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
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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:

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• 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
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FCOI: Vibronix Inc, Photothermal Spec Corp, Pulsethera
Color me bad: microbial pigments as virulence factors

George Y. Liu¹ and Victor Nizet²

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 ex pressed by microbial pathogens. (a) Staphylococchi, Staphylococcus aureus; (b) haematin in muratal haemocin or the Pseudomonas aeruginosa pigment; (c) violaxanthin, Chromobacterium violaceum; (d) granudene, Group B Streptococcus; (e) pyoverdin, Pseudomonas aeruginosa; (f) prastigxin, Serratia marcescens; (g) melanin, Cryptococcus neoformans

https://www.disassociated.com/2013/07/17/these-bacterial-artworks-just-might-go-viral/
Chromophore played an essential role in discovery of the first antibiotics in human history

http://broughttolife.science museum.org.uk/broughttolife/objects/display?id=11193
By 2050

Deaths from antimicrobial resistant infections and other causes in 2050

- Antimicrobial resistant infections: 10.0m
- Cancer: 8.2m
- Diabetes: 1.5m
- Diarrhoeal disease: 1.4m
- Road traffic accidents: 1.2m
- Measles: 130,000
- Cholera: 120,000
- Tetanus: 60,000

Figure adapted from CARBX annual report (2017).

HOT ZONE

by Ryan Maddox

Remember, Junior, say no to drugs.

Mr. and Mrs. MRSA

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

- Acinetobacter baumannii carbapenem-resistant
- Pseudomonas aeruginosa carbapenem-resistant
- Enterobacteriaceae carbapenem-resistant, ESBL-producing
- Enterococcus faecium vancomycin-resistant
- Staphylococcus aureus methicillin-resistant vancomycin-intermediate and resistant
- Helicobacter pylori clarithromycin-resistant
- Campylobacter spp., fluoroquinolone-resistant
- Salmonella fluoroquinolone-resistant
- Neisseria gonorrhoeae cephalosporin-resistant fluoroquinolone-resistant

Source: WHO

33 year gap
Nearly every antibiotic in use today is based on a discovery made more than 33 years ago. (clampycin in 1984)

55 year gap
for Gram-negatives (quinolones in 1962)

No newly approved classes of antibiotics have been discovered since 1962 for the most dangerous types of bacteria - Gram negatives.
No new classes at all discovered after 1964.

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].

  - Naftifine-treated: $\tau_2 = 0.16 \pm 0.07 \text{ s}$
  - MRSA: $\tau_2 = 0.39 \pm 0.07 \text{ s}$

- **CrtM mutant**: *S. aureus* with a mutation on dehydrosqualene synthase which is responsible for STX biosynthesis[2].
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,
- \( \tau_1 \) constant for first-order bleaching
- \( \tau_2 \) constant for 2nd-order bleaching

\( \tau_1 = \infty \)
\( \tau_2 = 0.16 \text{ s} \)

\[ R^2 = 0.99 \]
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

A

Retention time (min)

Normalized Abundance

- 0 min blue light
- 2.5 min blue light
- 5 min blue light
- 10 min blue light

m/z = 841.5 ([M+Na+]^+)

B

Retention time (min)

Normalized Abundance

- 10 min blue light
- 5 min blue light
- 2.5 min blue light
- 0 min blue light

m/z = 643.5 ([M+H]^+)

C

Blue light

M_w = 818.5

M_w = 642.5
470-nm light degrades STX and disturbs the membrane

This finding opens a panel of new opportunities …
Blue light is unable to eradicate MRSA completely!

