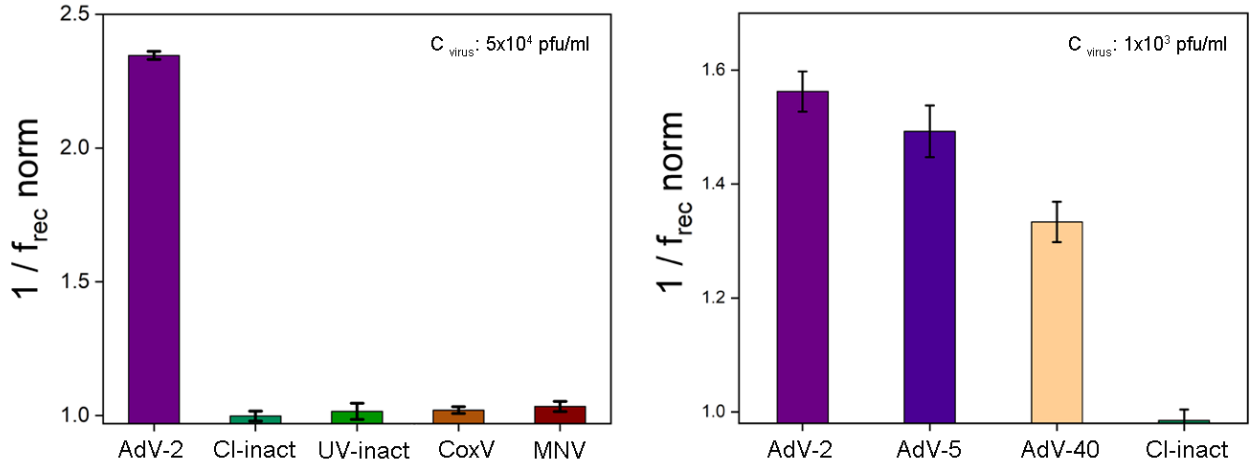


- Preliminary selectivity tests

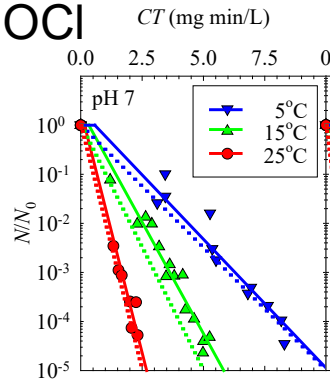


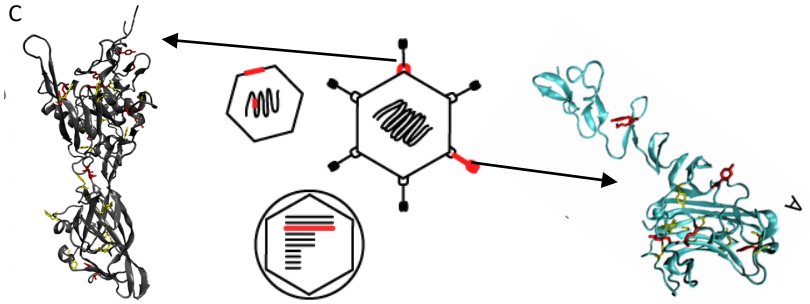
A.S. Peinetti, R.J. Lake, W. Cong, L. Cooper, Y. Wu, Y. Ma, G.T. Pawel, M.E. Toimil Morales, C. Trautman, L. Rong, B.J. Marinas, O. Azzaroni, **Yi Lu**, *Sci. Adv. (under revision)*

Inter/Transdisciplinary Research Approach

Kwanrawee Sirikanchana, Martin Page, Aimee Gall, Bernardo Vazquez, Dana Al-Qadi, Kelley Gonçalves, Shiliang Tian, Wen Cong, Ana S. Peinetti, Anisa Hardin
Joanna L. Shisler, Yi Lu

HOCl





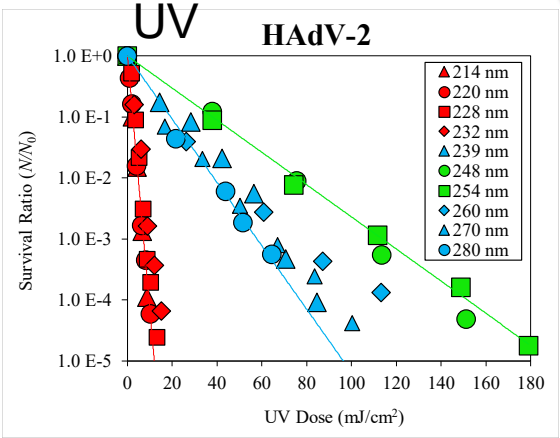
Protein/Genome transformation

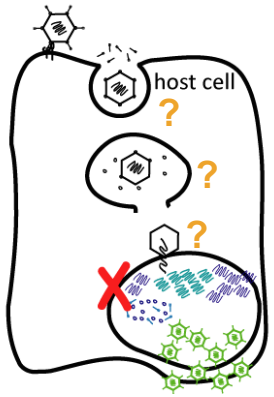
Inactivation kinetics

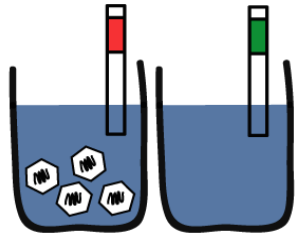
Replication cycle disruption

Sensor development

UV







Gall et al., *PLoS Pathog* **2015,** *11* (6), e1004867

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