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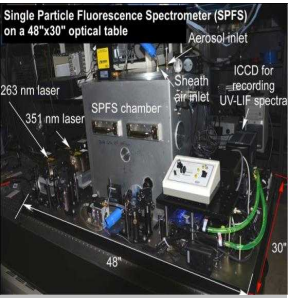

Changes in the Detectability and Viability of Aged Biological Particles

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Sandia National Laboratories

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U.S. DEPARTMENT OF ENERGY

NNSA National Nuclear Security Administration

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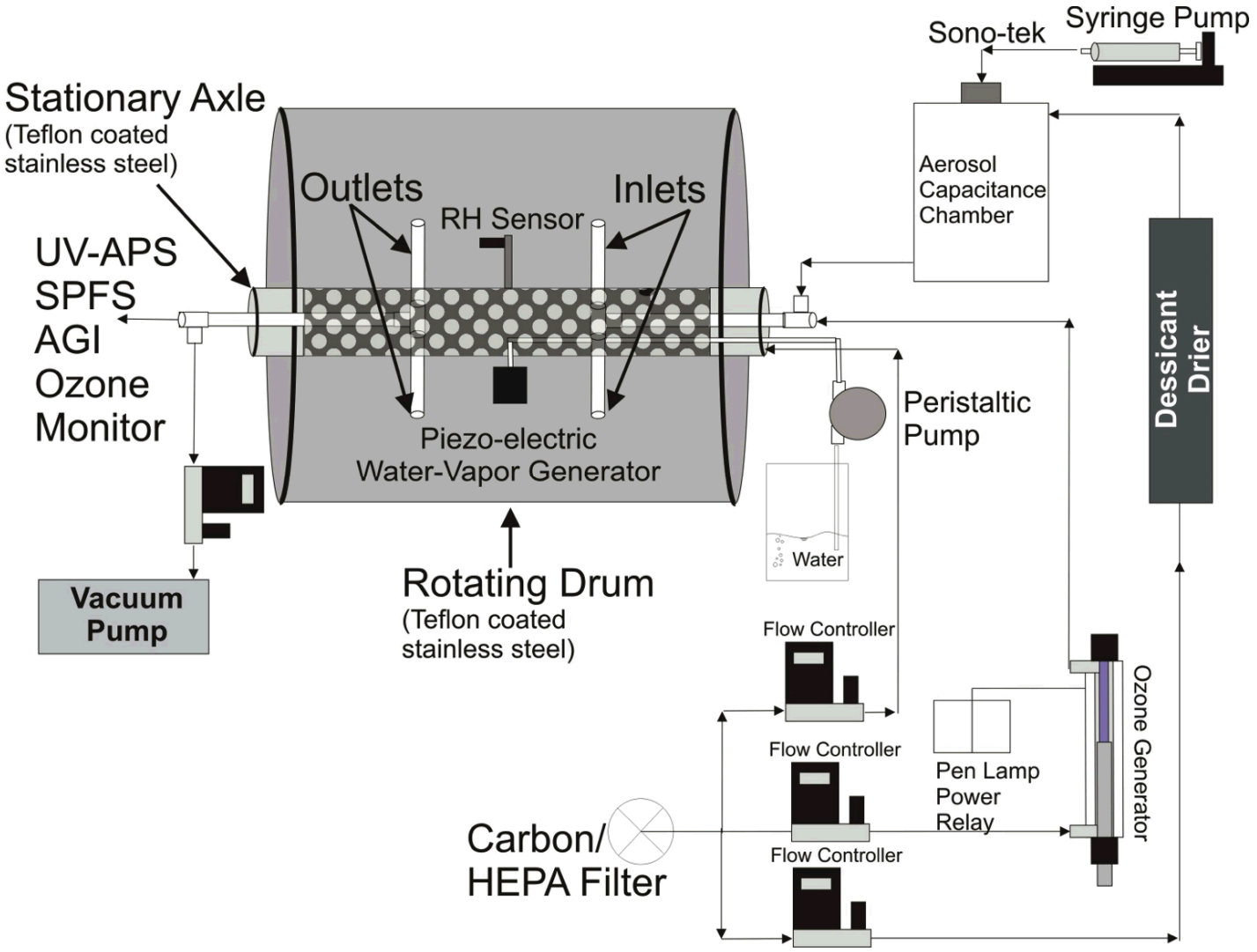
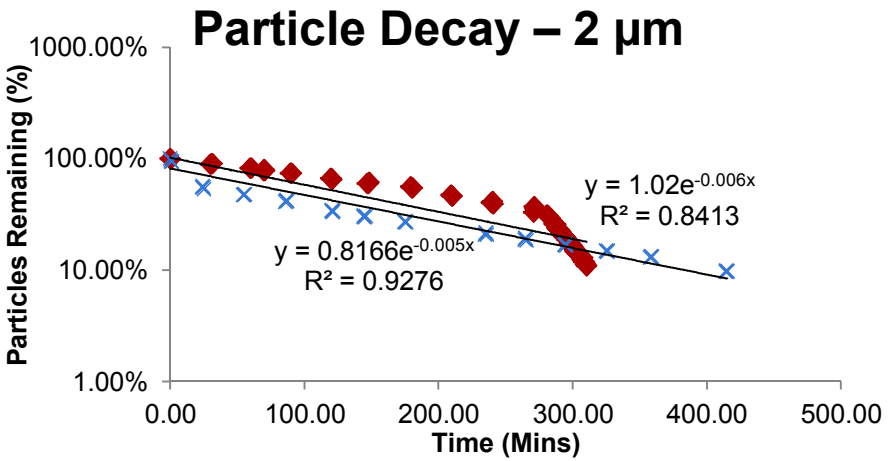
Background

- Primary Biological Aerosols (PBA)
  - May undergo chemical or physical changes in the atmosphere via differing processes
  - Open Air Factor (OAF) that includes ozone, humidity, VOCs, and temperature fluctuations.
  - Study effects in controlled environment and in outdoor natural aging environment.
- Potential benefits include:
  - Determining mechanisms and rates of change to feed into future models
  - Potential forensics for unique reactions with trace, geographically unique atmospheric compounds
  - Better understanding of variables that affect infectivity and detectability of bio-aerosols

