Metropolitan Water Reclamation District of Greater Chicago

# Welcome to the October Edition of the 2020 M&R Seminar Series

# NOTES FOR SEMINAR ATTENDEES

- All attendees' audio lines have been muted to minimize background noise.
- A question and answer session will follow the presentation.
- Please use the Chat feature to ask a question via text.
- The presentation slides will be posted on the MWRD website after the seminar.

**Benito J. Mariñas**, Ph.D.

Ivan Racheff Endowed Professor of Environmental Engineering

Dept of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign

Dr. Mariñas is Ivan Racheff Endowed Professor of Environmental Engineering, Department of Civil and Environmental Engineering (CEE), University of Illinois at Urbana-Champaign (UIUC) where he served as Department Head from 2014-2020. He is also Director of the Safe Global Water Institute (2013-present) at UIUC and served as Director of the NSF Science and Technology Center of Advanced Materials for the Purification of Water with Systems - WaterCAMPWS (2012-14).

Dr. Mariñas' research explores mechanistic aspects of chemical and ultraviolet light disinfection processes, chemistry and toxicity of nitrogenous disinfection by-product (N-DBP), with emphasis on N-DBP formation and control. He also does research on membrane technologies for the control of water-borne pathogens and chemical contaminants, with emphasis on the development of novel membrane materials.

Dr. Mariñas holds a B.S. degree in civil engineering from the Universidad Politecnica de Madrid, Spain (1982); and M.S. (1985) and Ph.D. (1989) degrees in sanitary and environmental engineering from the University of California at Berkeley. Before joining the CEE faculty at UIUC in 1995, he was a faculty member (1989-1995) at the School of Civil Engineering of Purdue University, West Lafayette, Indiana.

Dr. Mariñas was the recipient of the Arthur and Virginia Nauman Faculty Scholar award (1998- 2005) at the Dept. of CEE of UIUC. He is a Fellow of the Association of Environmental Engineering and Science Professors (2020), Member of the Academy of Distinguished Alumni Academy, Dept of CEE, University of California at Berkeley (2019), won Harold Munson Outstanding Teacher Award (1992), and Ross Judson Buck '07 Outstanding Counselor Award (1992) from the School of Civil Engineering at Purdue University, and making the List of Teachers Ranked as Excellent by their Students multiple times at the UIUC.

# Advances in Kinetics, Mechanisms, and Detection of Viral Pathogens Toward Developing Smart Water Disinfection Systems


Benito J. Mariñas

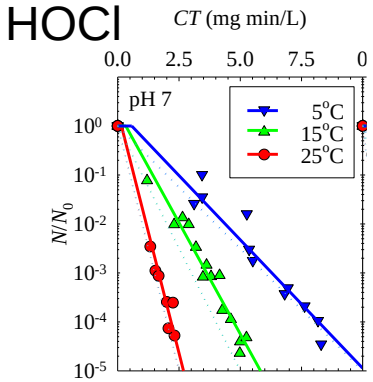
*Director*, Safe Global Water Institute (SGWI)

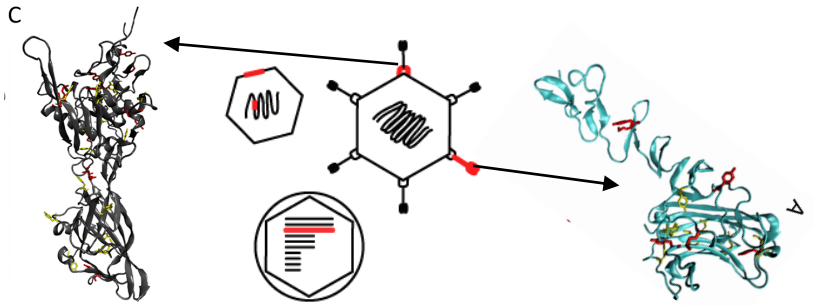
*Ivan Racheff Professor*, Department of Civil & Environmental Engineering

# 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



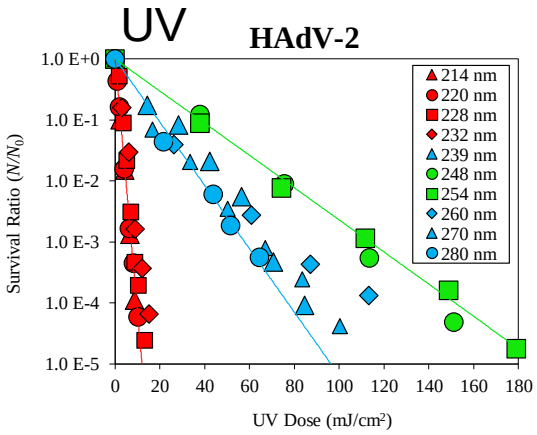


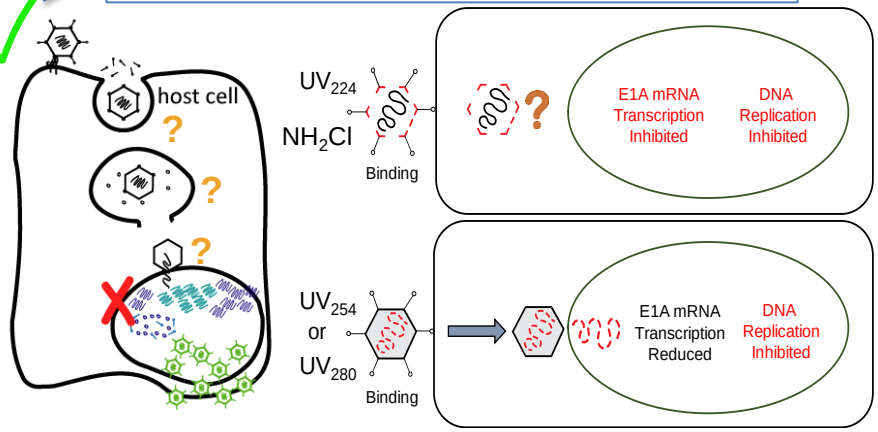
**Protein/Genome transformation**

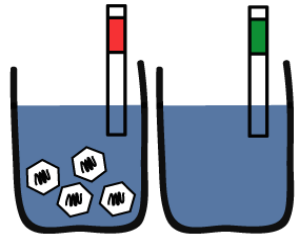
**Inactivation kinetics**

**Replication cycle disruption**

**Sensor development**



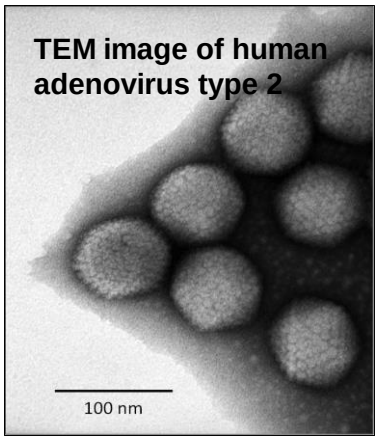
Gall et al., *PLoS Pathog* **2015,** *11* (6), e1004867

# Viral Pathogens, Disinfectants & Objectives

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

## Pathogens:

- **Human Adenovirus 2 (non-enveloped capsid ~90 nm; dsDNA ~36 kbp)**
- Coxsackievirus B5 (enterovirus;non-enveloped capsid ~30 nm; +ssRNA ~7.5 kb)

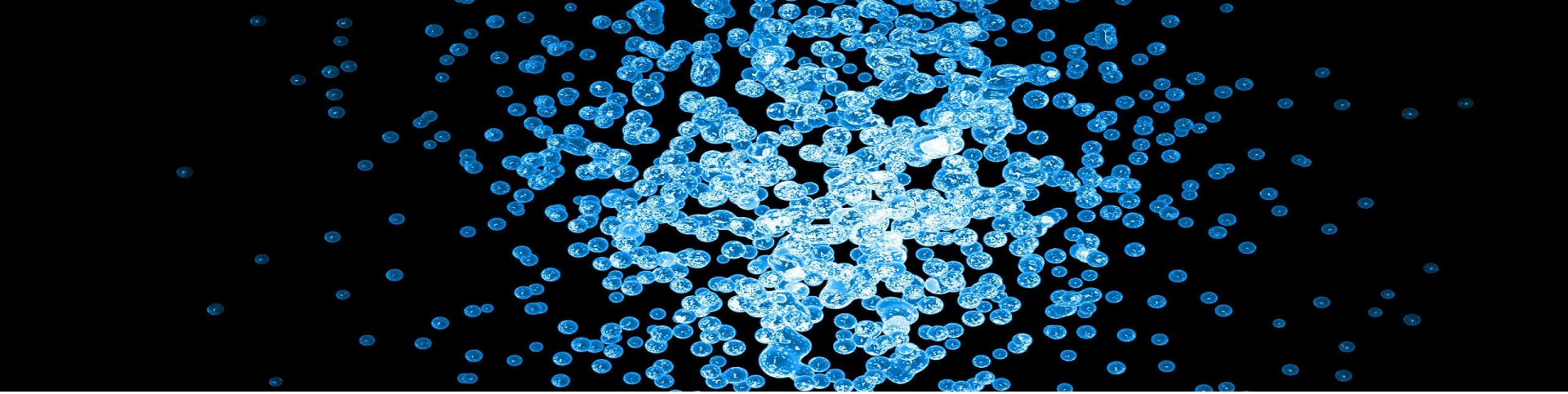



## Disinfectants:

- **Free chlorine**
- **Monochloramine**
  - 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)

