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Eliminating Drug-Resistant bacteria and Fungal Infections via Photo-Inactivation of Intrinsic Chromophores

Dr. Ji-Xin Cheng, Boston University

28th October 2022

Technical Group Executive Committee

Stephen T. C. Wong

TG Chair

Houston Methodist Neal Cancer Center and Weill Cornell Medical College

About Our Technical Group

Our technical group focuses on:

- the use of lasers in surgery or in other treatments of disease,
- optical spectroscopy and imaging as real-time diagnostic or study tools for therapeutic applications, and
- basic science studies of the mechanisms by which light affects tissue in adverse or therapeutic ways.

Our mission is to connect the 900+ members of our community through technical events, webinars, networking events, and social media.

Our past activities have included:

- Six previous webinars available for on-demand viewing at Therapeutic Laser Applications - Bio-Medical Optics (BMO) - The Optical Society (OSA) | Optica

Connect With Our Technical Group

Join our online community to stay up to date on our group's activities. You also can share your ideas for technical group events or let us know if you're interested in presenting your research.

Ways to connect with us:

- Our website at www.optica.org/BA
- On LinkedIn at www.linkedin.com/groups/8302285/
- On Facebook at www.facebook.com/groups/opticatherapeuticlaserapplications
- Email us at STWong@houstonmethodist.org or TGactivities@optica.org

Today's Speaker

Dr. Ji-Xin-Cheng

Boston University

Ji-Xin Cheng attended the University of Science and Technology of China (USTC) from 1989 to 1994. From 1994 to 1998, he carried out his PhD study on bond-selective chemistry at USTC. As a graduate student, he worked as a research assistant at Universite Paris-sud (France) on vibrational spectroscopy and the Hong Kong University of Science and Technology (HKUST) on quantum dynamics theory.

After postdoctoral training on ultrafast spectroscopy at HKUST, he joined Sunney Xie's group at Harvard University as a postdoc, where he spearheaded the development of CARS microscopy that allows high-speed vibrational imaging.

Cheng joined Purdue University in 2003 as Assistant Professor in Weldon School of Biomedical Engineering and Department of Chemistry, promoted to Associate Professor in 2009 and Full Professor in 2013. He joined Boston University as the Inaugural Theodore Moustakas Chair Professor in Photonics and Optoelectronics in summer 2017.

Eliminating Superbugs by Photo-bleaching of Intrinsic Chromophores

Ji-Xin Cheng

Moustakas Professor in Photonics and Optoelectronics

ECE, BME, Physics, Chemistry, MCBB, MSE

Boston University

jxcheng@bu.edu

FCOI: Vibronix Inc, Photothermal Spec Corp, Pulsethera

BOSTON UNIVERSITY

The Boston University photonics center

https://www.disassociated.com/2013/07/17/these-bacterial-artworks-just-might-go-viral/

Review

Cell PRESS

Color me bad: microbial pigments as virulence factors

George Y. Liu[1] and Victor Nizet[2]

[1] Division of Pediatric Infectious Diseases and Immunobiology Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA

[2] Department of Pediatrics and Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA, USA

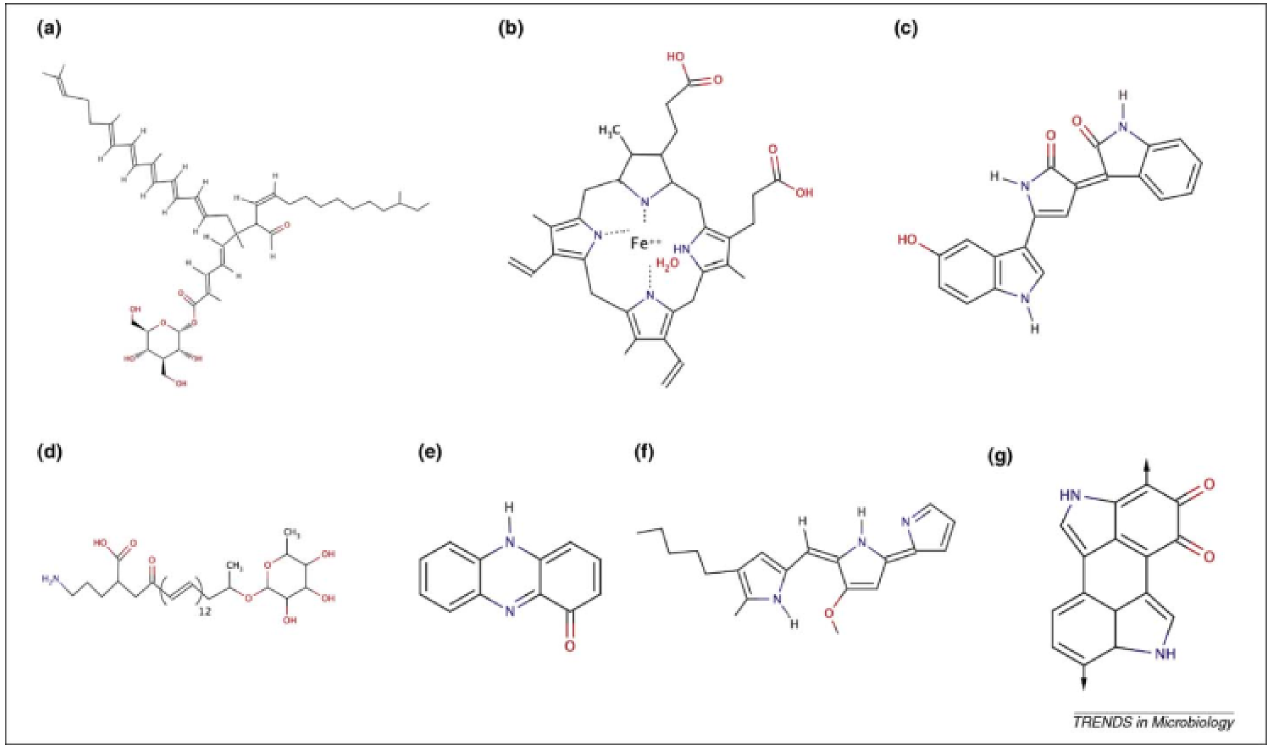
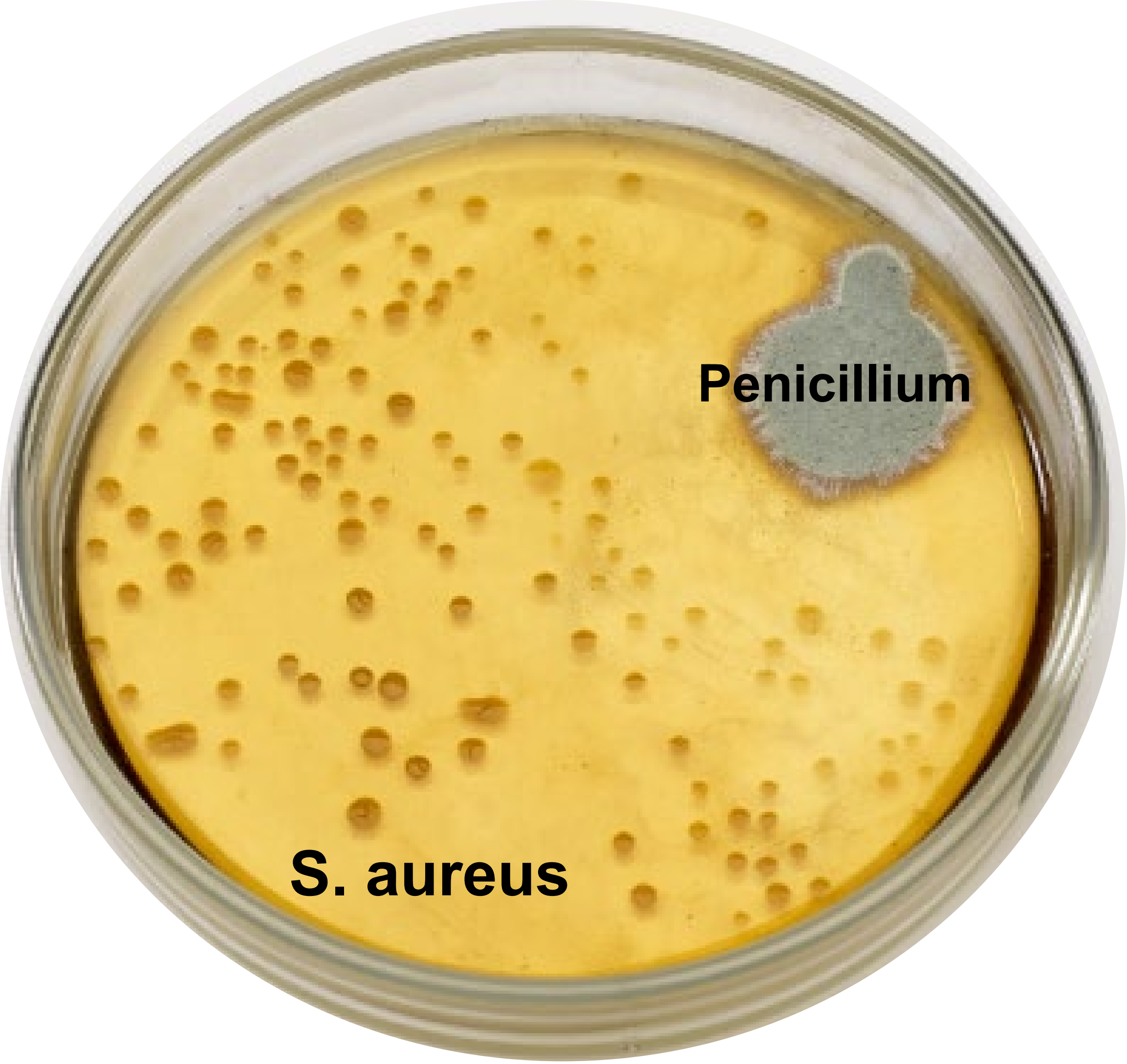


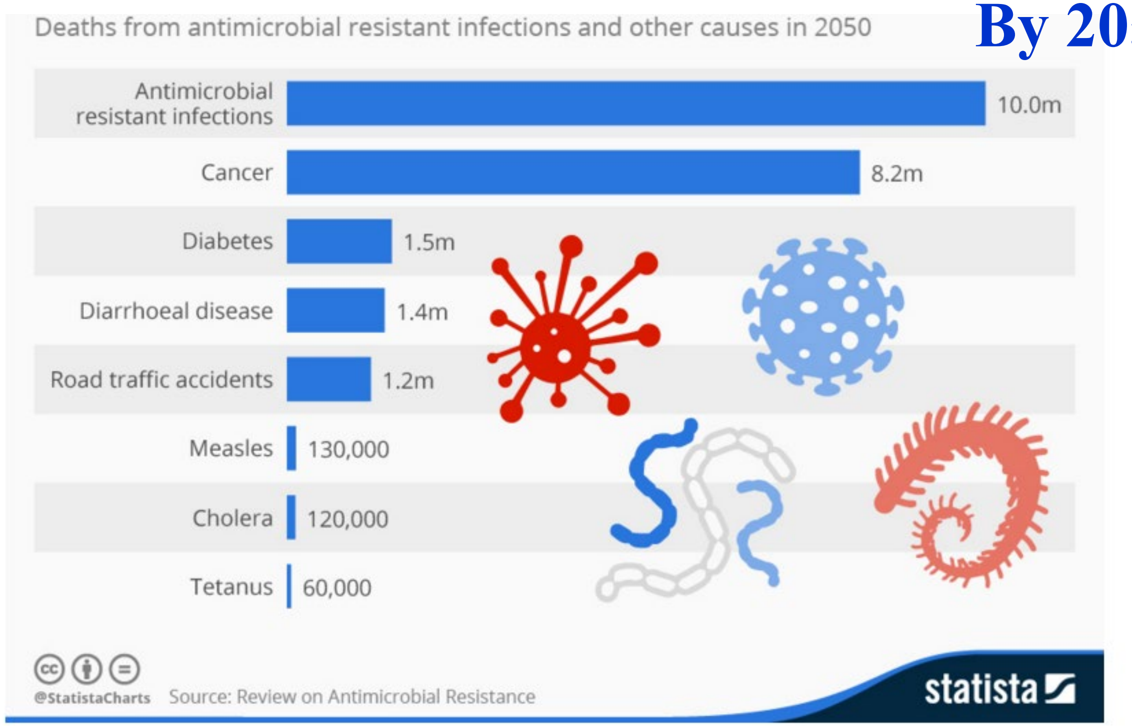
Figure 1. Diverse chemical structures of pigments expressed by microbial pathogens. **(a)** Staphyloxanthin, *Staphylococcus aureus*; **(b)** hematin in malarial hemazoin or the *Porphyromonas gingivalis* pigment; **(c)** violacein, *Chromobacterium violaceum*; **(d)** granadaene, Group B *Streptococcus*; **(e)** pyocyanin, *Pseudomonas aeruginosa*; **(f)** prodigiosin, *Serratia marcescens*; **(g)** melanin, *Cryptococcus neoformans*.

Chromophore played an essential role in discovery of the first antibiotics in human history



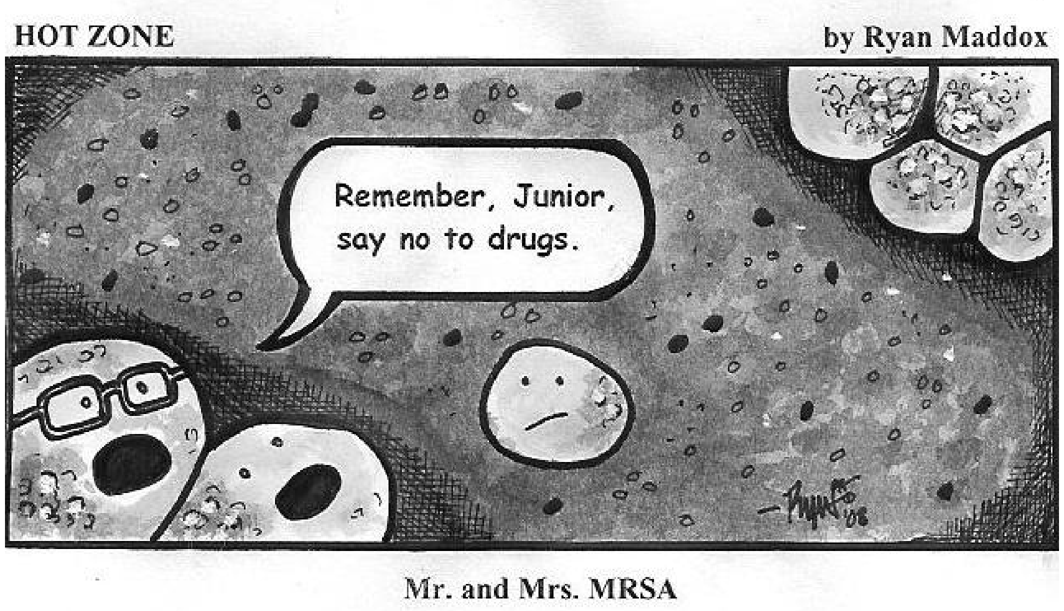
http://broughttolife.sciencemuseum.org.uk/broughttolife/objects/display?id=11193

By 2050



PRIORITY: CRITICAL	PRIORITY 2: HIGH	PRIORITY 3: MEDIUM
Acinetobacter baumannii carbapenem-resistant	**Enterococcus faecium** vancomycin-resistant	**Streptococcus pneumoniae** penicillin-non-susceptible
Pseudomonas aeruginosa carbapenem-resistant	**Staphylococcus aureus** methicillin-resistant vancomycin-intermediate and resistant	**Haemophilus influenzae** ampicillin-resistant
Enterobacteriaceae carbapenem-resistant, ESBL-producing	**Helicobacter pylori** clarithromycin-resistant	**Shigella spp.** fluoroquinolone-resistant
	Campylobacter spp. fluoroquinolone-resistant	
	Salmonellae fluoroquinolone-resistant	
	Neisseria gonorrhoeae cephalosporin-resistant fluoroquinolone-resistant	

Source: WHO



Discovery of novel antibiotics* is not keeping pace with the emergence of new superbugs