Goldberg Rotating Drum



Particle Loss and Test Matrix

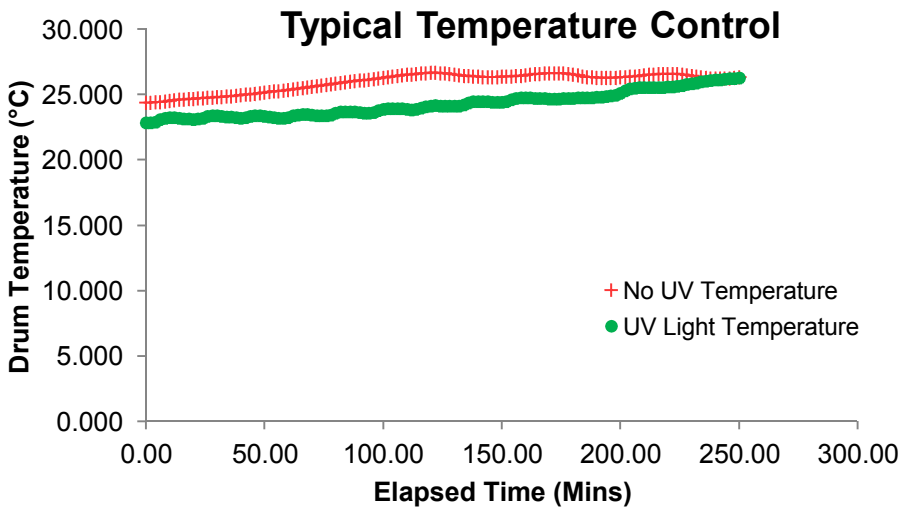


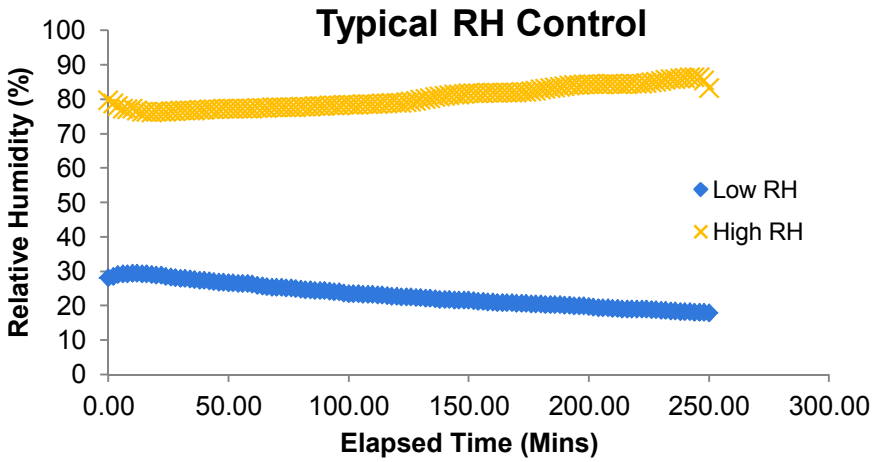
Completed Replicates

	MS-2	Bt Al Hakam
20% RH	2	2
80% RH	2	2
20% RH and UV	2	2
20% RH and O_3	2	2
80% RH and O_3	2	2

- Log Loss Rates:
  - 2 hours 51 minutes to 6+ hours depending on conditions
- Target 2 µm particle size
- Initially targeting RH, Ozone, and UV light
- Future Tests
  - Expanding numbers of simulants in future tests
  - Adding VOC toluene

Temperature and RH Control

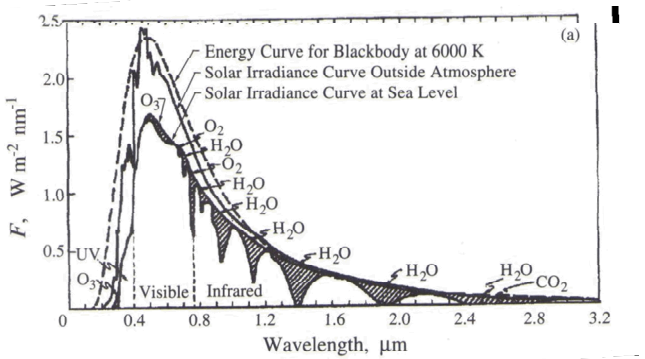


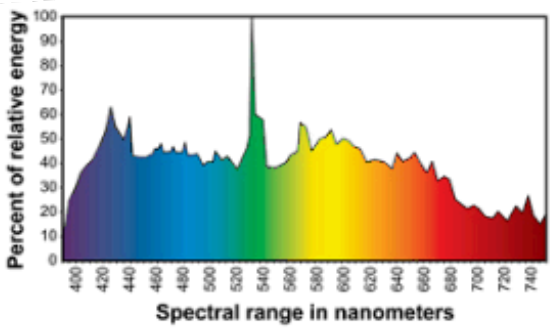


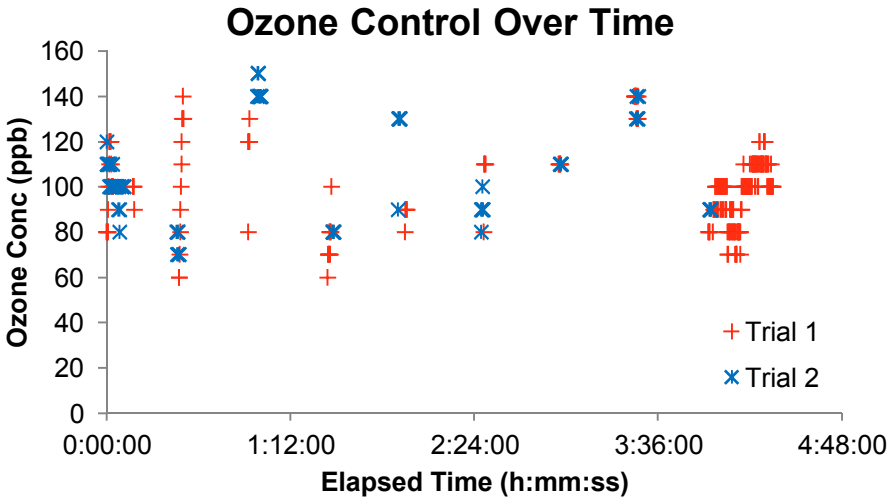
- Temperature control via HVAC systems
  - Vary from 23-28° C
- RH targets
  - High: 80% RH
  - Low: 20% RH
  - Wet dissemination causes increase in initial relative humidity

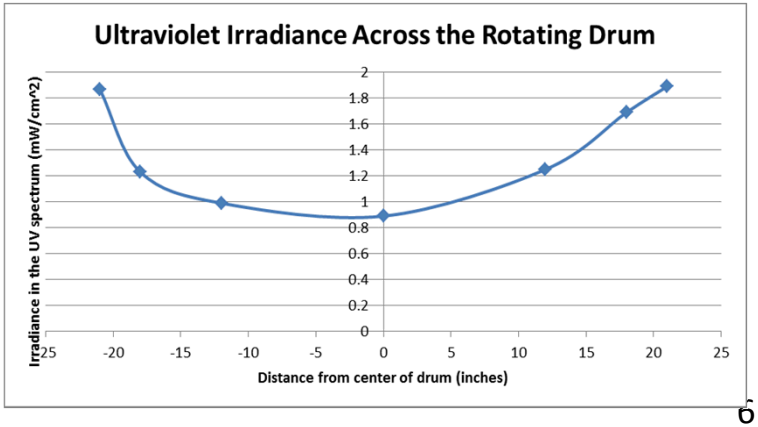
UV and Ozone Control

- UV produced by Mercury Halide lamps
- Ozone target: 100 ppb
  - Achieve 60-150 ppb sampling every 30 minutes

