Fungal Cell Barriers: Nanoparticle-Mediated Photoporation for Effective RNA Delivery

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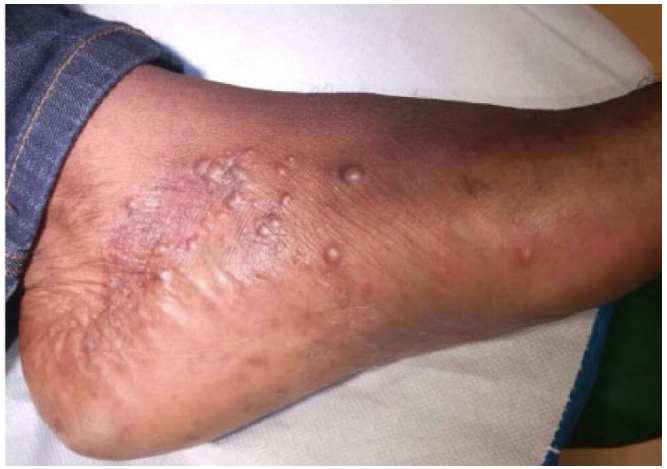
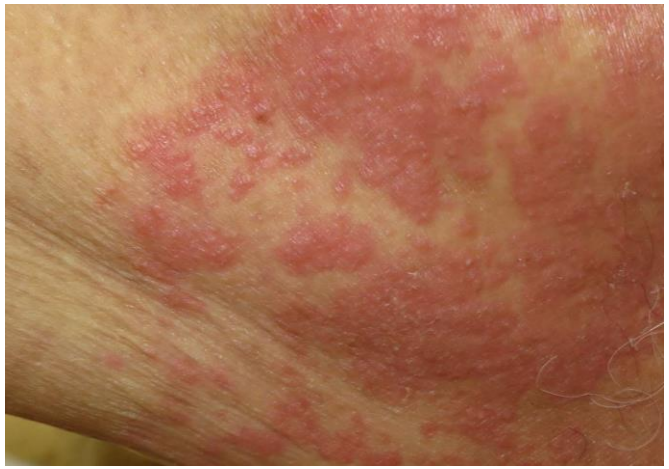
NEXT-GENERATION DELIVERY INNOVATIONS

Global Burden of Fungal Infections

- In the USA more than 75,000 hospitalizations and nearly 9 million outpatient visits occur every year for fungal diseases.
- Immunocompromise patients are particularly vulnerable to persistent fungal infections.
- WHO Fungal Priority Pathogens List

  Critical: *C. auris, C. albicans*
  High: *C. glabrata, C. parapsilopsis, C. tropicalis*
  Medium: *C. krusei*





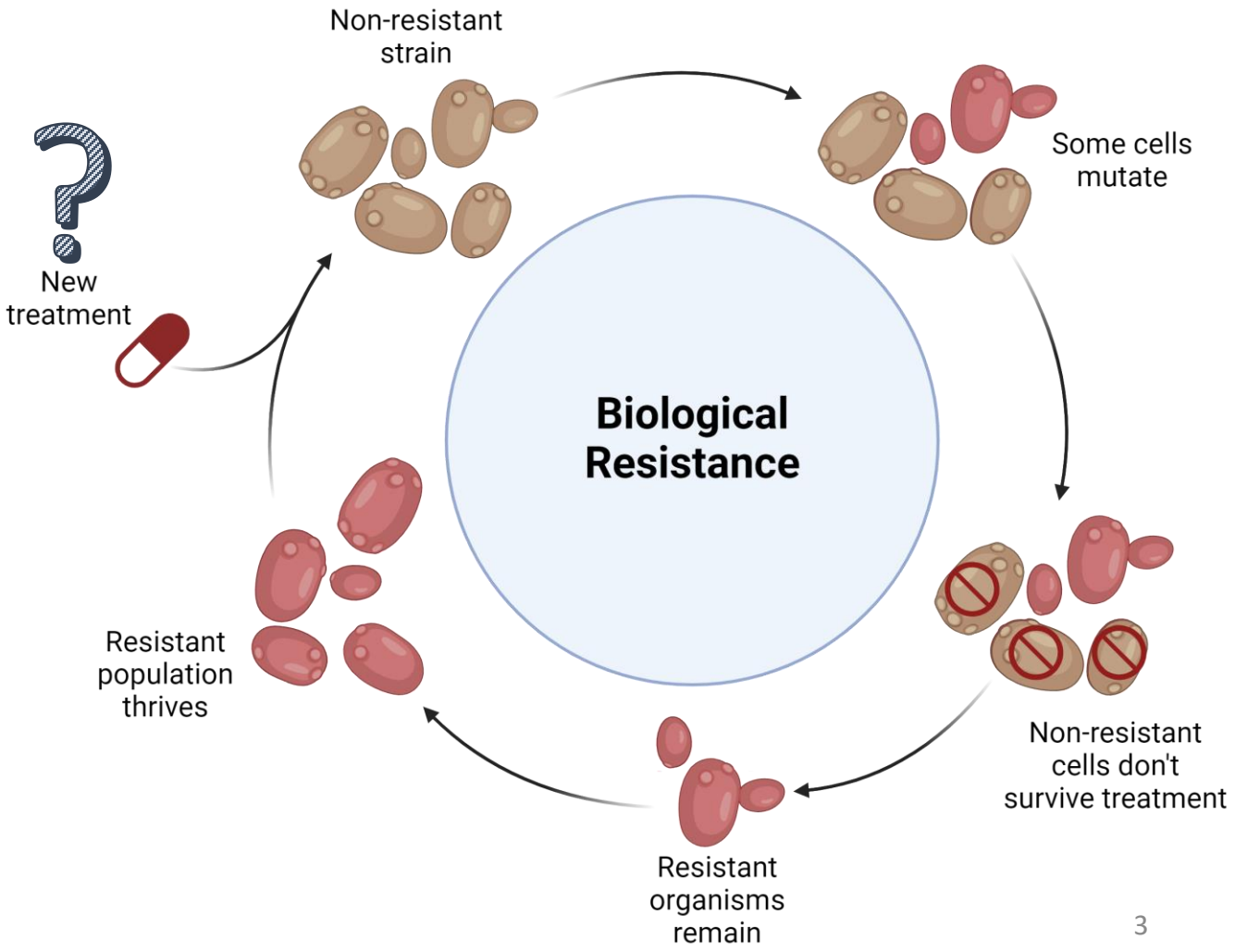
Sudbury et al. **2004** *Trends in Microbiology*

The cycle of biological resistance

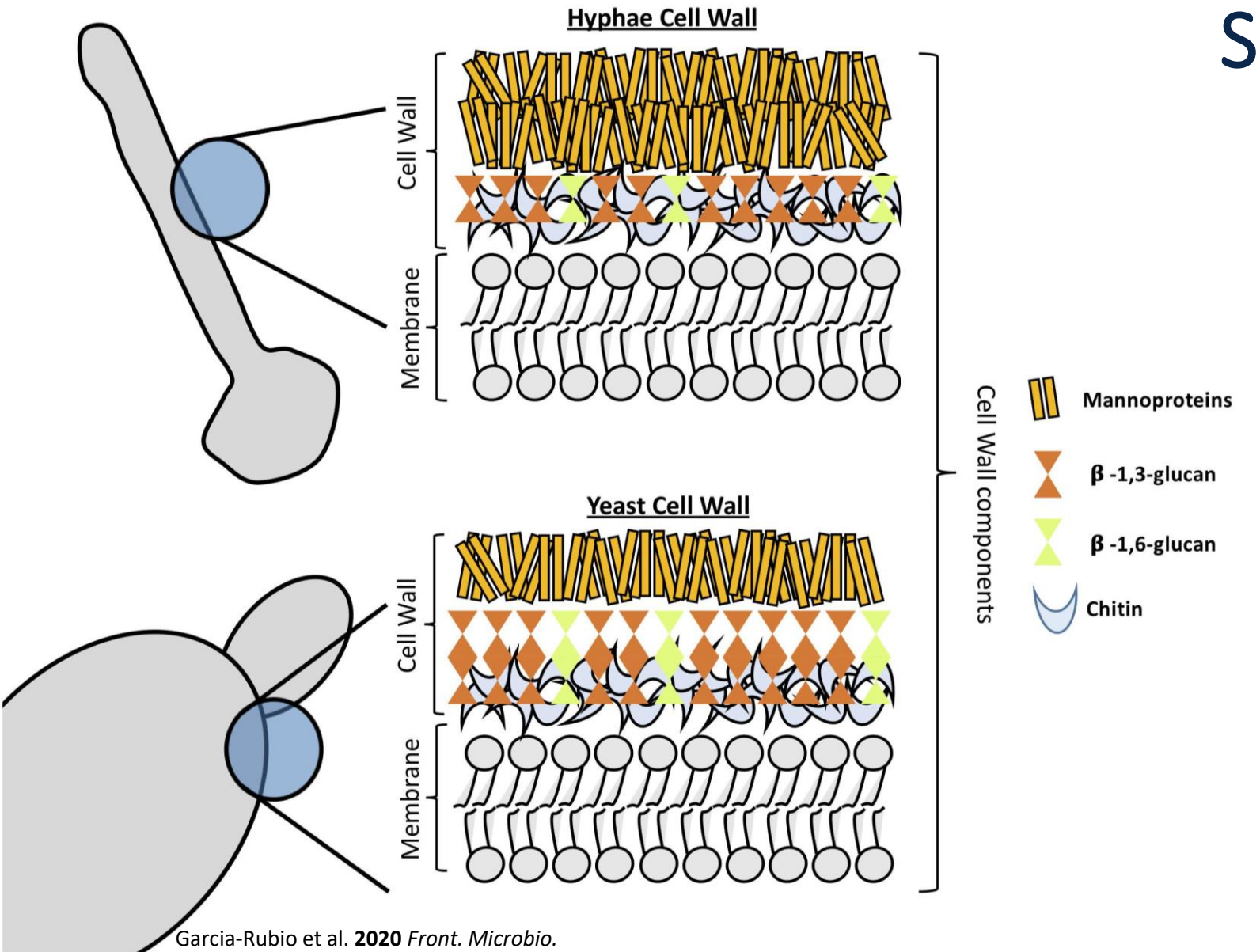
- Currently, there are only three major classes of antifungal drugs:

- Azoles
- Echinocandins
- Polyenes

- In contrast to antibiotics, **no new class of antifungals** has been developed in the **last 30 years**.