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


STX photolysis plus H$_2$O$_2$ synergistically kills MRSA

*** p<0.001, ns: not significant
STX Photolysis & H$_2$O$_2$ Effectively Eradicate:

a. Stationary phase

b. MRSA persisters

c & d. Biofilm
STX photolysis and ROS eradicate intracellular MRSA

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

STX photolysis and H$_2$O$_2$ eliminate MRSA-induced skin infection in vivo

Control
Fusidic acid
Blue light
Blue light + H$_2$O$_2$

Before treatment
After treatment
After sacrifice

Dilution factor

Control
Blue light + H$_2$O$_2$

log$_{10}$(CFU/mL)

*** $p<0.001$

STX photolysis and H$_2$O$_2$ eliminate MRSA-induced skin infection in vivo
SPIE Translational Research Award, Feb 2018

2018 Translational Research Best Paper Award
Presented to
PU-TING DONG

Sponsored by:
Beckman Laser Institute and Medical Clinic
Wellman Center for Photomedicine

2018 SPIE Photonics West
San Francisco, California USA
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*
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!

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.

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

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

1. Poration

SYTOX green (600 Da)

2. Increasing fluidity

3. PBP2a detachment

**Adv. Sci. 2020, 7:1903117**
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
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)

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**

Stationary-phase MRSA USA300; Intensity: 50 mW, 5 min;

Data: Mean ± standard deviation (N=3)
Photoinactivation of catalase inside *P. aeruginosa*

Data: Mean ± standard deviation (N=3)

Active catalase %

Stationary-phase *P. aeruginosa* 65 mW, 5 min

Untreated ns-410 nm ns-420 nm ns-430 nm ns-440 nm ns-450 nm ns-460 nm ns-480 nm
Mechanism: heme ring detachment

b Raman spectroscopy

- 410 nm exposed
- Untreated

Raman Intensity (a.u.)

Raman shift (cm$^{-1}$)

754 cm$^{-1}$
Nanosecond pulses are more effective than LED

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<td>Remaining catalase %</td>
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</table>
Photoinactivation of catalase sensitizes pathogenic bacteria to H$_2$O$_2$
Catalase mutant is highly sensitive to $\text{H}_2\text{O}_2$
Live/dead bacteria inside *P. aeruginosa* PAO-1 biofilms after different treatments

0 mM H₂O₂  |  6.6 mM H₂O₂  |  26.4 mM H₂O₂  |  52.8 mM H₂O₂
Photoinactivation of catalase assists macrophages to eliminate intracellular bacteria
Inactivation of catalase reduces *P. aeruginosa* burden in a *P. aeruginosa*-induced skin abrasion model.
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

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

Sebastian Jusuf et al, under review 2022
Fungi infections

Nosocomial bloodstream infections (BSI) are an important cause of infections in hospitalized patients. *Candida* infections account for the fourth most frequent infections in patients admitted to critical care units as evidenced in a multicenter study in 14 hospitals in the United States. A multicenter prospective observational study of several tertiary care centers in the United States revealed that *C. albicans* and *Candida* species isolated in BSI. Similar surveillance studies had reported rates of candidemia secondary to non-albicans *Candida* (NAC).
Photoinactivation of Catalase Sensitizes *Candida albicans* and *Candida auris* to ROS-Producing Agents and Immune Cells
Catalase photoinactivation and H2O2 synergistically reduce C. albicans burden in a mouse skin abrasion model.
Blue light deactivation of catalase suppresses candida hyphae development

Photochemistry & Photobiology  September 2022, DOI: 10.1111/php.13719
Catalase inactivation suppresses lipid metabolism
405 nm light alone suppresses fungal invasion in vivo

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Photochemistry & Photobiology
September 2022, DOI: 10.1111/php.13719
Cheng Ji-Xin Group:
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• Discovery
• Translation

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• NSF Chemical Imaging
• DoE
• DoD

Industry:
• Hologic
• Vibronix Inc
• Daylight Solutions
• Photothermal Spectroscopy Corp

Postdoc, PhD, visiting scholar positions available

Industrial partners: Vibronix Inc, Photothermal Spectroscopy Corp, Hologic, Pendar Technologies, Pulsethera, Daylight Solutions, Bruker (Germany), Refined Lasers (Germany), Axorus (Paris).

55 Faculty, $37 M grants in 2020
15 staff members