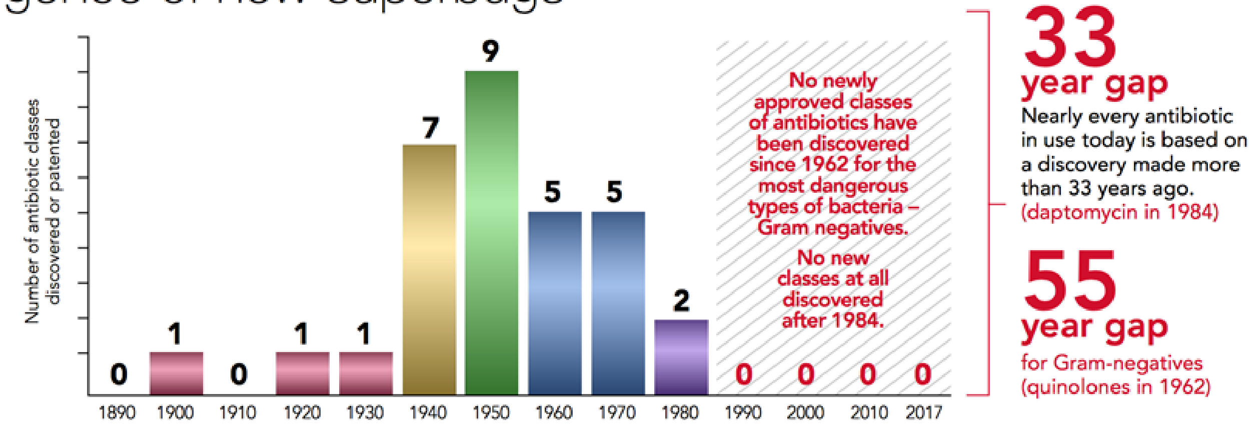
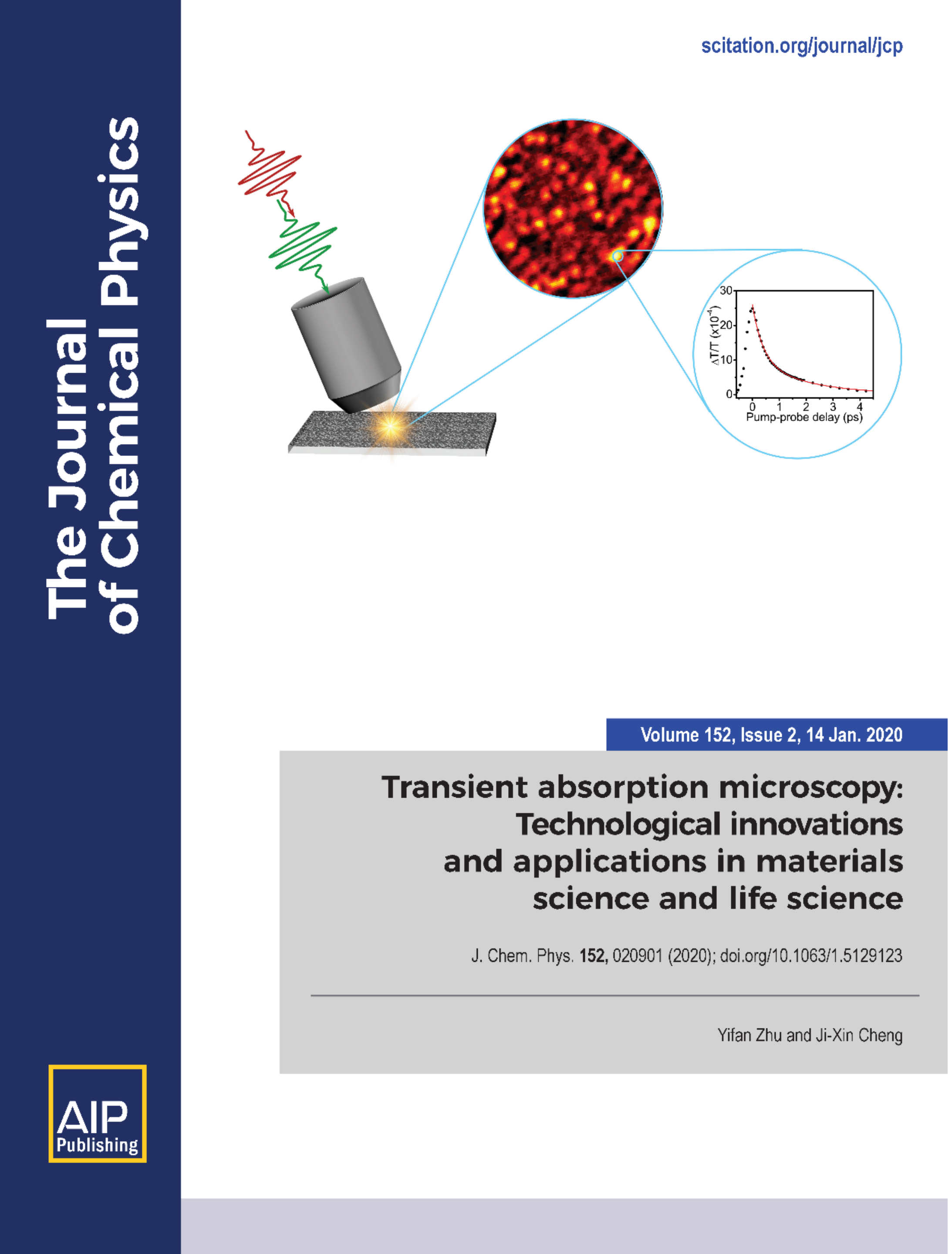
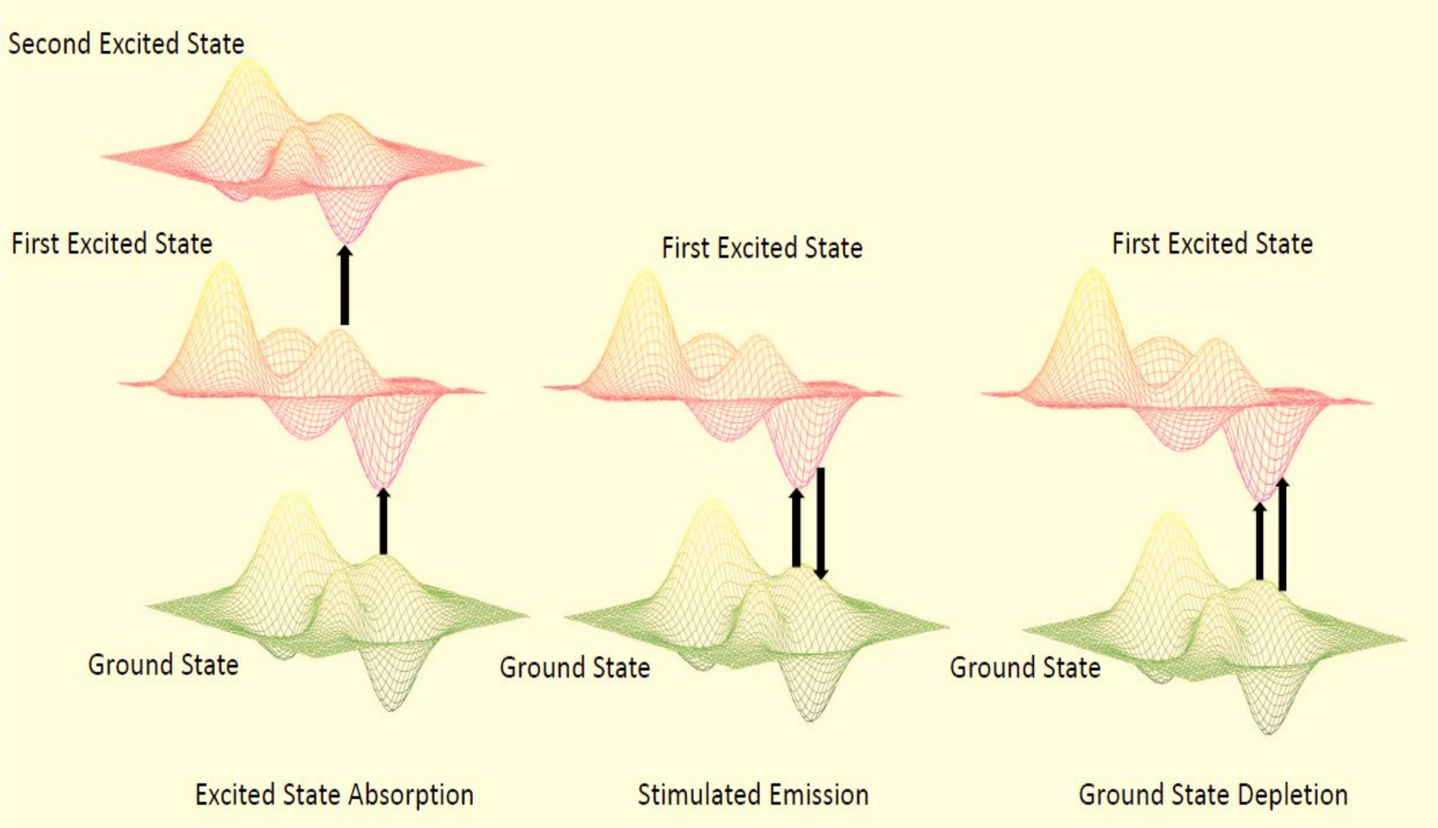


Figure adapted from CARBX annual report (2017).

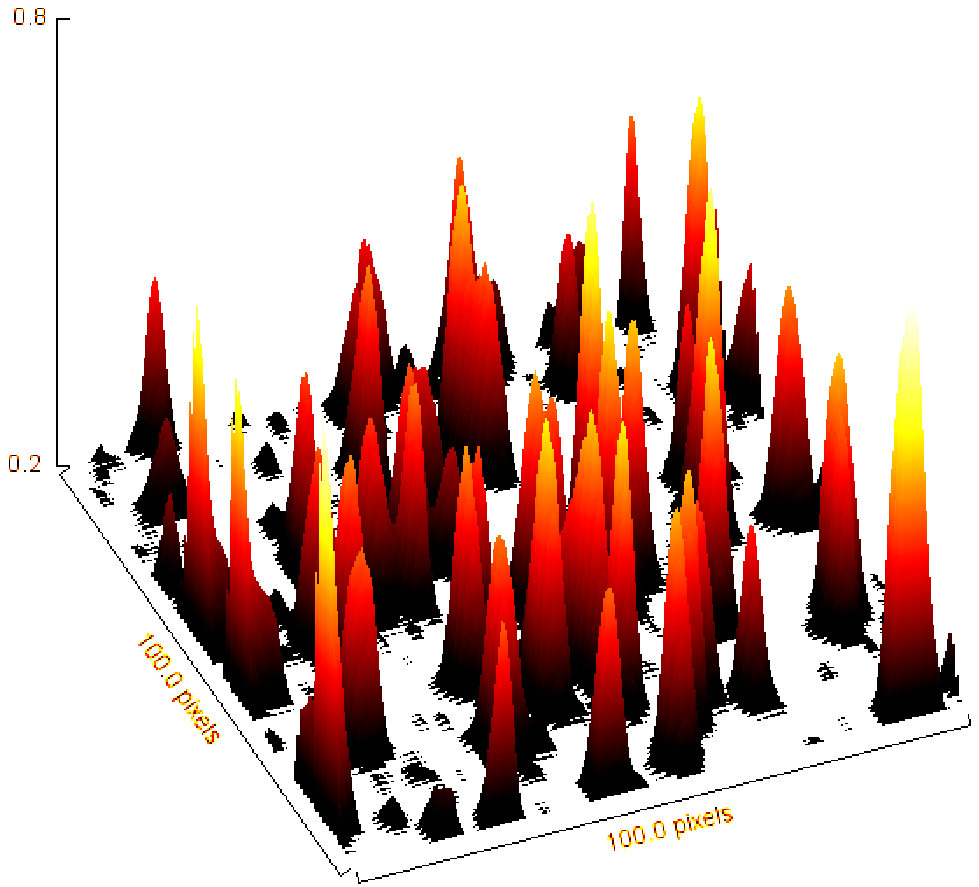


Transient Absorption Microscopy

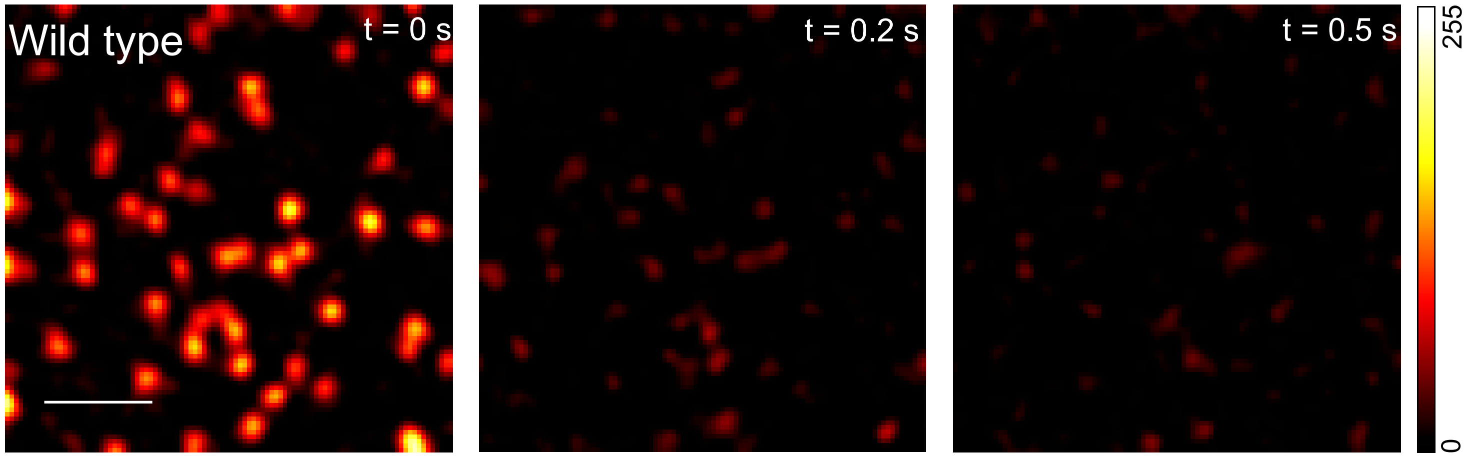
Imaging chromophores that do not fluorescence



Unexpected, Fast Photobleaching of MRSA under a Transient Absorption Microscope (2017)



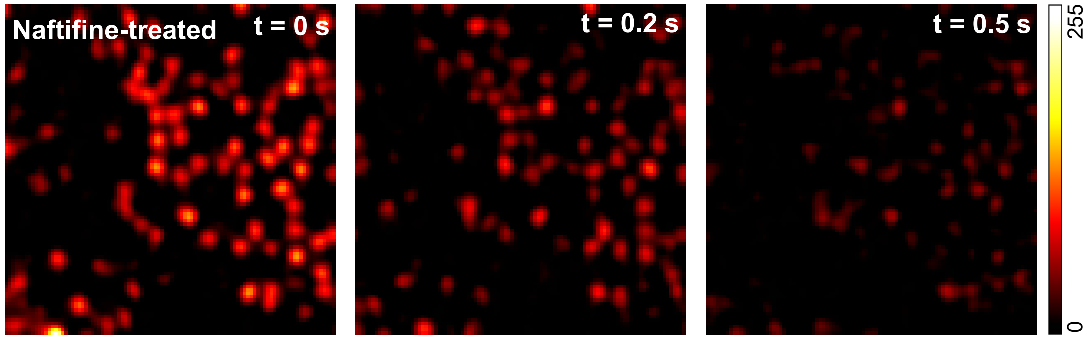
Sample: Methicillin-resistant S. Aureus (MRSA)

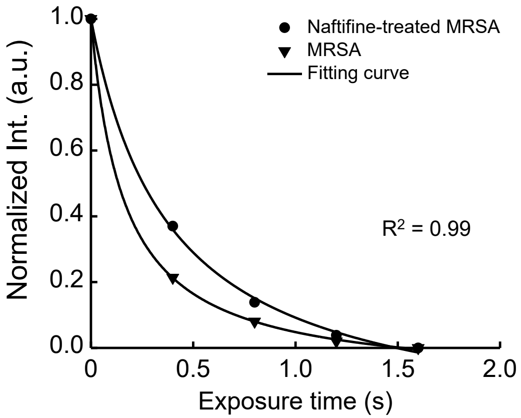


US patent issued in 2021

The golden pigment, staphyloxanthin (STX), is responsible for the photobleaching

- **Naftifine: FDA-approved antifungal drug to block the synthesis of STX[1].**

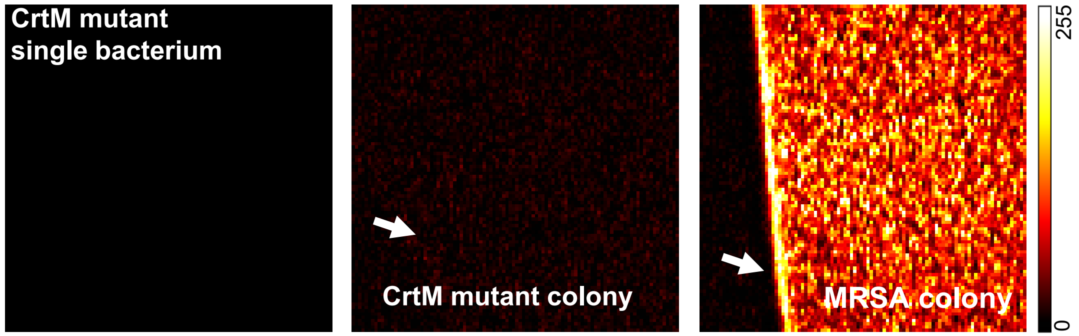




MRSA $\tau_2 = 0.16 \pm 0.07$ s

Naftifine-treated: $\tau_2 = 0.39 \pm 0.07$ s

- **CrtM mutant: *S. aureus* with a mutation on dehydrosqualene synthase which is responsible for STX biosynthesis[2].**



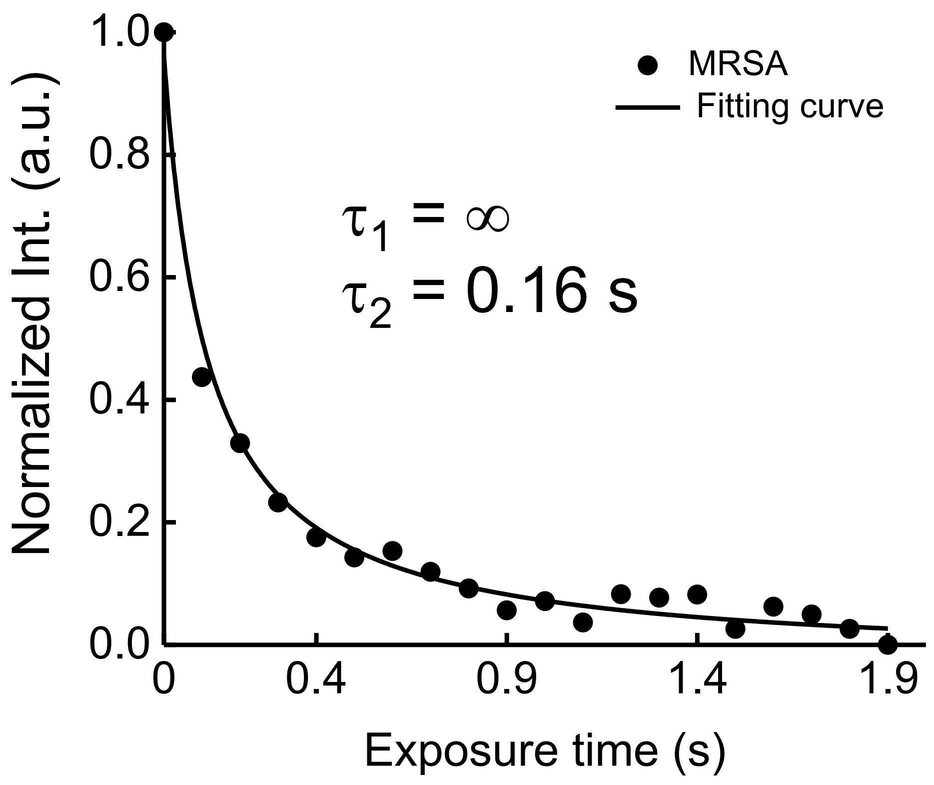
[1]. *Nature chemical biology* **12**, 174 (2016);
[2]. *Science* **319**, 1391 (2008).

Second-order Bleaching

The photobleaching model

$$y = y_0 + A * \frac{exp\left(-\frac{t}{\tau_1}\right)}{1 + \frac{\tau_1}{\tau_2} * \left(1 - exp\left(-\frac{t}{\tau_1}\right)\right)}$$

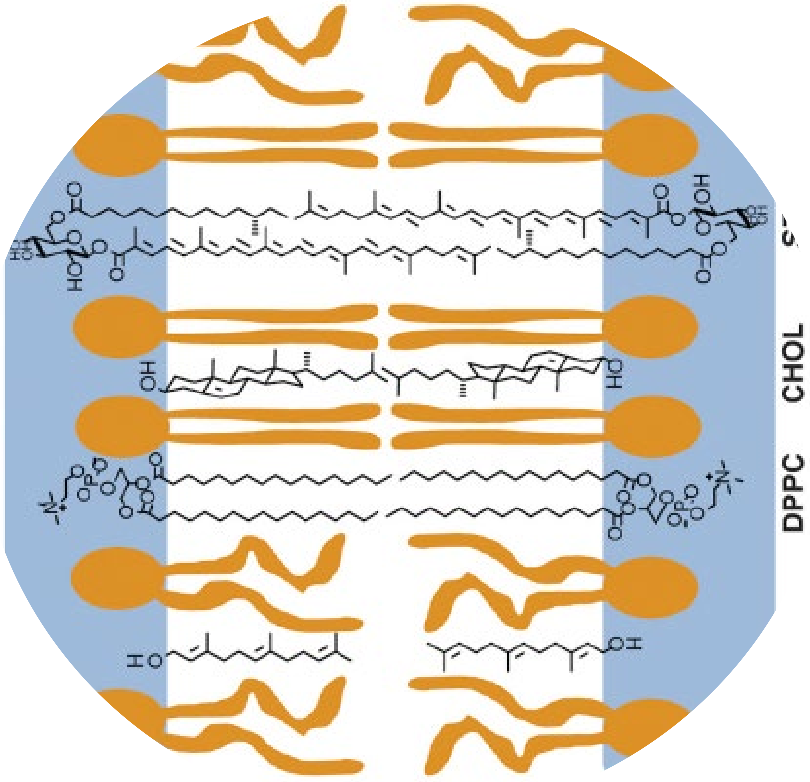
t duration of light irradiation,
y signal intensity,
y_0 and A are constants,
τ_1 constant for first-order bleaching
τ_2 constant for 2nd−order bleaching