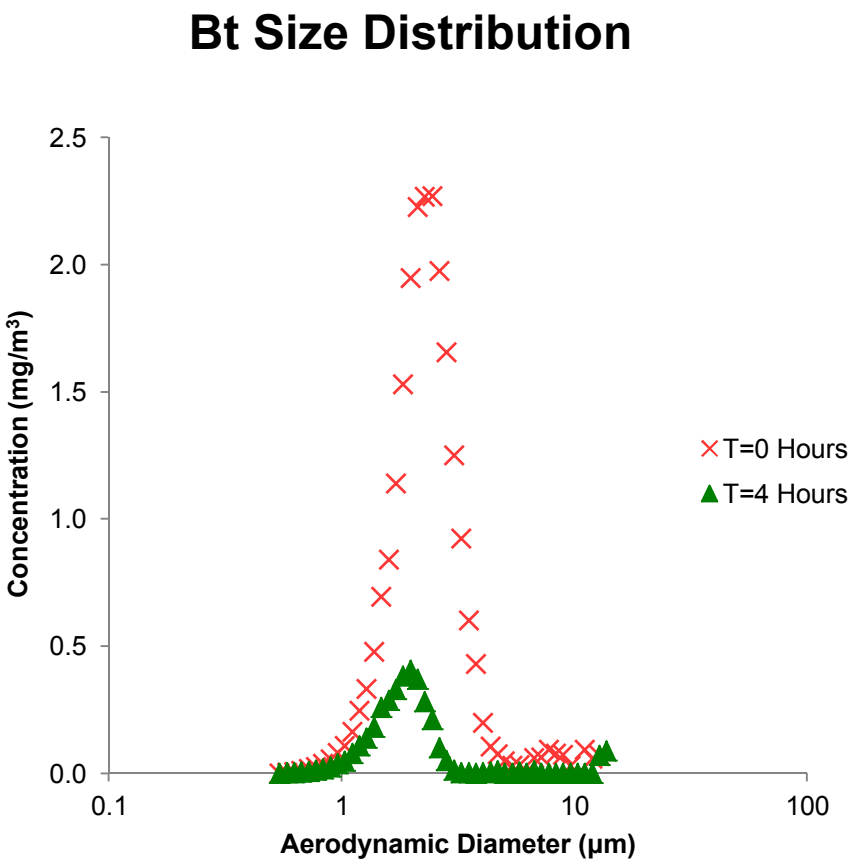






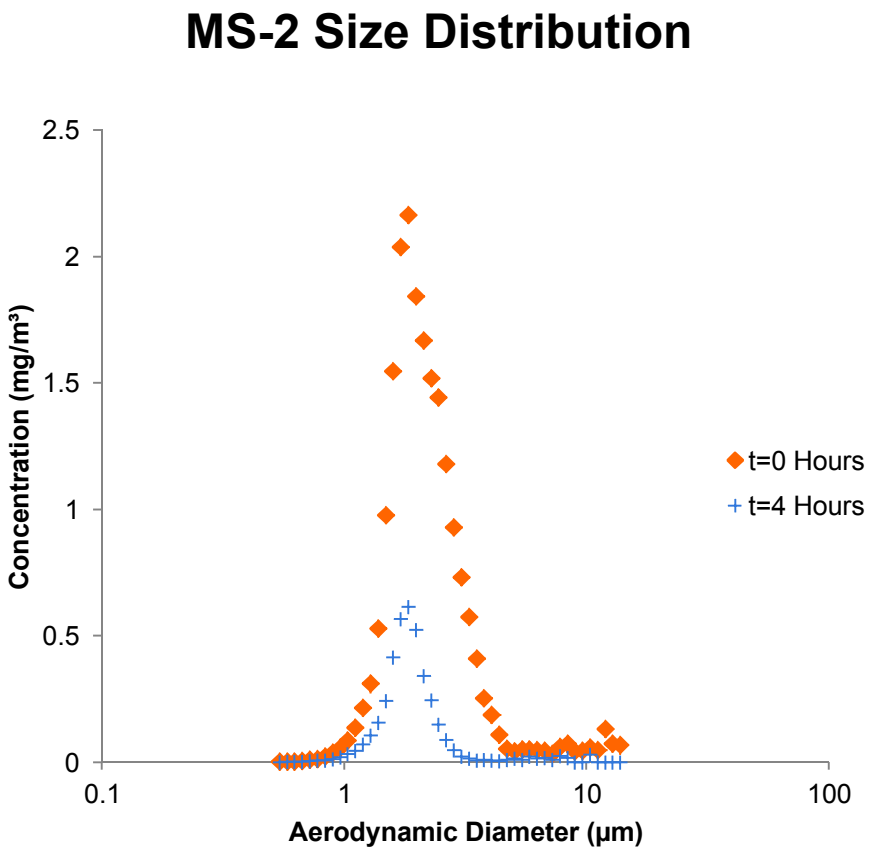


Bacillus thuringiensis al Hakam Aerosol



- $MMAD_0$= 2.32 µm ±0.0754
- $MMAD_4$= 1.88 µm ±0.0439
- Track mass concentration using APS to account for physical losses during test
- Grown in Difco Sporulation Medium (DSM) and washed three times in ethanol
- Resuspended in DI H_2O and aerosolized