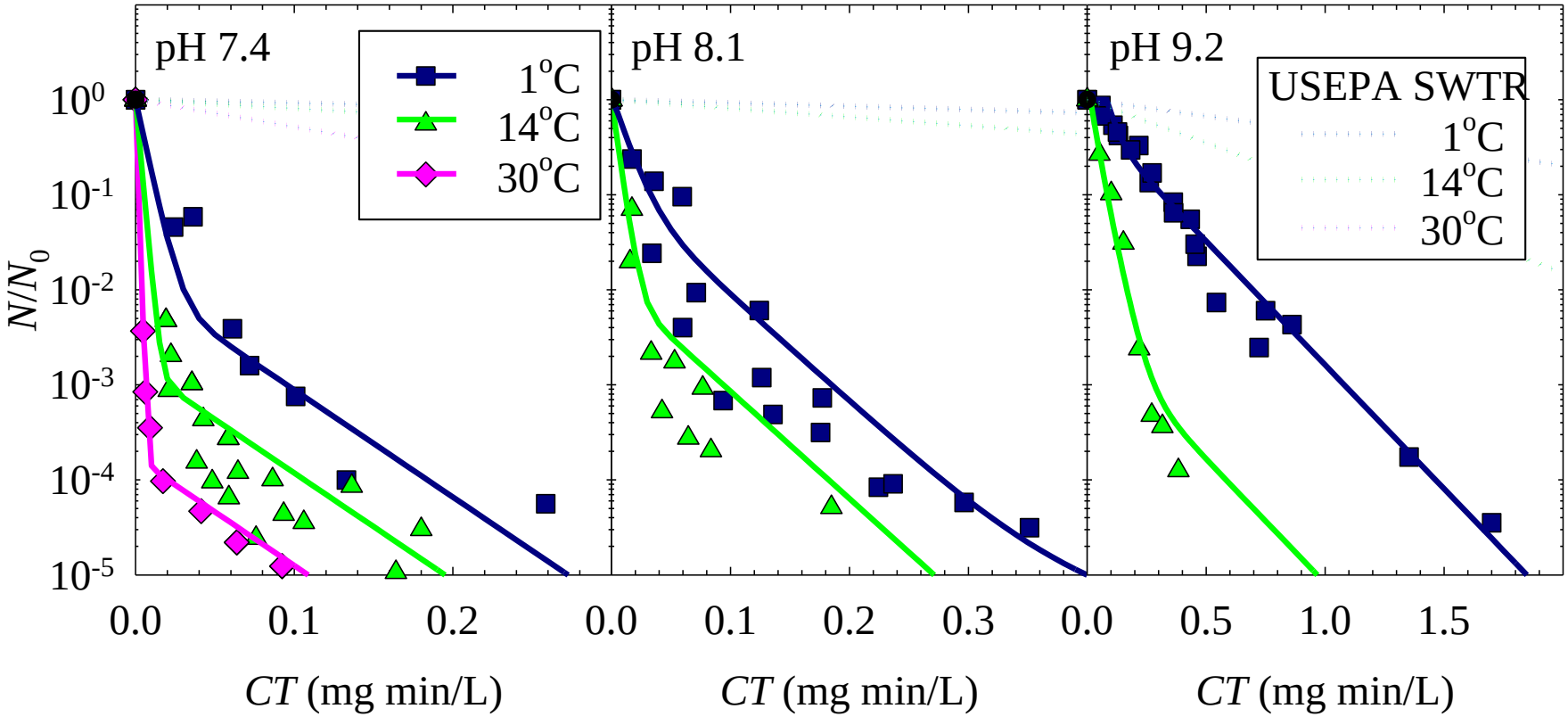
## Broad Objective:

- **Have better control of viruses in drinking water**
  - Determination of inactivation kinetics
  - Inactivation mechanisms
  - Sensitive methods to detect infectious viruses (ultimately including those for which cell culture is not available, like human norovirus)