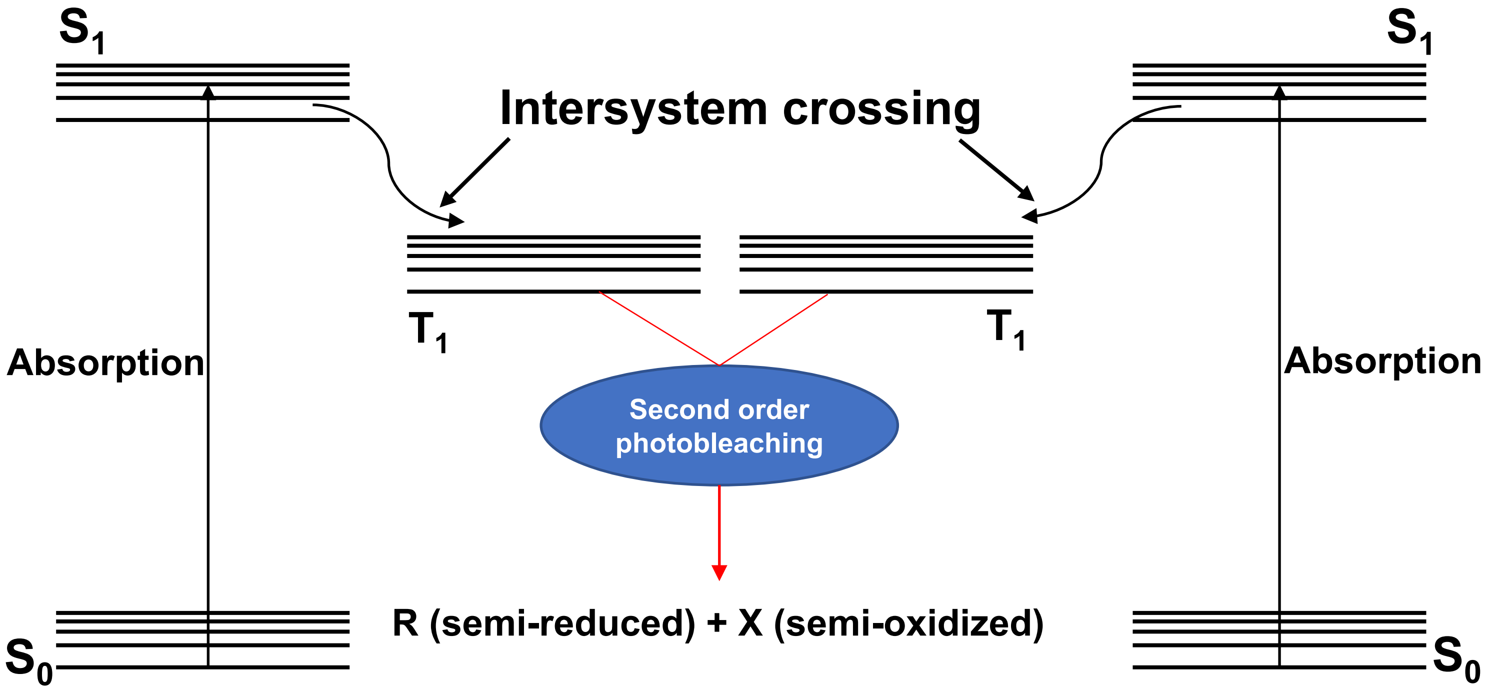


R^2=0.99

Second-order Photobleaching

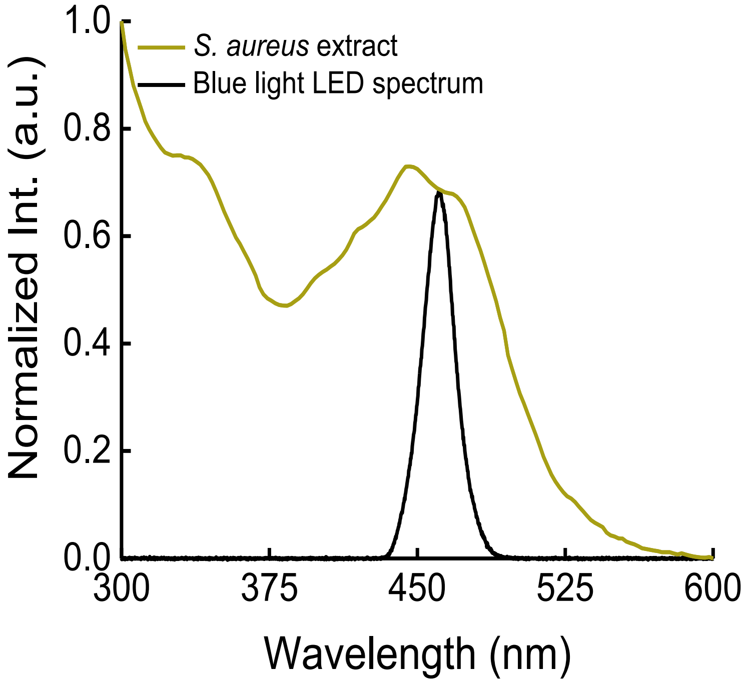
Chemistry & Biology *21*, 1557-1563, 2014

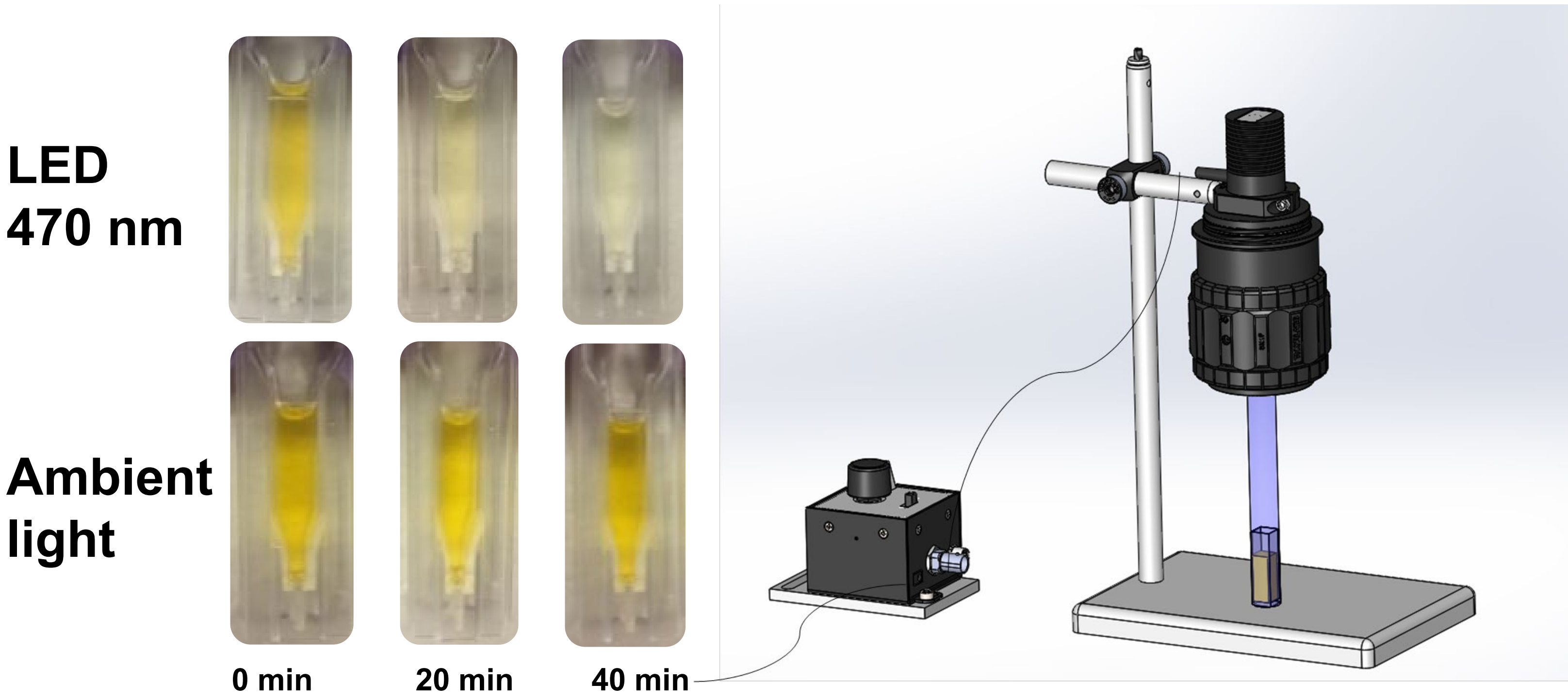




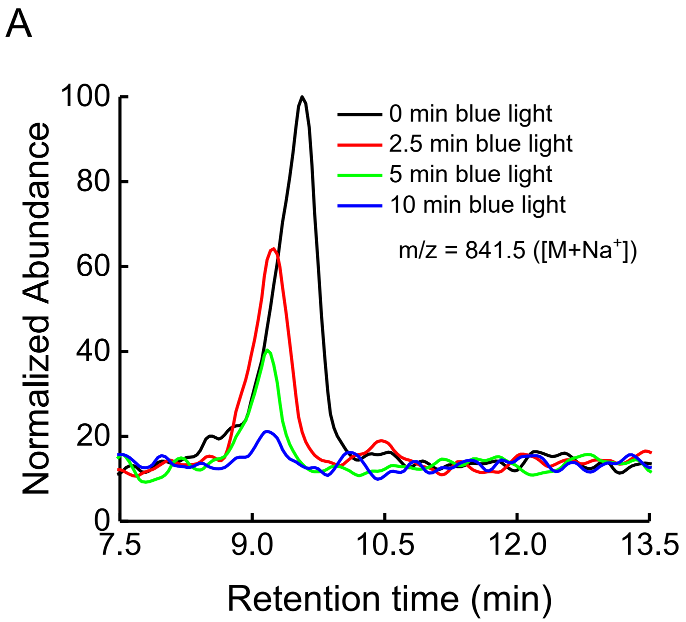
- Staphyloxanthin (STX) resides in membrane via dimer structure;
- Photobleaching of STX is more efficient with pulses.

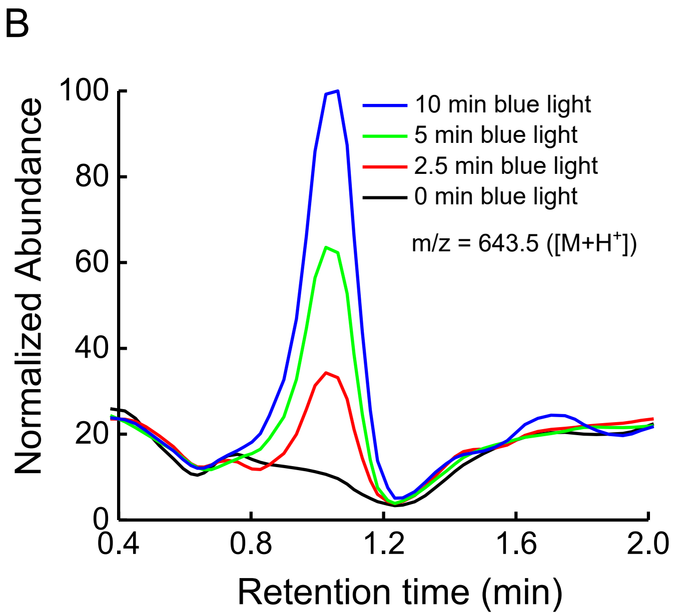
Optimal wavelength to bleach STX is around 470 nm

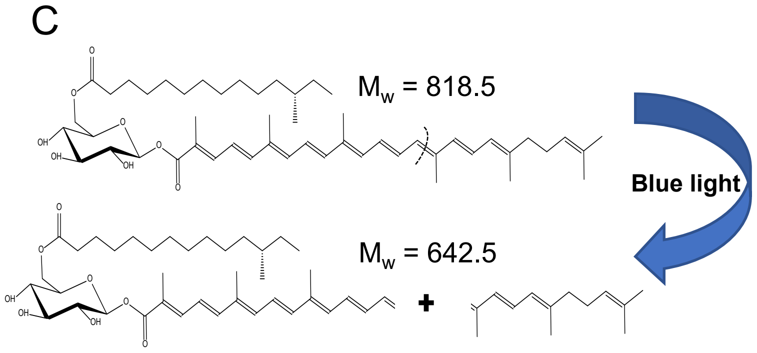




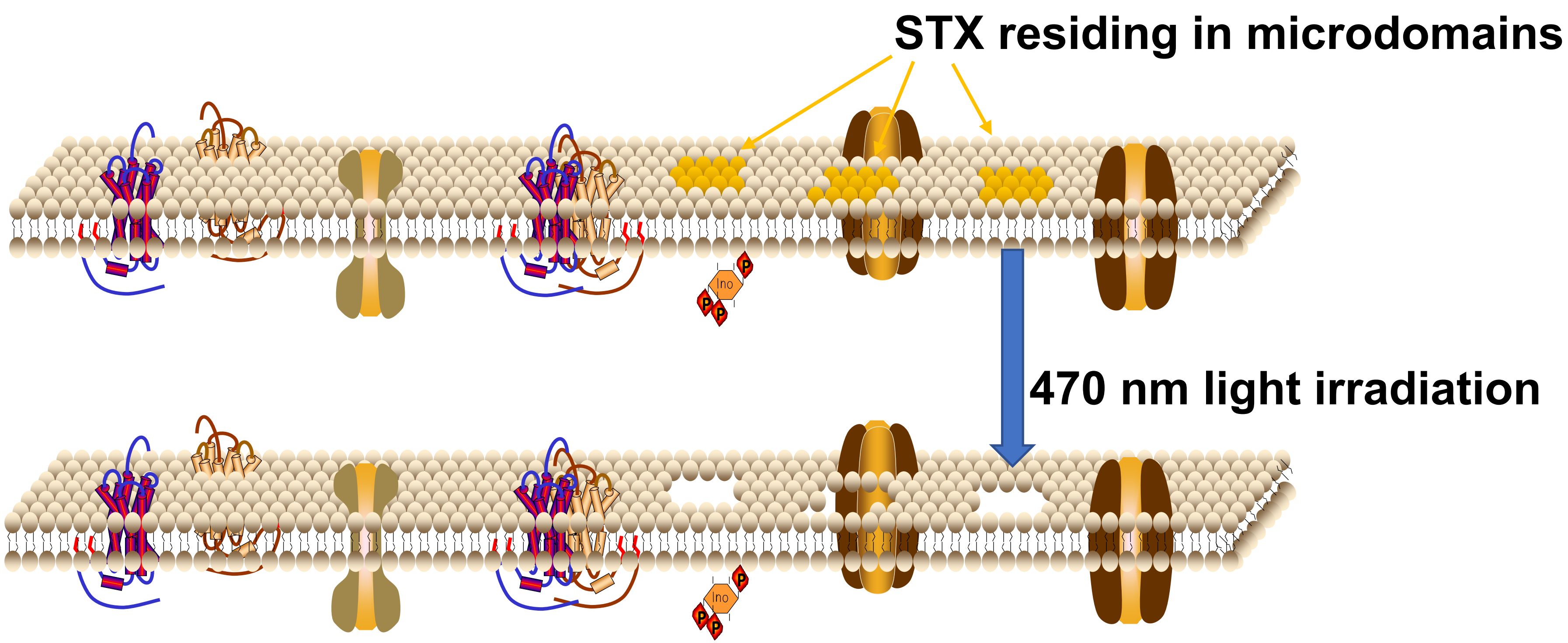
Mass Spectrometry Unveils Photolysis of STX





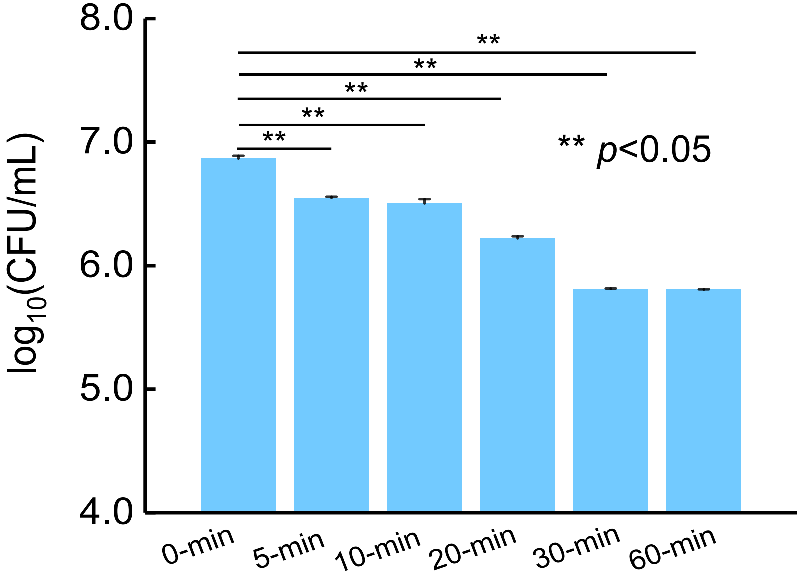


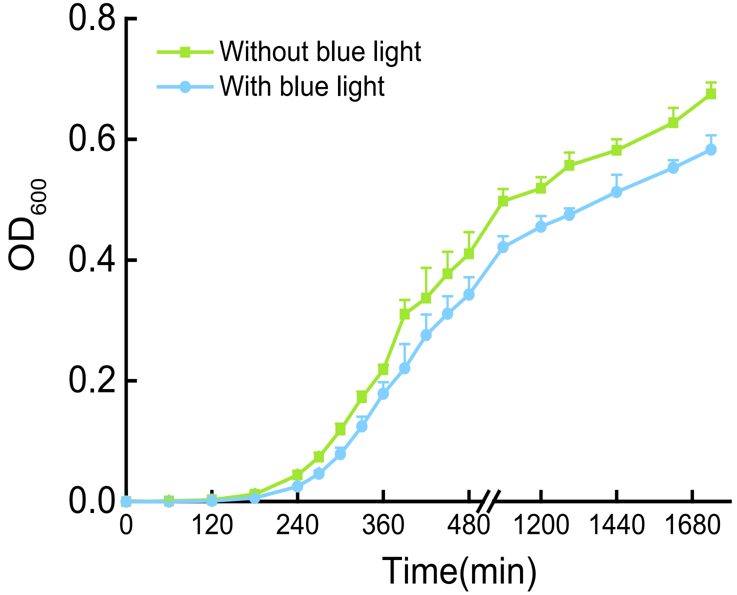
470-nm light degrades STX and disturbs the membrane



This finding opens a panel of new opportunities …

470-nm light eradicates MRSA incompletely





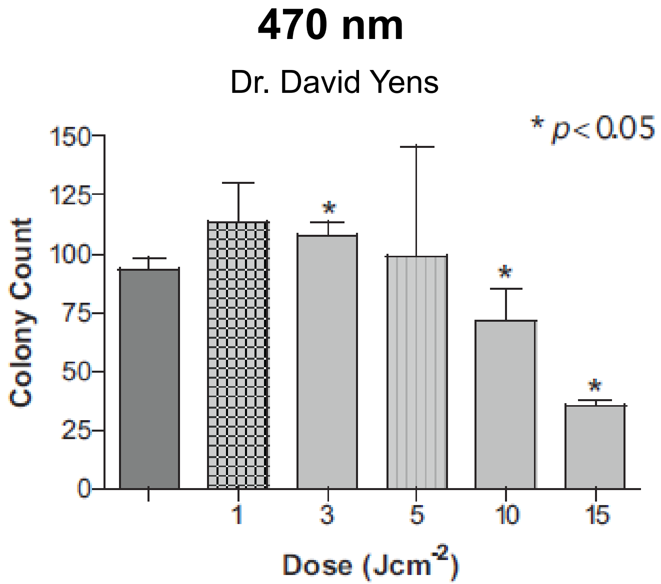


FIG. 5. Bactericidal effects of 470-nm light: *Staphylococcus aureus.*

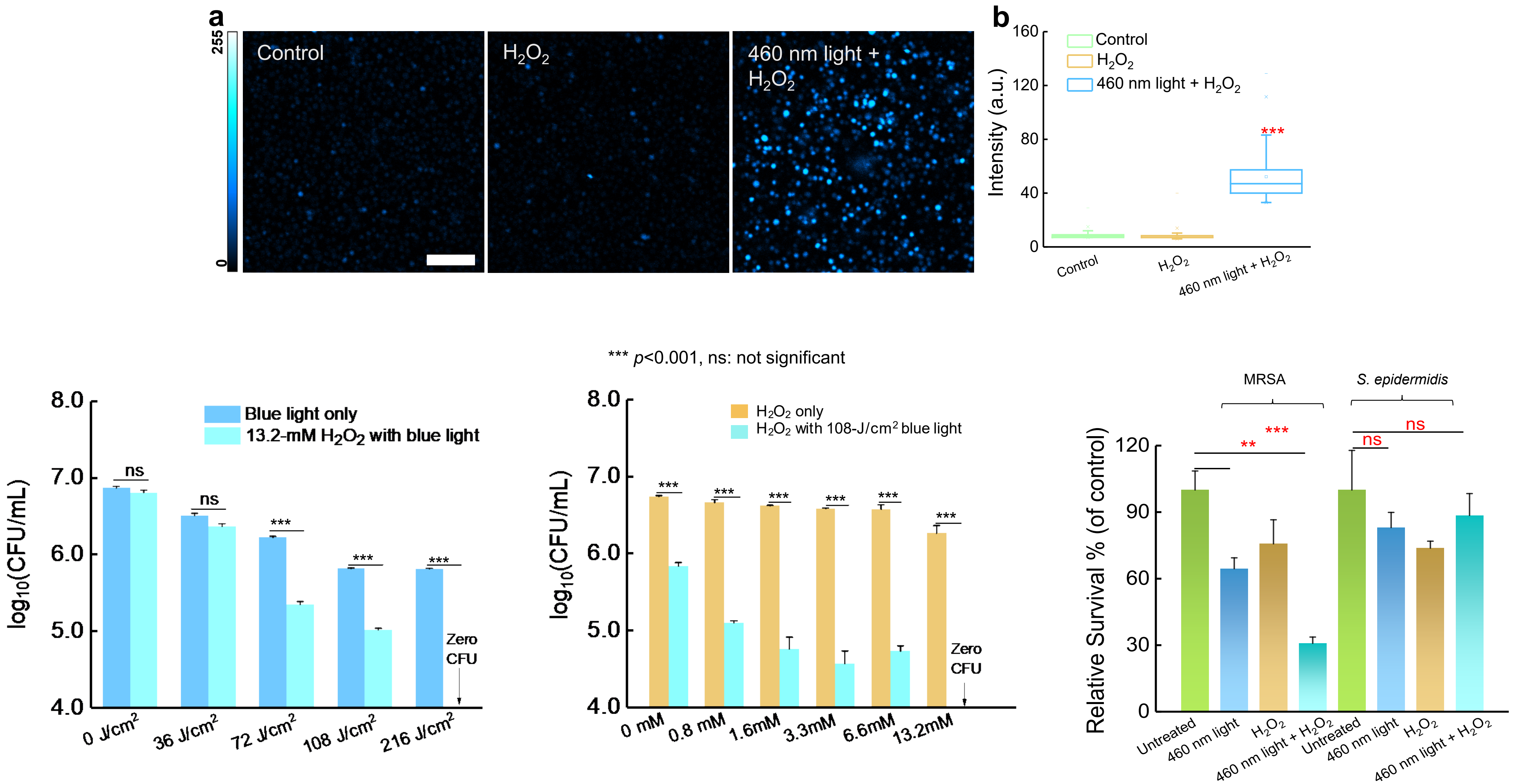
Blue light is unable to eradicate MRSA completely!