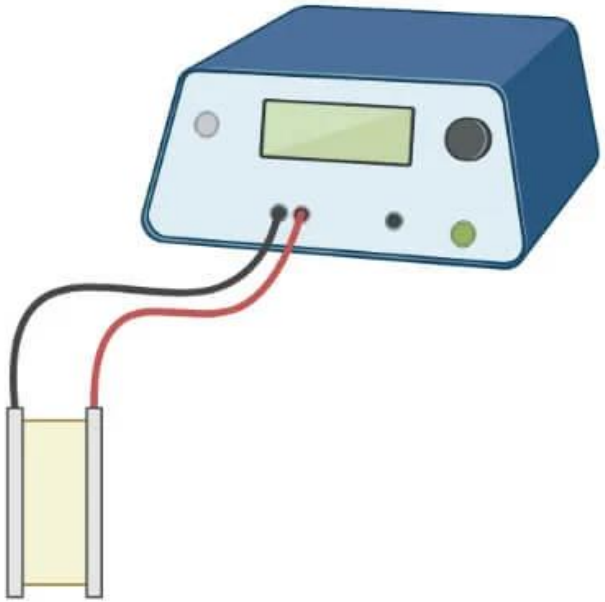
Cell Wall: Serve and Protect

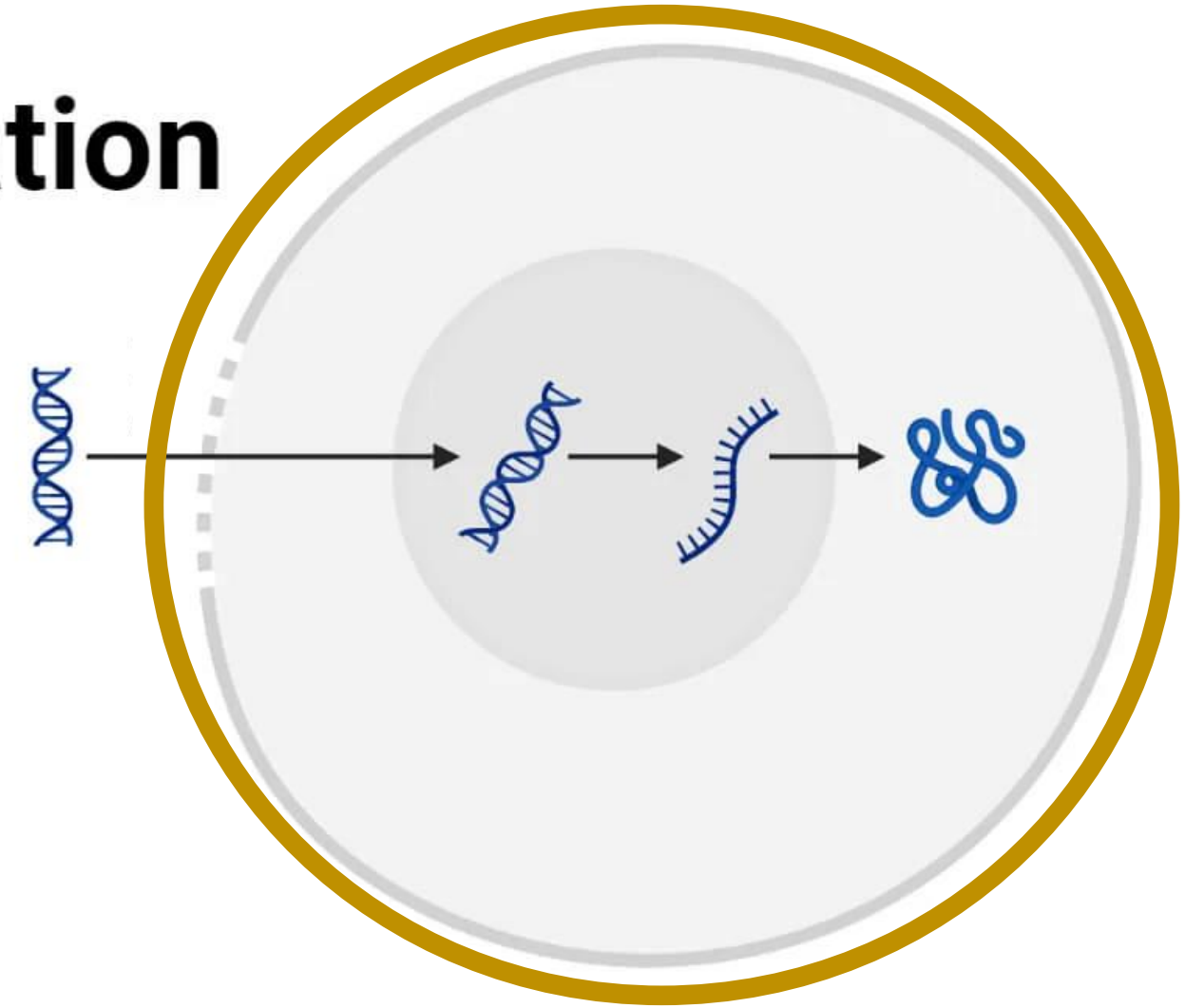


- Rigid barrier that protects the cell
- Not amenable to chemical delivery using nanoparticles!
- What about endocytosis?

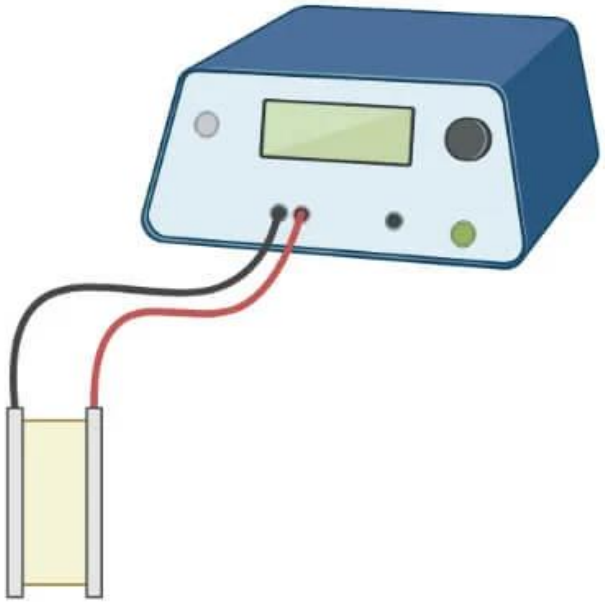
Garcia-Rubio et al. **2020** *Front. Microbio.*