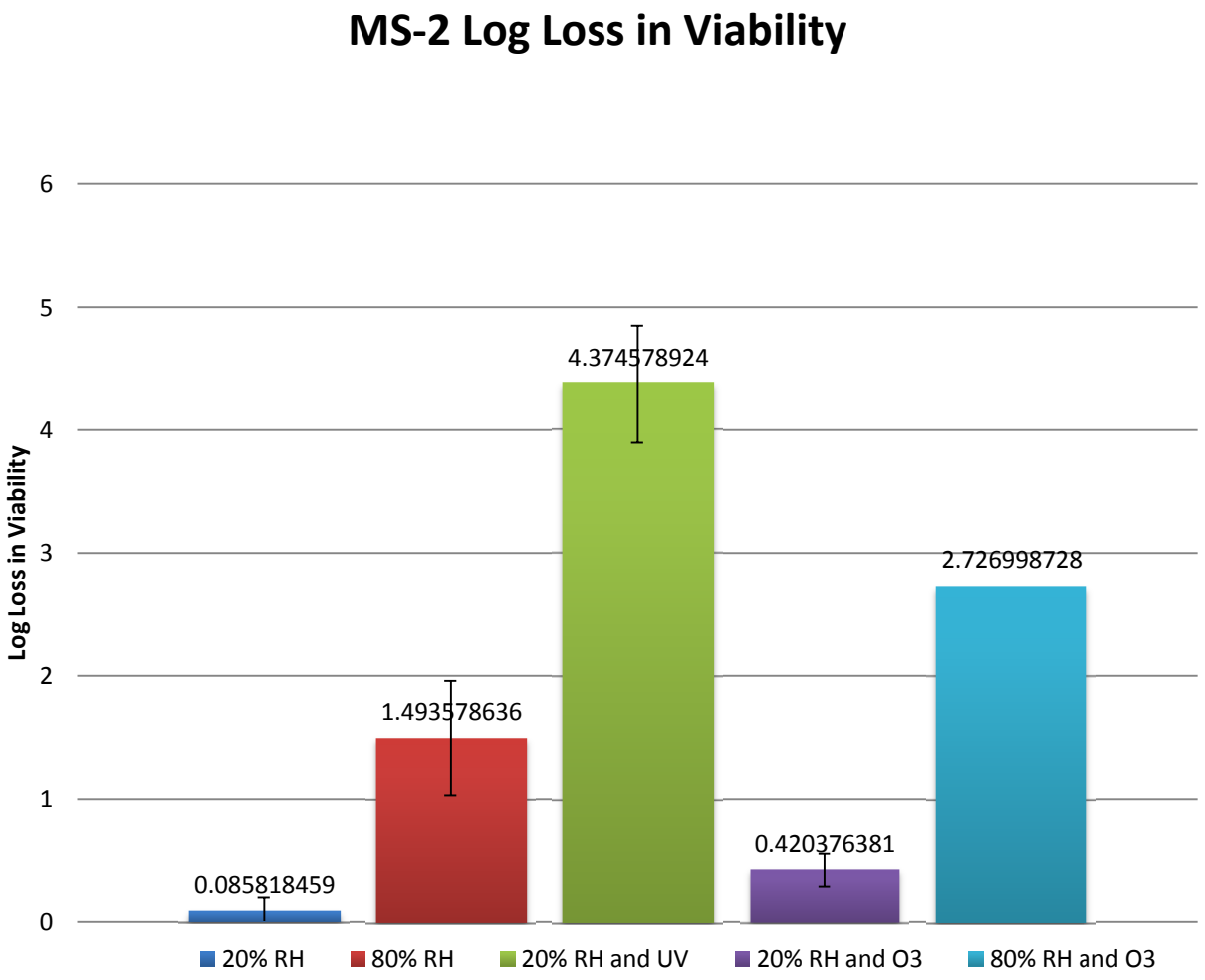
MS-2 Bacteriophage Aerosolization



- $MMAD_0$= 2.25 µm ±0.2171
- $MMAD_4$= 1.91 µm ±0.5226
- Track mass concentration using APS to account for physical losses during test
- Grown in *E. Coli* 15597 in Escherichia Medium 271 Growth Medium
- 0.45 µm-filtered lysate and aerosolized

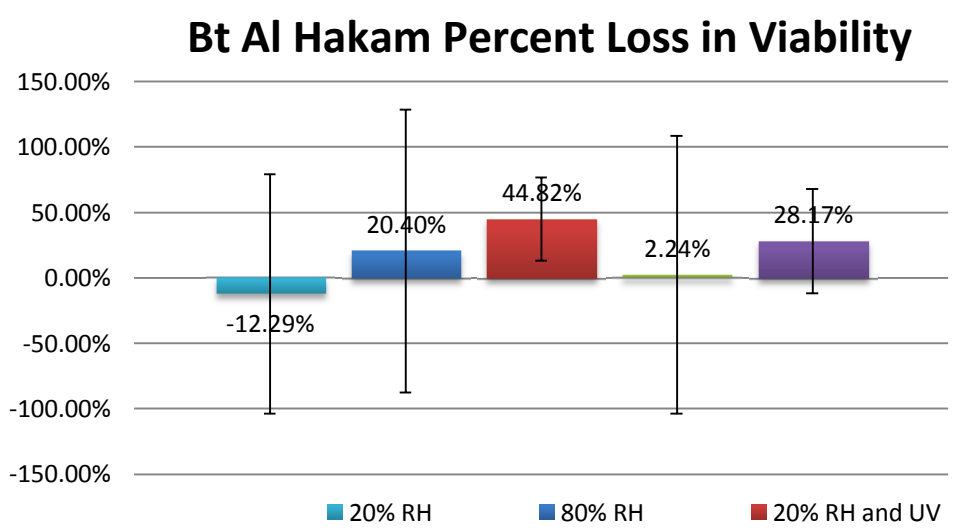
MS-2 Loss in Viability

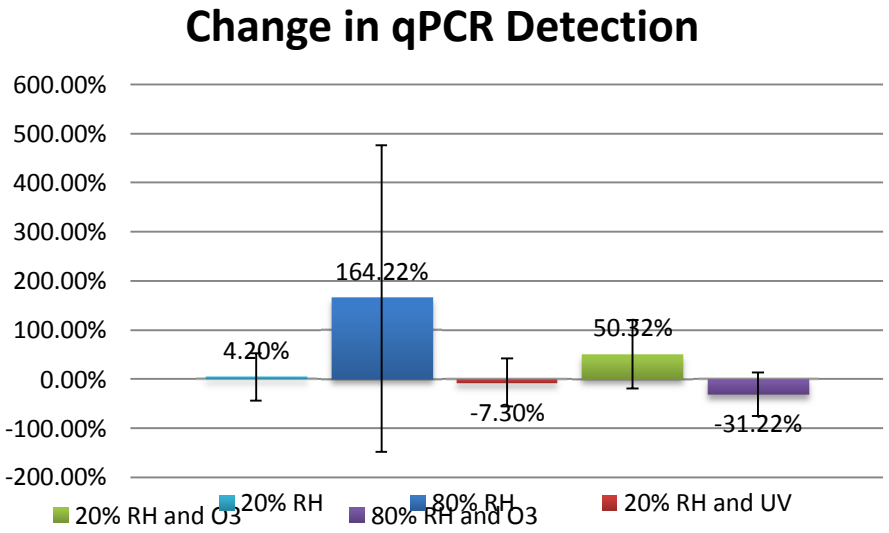
- Samples collected after four hours in AGI-30 impinger
- Normalized for mass concentration in drum
- Ozonolysis is RH dependent
- Largest decrease in presence of UV light



Aging in *Bt* Al Hakam

- Small changes (<1 log) in *Bacillus thuringiensis* viability under all conditions over 4 hours
  - Likely most sensitive to UV degradation
- Wide variations in PCR detectability
  - Cleaner preparation than previous studies
  - Need for additional replicates