MRSA recovers in 30 min after being exposed to 470-nm light!

[1]. Enwemeka *et al.*, *Photomedicine and Laser Surgery* 27:221-226 (2009).

[2]. Guffey JS et al., *Photomed Laser Surg*. 2006 Dec;24(6):684-8.

STX photolysis plus H_2O_2 synergistically kills MRSA

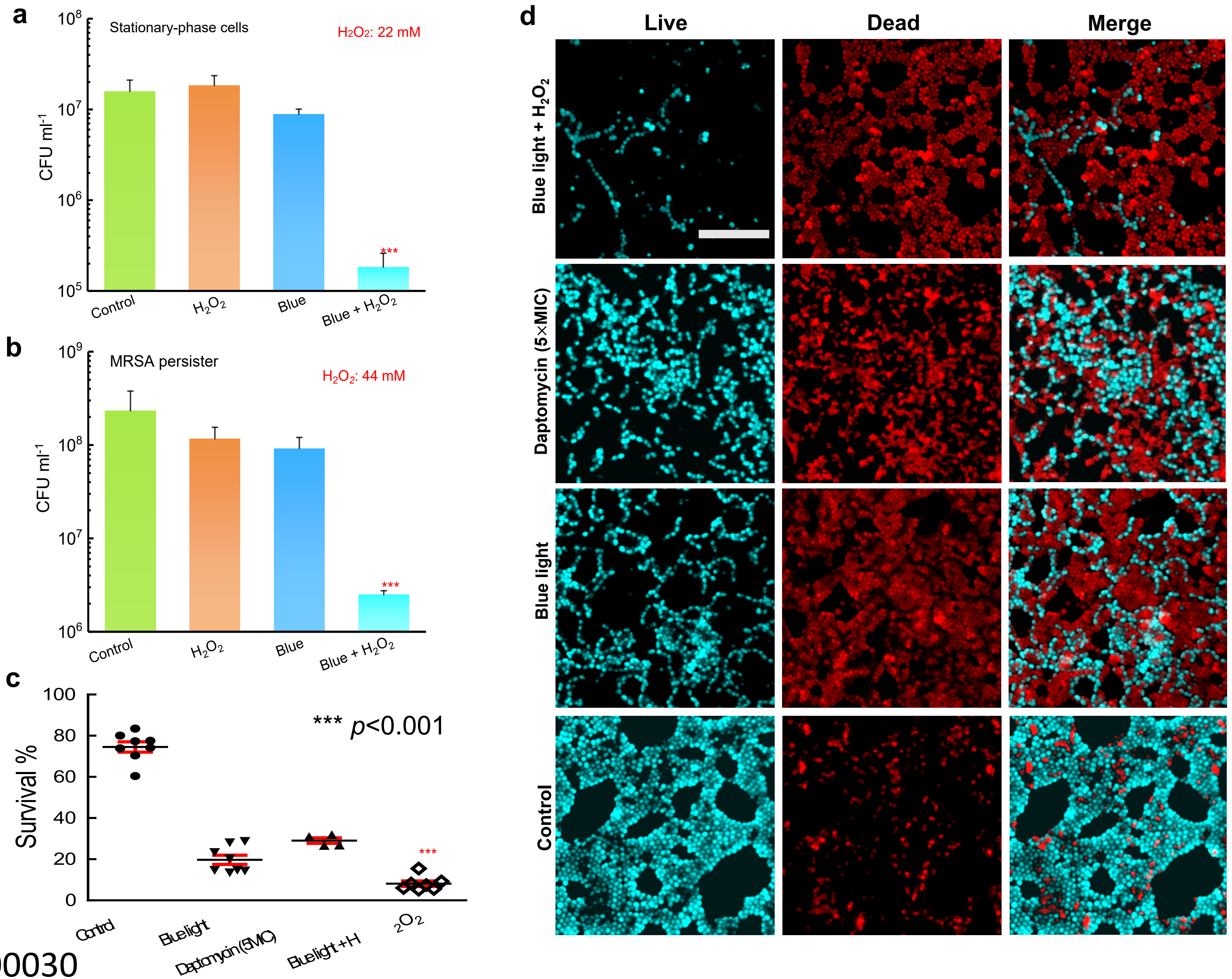


STX Photolysis & H_2O_2 Effectively Eradicate:

a. Stationary phase

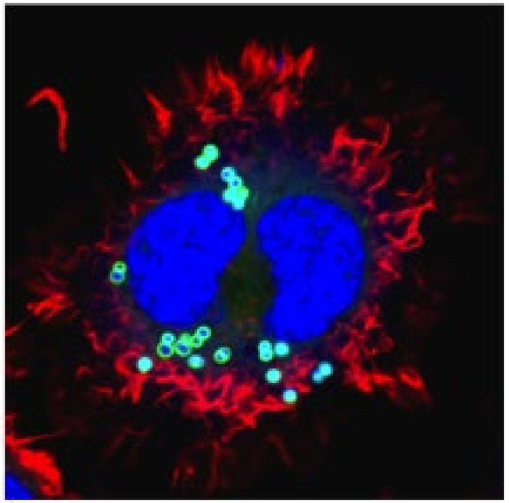
b. MRSA persisters

c & d. Biofilm



Advanced Science, 2019, 6: 1900030

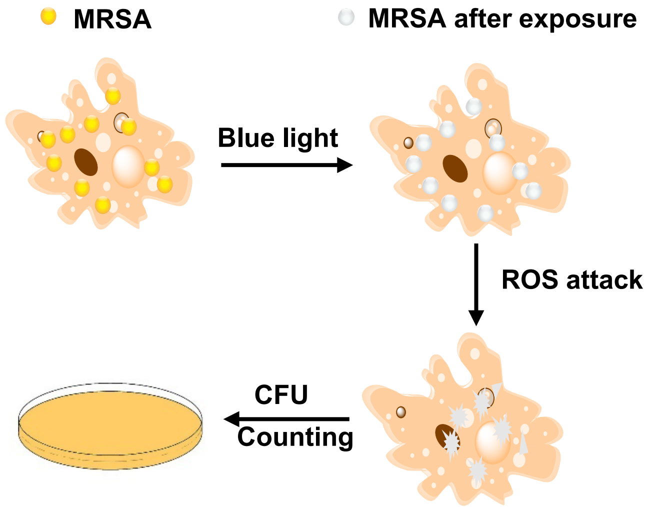
STX photolysis and ROS eradicate intracellular MRSA



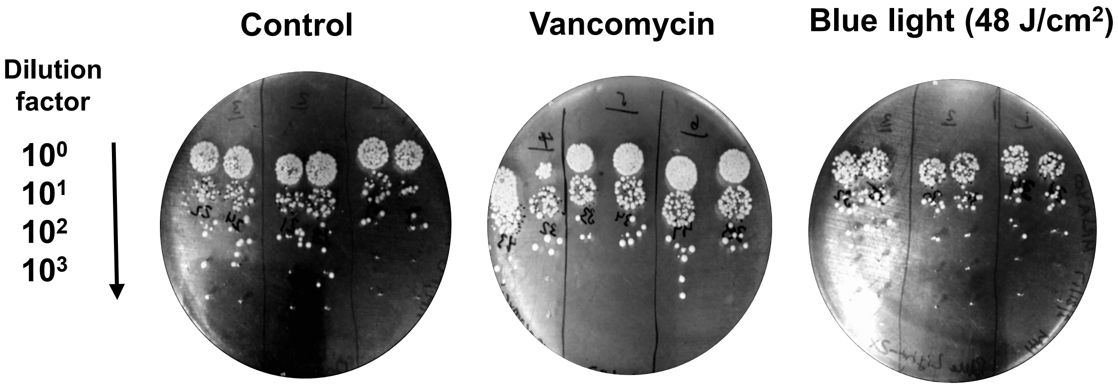
Intracellular *Staphylococcus aureus* within dendritic cells

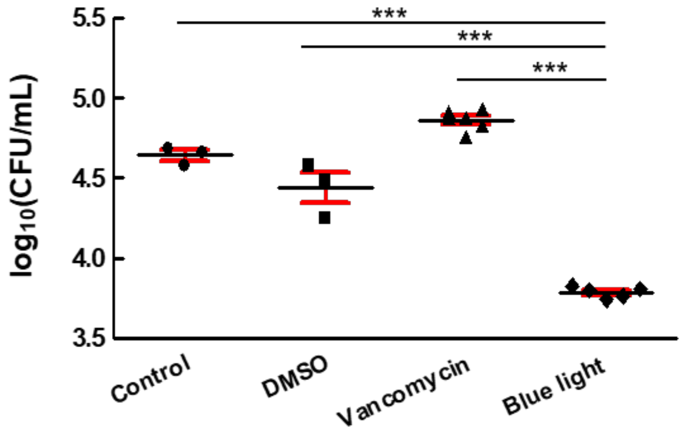
"Survival of *S. aureus* within host cells may provide a reservoir relatively protected from antibiotics……"

***Nature* 527, 323 (2015)**



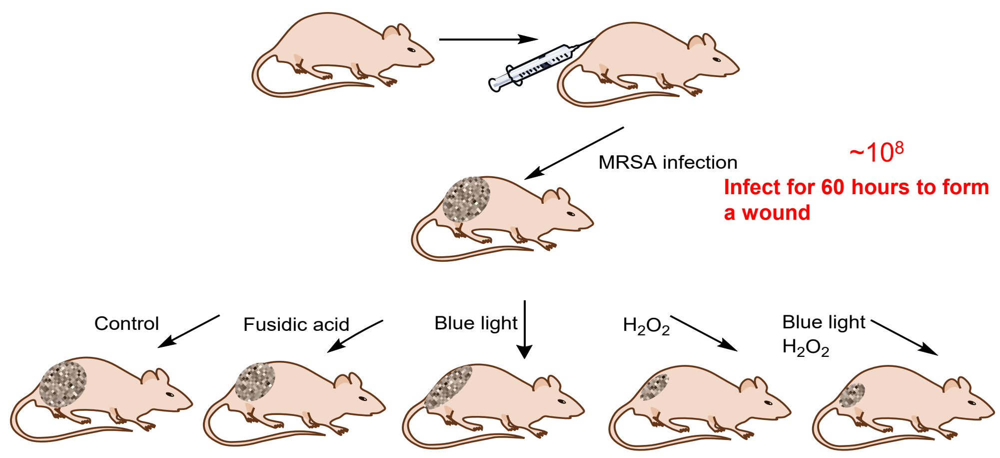
*** p<0.001

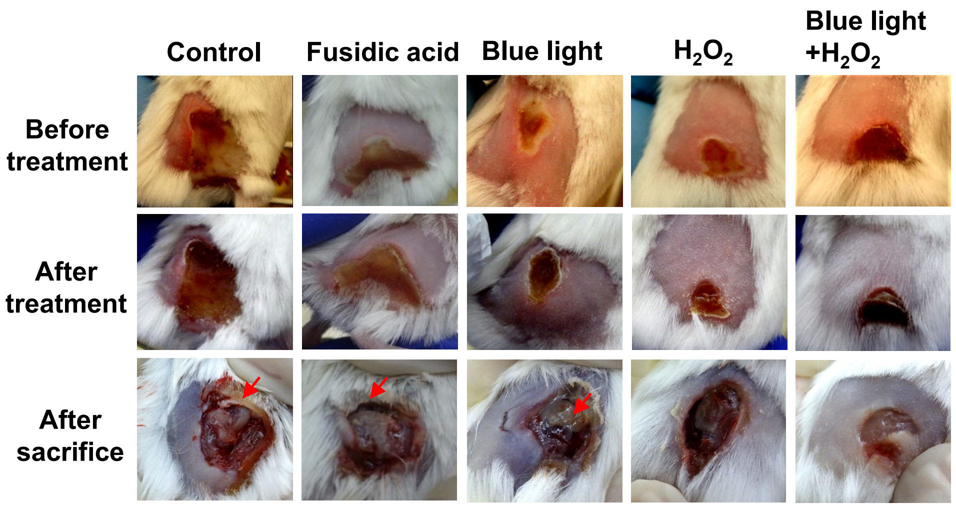


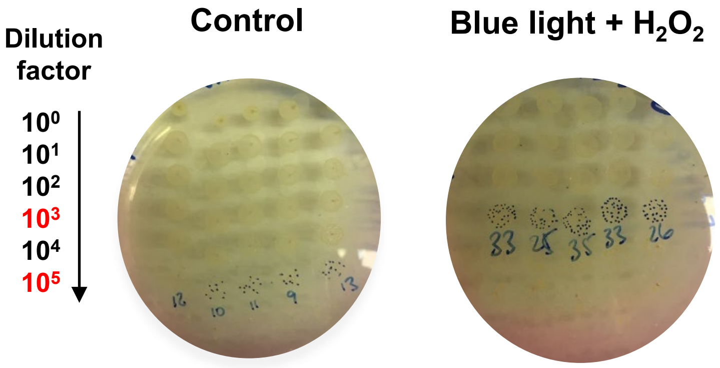


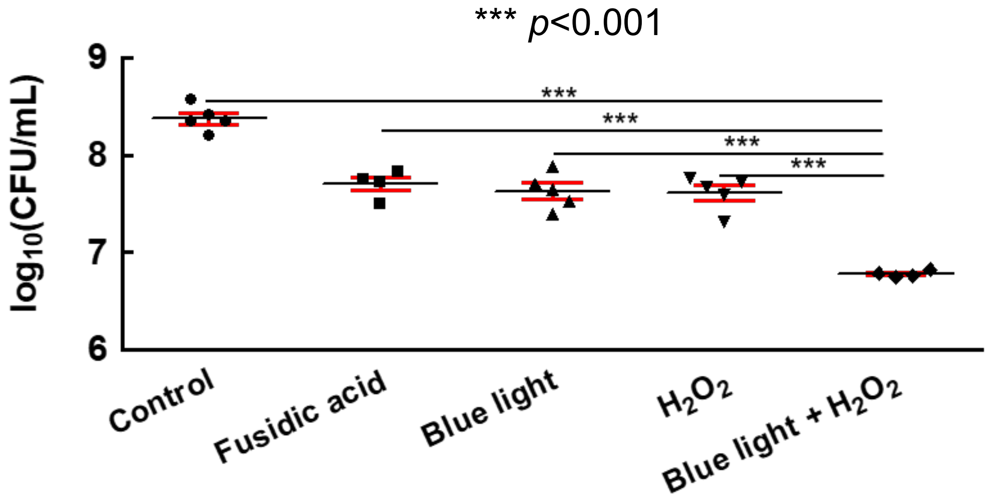
Advanced Science, 2019, 6: 1900030

STX photolysis and H_2O_2 eliminate MRSA-induced skin infection *in vivo*









Advanced Science, 2019, 6: 1900030

SPIE Translational Research Award, Feb 2018

Advanced Science, 2019, 6: 1900030

FULL PAPER

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Phototherapy

www.advancedscience.com

Photolysis of Staphyloxanthin in Methicillin-Resistant *Staphylococcus aureus* Potentiates Killing by Reactive Oxygen Species

Pu-Ting Dong, Haroon Mohammad, Jie Hui, Leon G. Leanse, Junjie Li, Lijia Liang, Tianhong Dai, Mohamed N. Seleem, and Ji-Xin Cheng**

US 11,013,933 B2, issued 5/25/2021

How Light Turns Ordinary Hydrogen Peroxide into a MRSA Treatment

BU engineers have invented a new blue light therapy that can kill MRSA without antibiotics

By Kat J. McAlpine. Photos by Jackie Ricciardi.

As a kid, I skinned my knees on a range of surfaces, from our asphalt driveway, to wood chips on the playground, to the concrete deck of our town pool. I usually cried, not because of the fall itself, but because I knew any scrape deep enough to bleed would attract the attention of my parents and cause them to reach into the medicine cabinet for that dreaded bottle of hydrogen peroxide. Oh, the stinging!

Photonics researchers at Boston University have developed a drug-free treatment for tough-to-treat

But now, a few decades later, I've finally found a reason to appreciate hydrogen peroxide. It turns out that it's powerful enough to kill a particularly lethal kind of antibiotic-resistant bacteria—as long as it's combined with a blue LED light or laser.

Hello sir,
Your work researching blue light and MSRA may save thousands of lives every year.

Personally, I’ve seen the results with an MSRA skin infection that would not heal for 2 months. I’m young and healthy but the infection kept getting worse.

I know my case is not scientifically provable, but please accept my sincere thanks and my enthusiastic support. Keep up the good work. 2 photos showing my improvement over 72 hours after **3x day 3 minute 460nm LED exposure** using standard SMD5050 LED chips, followed by **1 wipe of 3% hydrogen peroxide**. The results are incredible.


Before treatment


After treatment

Three new advances to further transform the accidental discovery into a platform for MRSA treatment

1. Pulsed laser dramatically improves STX photolysis efficiency and depth

2. STX photolysis disassembles MRSA membrane micro-domains

3. STX photolysis potentiates conventional antibiotics

FULL PAPER **Adv. Sci. 2020, 7: 1903117**

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Photo-Disassembly of Membrane Microdomains Revives Conventional Antibiotics against MRSA

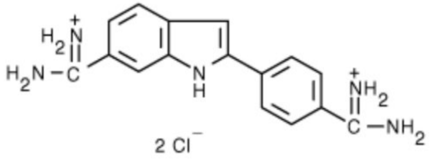
*Jie Hui, Pu-Ting Dong, Lijia Liang, Taraknath Mandal, Junjie Li, Erlinda R. Ulloa, Yuewei Zhan, Sebastian Jusuf, Cheng Zong, Mohamed N. Seleem, George Y. Liu, Qiang Cui, and Ji-Xin Cheng**

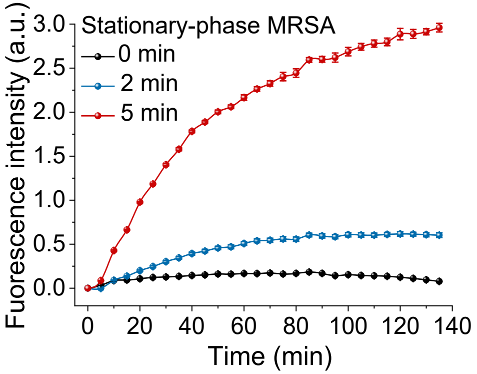
Three distinctive mechanisms of remodeling MRSA membrane

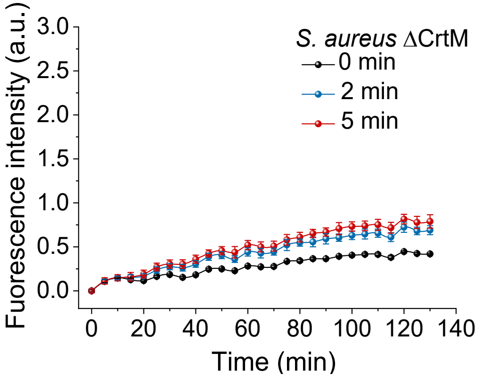
Adv. Sci. 2020, 7:1903117

1. Poration

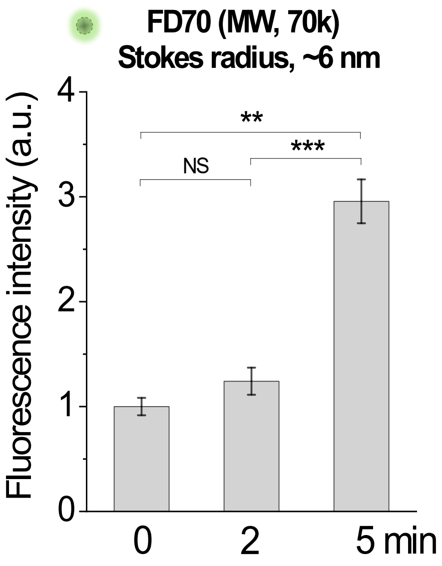
SYTOX green (600 Da)

