# Optimization of spirulina for biomanufacturing and the delivery of protein therapeutics

LUMEN BIOSCIENCE

**Mark Heinnickel**
**Principal Scientist**

Agenda:

1. Introduction to Lumen Bioscience

2. Therapeutic Optimization

3. Recent Application

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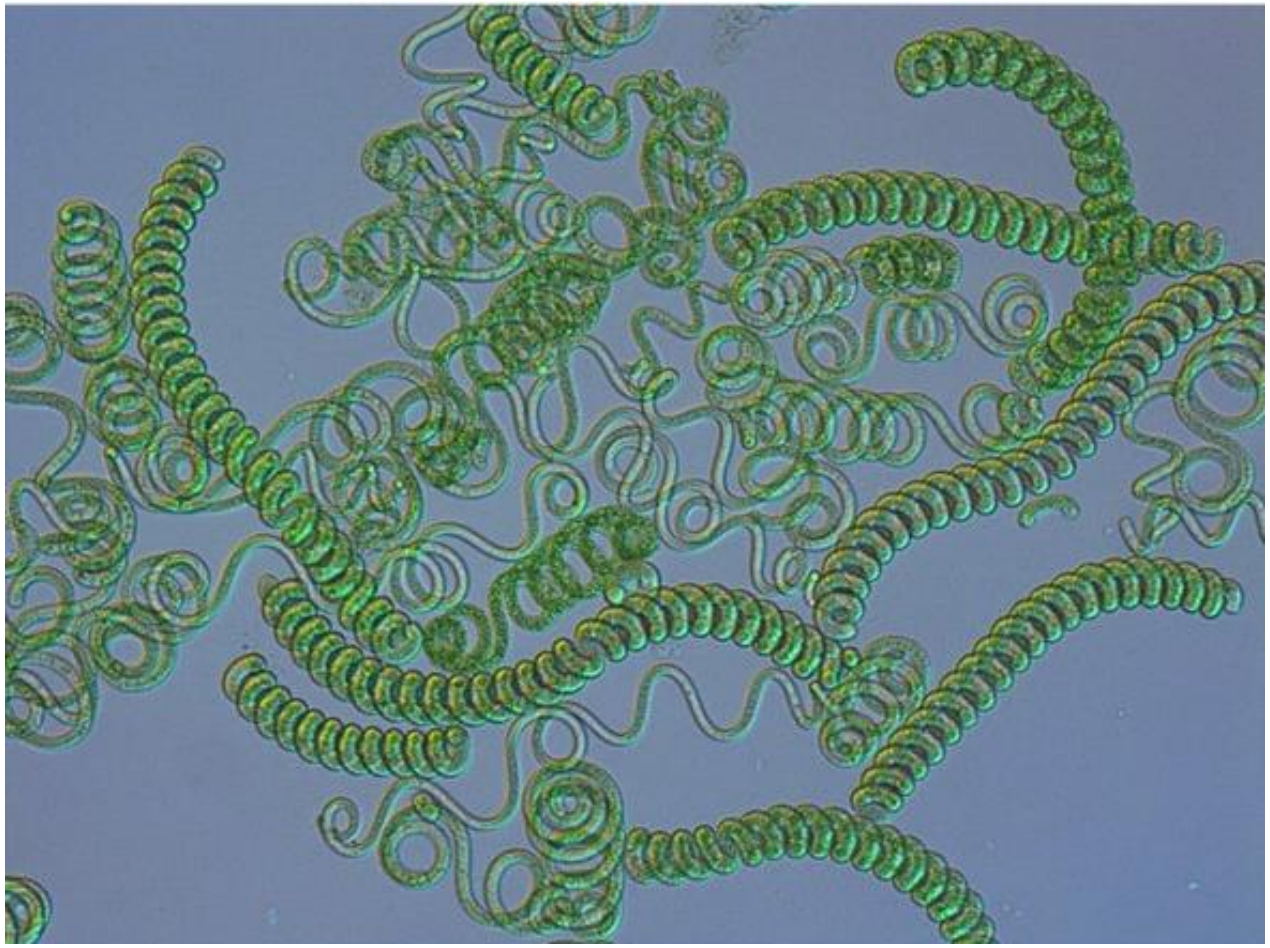
# Spirulina is cultivated globally as a food