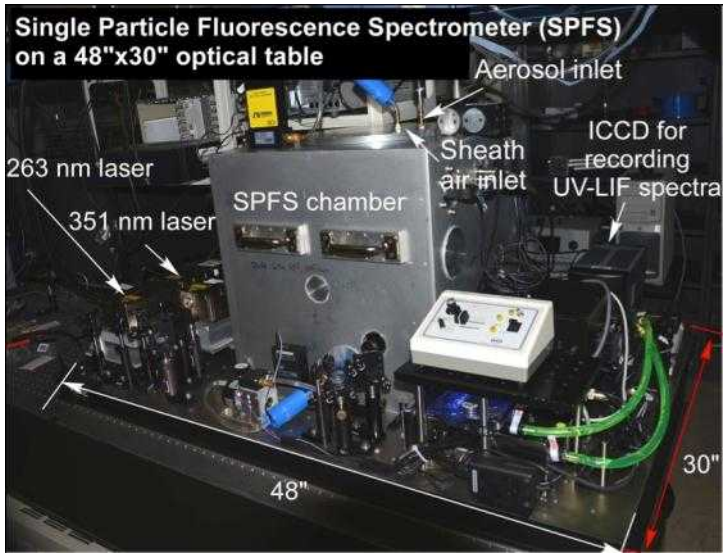


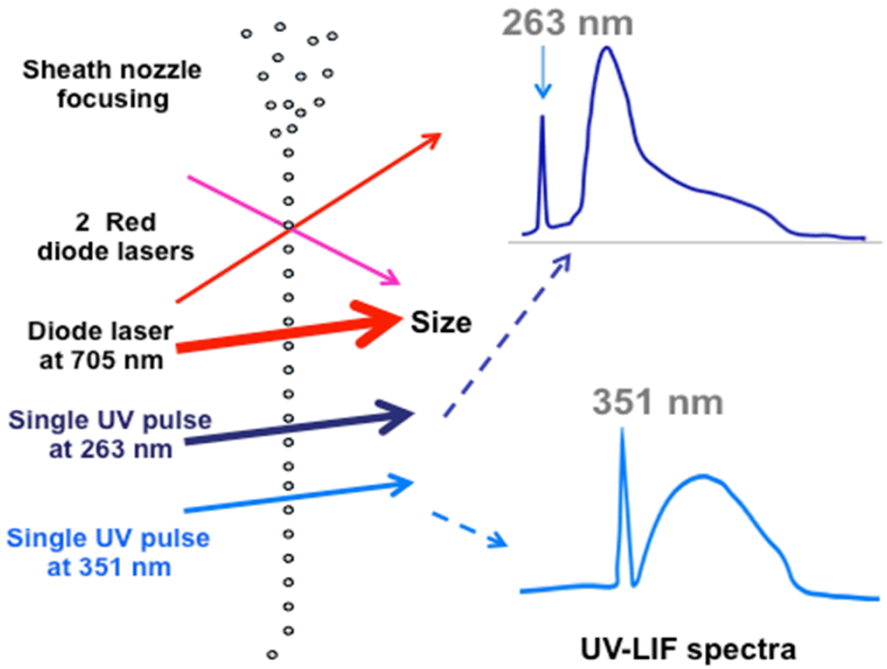


Single Particle Fluorescence Spectrometer (SPFS)

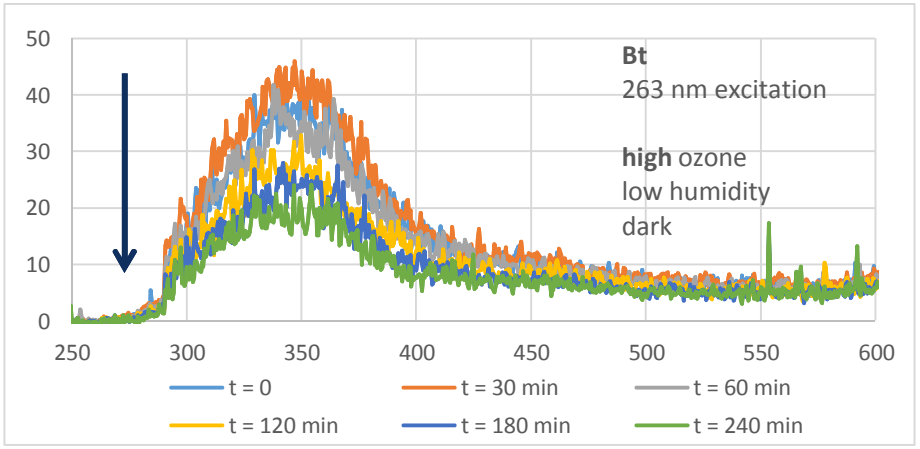
Sandia National Laboratories

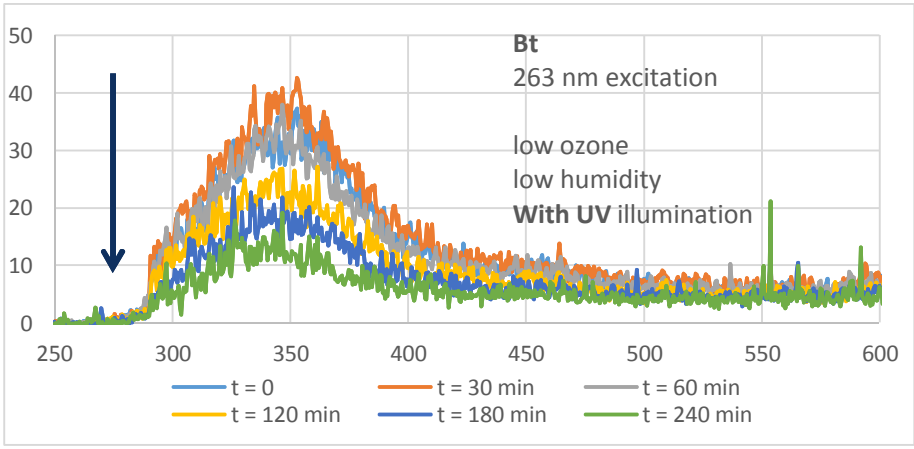
- Army Research Laboratory
  - Has been used for determination of fluorescence cross section of agents, simulants, and ambient aerosol characterization