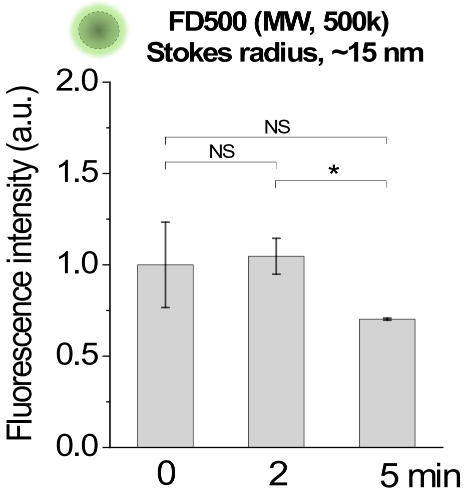




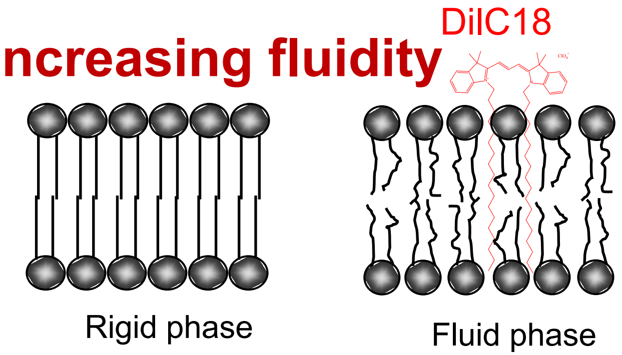


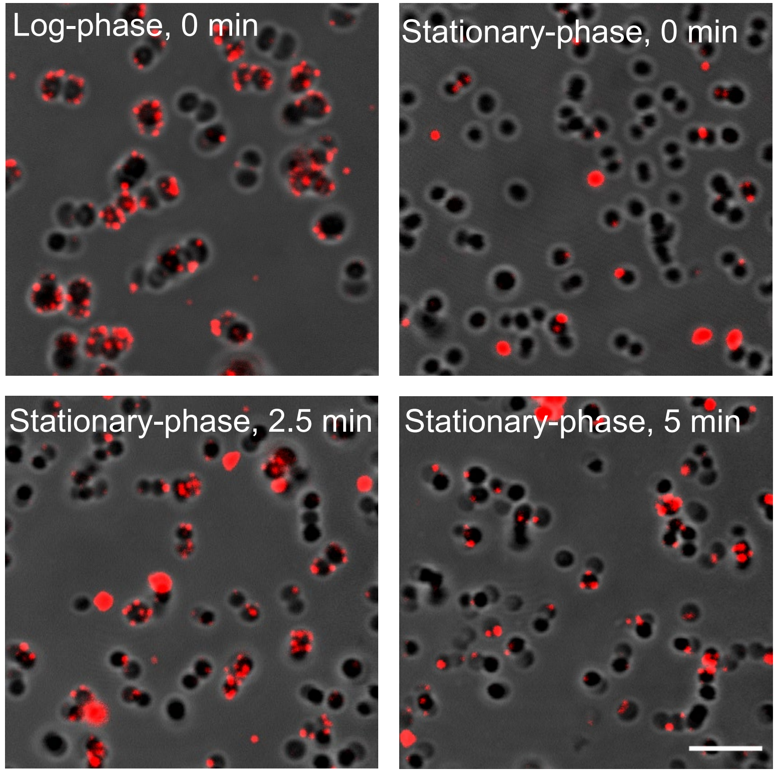
FITC-Dextran





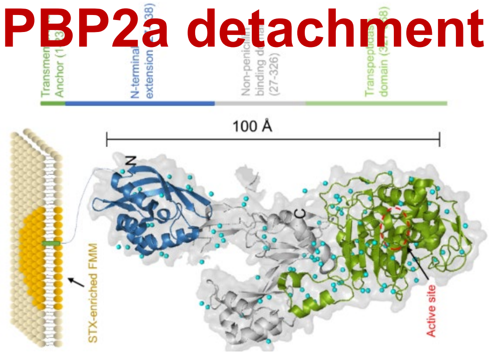
2. Increasing fluidity

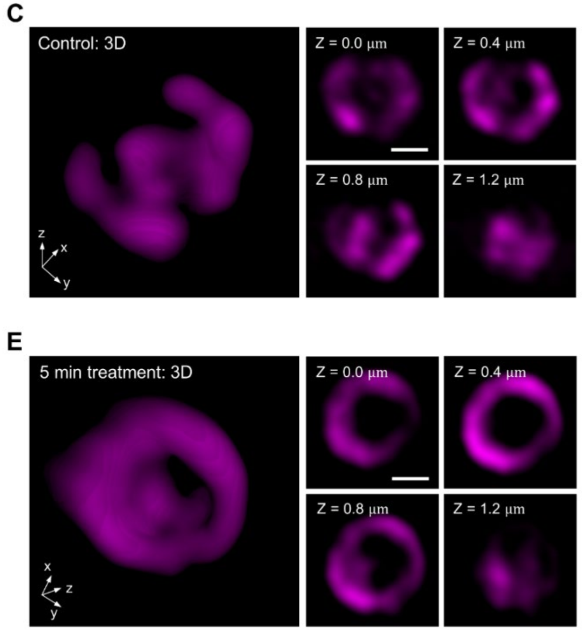


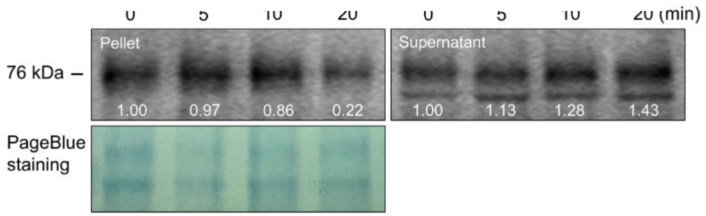


Red: DiIC 18; Grey: transmission

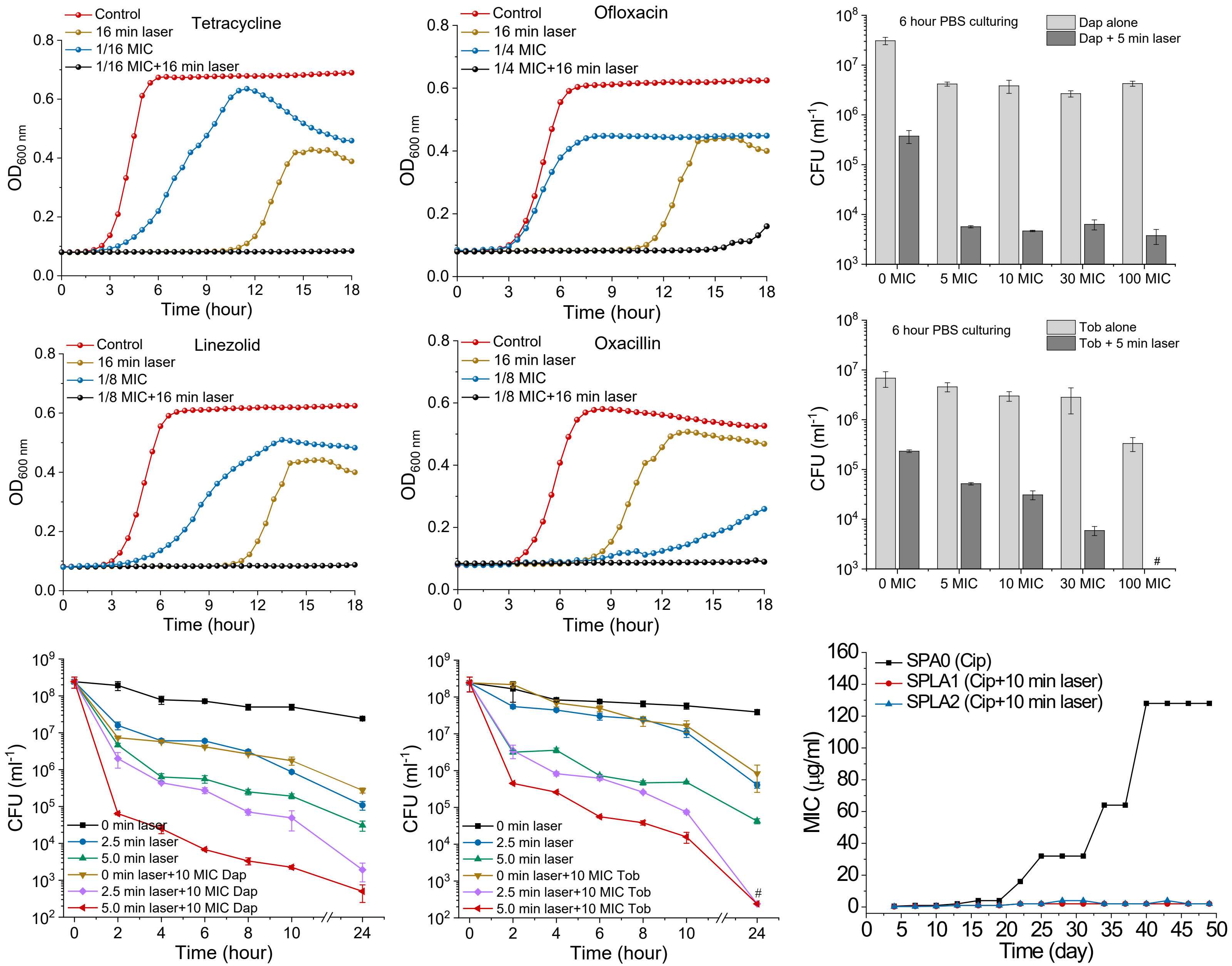
3. PBP2a detachment







Photolysis of STX sensitizes broad-category antibiotics against MRSA



Adv. Sci. 2020, 7:1903117

Eradication of multi-drug resistant pathogens via photo-inactivation of a detoxifying enzyme

US 11,110,296 B2 issued 9/7/2021, Licensed to Pulsethera Inc, Founded 2019

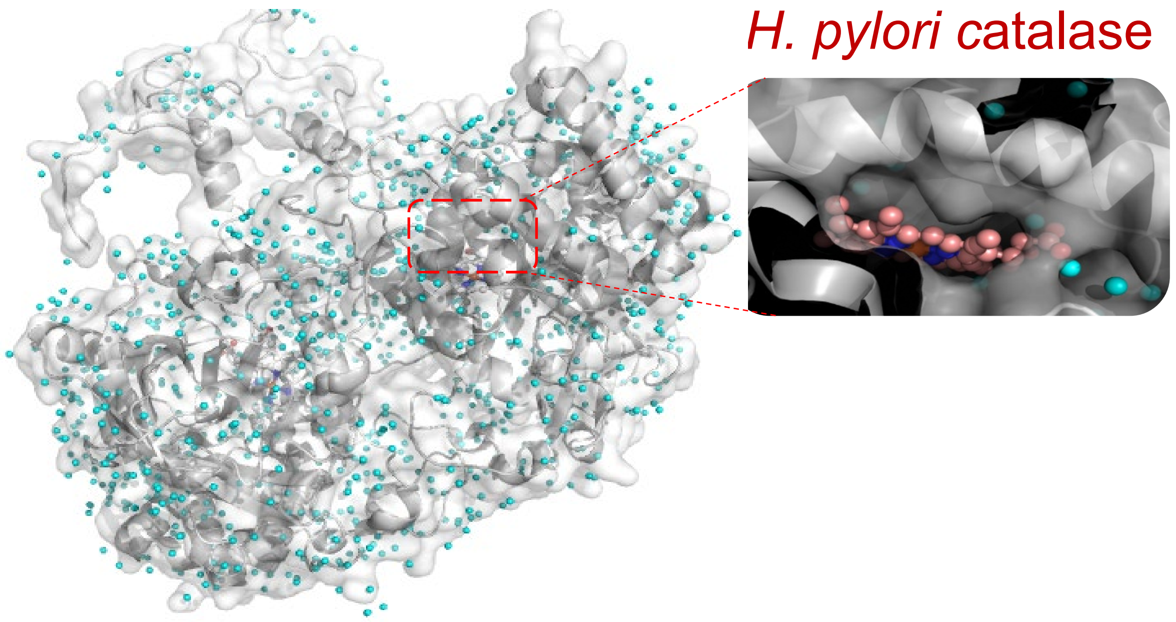
Life can be seen as a balance between metabolic rate and a cell's ability to detoxify reactive oxygen species (ROS)[1]

[1] *Science* 334, 6058, 915-916.

Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + .OH + OH^-$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + .OOH + H^+$$



Most pathogens use catalase to convert excess H_2O_2 into water and O_2 to maintain a H_2O_2 concentration below 20 nM.

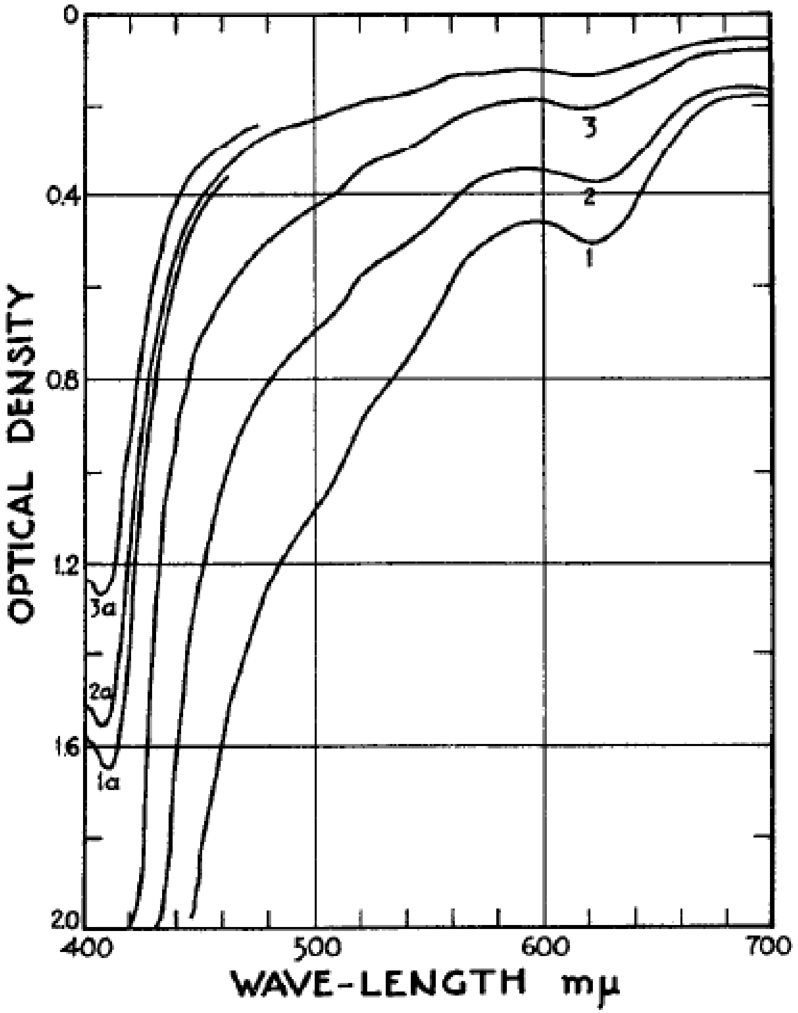
Catalase can be inactivated by photons

ON THE ABSORPTION SPECTRUM OF CATALASE*

By KURT G. STERN

(From the Laboratory of Physiological Chemistry, Yale University School of Medicine, New Haven)

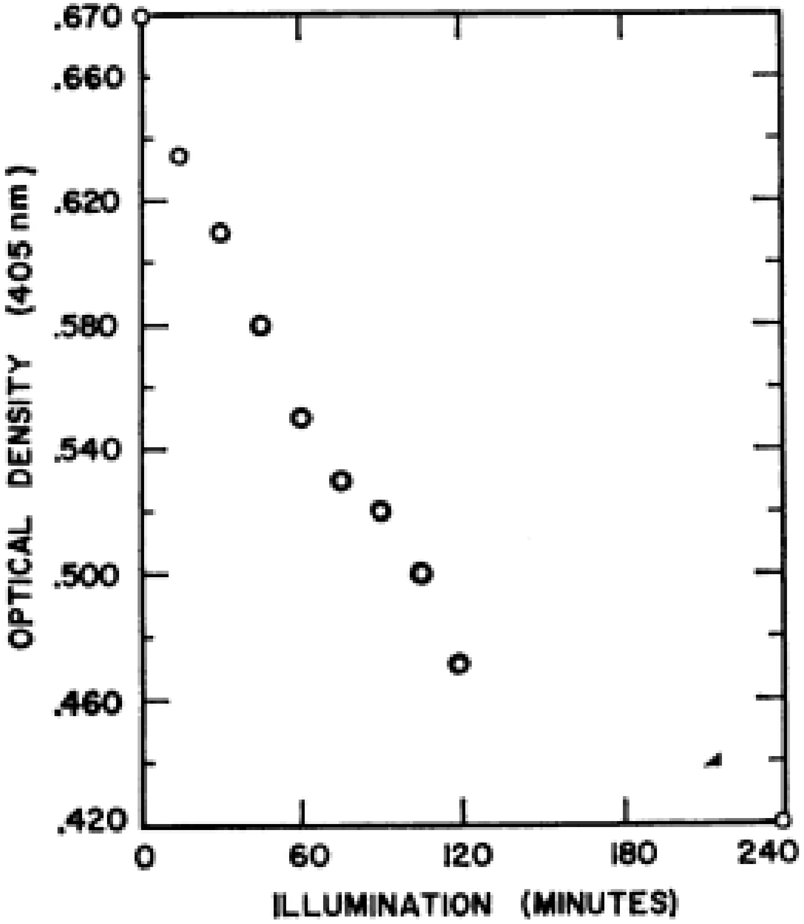
(Received for publication, June 22, 1937)



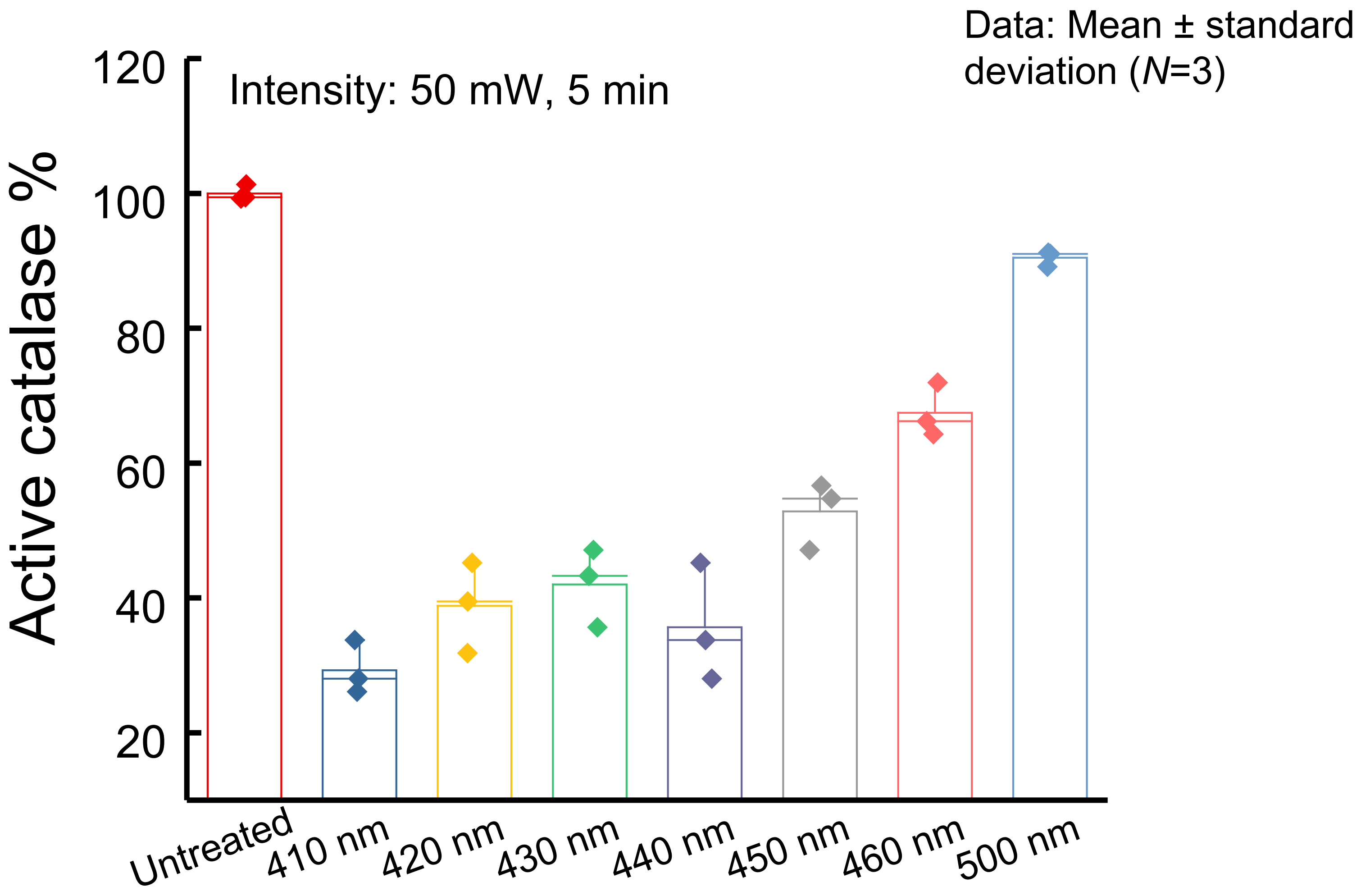
Science 150(3692), 74, 1965

Catalase Photoinactivation

Abstract. *The enzymatic activity of catalase is lost during exposure to sunlight in the presence of oxygen. A simultaneous decline occurs in the absorption peak at 405 nanometers.*