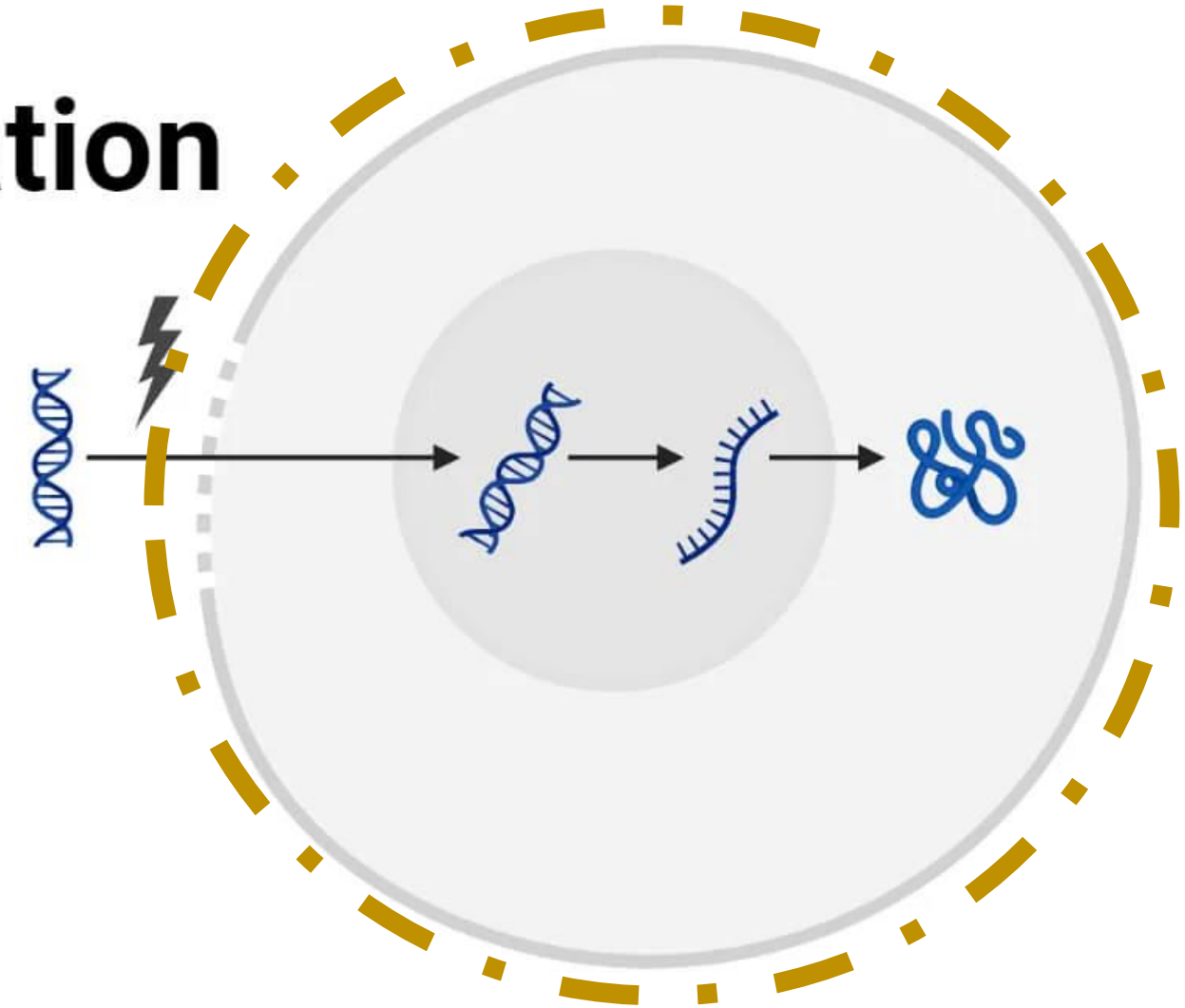
Electroporation





Electroporation



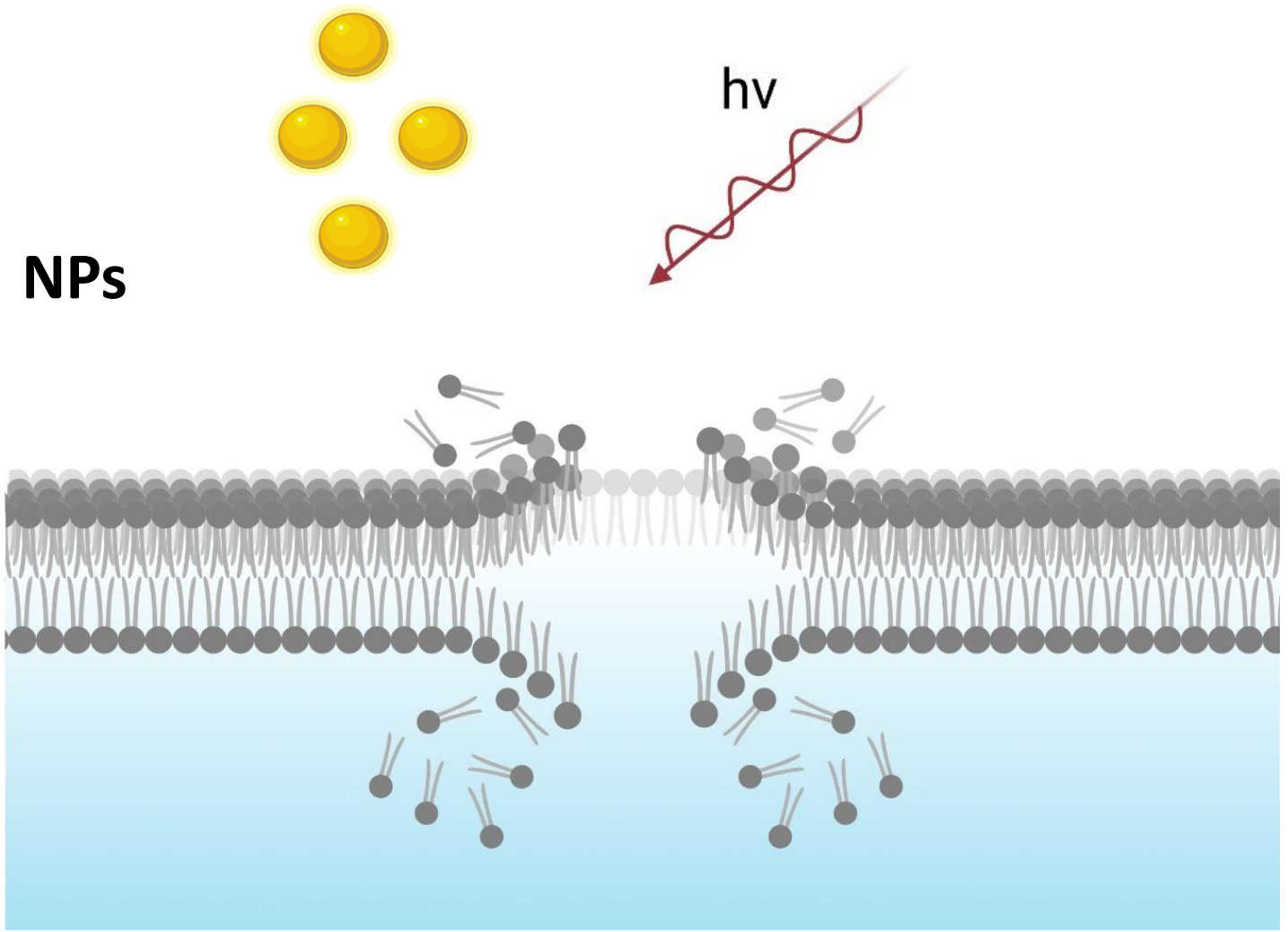


Has our field forgotten about fungal drug delivery?

Aim: To design a new approach for treating fungal infections that is simple, effective, and patient-friendly, using a novel antifungal and an innovative drug delivery strategy.

Photoporation

- **<u>Photoporation:</u>** the process of generating temporary pores in cells using lasers (pulsed).

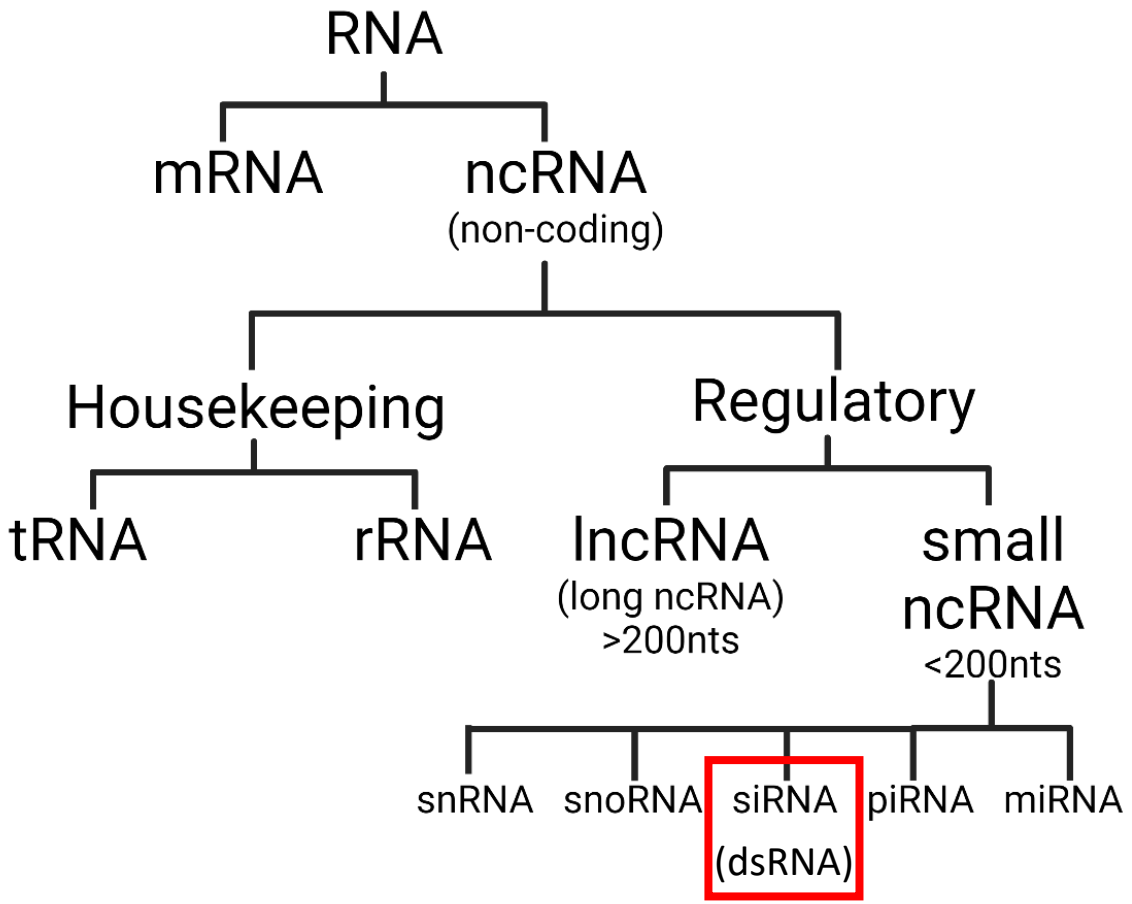


McGraw *et al.* **2020** *ACS Appl. Bio Mat.*, 3, 6167-6176

- Delivery Method 
- Novel Antifungal drug
- Organisms model

Alternative Strategy: Combating resistance through RNA

- Utilizes host machinery to cause or silence protein production
- Can be designed to be gene-specific to minimize off-target effects
- Multiple new therapies introduced over the last ~5 years (barely explored in fungal cells)



- Delivery Method 
- Novel Antifungal drug 
- Model Organism

Model organisms

- Saccharomyces cerevisiae (BY4741)

Non-pathogenic

Wealth of information available

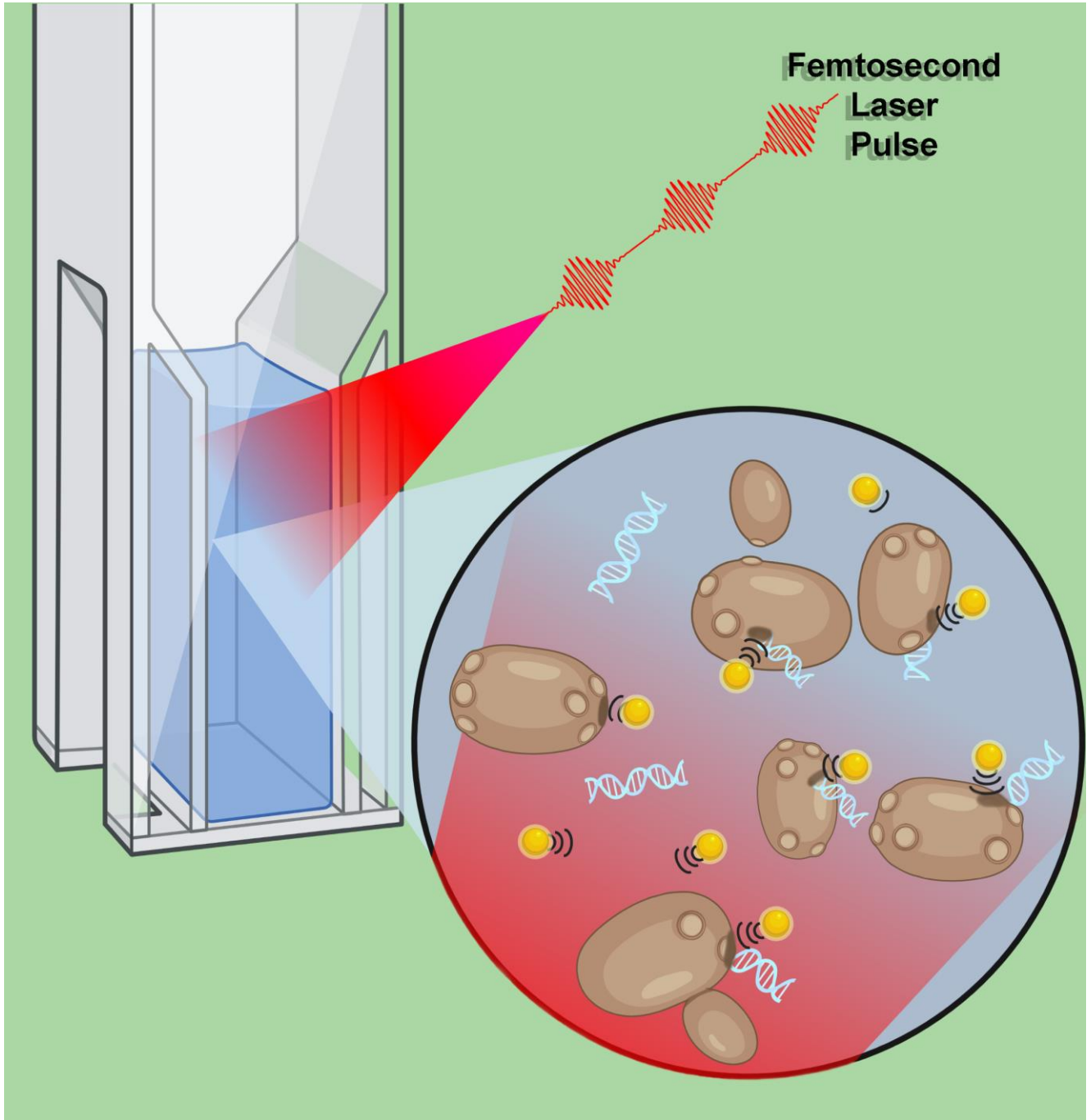
- *Candida albicans* (90028)
  - Clinically relevant
  - Well-studied
  - Safe…er