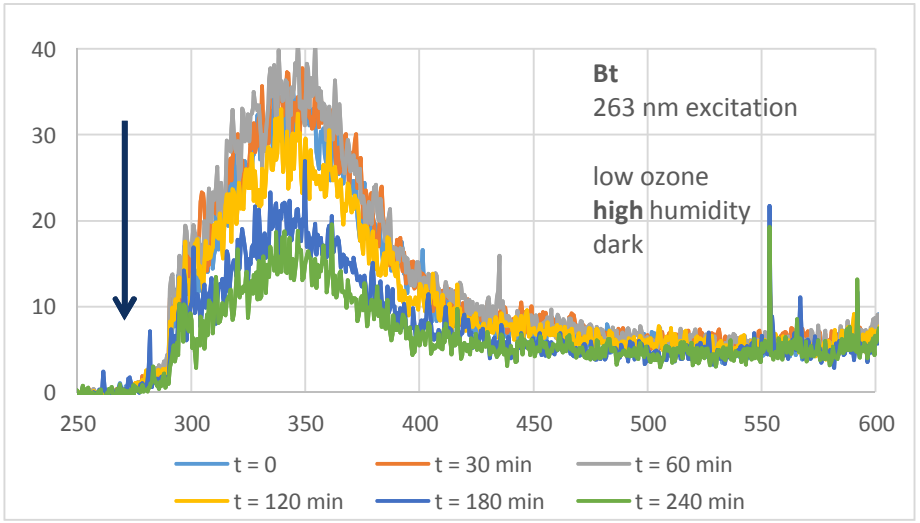




Bacillus thuringiensis al Hakam – 263 nm

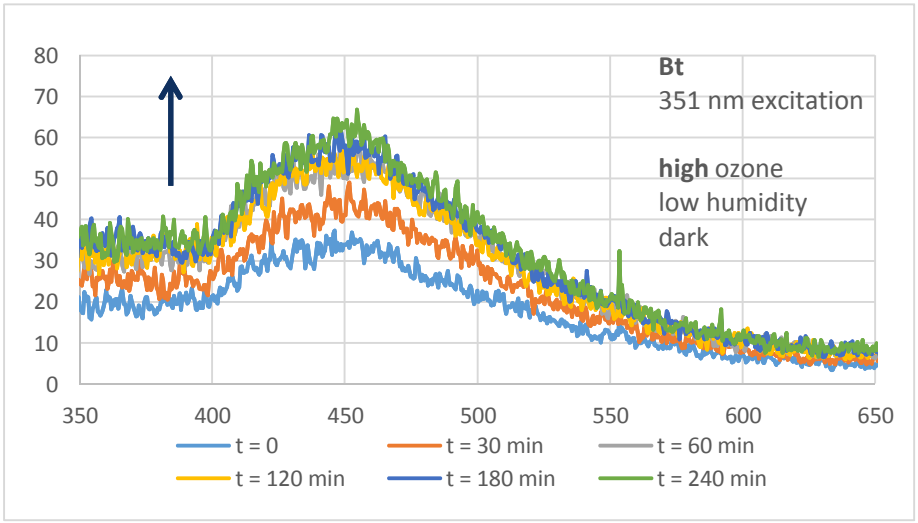


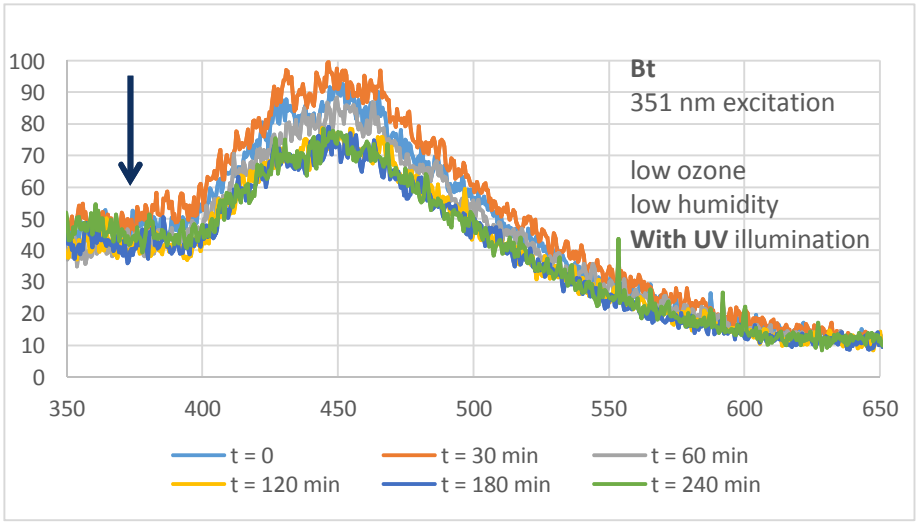


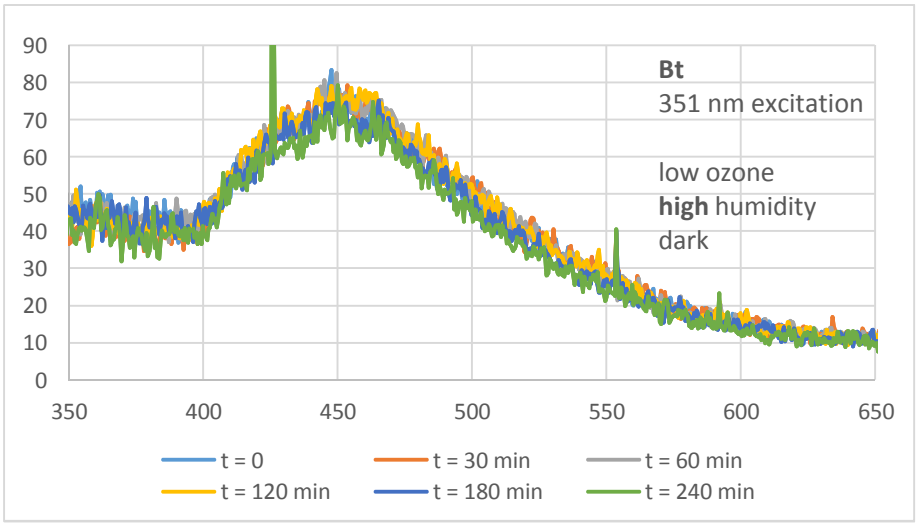


- **Decrease** of signal in presence of UV light, humidity, and ozone
- **More rapid decrease** of signal in presence of UV irradiation
- **Slower** degradation of signal from high humidity alone