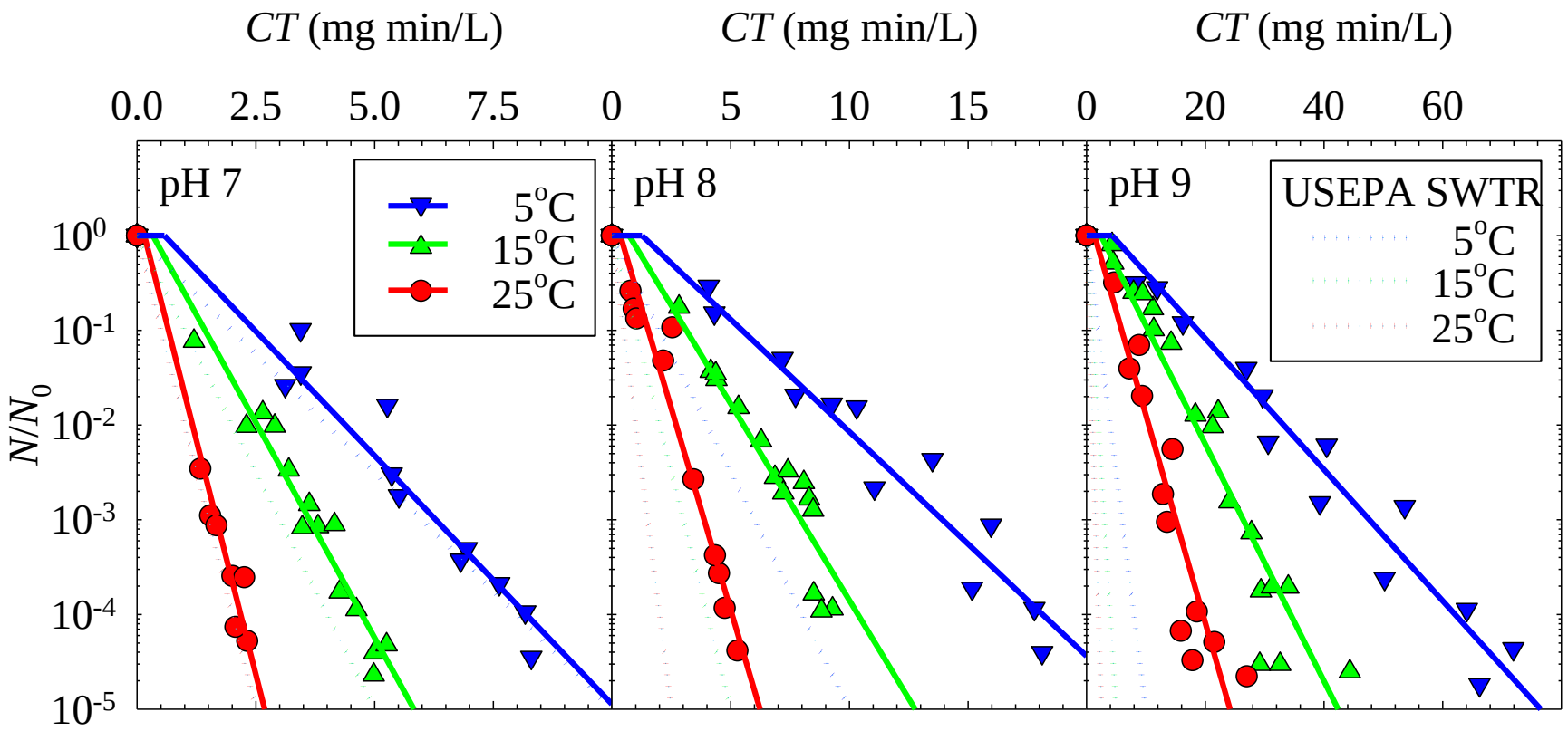
# INACTIVATION KINETICS

# Inactivation of Human Adenovirus 2 with Free Chlorine



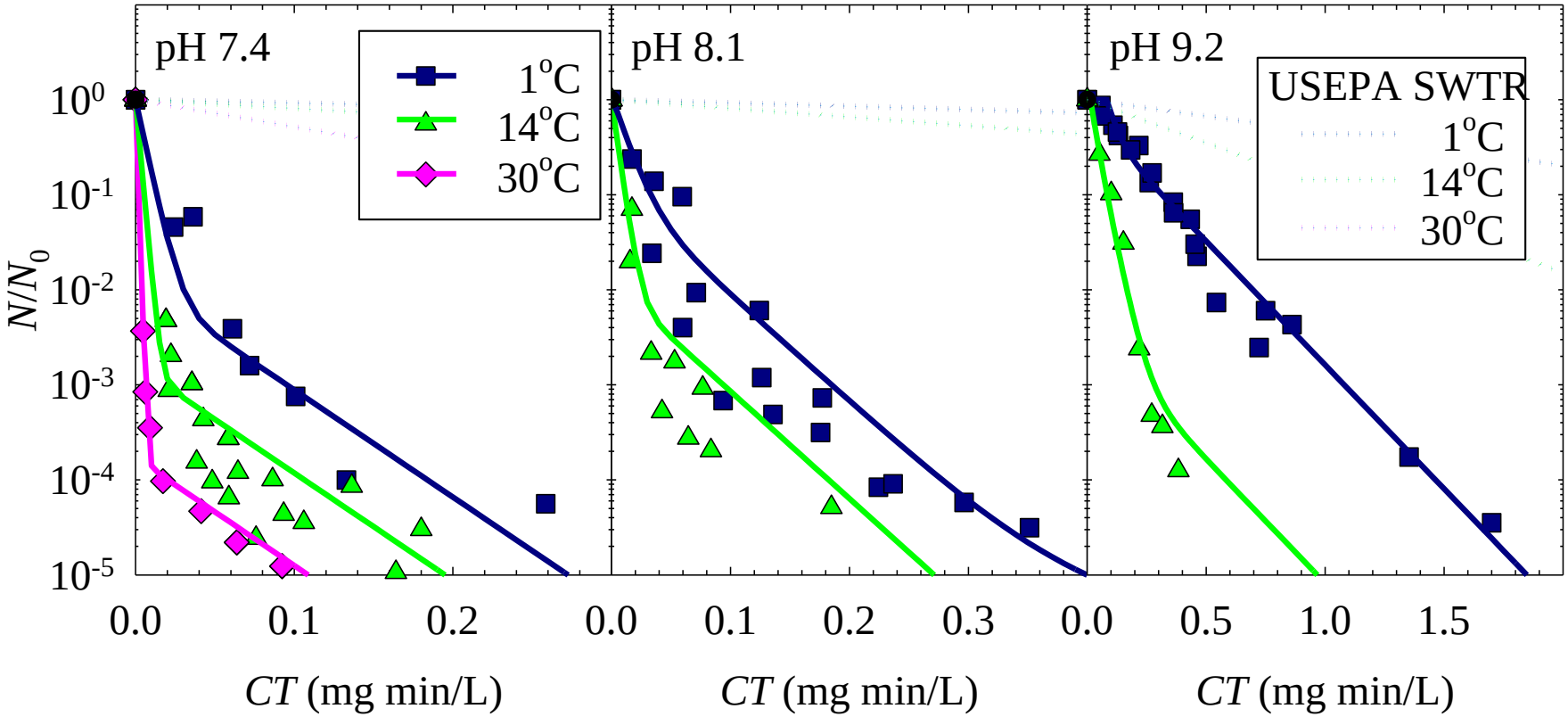

M.A. Page, J.L. Shisler, B.J. Marinas, *Wat. Res.*, 2009, 43, 2916-2926

# Inactivation of Coxsackievirus B5 with Free Chlorine



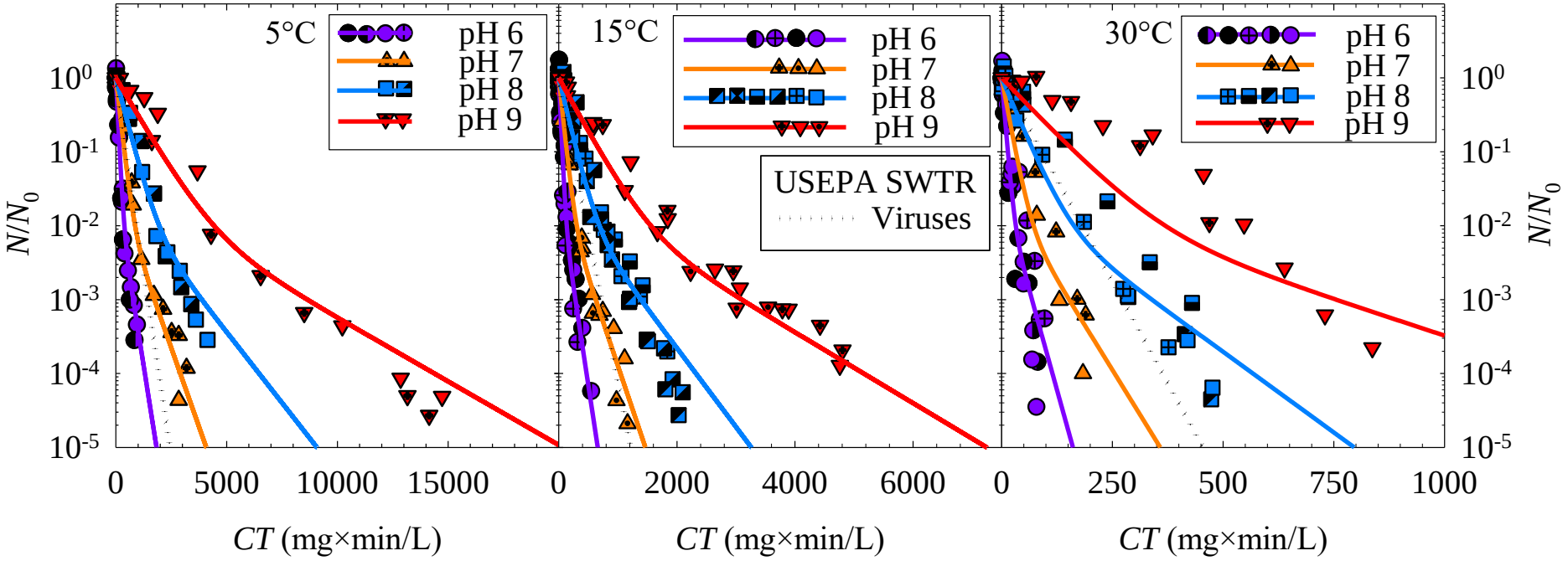

A. Hardin, W. Cong, B.J. Marinas, *in preparation*

# Inactivation of Human Adenovirus 2 with Free Chlorine

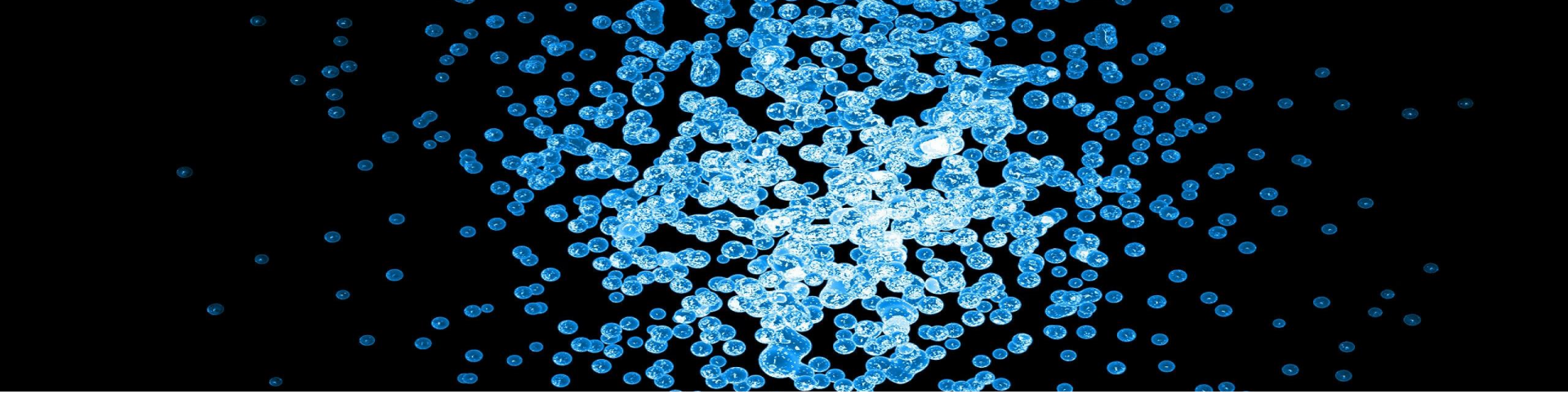



M.A. Page, J.L. Shisler, B.J. Marinas, *Wat. Res.*, 2009, 43, 2916-2926

# Inactivation of Human Adenovirus 2 with Monochloramine




A.M. Gall, J.L. Shisler, B.J. Marinas, *Environ. Sci. Technol. Lett.*, 2016, 3, 185-189