Roetzer et al. **2020** *Front. Microbio.*

- Delivery Method
- Novel Antifungal drug
- Model Organism

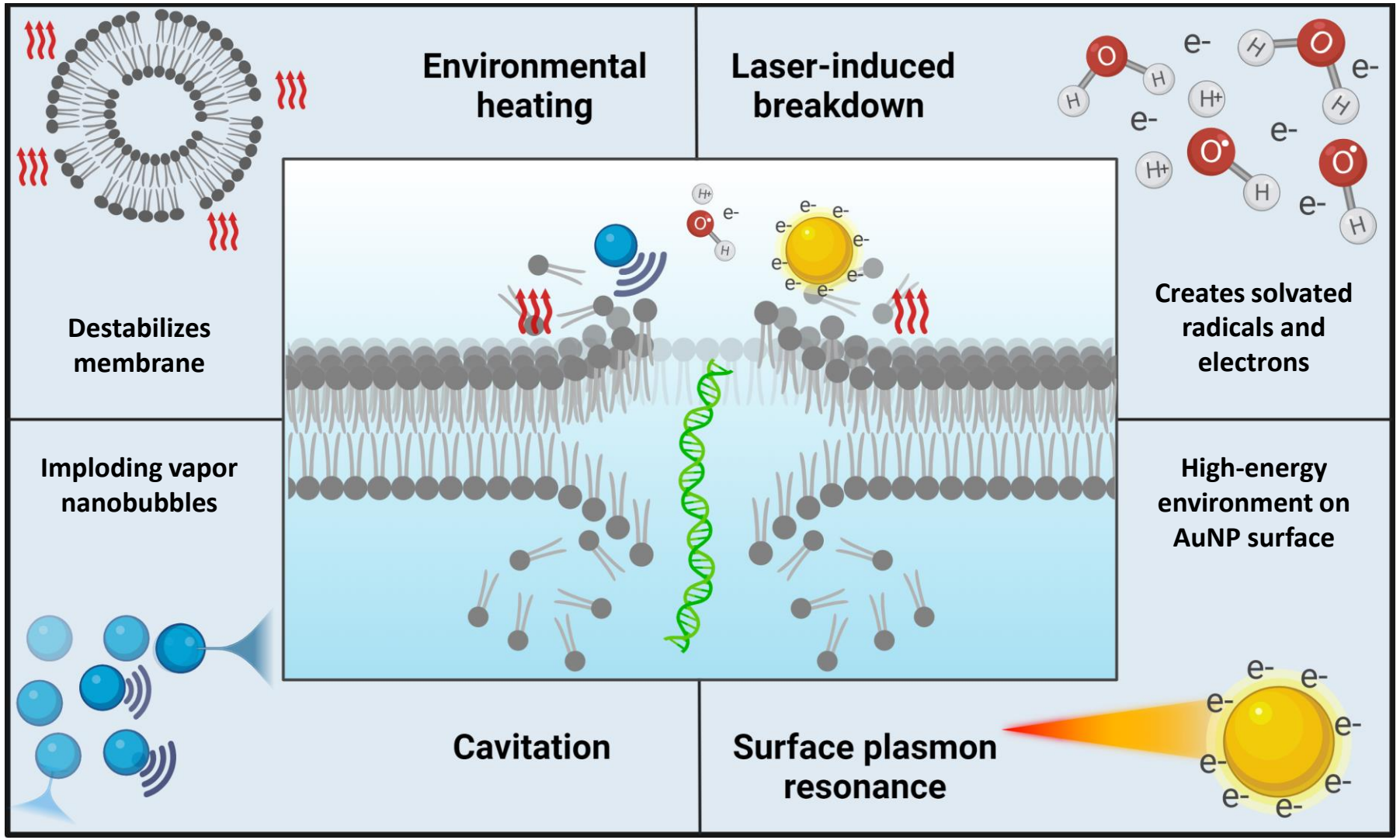
Nanoparticle Assisted Photoporation

- **<u>Nanoparticles can amplify photoporation effects.</u>**
- Delivering nucleic acids, <u>not</u> gold nanoparticles.
- First work in fungal cells



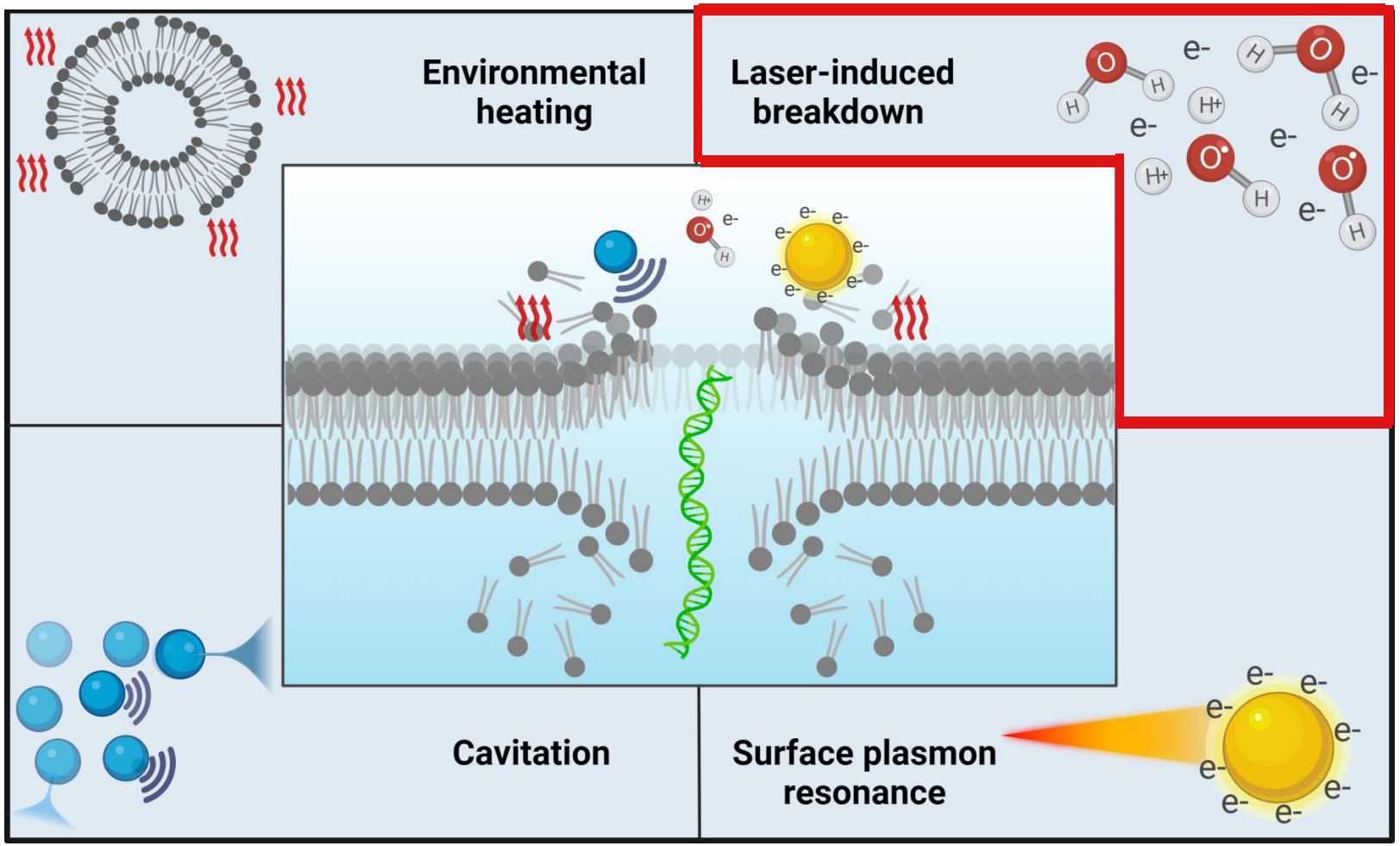
McGraw *et al.* **2020** *ACS Appl. Bio Mat.*, 3, 6167-6176

Photoporation mechanisms when nanoparticles are present



McGraw *et al.* **2024** *Nanoscale Adv.*, 6, 5007-5019

Photoporation mechanisms



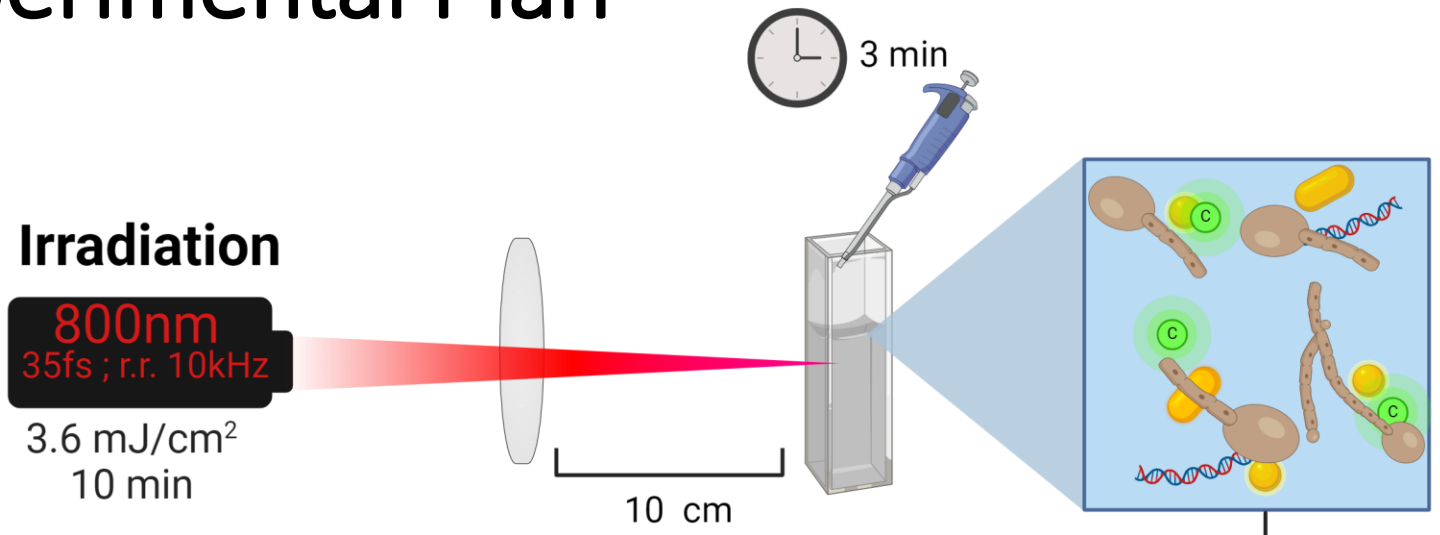
Laser-induced Breakdown:

$H_2O + h\nu \rightarrow H^+ + \bullet OH + e^-_{aq}$

- These reactive chemical species cascade into other effects!