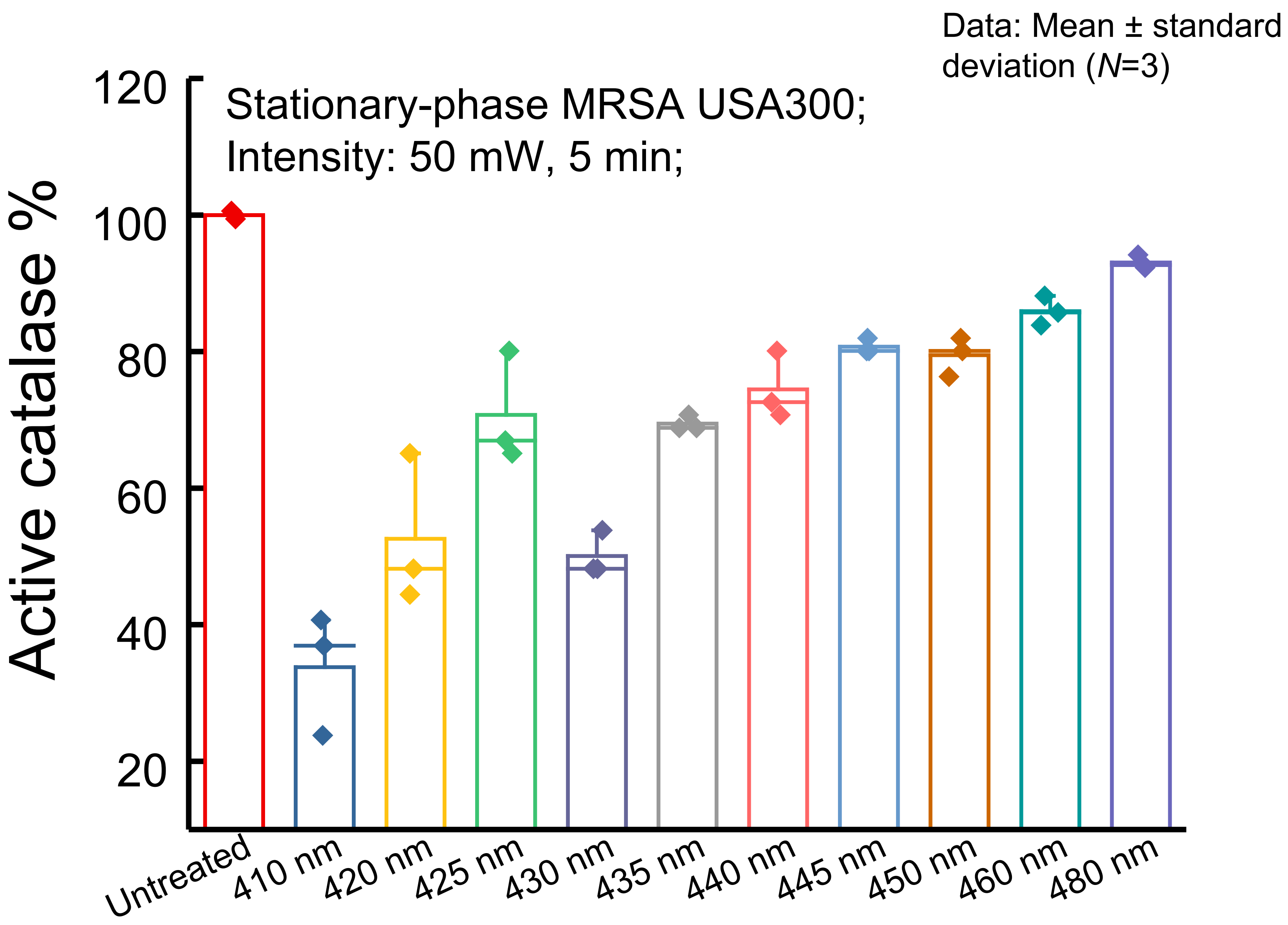


Photoinactivation of catalase (2.5 U/ml) solution



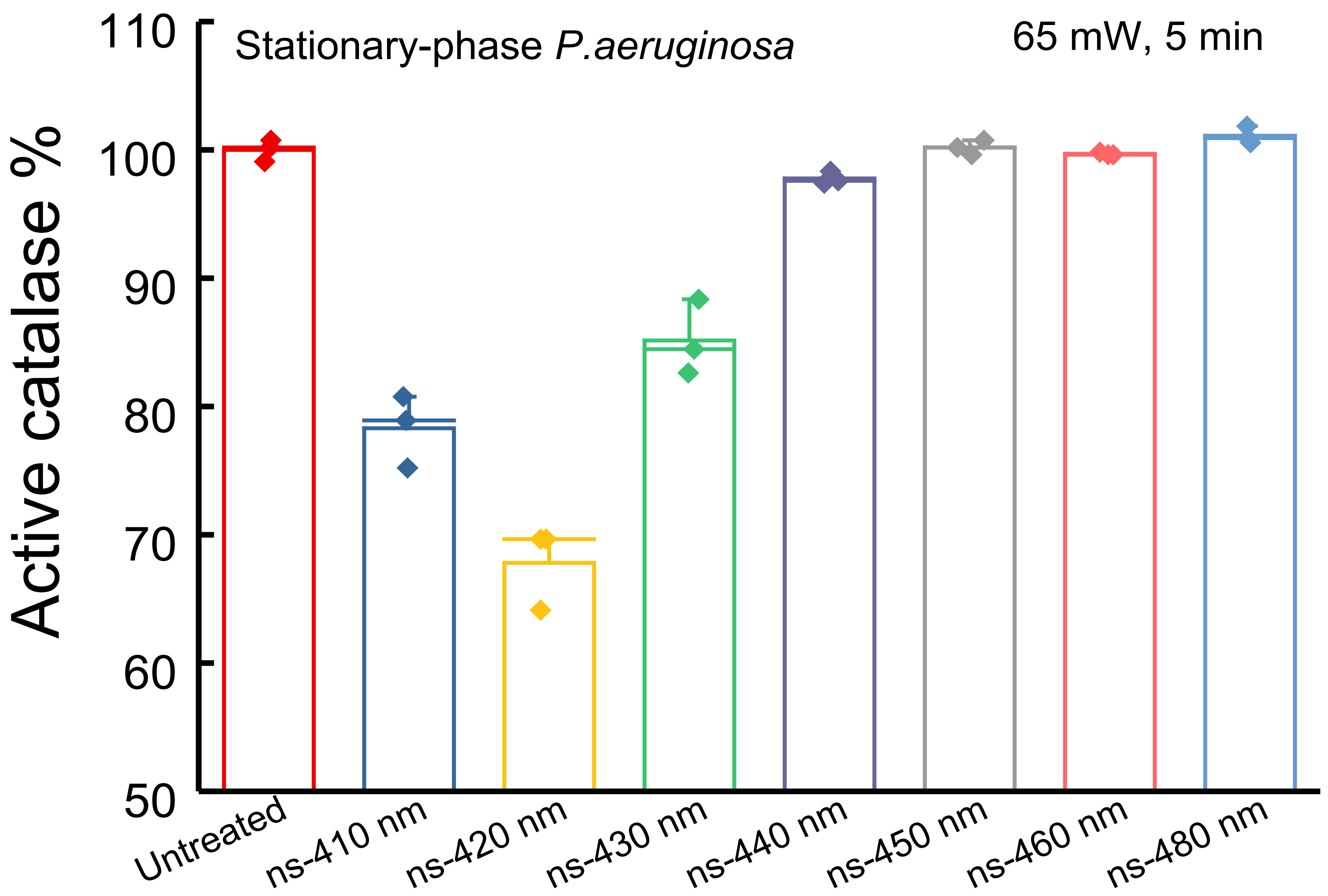
- Under the same dose exposure, 410 nm demonstrated the highest percent of photoinactivation of catalase;

Photoinactivation of catalase inside MRSA USA300



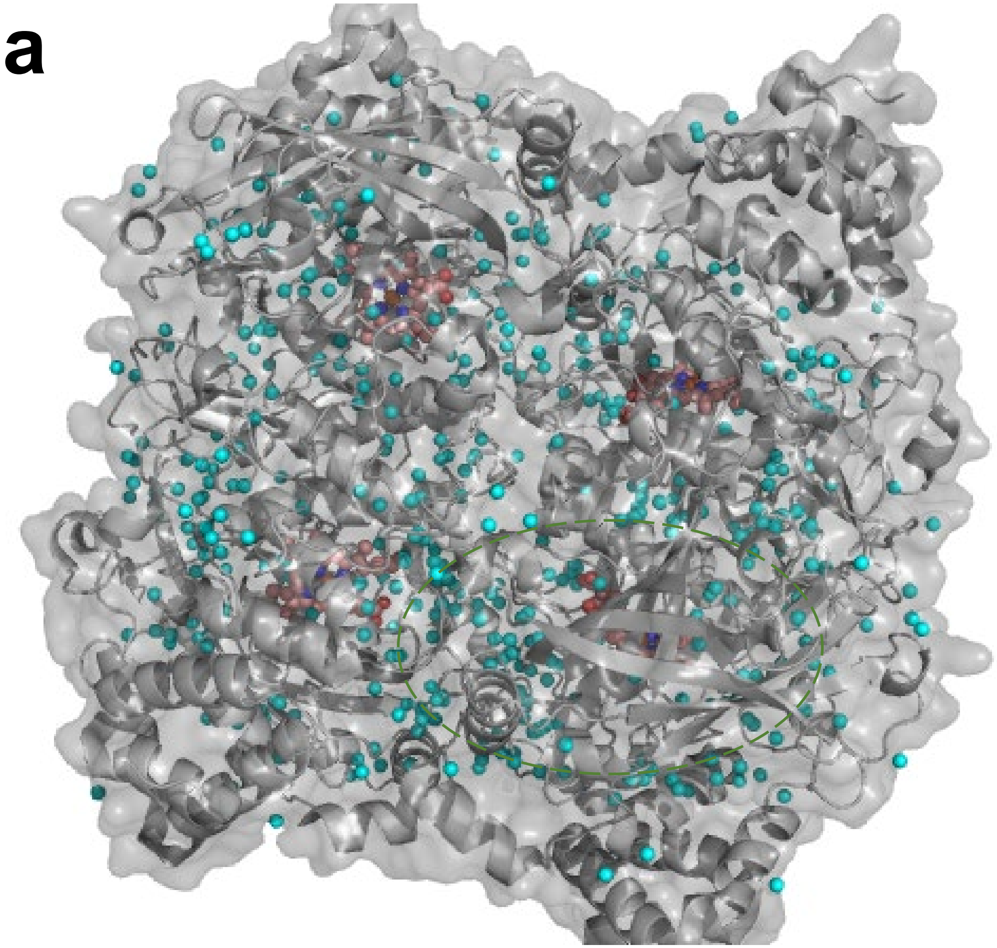
Photoinactivation of catalase inside *P. aeruginosa*

Data: Mean ± standard deviation (*N*=3)