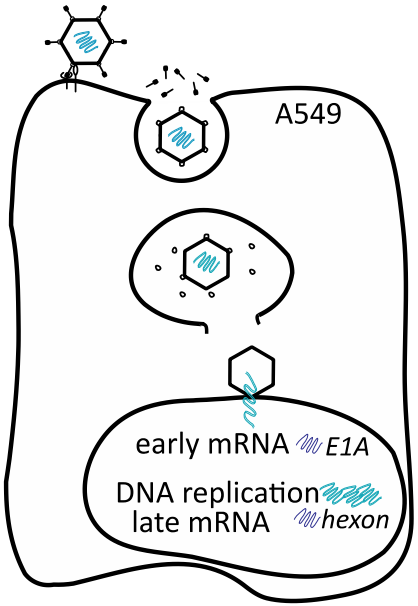
# INACTIVATION MECHANISMS: LIFE CYCLE STEP INHIBITION

# HAdV-2 and CoxV-B5 Replication Cycles

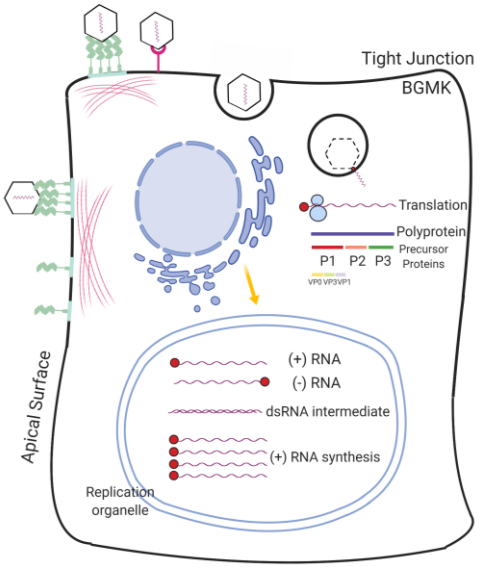
Selected HAdV-2 (90 nm, ~36,000 bp) Replication cycle events:

- Attachment (fiber to CAR, penton base to integrin?) – 0 h p.i.
- Internalization with endosome
- 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.

HAdV-2




CoxV-B5




Selected CoxV-B5 (30 nm, ~7,500 b) replication cycle events:

- Attachment (VP2&3 to DAF VP1, VP3, VP4 to CAR?) – 0 h p.i.
- Internalization with endosome
- Virion uncoating
- RNA release from endosome
- RNA synthesis 4-5 h p.i
- Viral lytic and non-lytic (vesicle) release – 6 h p.i.

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

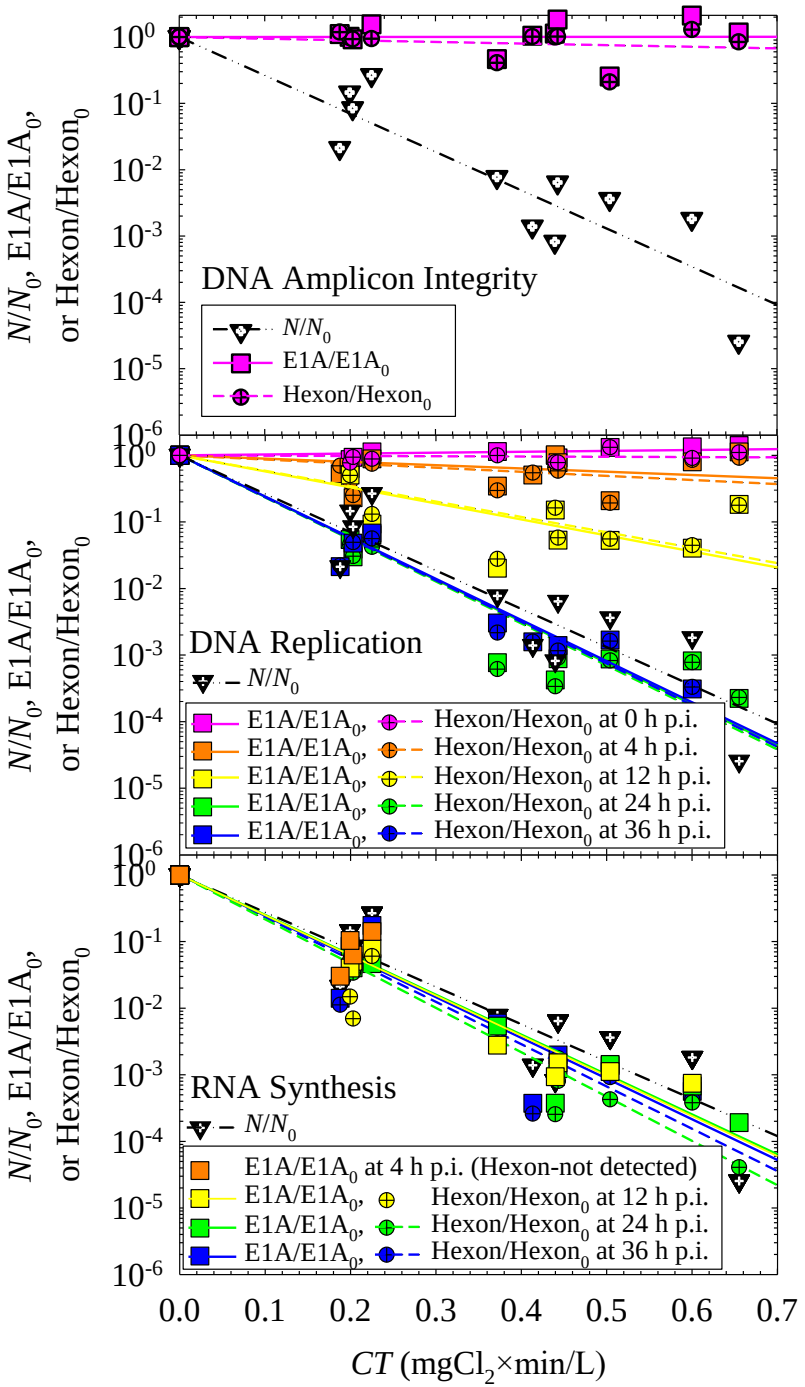
HAdV-2 genome showing selected amplicons



CoxV-B5 genome showing selected amplicons

# Comparison of Free Chlorine Inactivation of Human Adenovirus 2 with relative quantity of early (E1A) and late (hexon) DNA and RNA