McGraw *et al.* **2024** *Nanoscale Adv.*, 6, 5007-5019

Experimental Plan



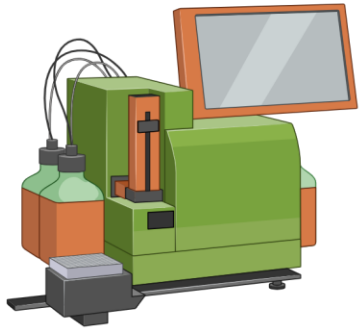
Irradiation Mix

-*S. cerevisiae* or *C. albicans*

-AuNR or AuNS

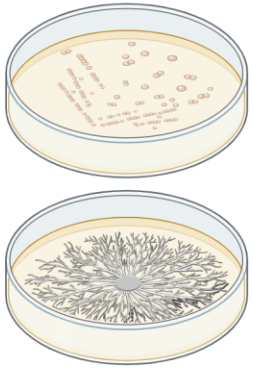
-Dye or dsRNA

Analysis

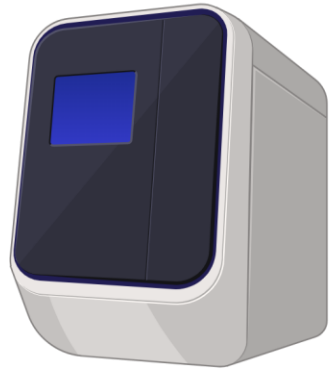


Flow Cytometry

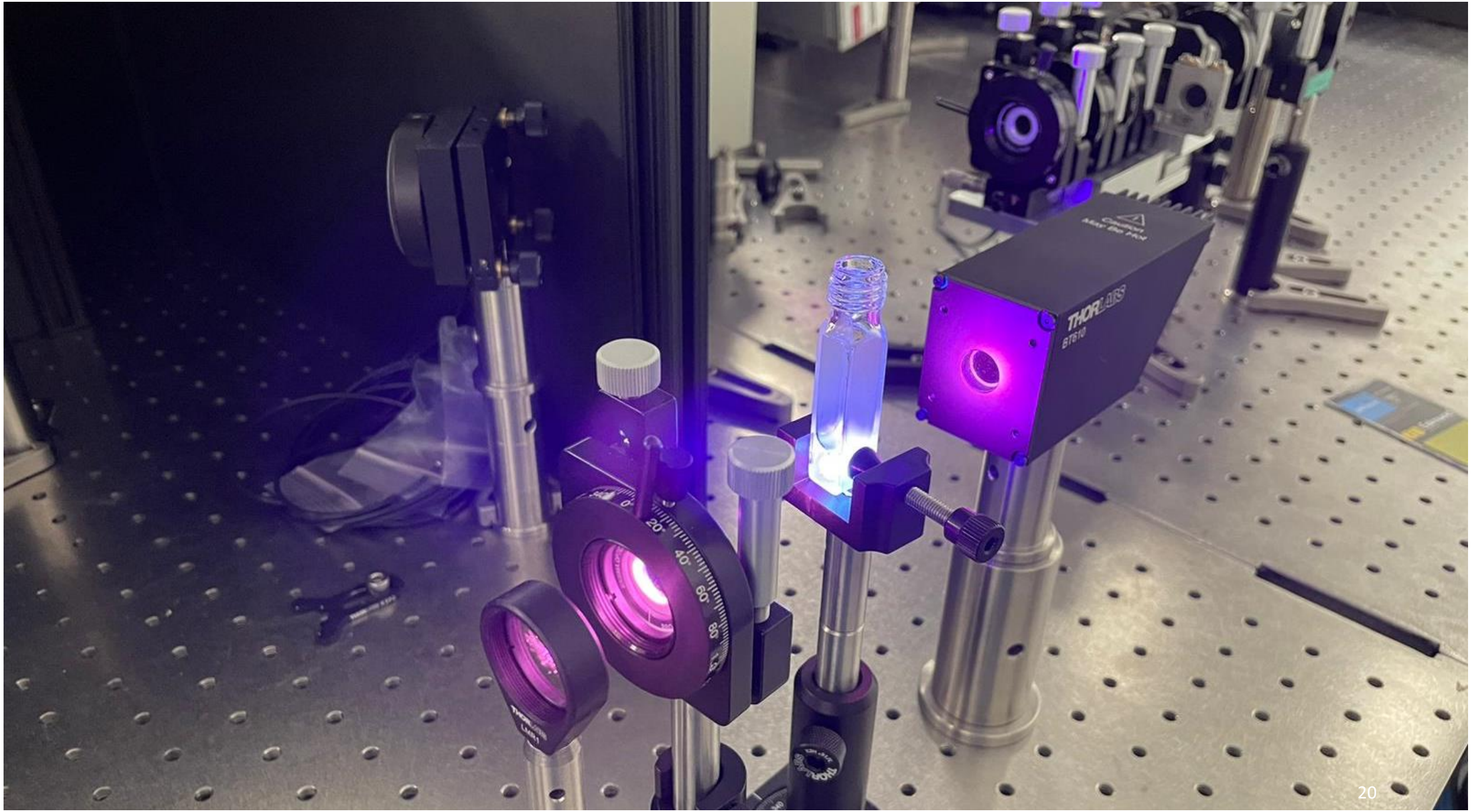
Microscopy



Colony Growth



RT-qPCR

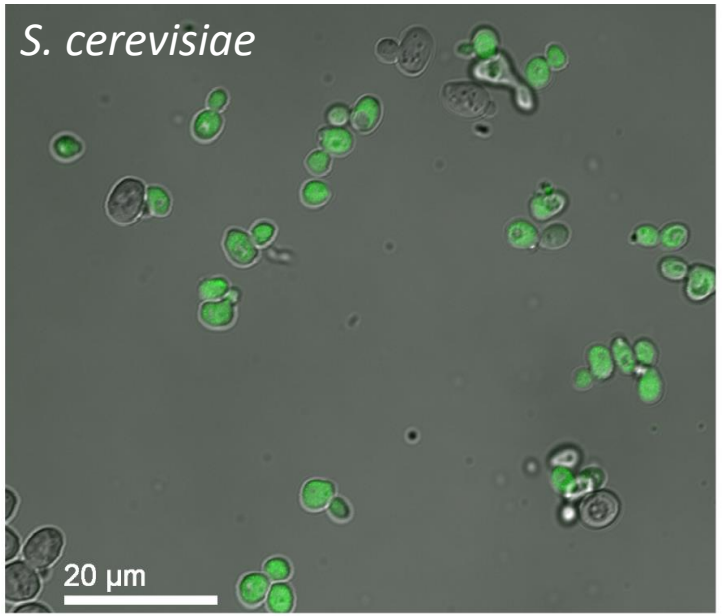

THORLABS
BTB10
20
40
60
80

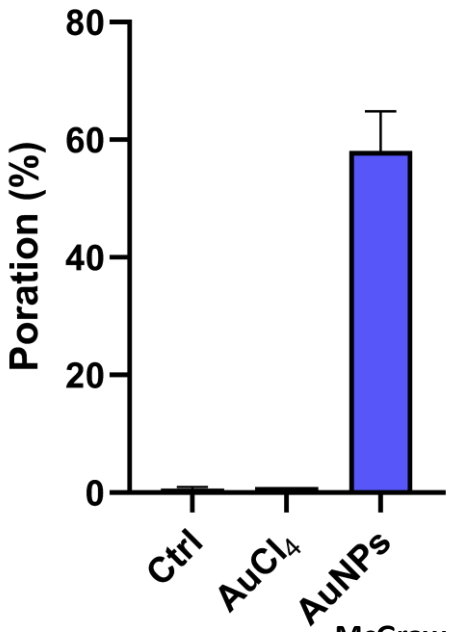
Poration Rate

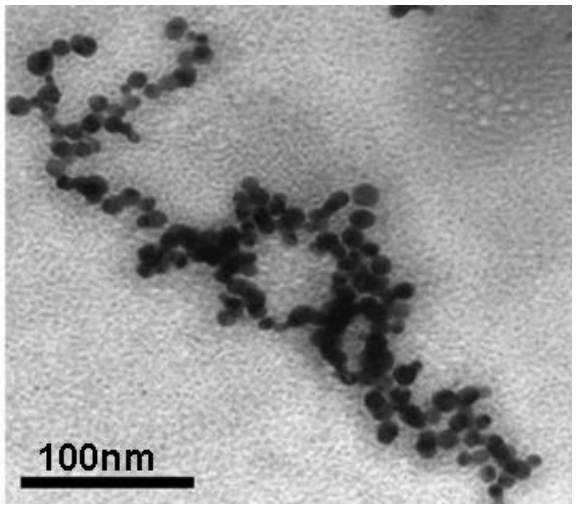
- Uptake of cell-impermeable fluorescent dye
- Quantified through flow cytometry
  Live, single, fluorescent cells
- Confirmed through microscopy

porations rates (aprox. 70%)

- AuNPs, not just Au, are needed for poration



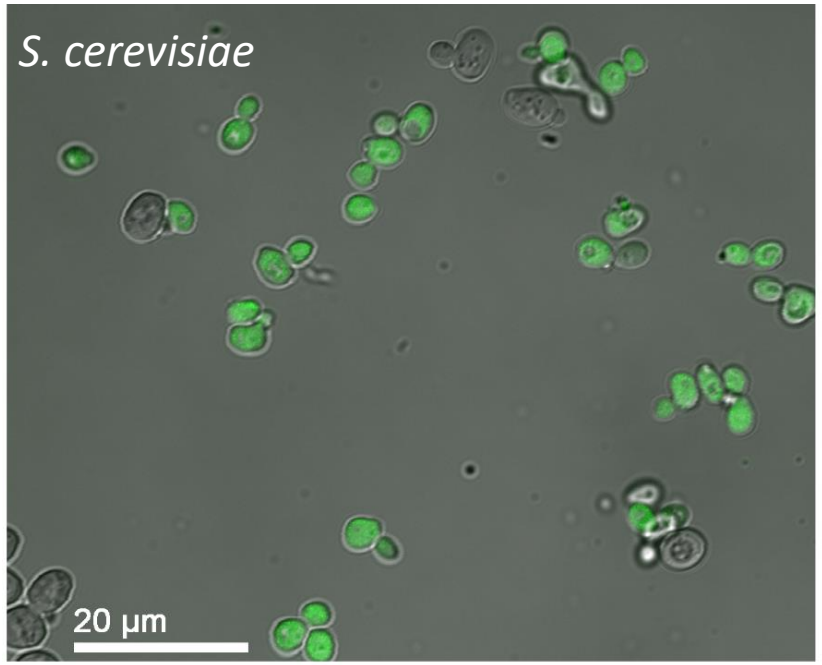


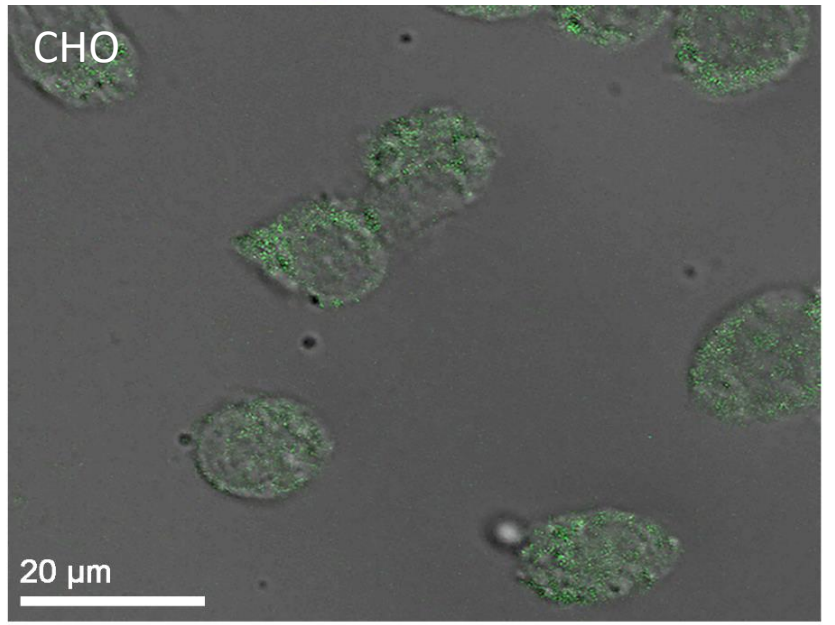


McGraw *et al.* **2020** *ACS Appl. Bio Mat.*, 3, 6167-6176

Mammalian analog

- Yeast cell wall helps preserve viability
- Effect on mammalian cells studied to understand how lacking a cell wall impacts poration
  - Chinese Hamster Ovary (CHO) cells
- “Best of both worlds” approach