# Spirulina: a new biotechnology platform



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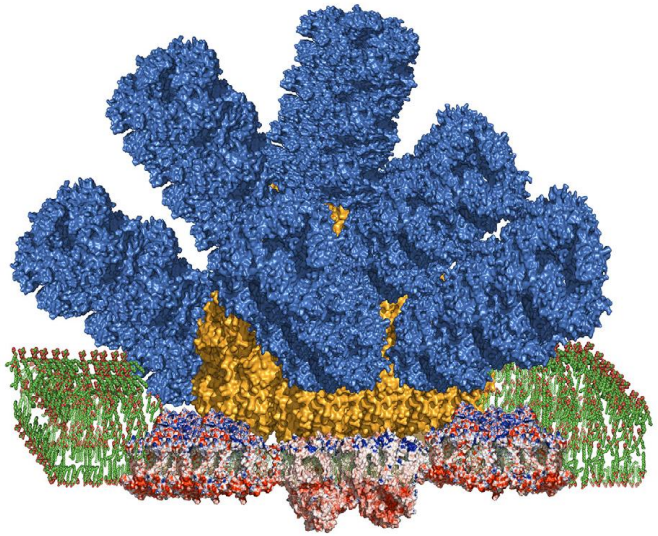
# Cyanobacteria are excellent at producing specific polypeptides at high levels

| Complex | Function | Protein(s) | %AFDW | Organism |
|---|---|---|---|---|
| Phycobilisomes | Light harvesting | CpcA/CpcB | 24% | *Synechococcus elongatus* |
| RubisCO | Carbon Fixation | RbcS/RbcL | 3% | Global Average, marine phytoplankton |

**Image of phycobilisome/reaction center supercomplex**



*Blankenship. (2015) PNAS 112 p 13751*

*Myers and Kratz (1955) J. Gen Physiol 39 pp 11*
*Bar-On and Milo (2019) Proc Natl Acad Sci 116 pp 4738*

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# Spirulina genetic engineering solved

(12) **United States Patent**
**Takeuchi et al.**

(10) **Patent No.: US 10,131,870 B2**
(45) **Date of Patent: Nov. 20, 2018**

(54) **TARGETED MUTAGENESIS IN *SPIRULINA***

(71) Applicant: **Lumen Bioscience, Inc.**, Seattle, WA (US)

(72) Inventors: **Ryo Takeuchi**, Seattle, WA (US); **James Roberts**, Seattle, WA (US)

(73) Assignee: **LUMEN BIOSCIENCE, INC.**, Seattle, WA (US)

| | | |
|---|---|---|
| WO | WO-2009/098089 A2 | 8/2009 |
| WO | WO-2010/048568 | 4/2010 |
| WO | WO-2010/075440 | 7/2010 |
| WO | WO-2012/087963 | 6/2012 |
| WO | WO-2012/087982 | 6/2012 |
| WO | WO-2013/116517 | 8/2013 |
| WO | WO-2014/164232 | 10/2014 |
| WO | WO-2014/164566 | 10/2014 |