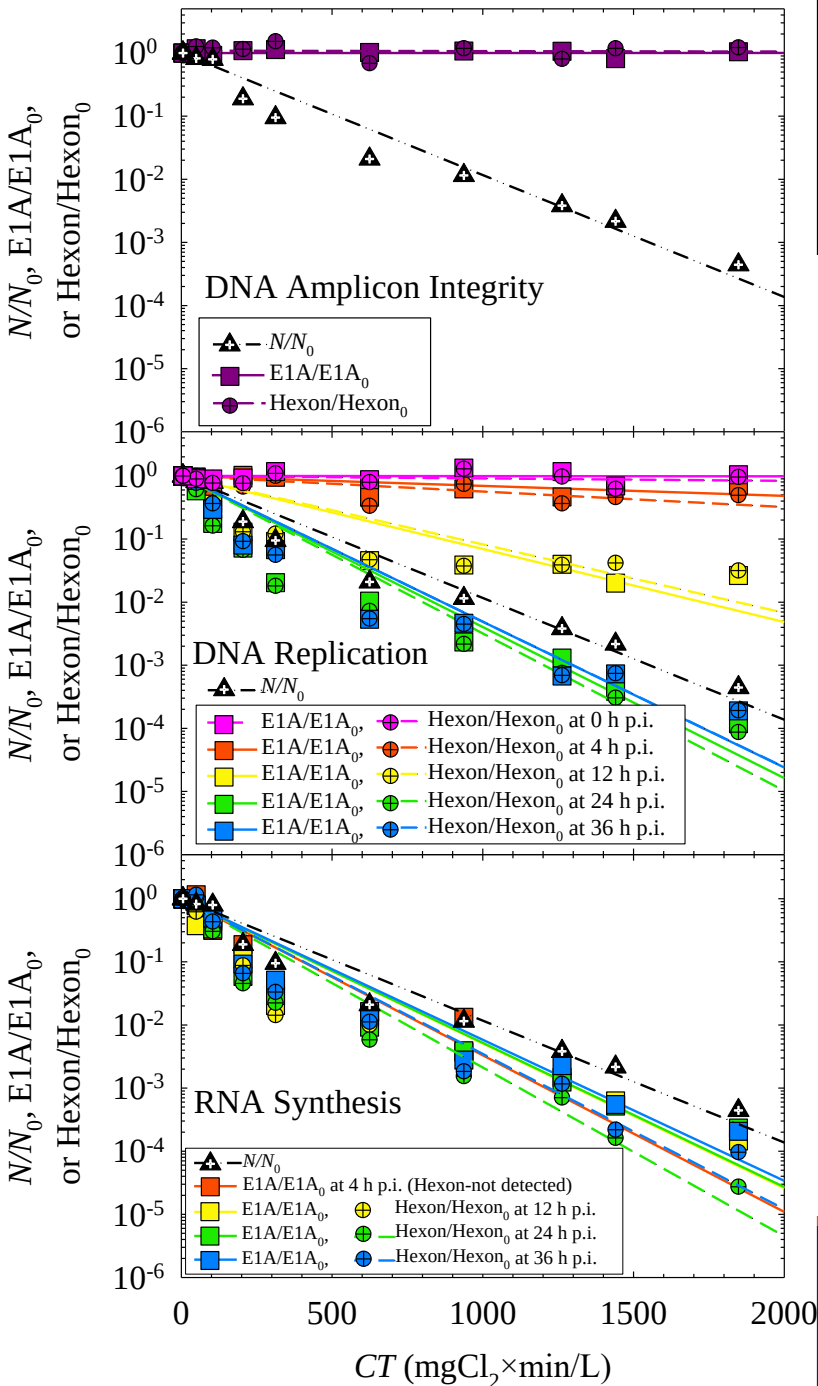
- Experiments performed at pH 7, 15°C
- Adenovirus inactivation at levels up to 99.99% did not result in loss of amplicon integrity or ability to attach
- However, synthesis of viral DNA and early E1A and late hexon gene transcription were inhibited




A.M. Gall, J.L. Shisler, B.J. Marinas, *Environ. Sci. Technol.*, 2015, 49, 4584-4590

# Comparison of Monochloramine Inactivation of Human Adenovirus 2 with relative quantity of early (E1A) and late (hexon) DNA and RNA

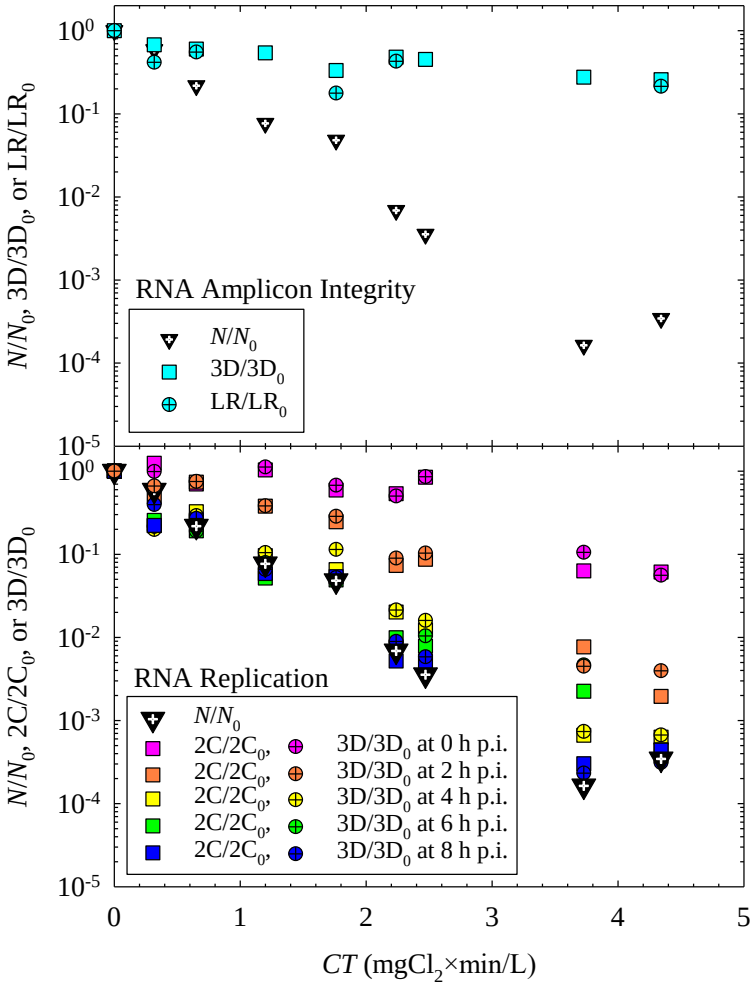
- Experiments performed at pH 8, 15ºC
- Adenovirus inactivation at levels up to 99.99% did not result in loss of amplicon integrity or ability to attach
- However, synthesis of viral DNA and early E1A and late hexon gene transcription were inhibited




A.M. Gall, J.L. Shisler, B.J. Marinas, *Environ. Sci. Technol. Lett.*, 2016, 3, 185-189

# Comparison of Free Chlorine Inactivation of Coxsackievirus B5 with relative quantity of RNA

- Experiments performed at pH 7, 15ºC
- In contrast to the observation for adenovirus, there is measurable loss of amplicon integrity and ability to attach at higher Coxsackievirus B5 inactivation levels
- Similar to the observation for synthesis of DNA genes for adenovirus, synthesis of viral RNA genes for Coxsackievirus B5 were inhibited




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

2C - 106 b - translate into protein 2C which rearranges the cellular membranes for the purpose of viral replication.
3D -149 b - translate into protein 3D, which is an RNA-dependent RNA polymerase
LR (long range) - 1235 b located near the end of the genome

# HAdV-2 and CoxV-B5 Replication Cycles

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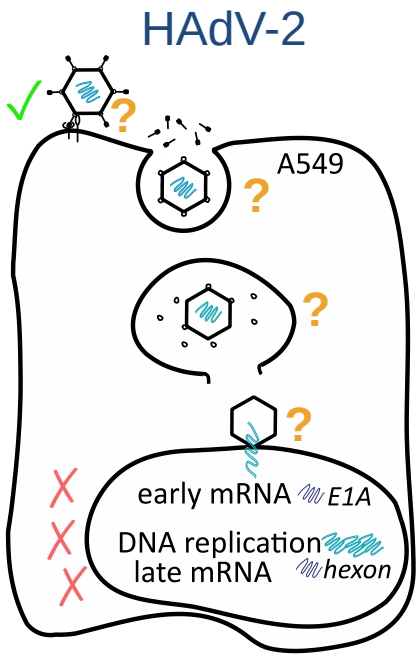


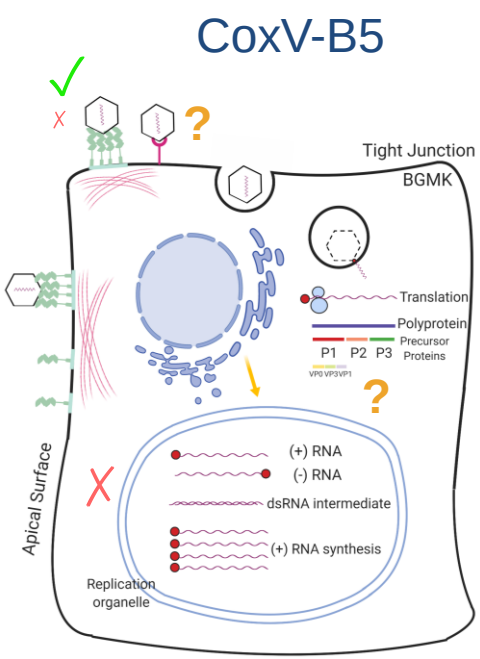


Selected CoxV-B5 (30 nm, ~7,500 b) replication cycle events:

- Attachment (VP2,VP3 to DAF, VP1, VP3, VP4 to CAR) – 0 h p.i.
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- Virion uncoating
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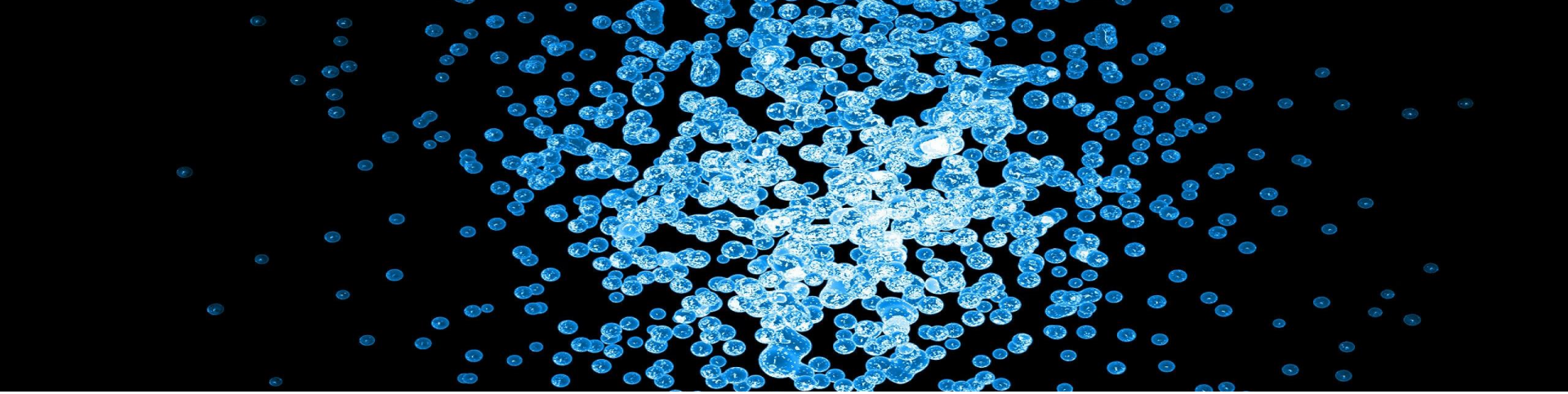
HAdV-2 genome showing selected amplicons



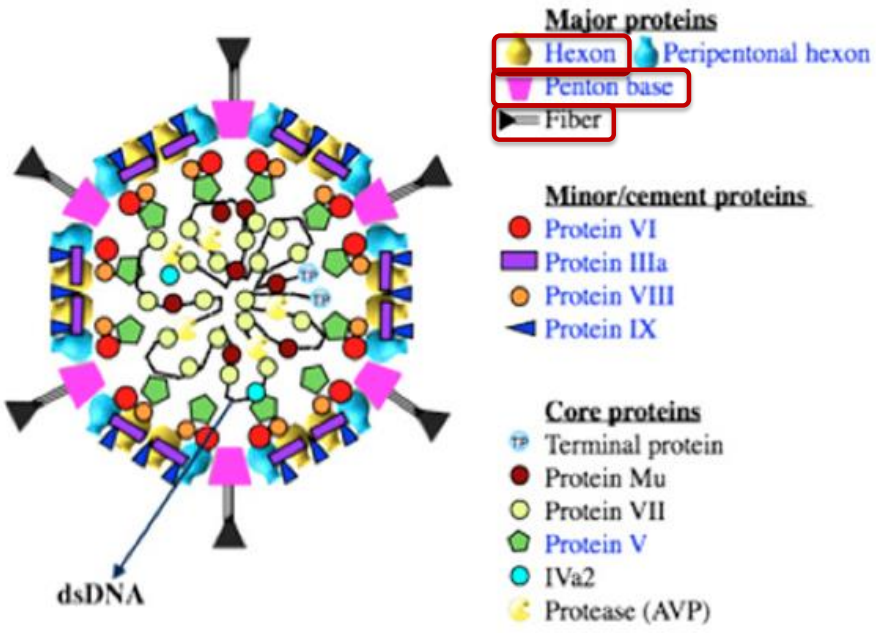
CoxV-B5 genome showing selected amplicons



# INACTIVATION MECHANISMS: CAPSID PROTEIN TRANSFORMATION

# Modifications of Human Adenovirus 2 Capsid Proteins with Free Chlorine

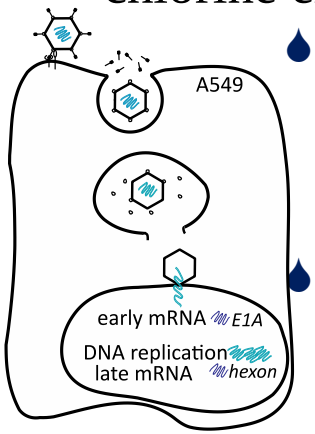
- Proteins targeted were: fiber, penton base, hexon
- The genes of the adenovirus fiber, penton base, and hexon were expressed in gene-modified *E. coli*
- 1 uM of each protein monomer (with 571, 582, 967 residues) was reacted with 10, 100, 1,000 uM free chlorine for 5 min (assuming no chlorine decay *CT*=3.6, 36, 360 mg min/L) before quenching with excess sodium thiosulfate
- Proteins digested (Trypsin) and residues analyzed by LC-MS/MS



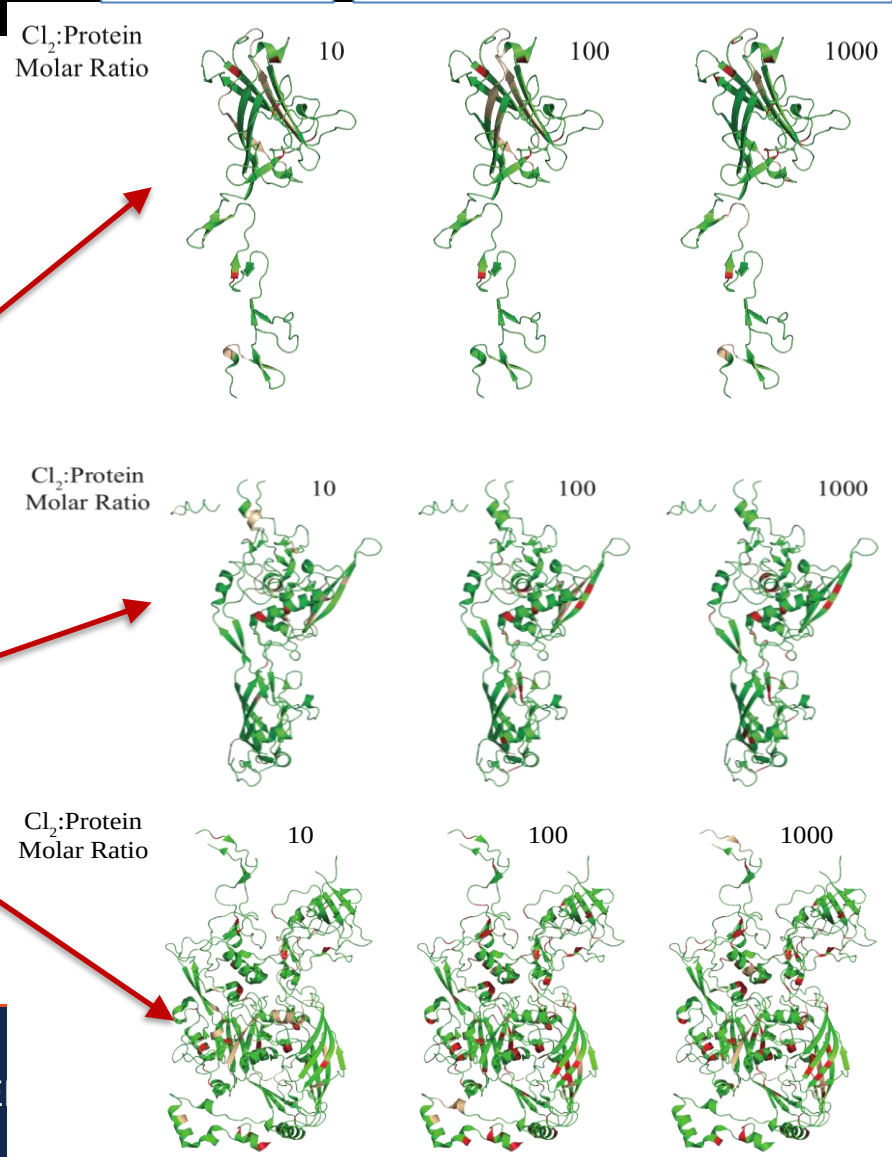

(Reddy and Nemerow, 2014)

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

- Ribbon diagrams showing modified residues (0.5-5%) in red (unmodified residues shown in green; peptides not detected shown in tan; 2-12%) with chlorine exposure:
  - Top: fiber tail, shaft, and head domains: CAR D1 binding & flexible shaft regions at mid and high *CT*;
  - Middle: penton base regions involved in pentamer formation, RGD loop, RGD integrin binding regions at lowest *CT*;
  - Bottom: hexon N terminus, viral jellyrolls, extended loops, and the viral jellyroll connector at all *CT*s.