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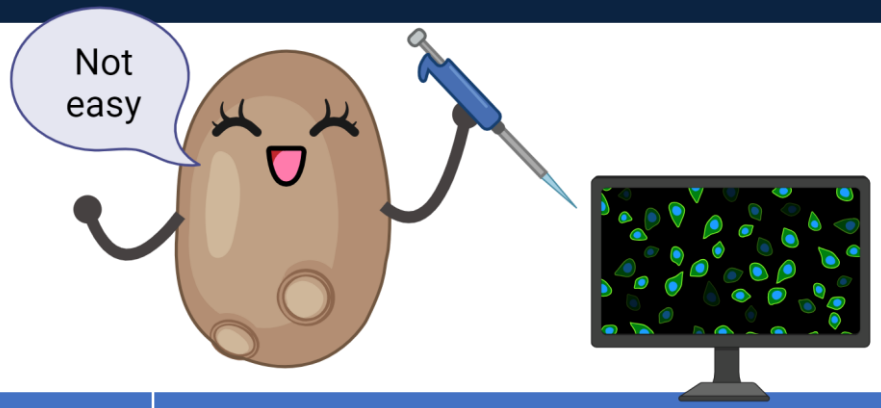
Summary of optimized experimental parameters

Focus	Variable	Tested
Laser Parameters	Irradiation time	5, 10, 20, 30 min
	Fluence (power)	0.8, 2.0, 2.8, 3.6, 4.4 mJ/cm^2
	Wavelength	400, 800 nm
AuNP Parameters	Shape	Spheres, Rods
	Size	10, 20, 50 nm
	Concentration	$8x10^{10}$, $4x10^{10}$, $0.8x10^{10}$ AuNP/mL

Final optimization conditions



Focus	Variable	Tested
Laser Parameters	Irradiation time	**10 min**
	Fluence (power)	**3.6 mJ/cm^2**
	Wavelength	**800 nm**
AuNP Parameters	Shape	**Spheres**
	Size	**50 nm**
	Concentration	**8x10^{10} AuNP/mL**

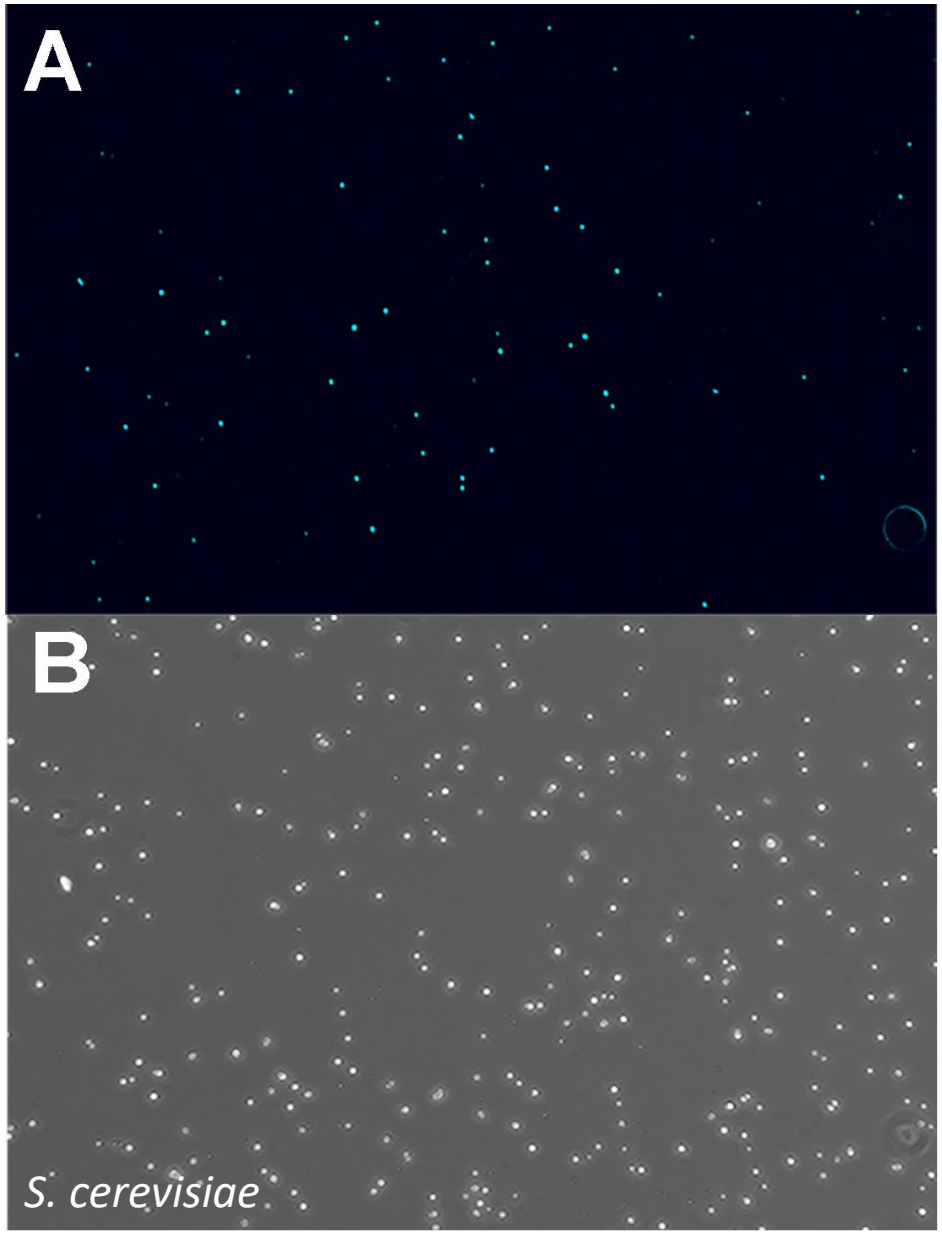
- Times: 4
- Fluences: 5
- Wavelengths: 2
- Shapes: 2
- Sizes: 3
- Concentrations: 3

→

- Years: 3
- Papers: 3
- Two funded grant applications

Delivery of Nucleic Acids

- Delivery of plasmid DNA tagged with BOBO-1 Iodide
- Fluorescence microscopy:
  - A) DAPI (bright blue) signal from porated cells (~40%)
  - B) Healthy morphology
- Successful delivery of a large molecule

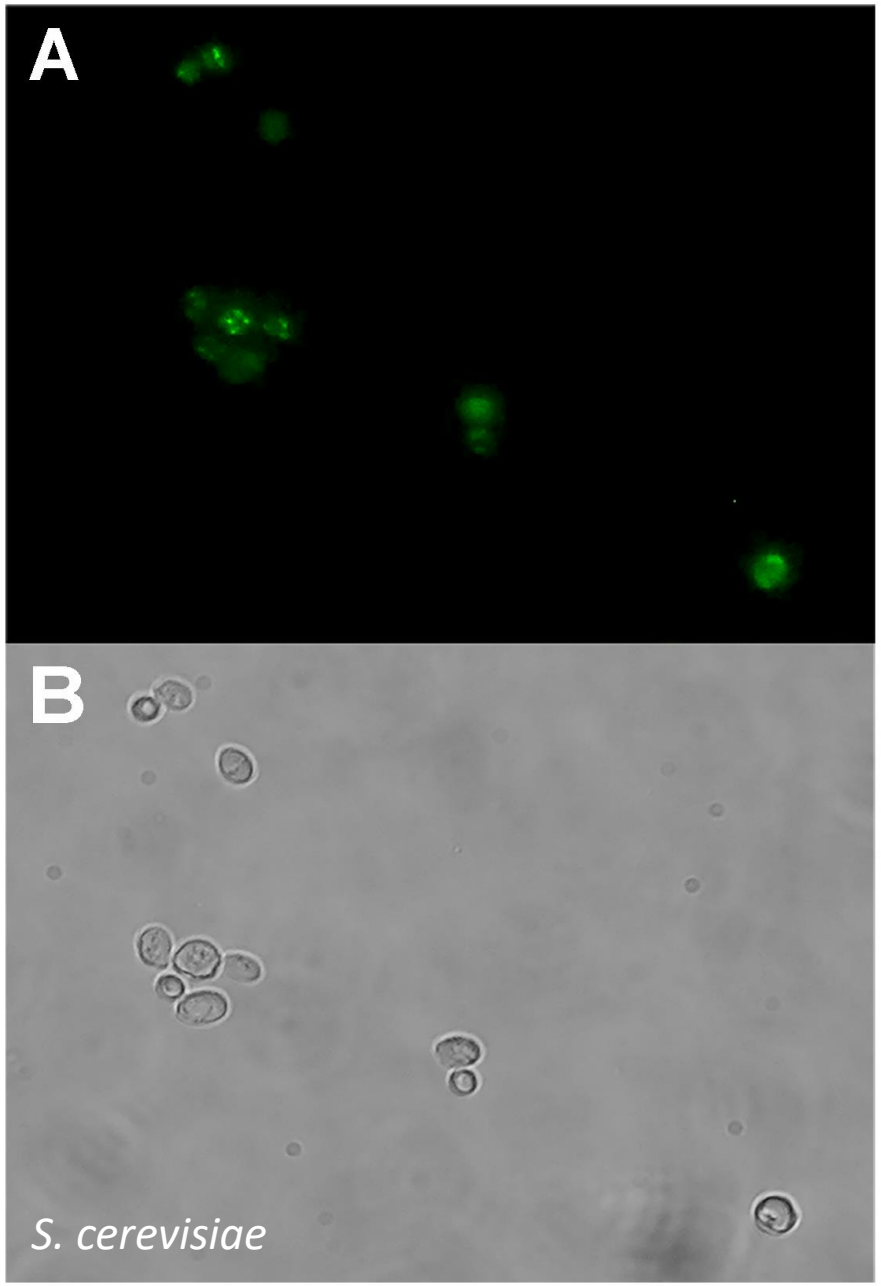


McGraw *et al.* **2020** *ACS Appl. Bio Mat.*, 3, 6167-6176

Nucleic acids remain functional

- Mitochondrial GFP (pYX142-mtGFP)
  - Selection marker: LEU2
- Porated cells were able to grow on leucine-dropout media