OTHER PUBLICATIONS

Ballicora et al, "ADP-Glucose Pyrophosphorylase, a Regulatory


nature biotechnology

ARTICLES

https://doi.org/10.1038/s41587-022-01249-7

Check for updates

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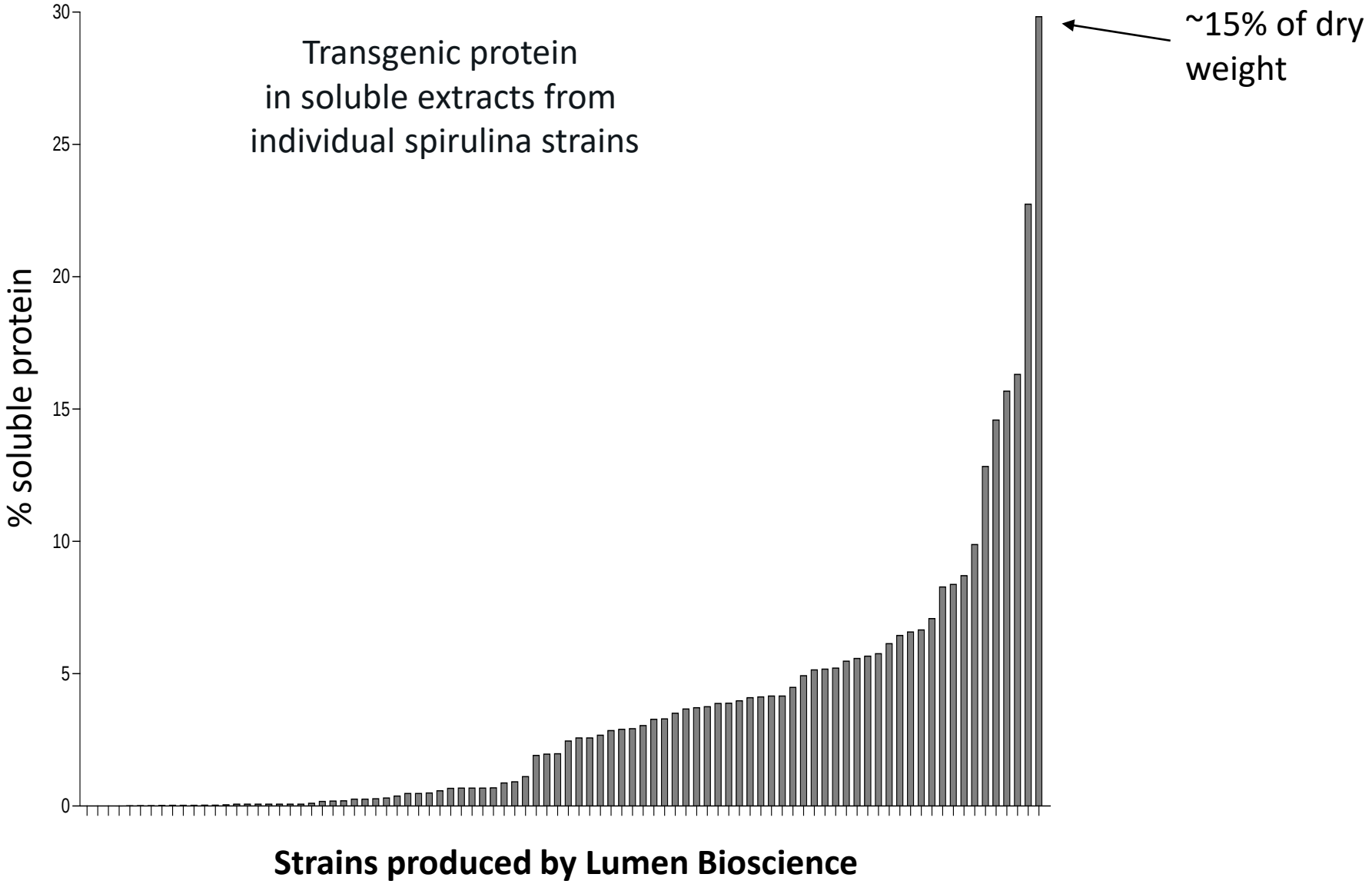
## Development of spirulina for the manufacture and oral delivery of protein therapeutics

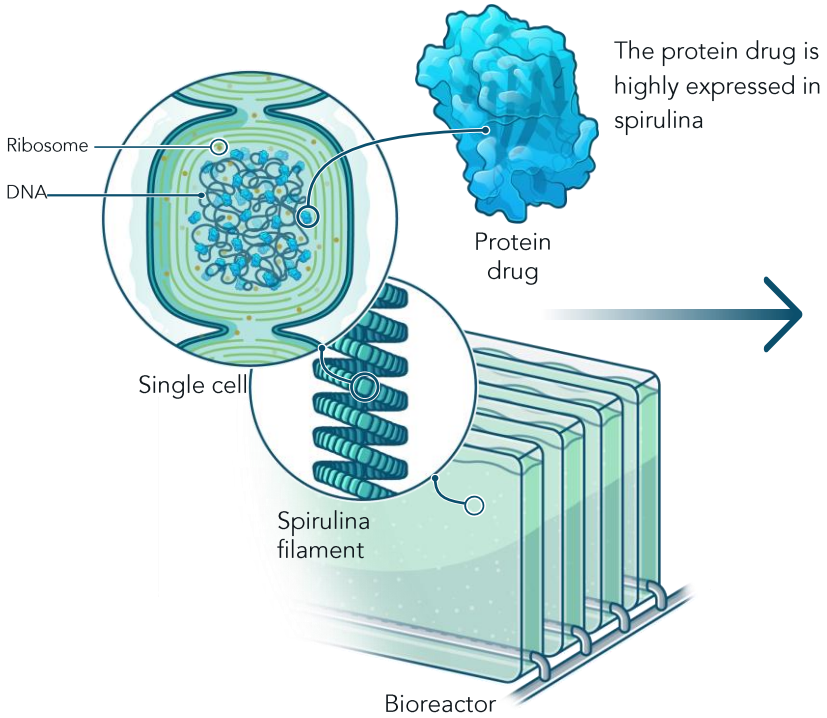
# Recent candidate strains show high productivity