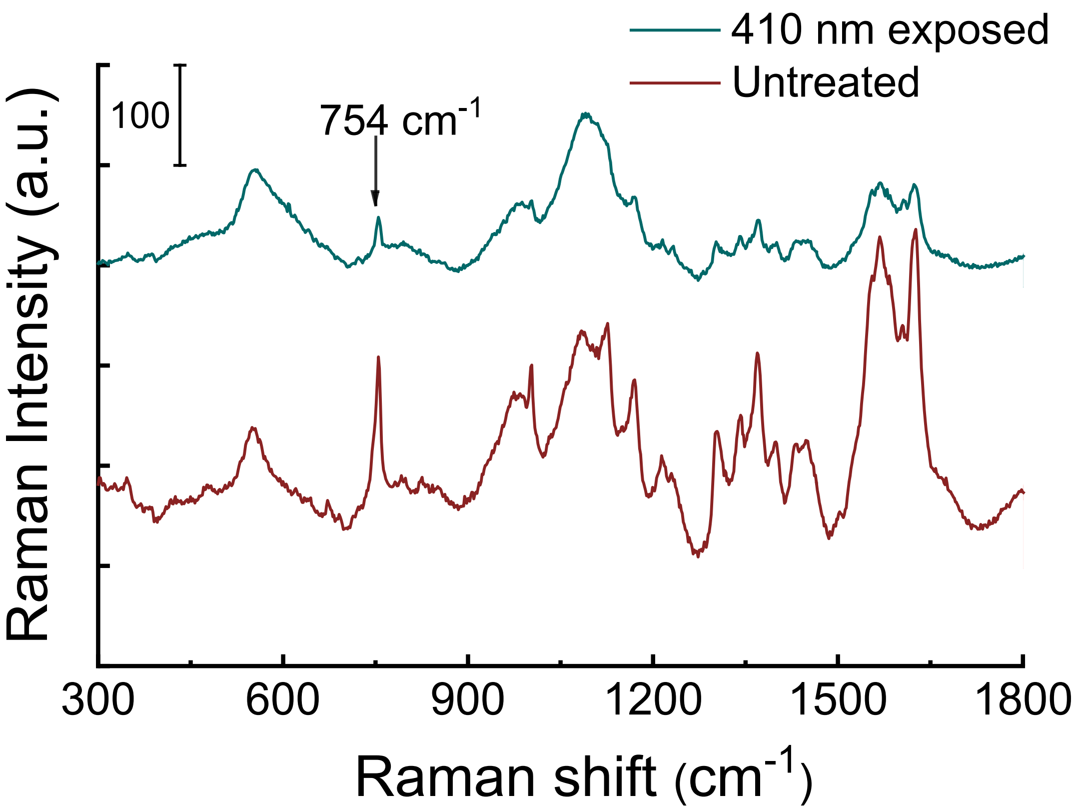


Mechanism: heme ring detachment

a



b Raman spectroscopy

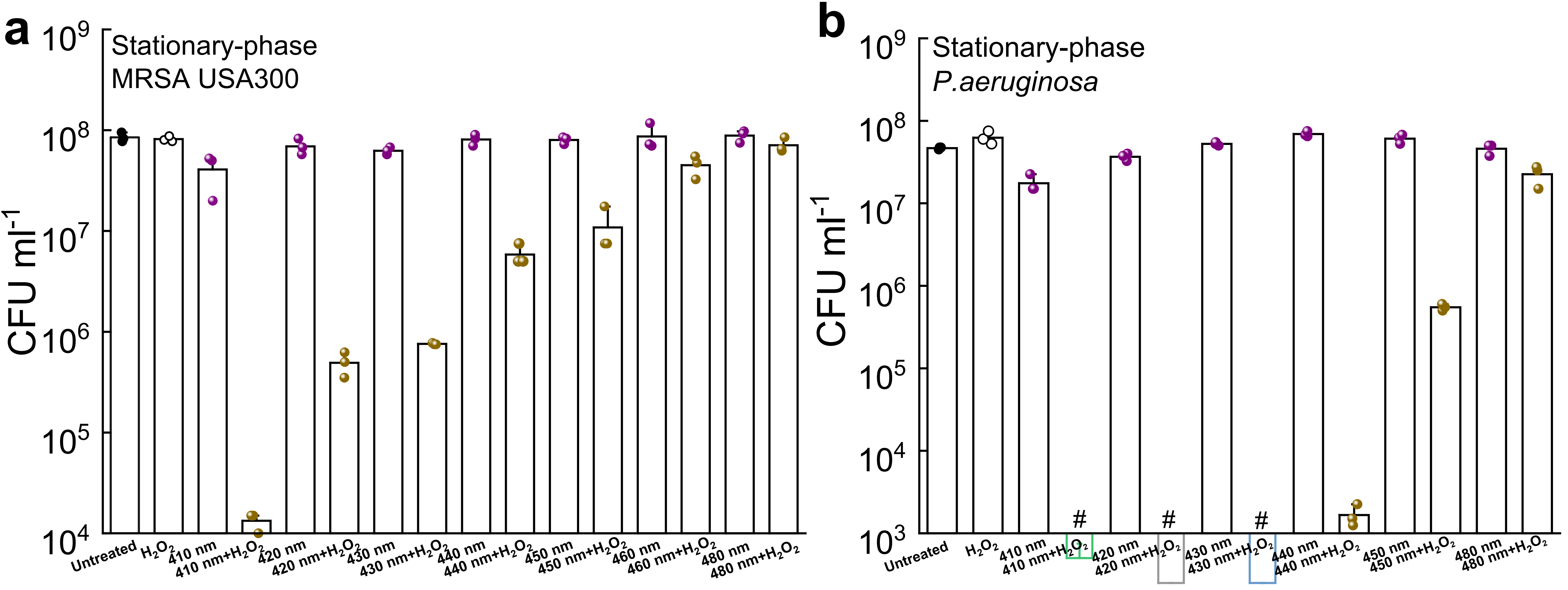


Nanosecond pulses are more effective than LED

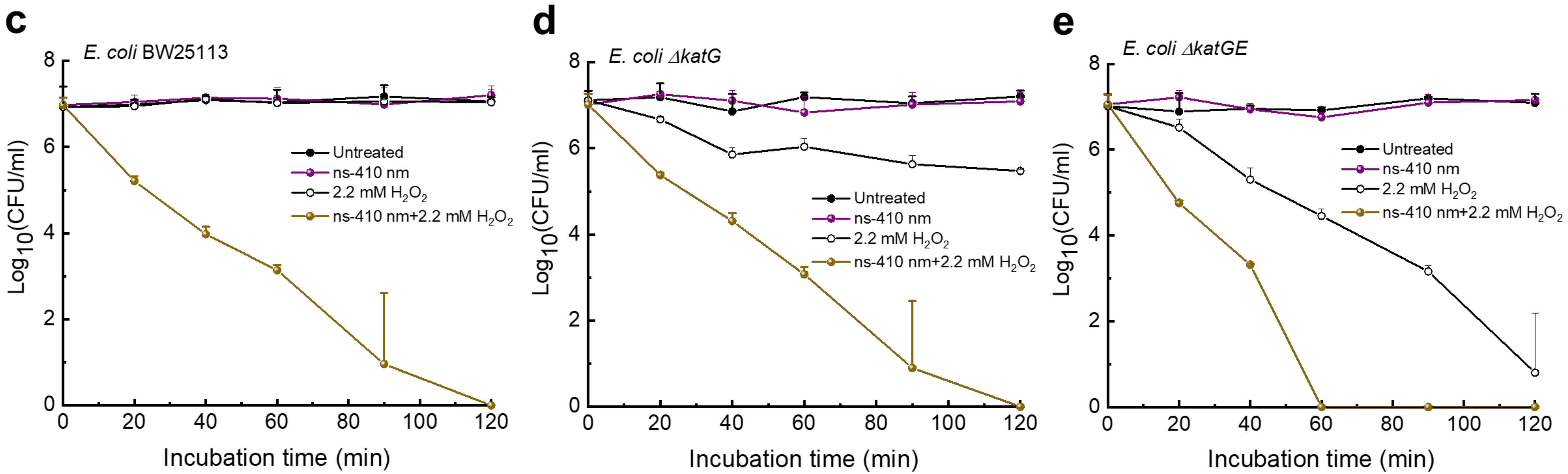
CW-410 nm (min) **ns-410 nm (min)**

Control 2 5 10 15 2 5 10 15

Photoinactivation of catalase sensitizes *pathogenic bacteria* to H_2O_2

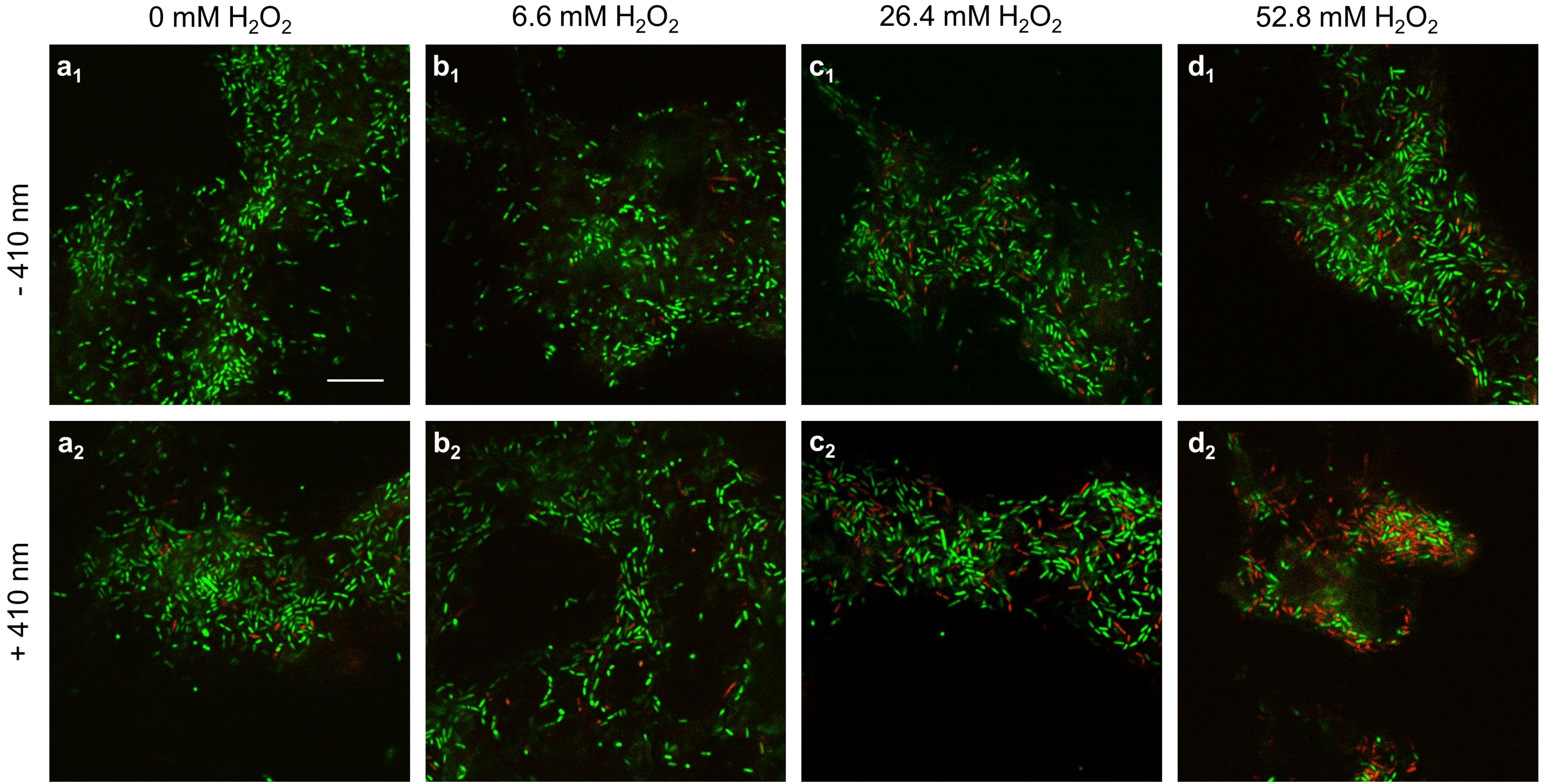


Catalase mutant is highly sensitive to H_2O_2



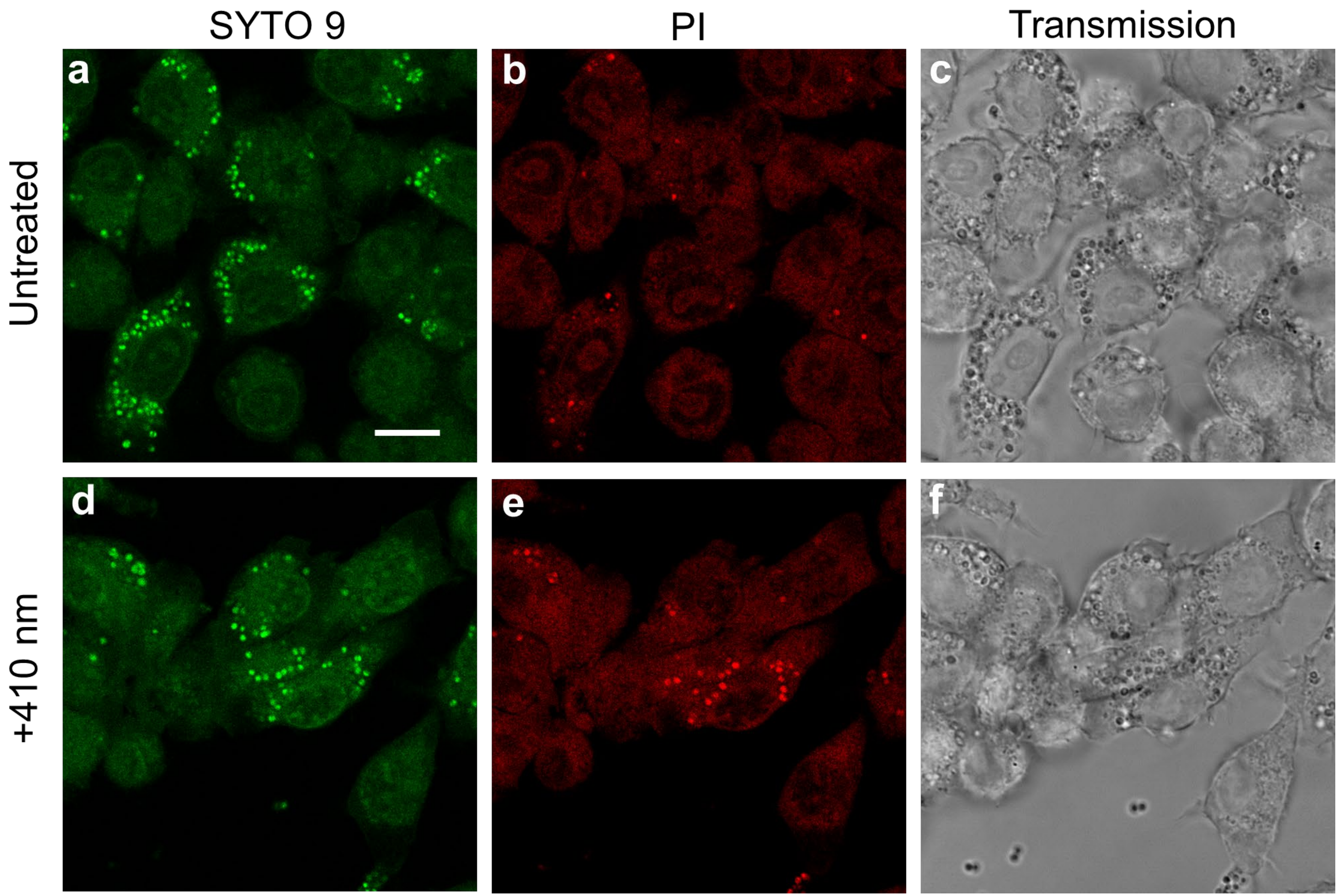
JCI Insight 2022

Live/dead bacteria inside *P. aeruginosa PAO-1* biofilms after different treatments



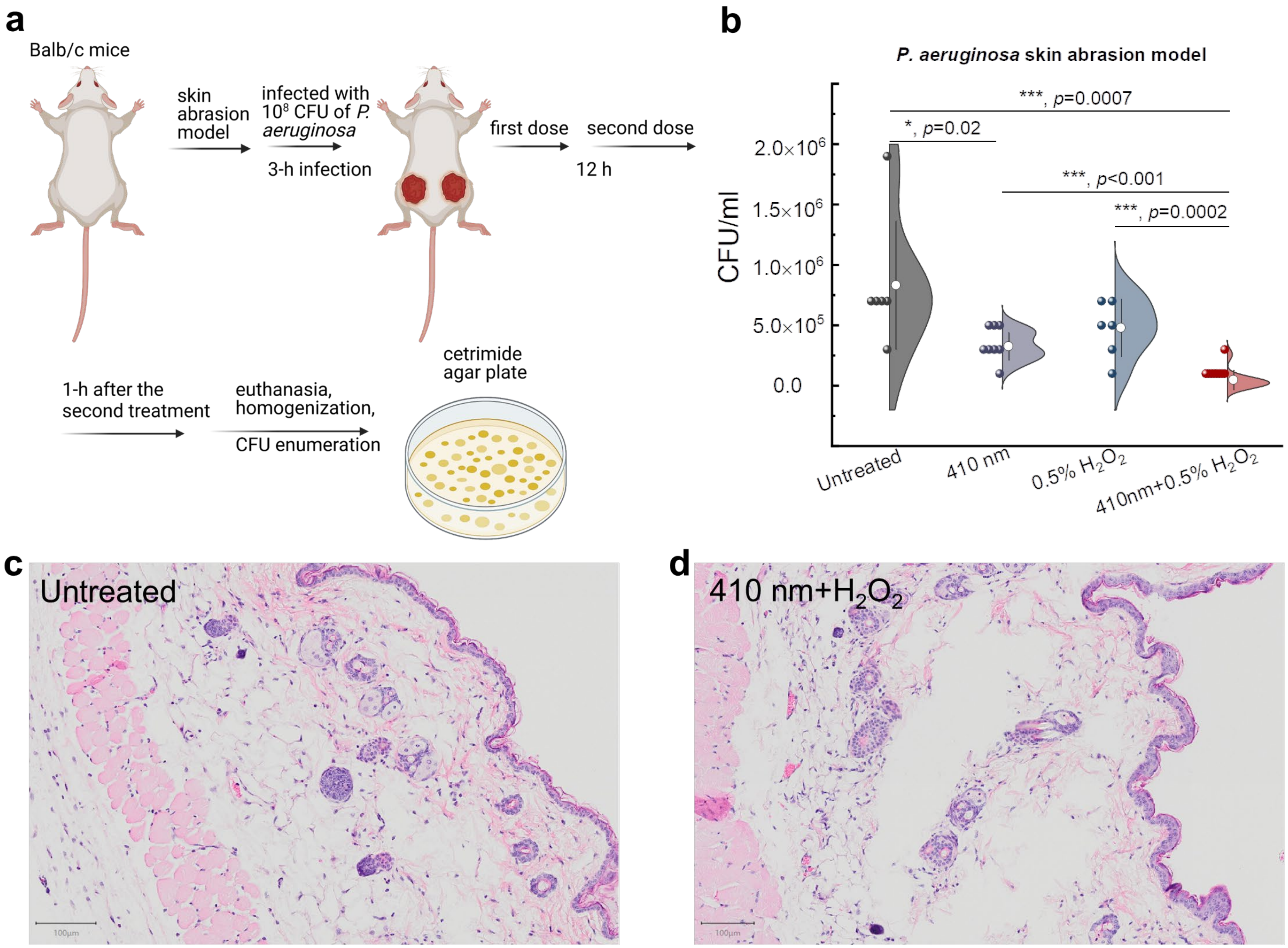
JCI Insight 2022

Photoinactivation of catalase assists macrophages to eliminate intracellular bacteria



JCI Insight 2022

Inactivation of catalase reduces *P. aeruginosa* burden in a *P. aeruginosa*-induced skin abrasion model

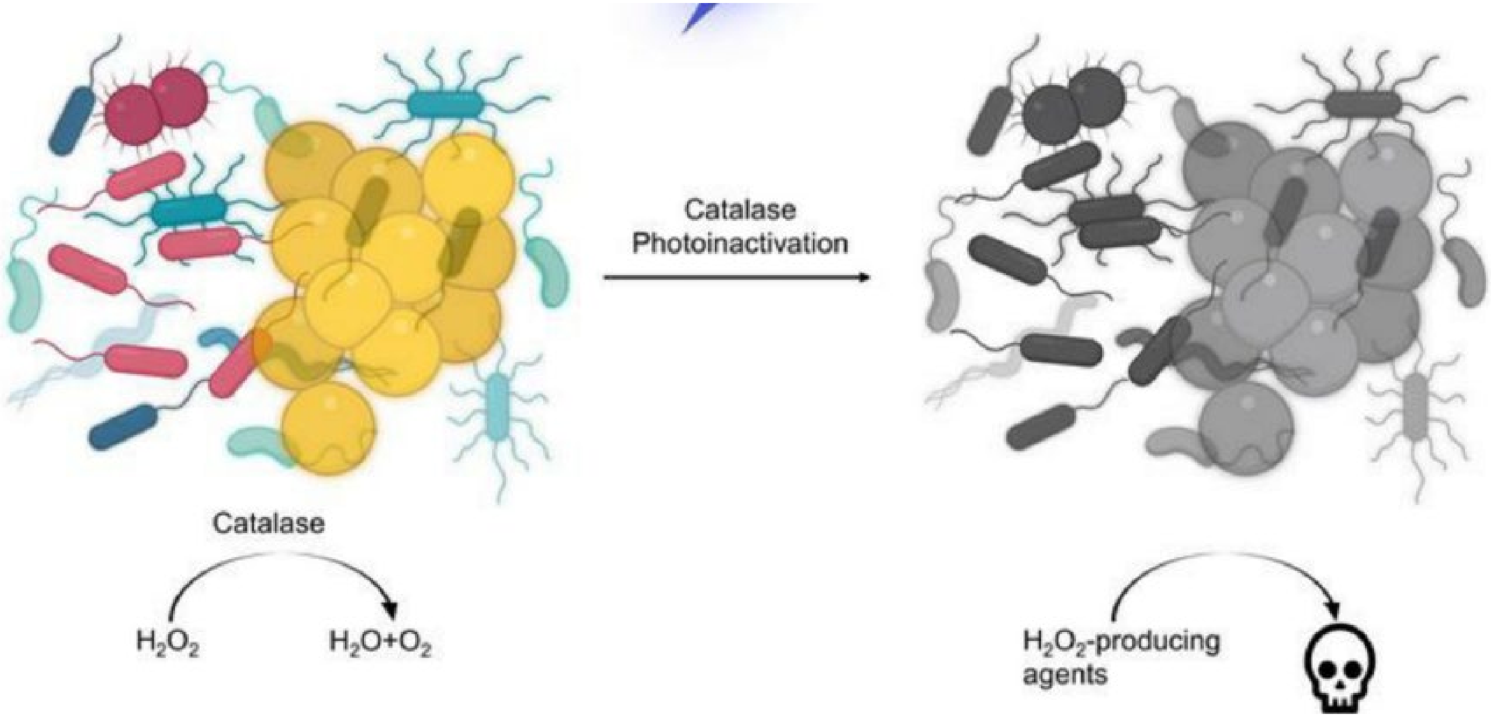


JCI Insight 2022

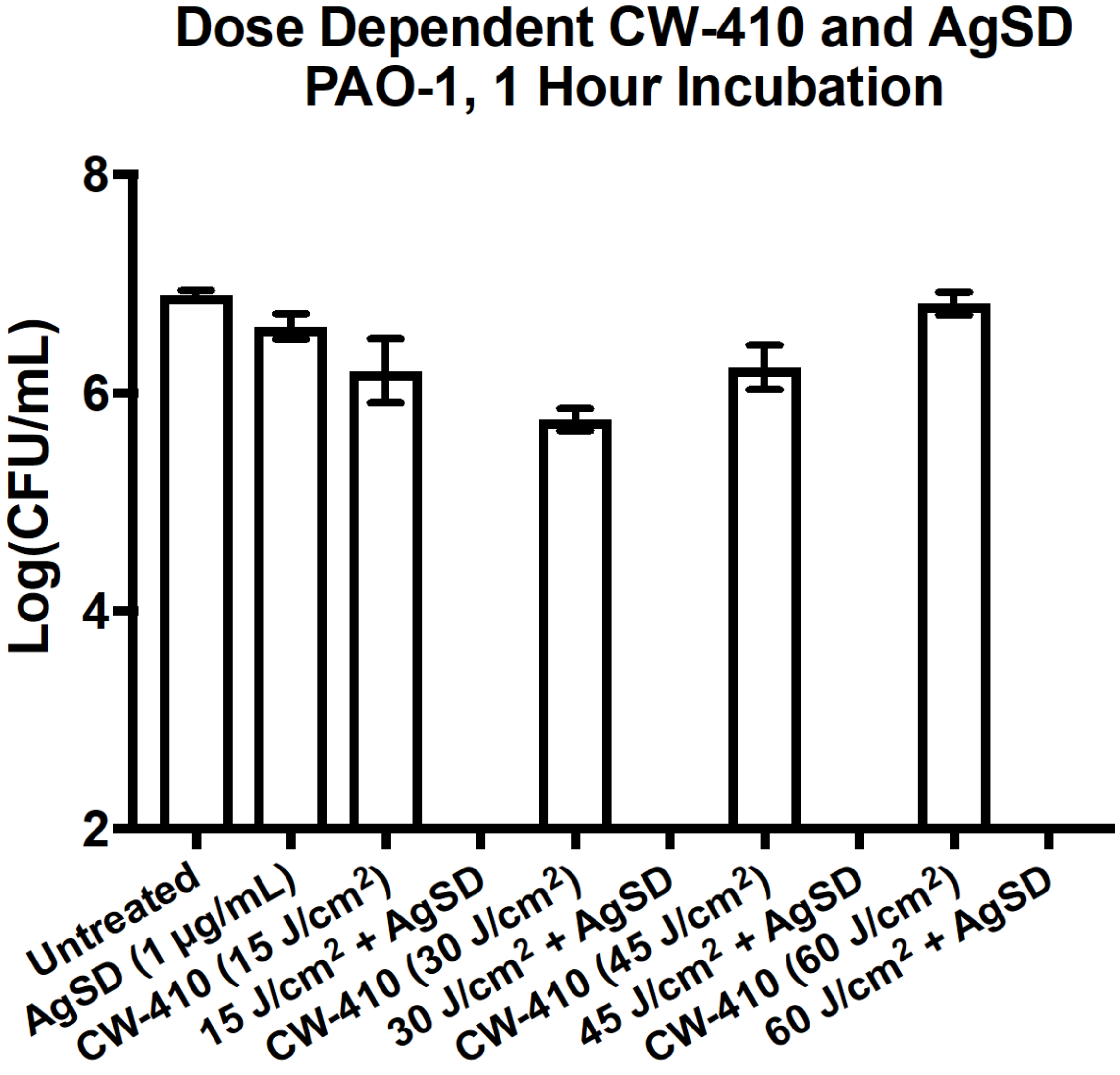
Photoinactivation of catalase sensitizes a wide range of bacteria to ROS-producing agents and immune cells

Pu-Ting Dong, … , George Y. Liu, Ji-Xin Cheng

JCI Insight. 2022;7(10):e153079. https://doi.org/10.1172/jci.insight.153079.



Silver Sulfadiazine (AgSD) Exhibits Improved Synergy at Very Low 410-nm Light Dosages



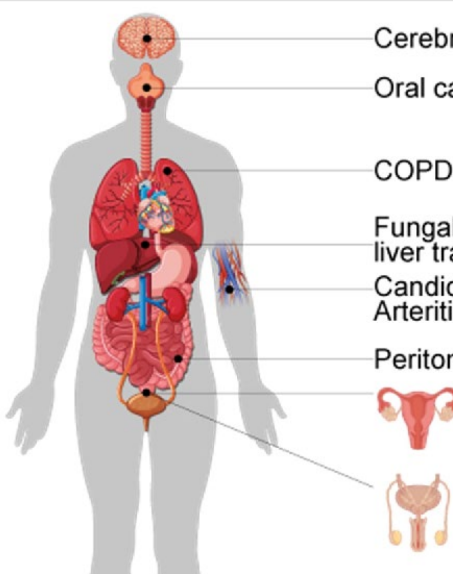
Sebastian Jusuf et al, under review 2022

Fungi infections

http://blogs.discovermagazine.com/crux/2019/04/23/candida-auris-albicans-fungus-infections-resistant/#.XY4iakZKiUk

P R Health Sci J. 2010 March ; 29(1): 26–29.