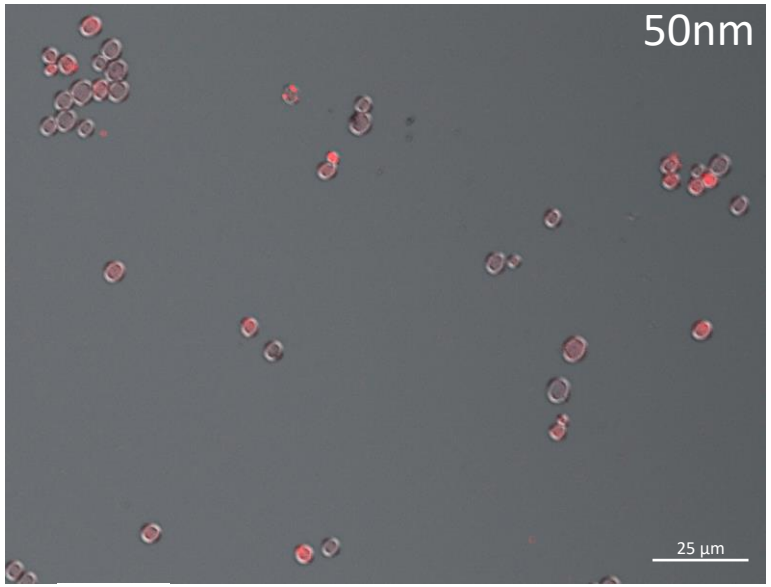
- **Suggests irradiation doesn't damage plasmid integrity**

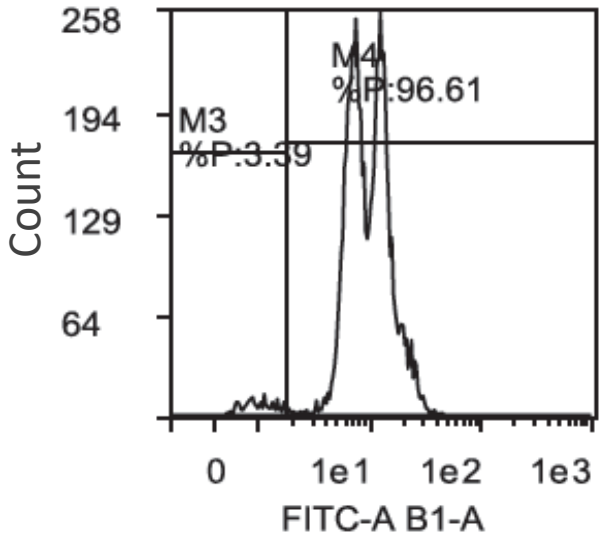


McGraw *et al.* **2020** *ACS Appl. Bio Mat.*, 3, 6167-6176

Photoporation of *C. albicans* with ATTO-siRNA

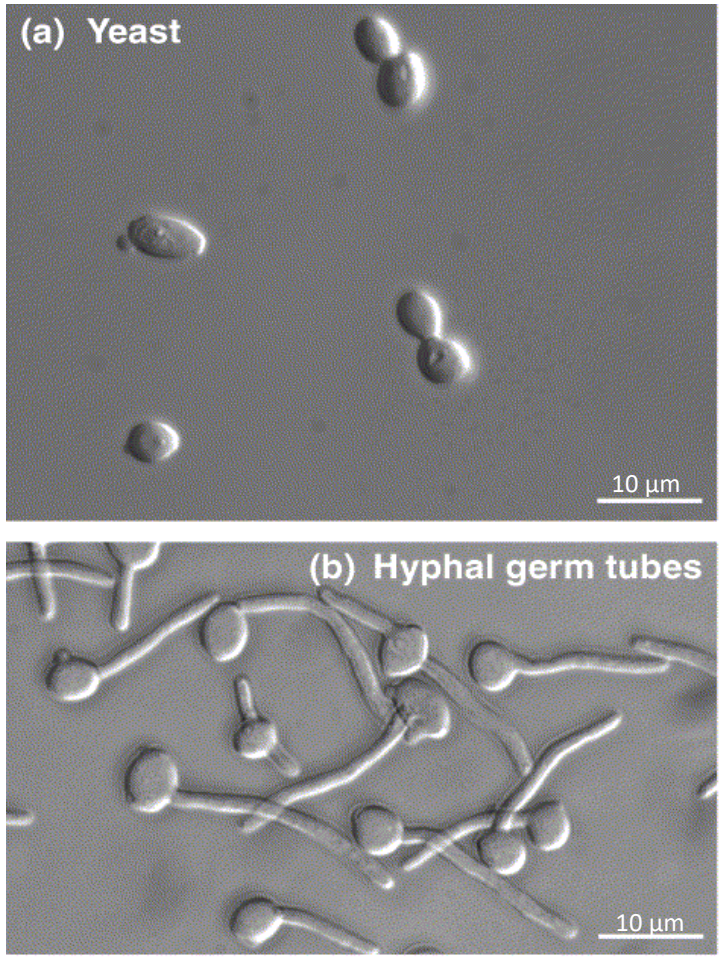
- Slightly different cell wall composition
- Concentration-dependent delivery
  - dsRNA (15 ng/µL): ~90%