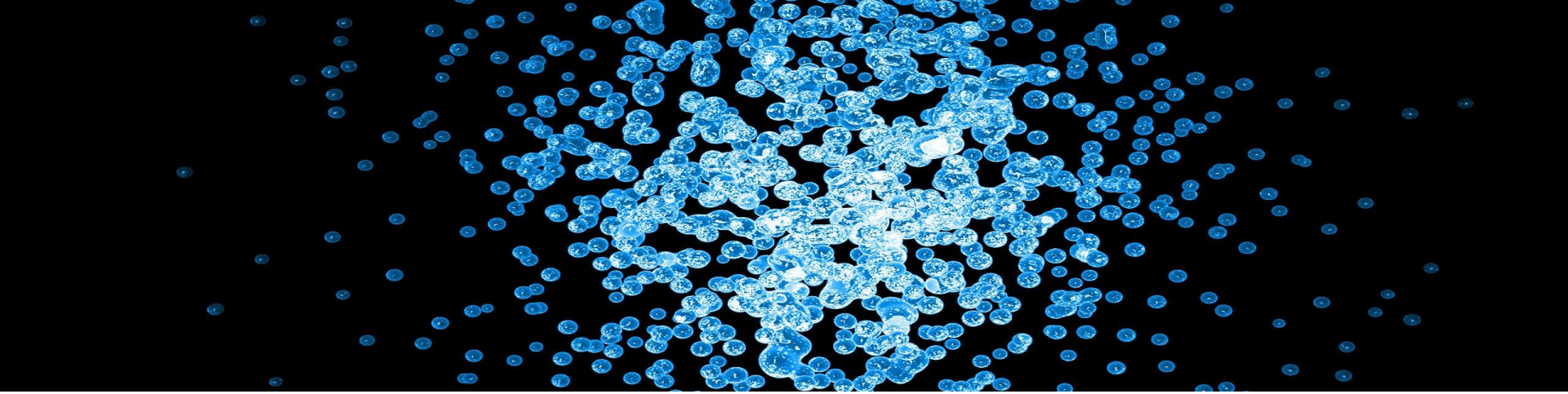



Met≈Cys>Trp | Met≈Cys>His>Trp>Tyr>Asn≈Gln




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

SAFE GLOBAL WATE

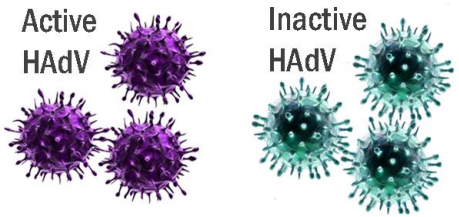


# INFECTIOUS VIRUS DETECTION: APTAMER-BASED SOLID STATE NANOPORE SENSOR DEVELOPMENT

# *In vitro* selection of an aptamer toward infectious HAdV-2

## Target preparation

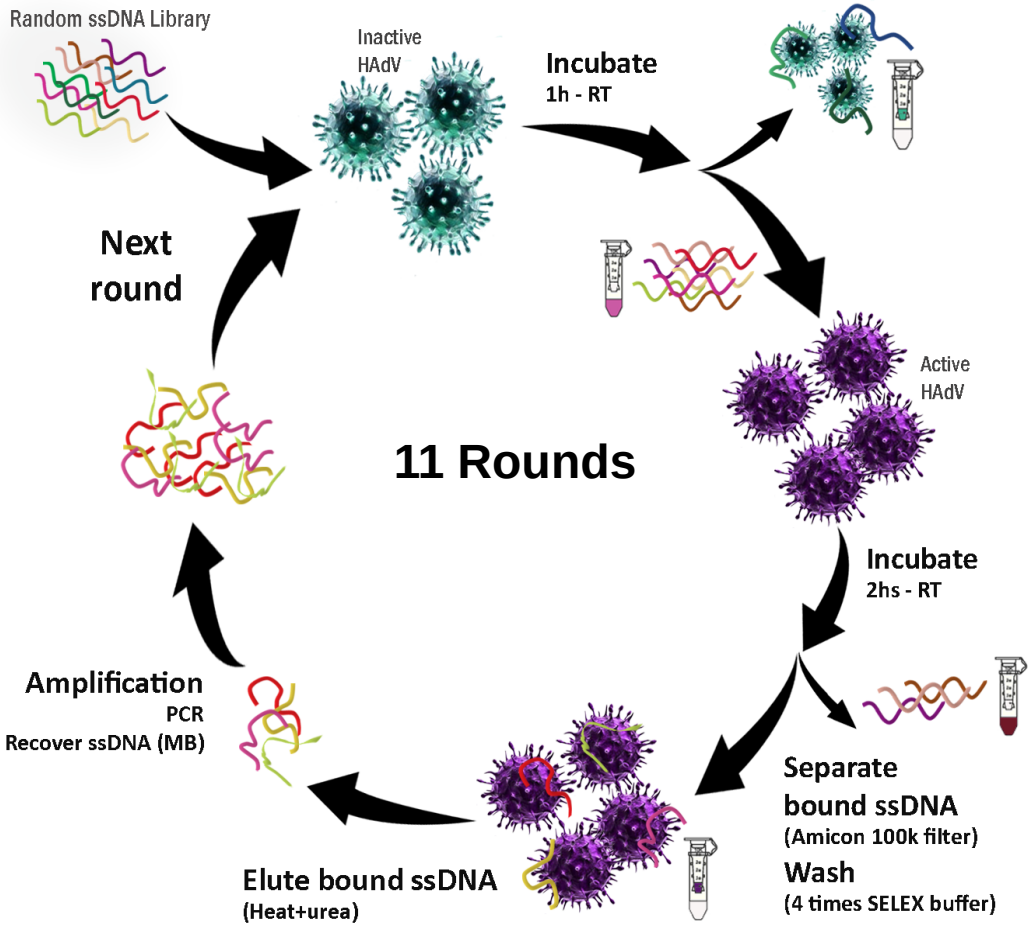
Human Adenovirus 2




Free Chlorine
pH 8.15, 22°C
*CT*≈2.3 mg min/L

## DNA library

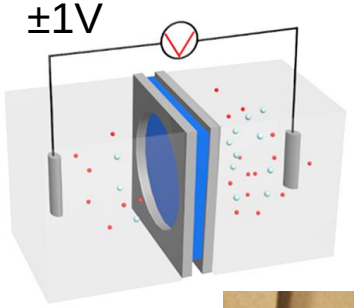
$10^{14}$ sequences
45 random nucleotides

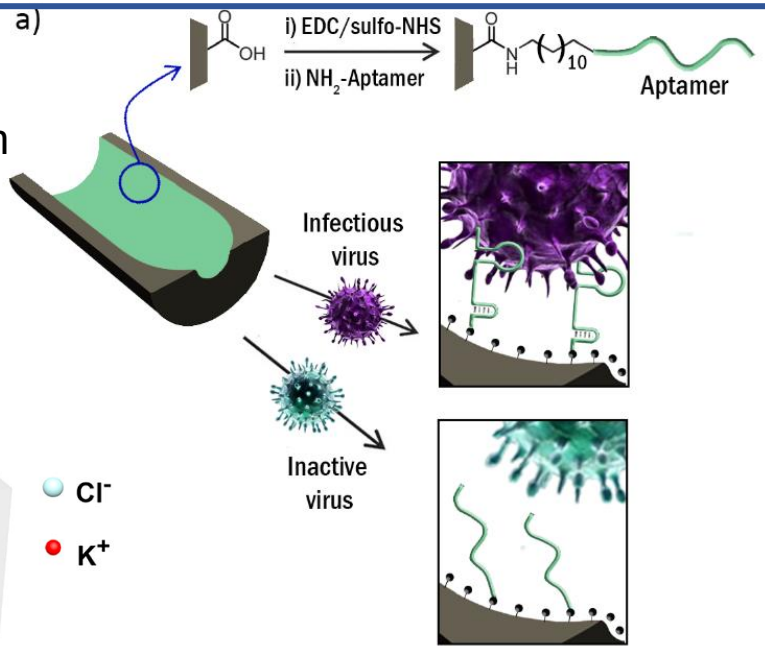


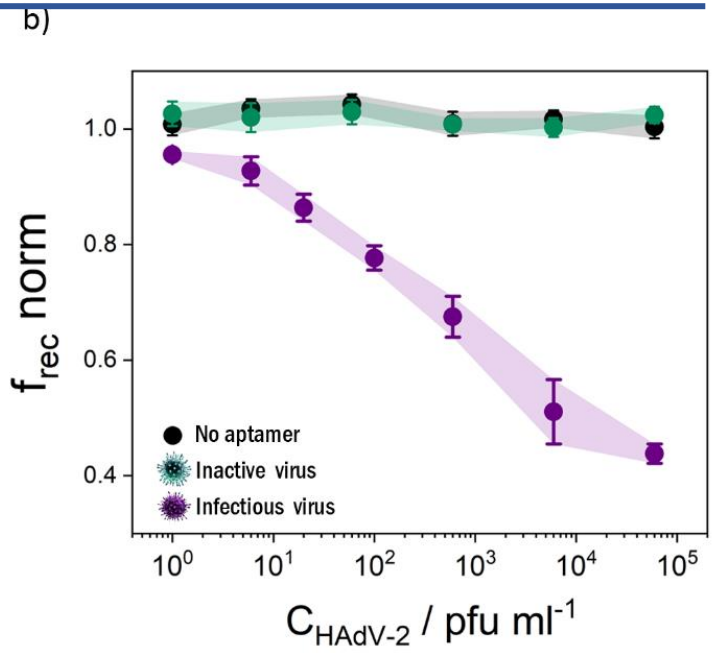

A.S. Peinetti, W. Cong, A. Gall, B.J. Marinas, O. Azzaroni, **Y. Lu**, *submitted*