Bacillus thuringiensis al Hakam – 351 nm

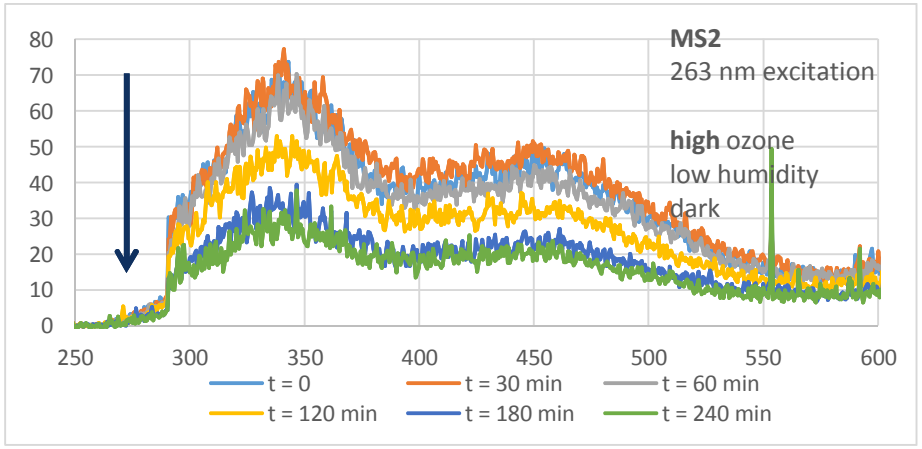


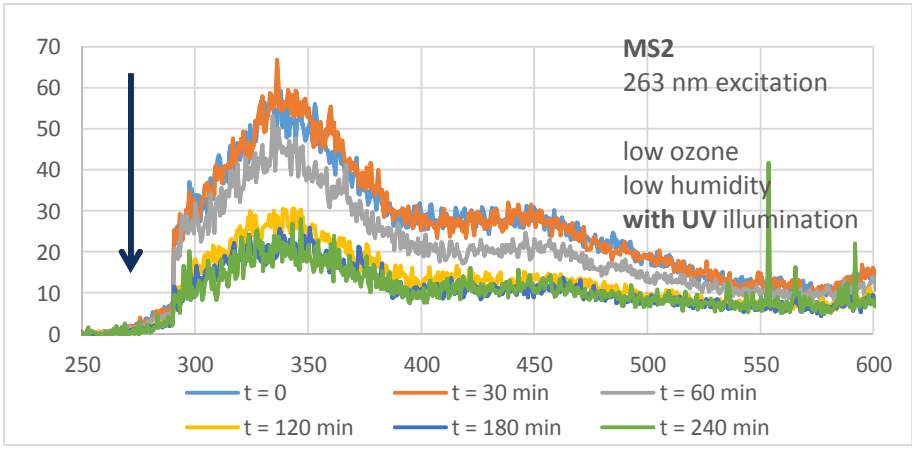


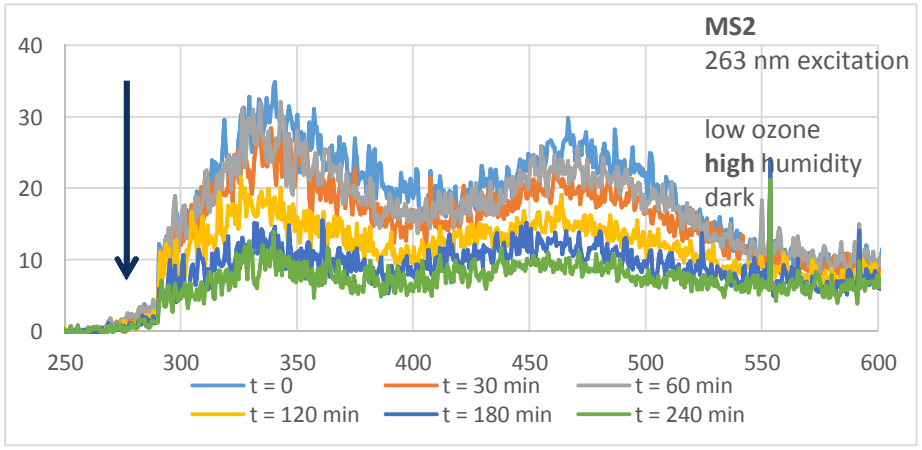


- **Decrease** of signal in presence of UV light
- **Increase** of signal in presence of ozone
- **No effect** of humidity
- Previously observed with 8-mer thrombospondin peptide