Gene of Interest: Enhanced Filamentous Growth Factor 1

- ***EFG1*** plays a key role in the complicated filamentation process

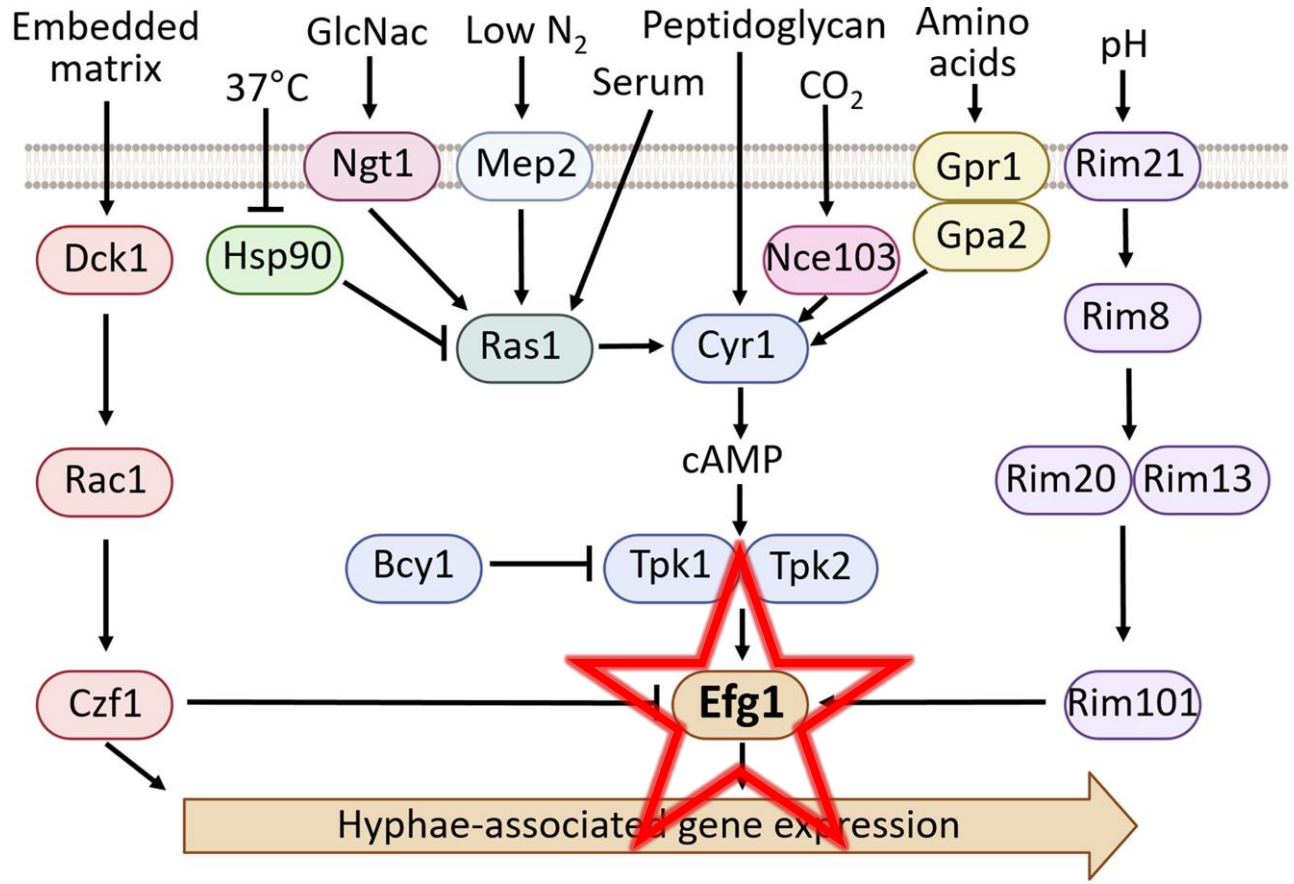


Glazier. 2022. *Front Cell Microbio.* 12:855229

Gene of Interest: Enhanced Filamentous Growth Factor 1

- *EFG1* plays a key role in the complicated filamentation process

- Our conditions:
  - 37 °C
  - Serum

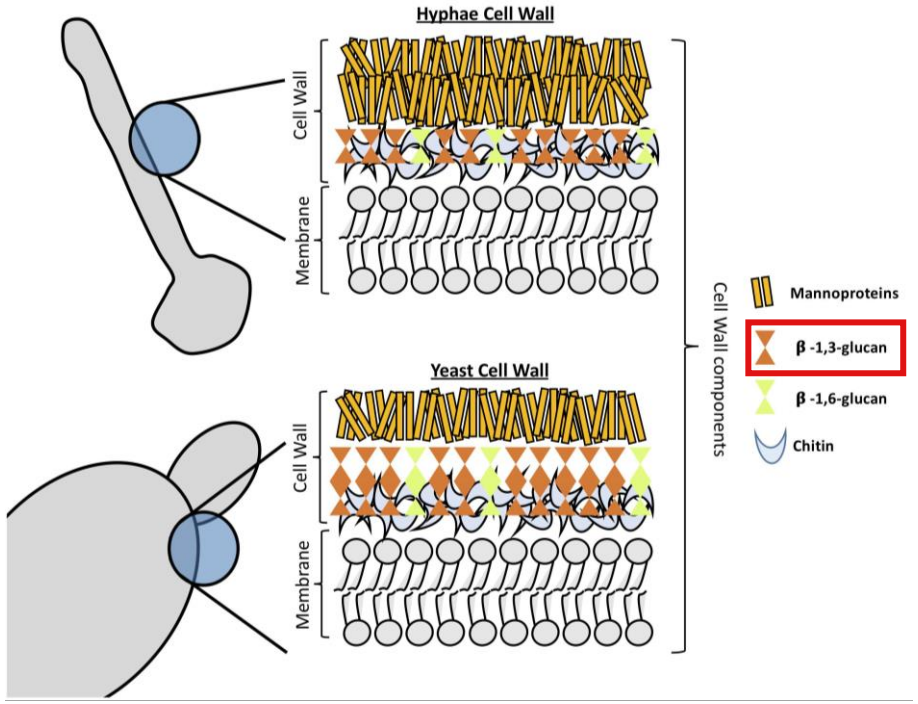


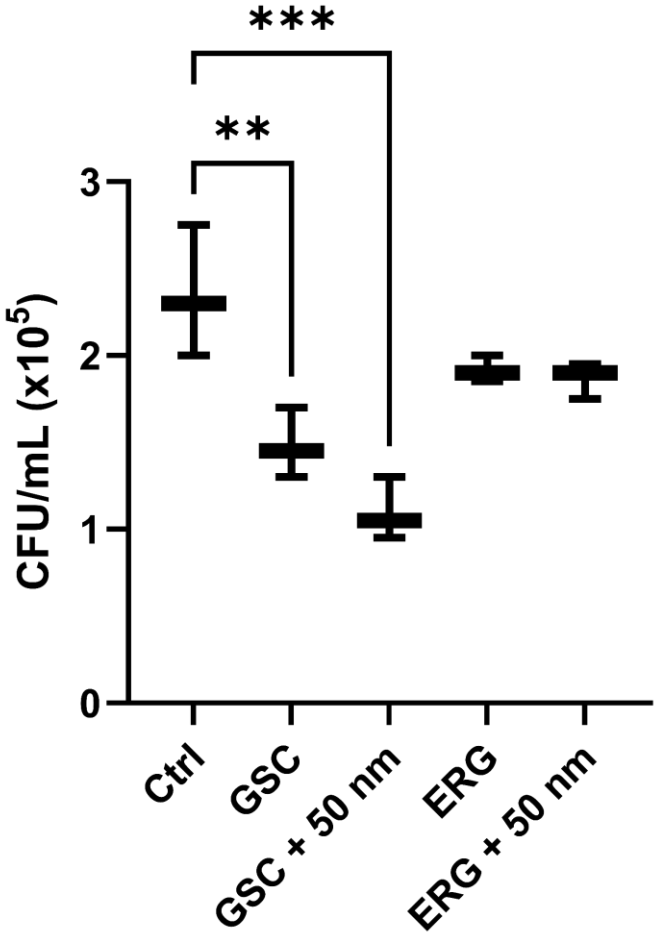
Glazier. 2022. *Front Cell Microbio.* 12:855229

Knockdown of essential cell wall/membrane components

- *ERG1:*
  - Membrane fluidity and integrity

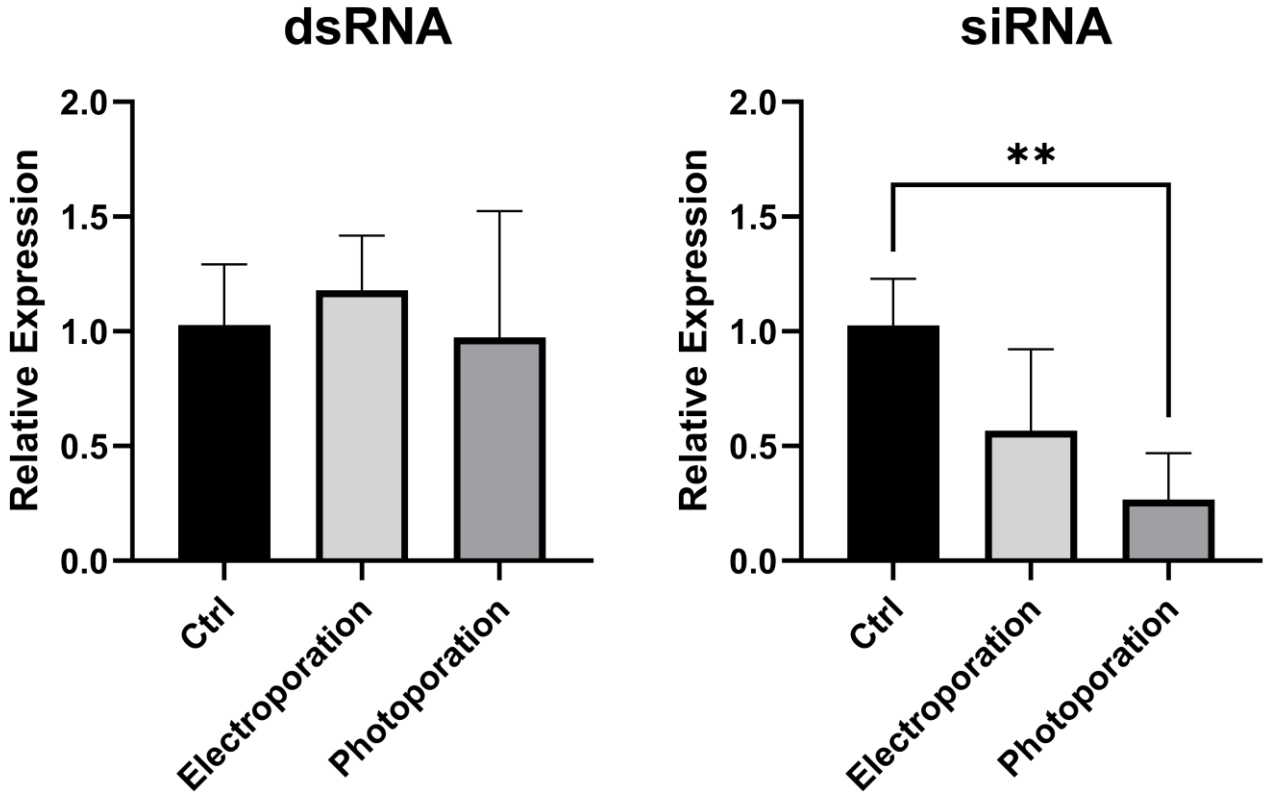
- *GSC1*: β-1,3-glucan synthase





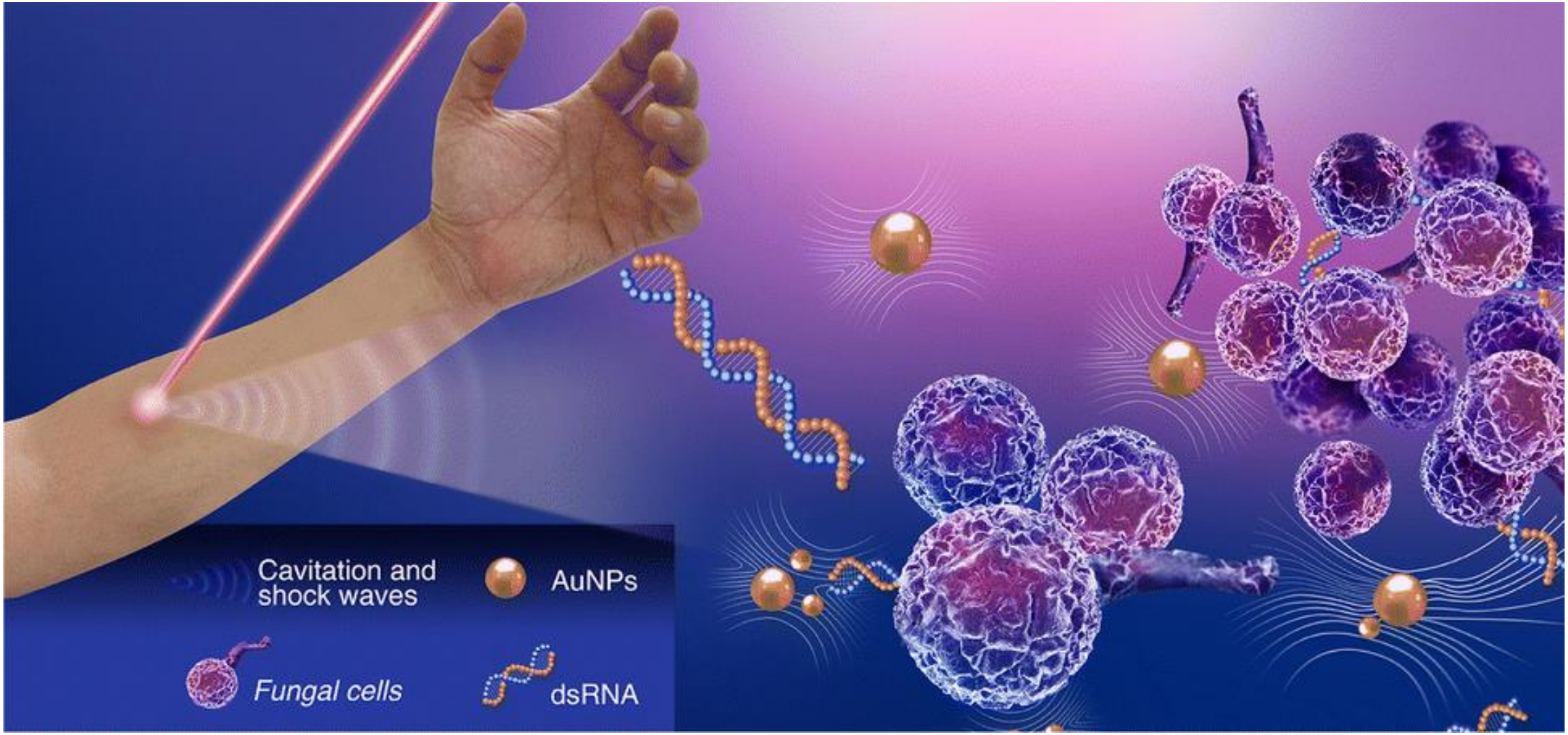
Delivery of siRNA is more effective for *EFG1* knockdown

- First comparison of dsRNA and siRNA
- More effective than electroporation



Sabita Gyawali
Dr. Jeffery Coleman

(**) $p < 0.002$
(ns) $p > 0.12$ (ANOVA)


Cavitation and shock waves
AuNPs
Fungal cells
dsRNA

Final conclusions

- Successfully validated the use of **dsRNA** and **siRNA** as gene silencing tools in *Candida albicans*.
- **Photoporation** was used for the **first time in fungal cells** as a delivery method for siRNA.
- Results support the potential of RNAi-based strategies for **functional genomics** and **antifungal research** in *C. albicans*.

What's next?

Thank you!

Questions?

Dr. Adriana Avila Flores
Collin Wall
Aanchal Bhatia
Maede Fekri
Chris Deveau
Dr. Nitish Kunte

Dr. Erin McGraw

Collaborators:
Dr. Guillaume Laurent (Physics)
Dr. Jeff Coleman (Plant pathology)
Dr. G. Mills (Chemistry)

Figures made using bioRender