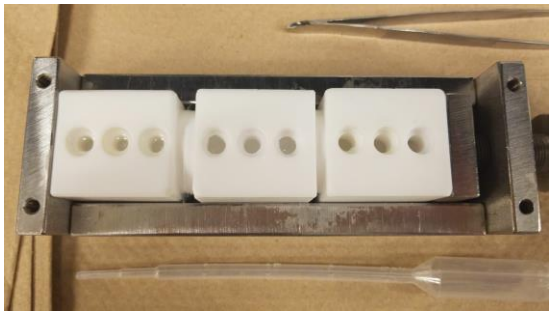
# Solid State Nanopore Sensor

- 2 hr equilibration with viruses
- 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**

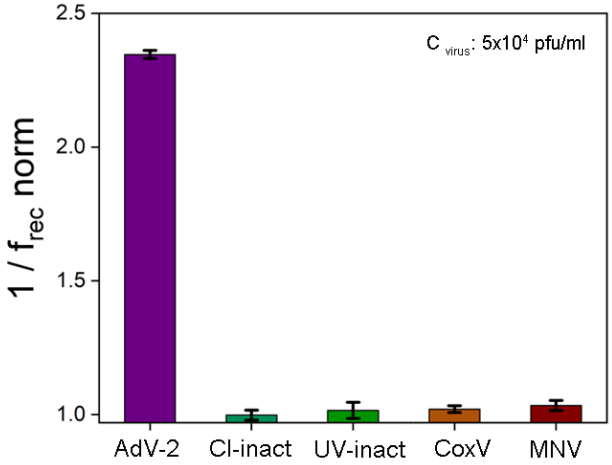

G. Perez-Mitta, A.S. Peinetti, M.L. Cortez, M.E. Toimil-Molares, C. Trautmann, Omar Azzaroni, *NanoLett.* 2018, 18, 3303-3310
A.S. Peinetti, W. Cong, A. Gall, O. Azzaroni, B.J. Marinas, **Y. Lu**, *submitted*

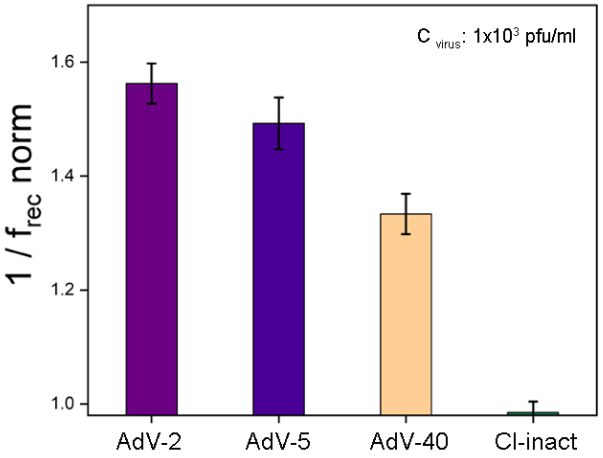
# Solid State Nanopore Sensor

- Comparison to inactivation data by plaque assay

| Sample # | % inactivation | Method; concentration (mean ± SD, n=3, pfu/ml) | |
|---|---|---|---|
| | | Nanopore system | Plaque-assay |
| 1 | 90 | 528 ± | 613 ± |
| 2 | 99 | 101 ± 26 | 94 ± |
| 3 | 99.9 | 12 ± 2 | 9.2 ± |

- Preliminary Selectivity Tests




- No background interference (same results with buffer, tap water, WW effluent)

G. Perez-Mitta, A.S. Peinetti, M.L. Cortez, M.E. Toimil-Molares, C. Trautmann, Omar Azzaroni, *NanoLett.* 2018, 18, 3303-3310
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# Inter/Transdisciplinary Research Approach

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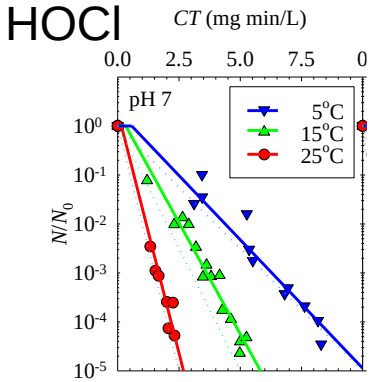
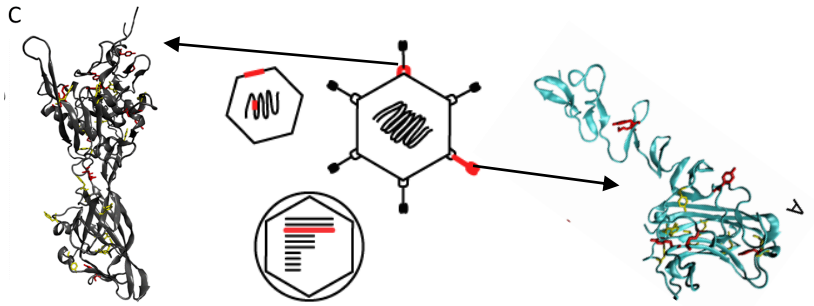


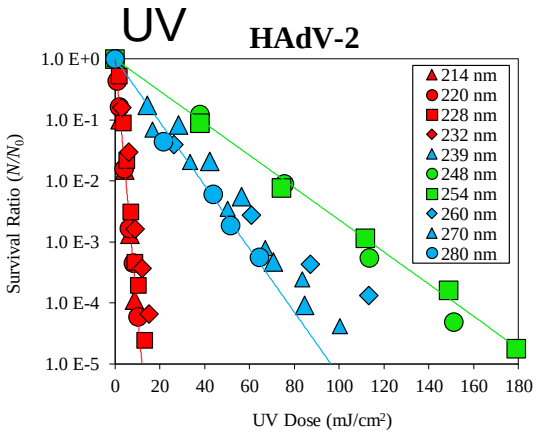


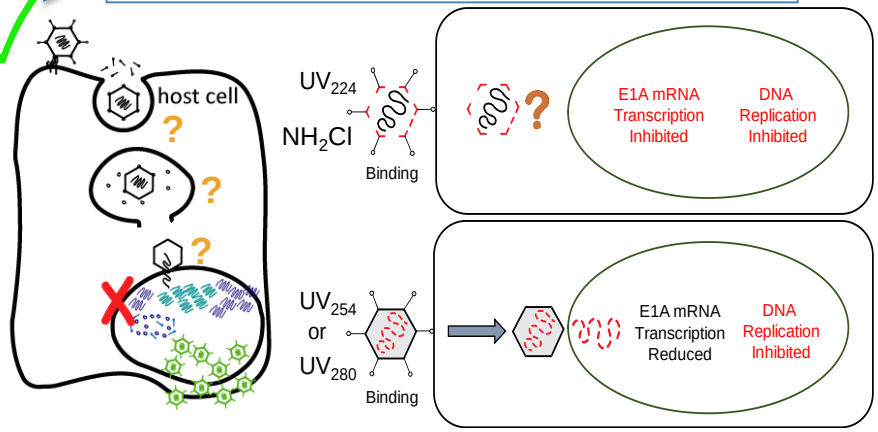
**Protein/Genome transformation**

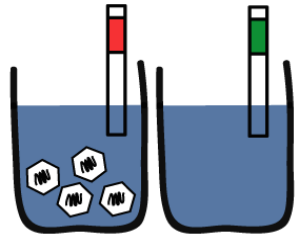
**Inactivation kinetics**

**Replication cycle disruption**

**Sensor development**



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

# THANK YOU!

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