Nosocomial bloodstream infections (BSI) are an important cause o
in hospitalized patients. *Candida* infections account for the fourth r
patients admitted to critical care units as evidenced in a multi-cente
hospitals in the United States.[1] A multicenter prospective observati
several tertiary care centers in the United States revealed that *C. albic*
Candida species isolated in BSI.[2] Similar surveillance studies had re
of candidemias secondary to non-albicans *Candida* (NAC).[3]



Candida auris: A

David Sears*, Brian S

Division of Infectious Diseases, Univ

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hus, Denmark

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Healthcare-associated
Outbreak
Infection control

The New York Times

To Fight Deadly Candida Auris, New York State Proposes New Tactics

A bed at Mount Sinai Hospital in Brooklyn. Most of New York's 331 cases of C. auris infection have been concentrated in Brooklyn and Queens. Hilary Swift for The New York Times

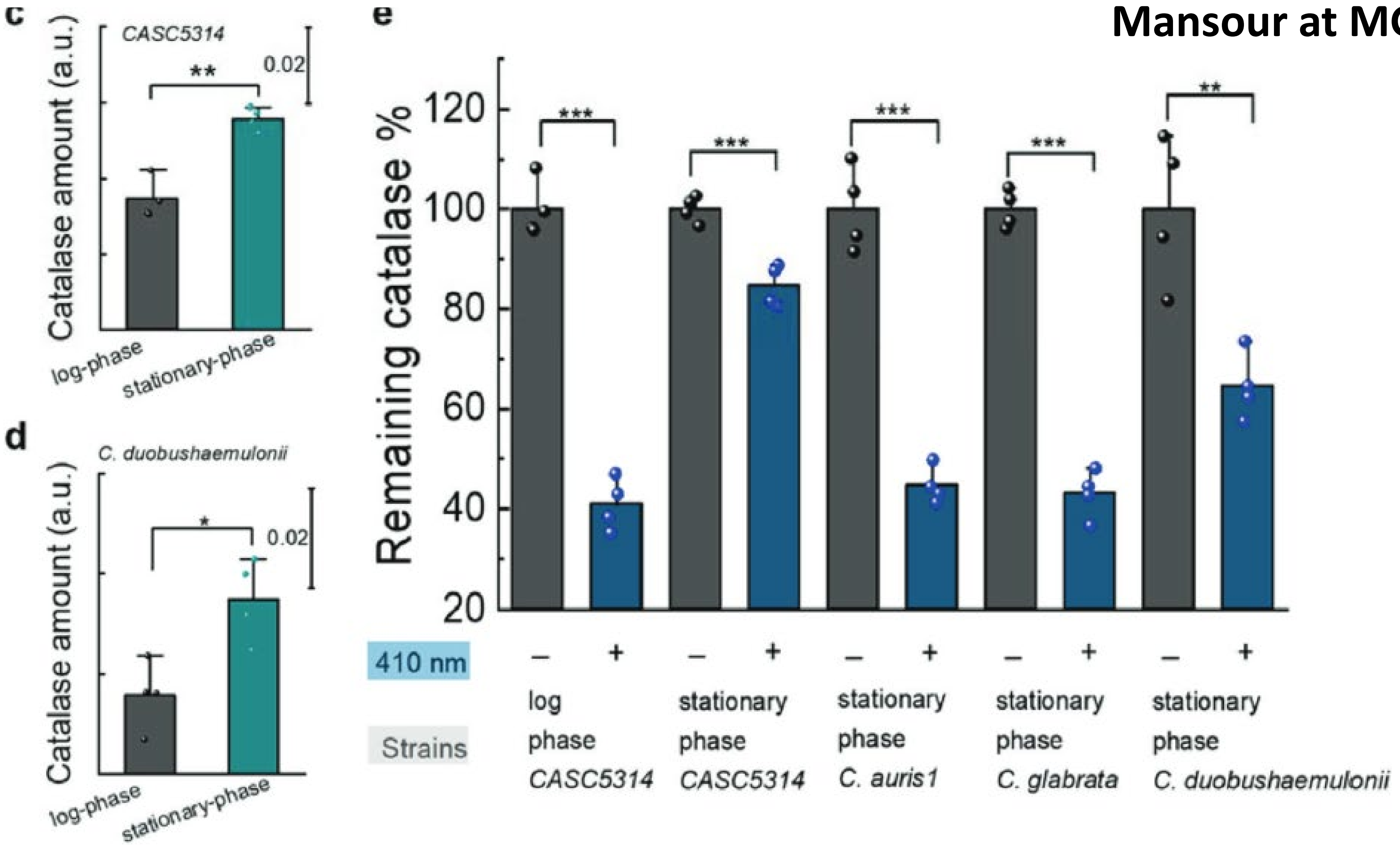
By Matt Richtel

May 23, 2019

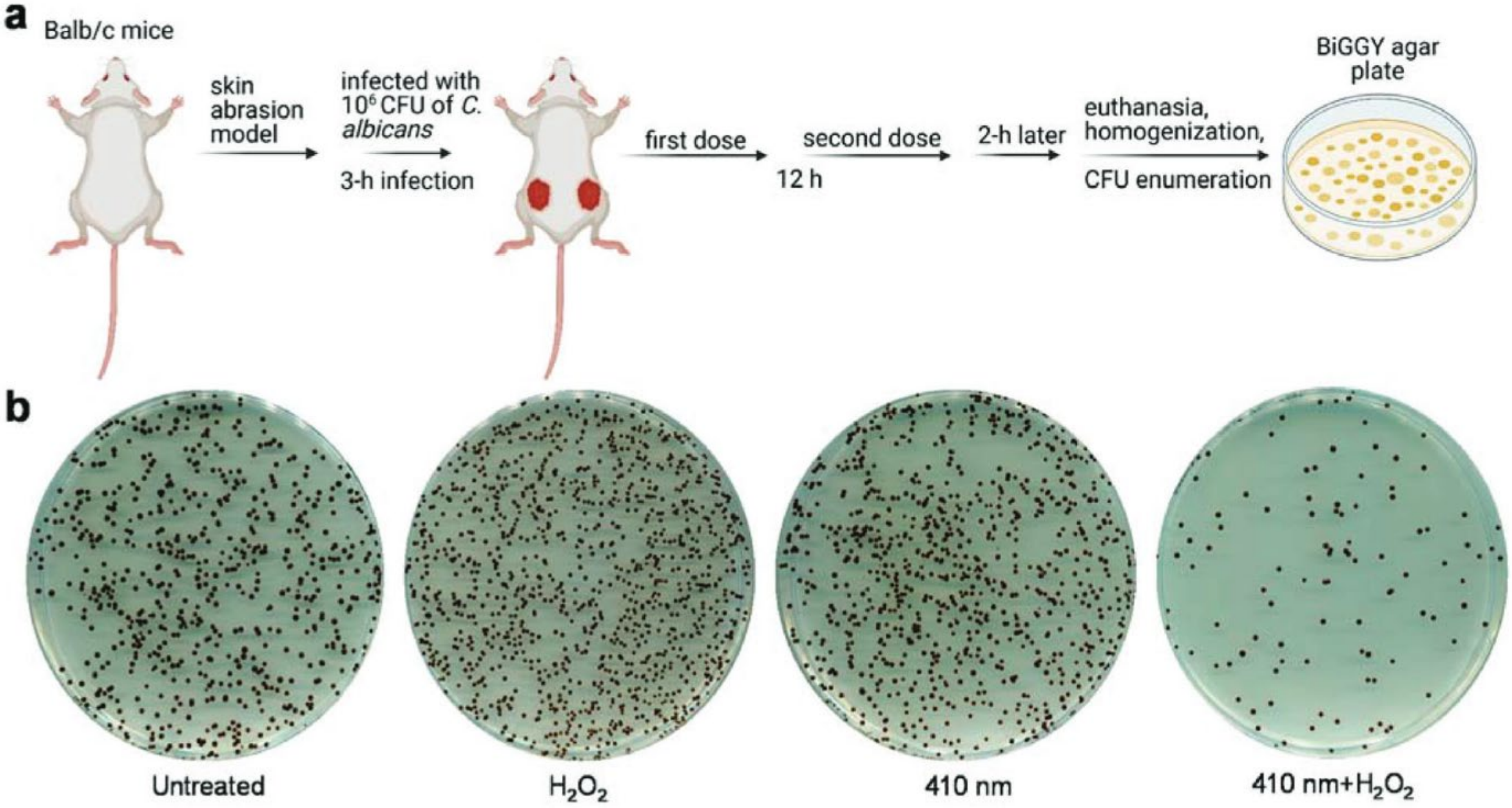
New York State health officials are considering rigorous new
requirements for hospitals and nursing homes to prevent the

Photoinactivation of Catalase Sensitizes *Candida albicans* and *Candida auris* to ROS-Producing Agents and Immune Cells

Advanced Science 2022, 9, 2104384
Collaborator: Michael Mansour at MGH

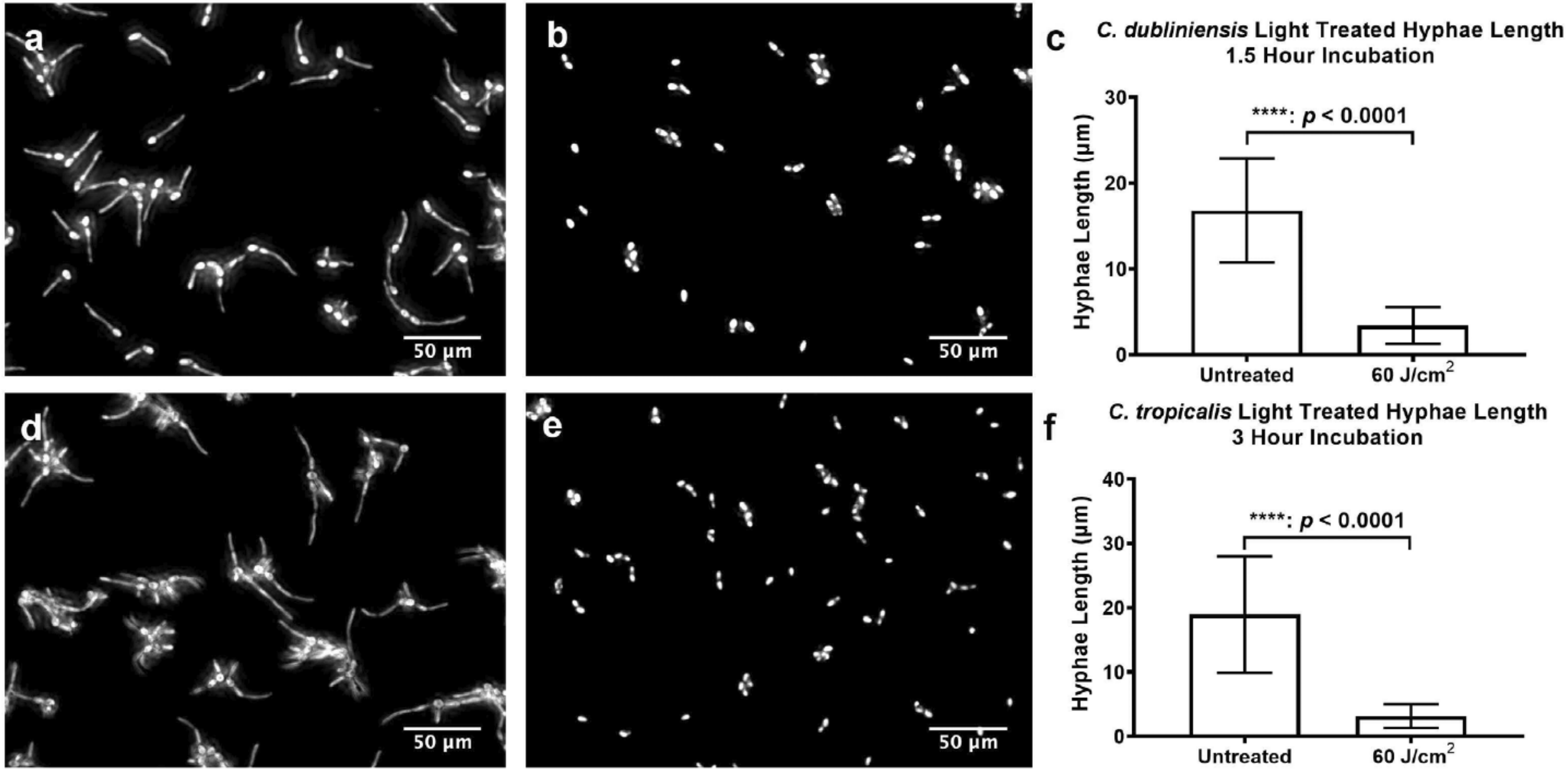


Catalase photoinactivation and H2O2 synergistically reduce C. albicans burden in a mouse skin abrasion model



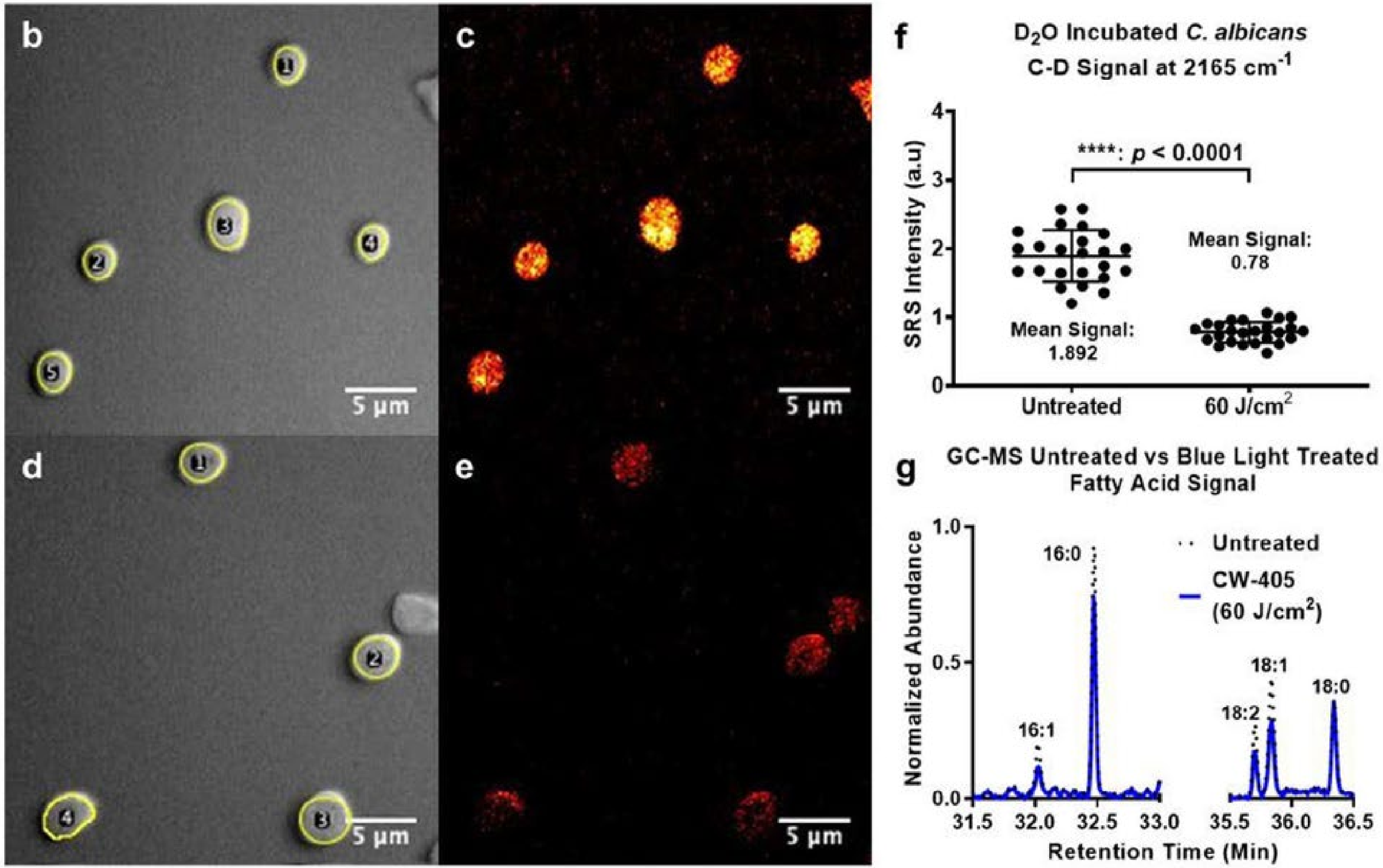
Advanced Science 2022, 9, 2104384

Blue light deactivation of catalase suppresses candida hyphae development



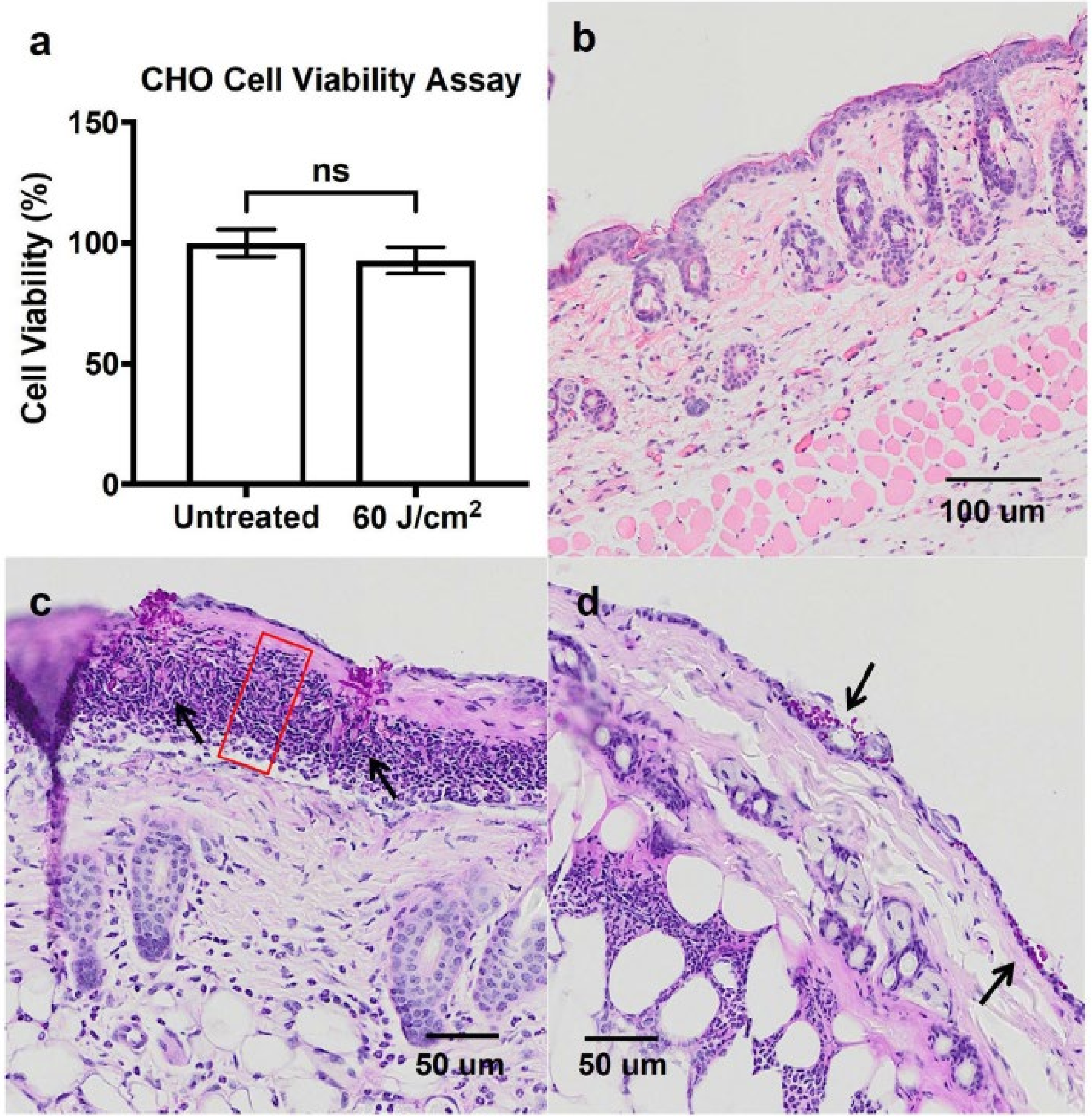
Photochemistry & Photobiology September 2022, DOI: 10.1111/php.13719

Catalase inactivation suppresses lipid metabolism



Photochemistry & Photobiology September 2022, DOI: 10.1111/php.13719

405 nm light alone suppresses fungal invasion in vivo



Photochemistry & Photobiology
September 2022, DOI: 10.1111/php.13719

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