MS-2 Bacteriophage – 263 nm

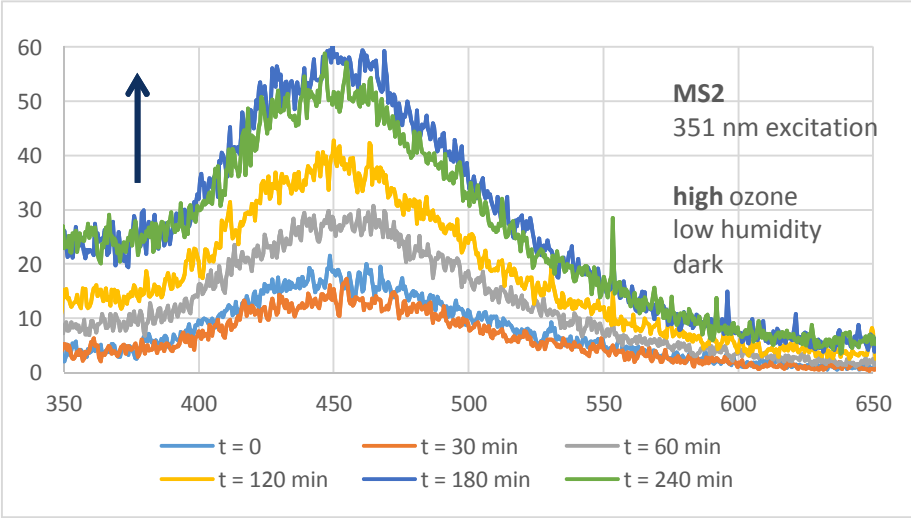


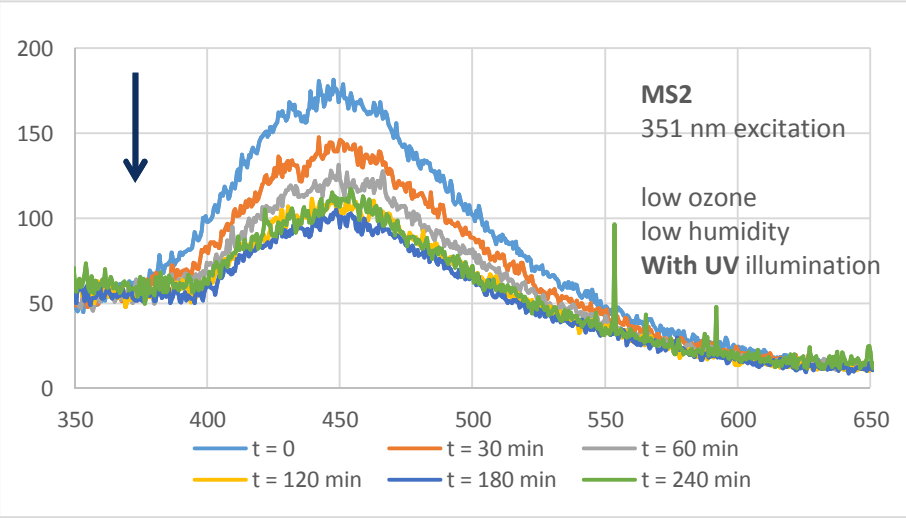


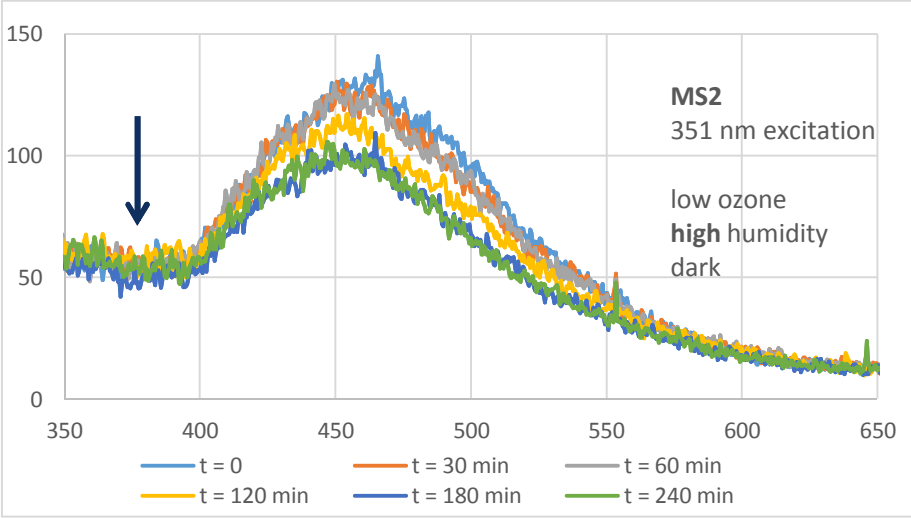


- **Decrease** of signal in presence of UV light, humidity, and ozone
- **More rapid decrease** of signal in presence of UV irradiation

MS-2 Bacteriophage – 351 nm







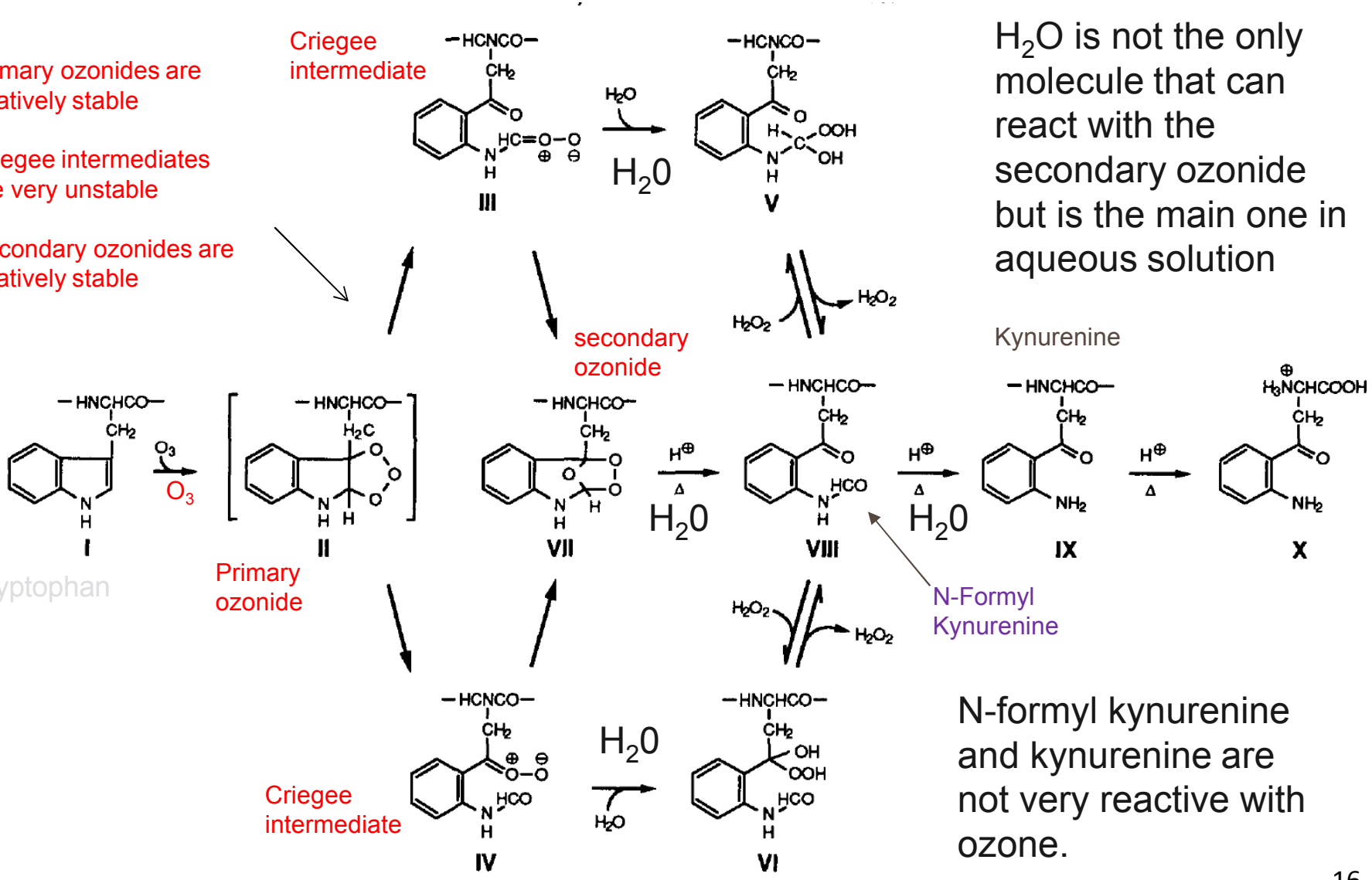
- **Decrease** of signal in presence of UV light and humidity
- **Increase** of signal in presence of ozone
- Previously observed with 8-mer thrombospondin peptide

Potential Chemical Pathway

Primary ozonides are relatively stable

Criegee intermediates are very unstable

Secondary ozonides are relatively stable



H_2O is not the only molecule that can react with the secondary ozonide but is the main one in aqueous solution

N-formyl kynurenine and kynurenine are not very reactive with ozone.

Conclusions

- UV irradiation driver in aging of both MS-2 bacterio-phage and *Bacillus thuringiensis* al Hakam particles
- Further evidence of kynurenine pathway in tryptophan ozonolysis
  - Increase in 365 nm wavelength in presence of ozone
- Preparation methodology affects aging
  - Amount of DNA in sample
  - Initial fluorescence signal
- Further replicates will allow determination of degradation rates

Thank You

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