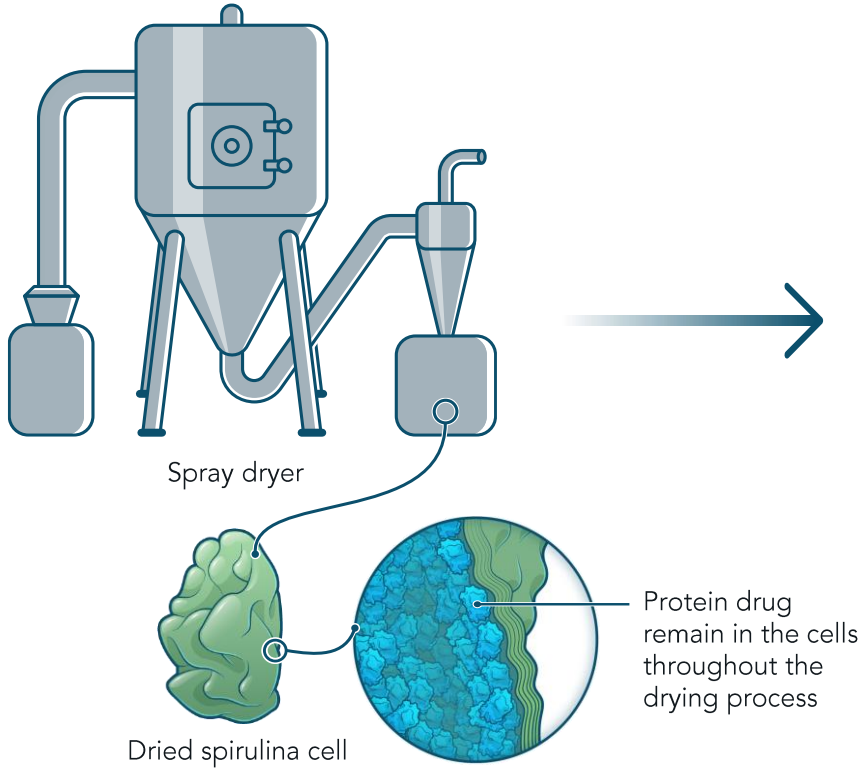
# Lumen platform manufacturing

## Simple, scalable manufacturing

**01** Harvest spirulina semi-continuously; only minerals, light, water, and air are needed

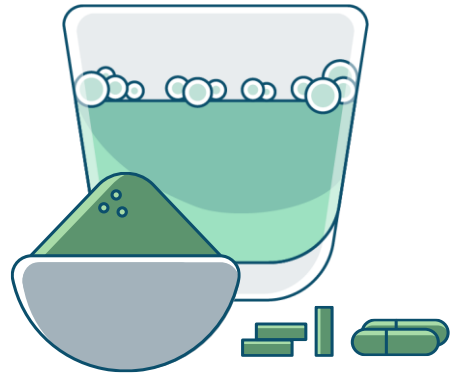



**02** Spray-dry the biomass




**03** Resuspend in water and administer orally (optionally formulate or encapsulate)

No cold chain
No needles
No medical personnel

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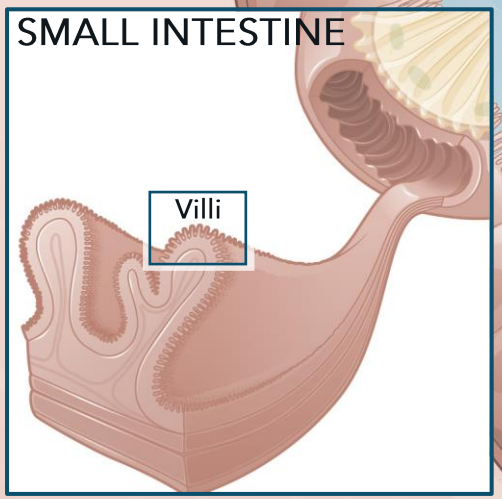


Antibodies and other biologics released in the intestine

Biologic drugs include:

Antibodies

Enzymes

Signaling proteins

Treatments for:

Infectious diseases

Metabolic diseases

Inflammatory diseases

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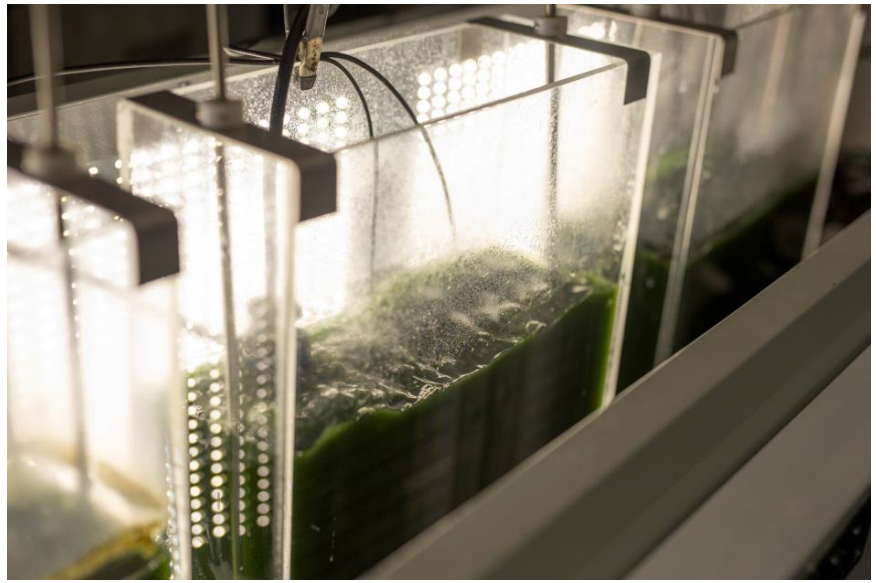
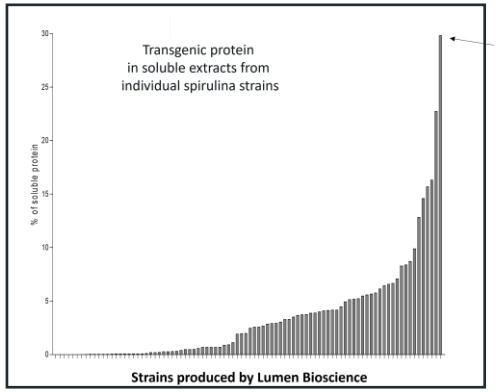
# Lumen's objective is to optimize/expression potency in *Arthrospira*

- Therapeutic proteins are far more valuable than pigments or fuel
- To produce a useful therapeutic, yield/biomass needs to be optimized
- Growth is a secondary concern






**Western blotting/ enzyme assays**

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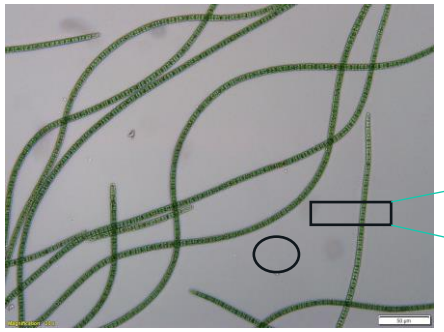
# Growing phototrophic organisms represents unique challenges in the laboratory

Small scale to large scale comparisons suffer from several disadvantages:

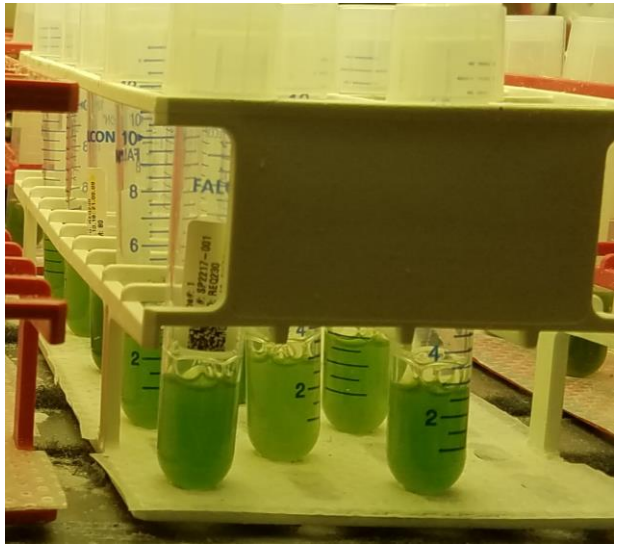
1. Light Intensity
2. Mixing Rates
3. pH values



*Arthrospira platensis*

$HCO_3^- \rightarrow CO_2 + OH^-$

*(photosynthesis)*



*Outdoor photobioreactors from the AzCATI facility*

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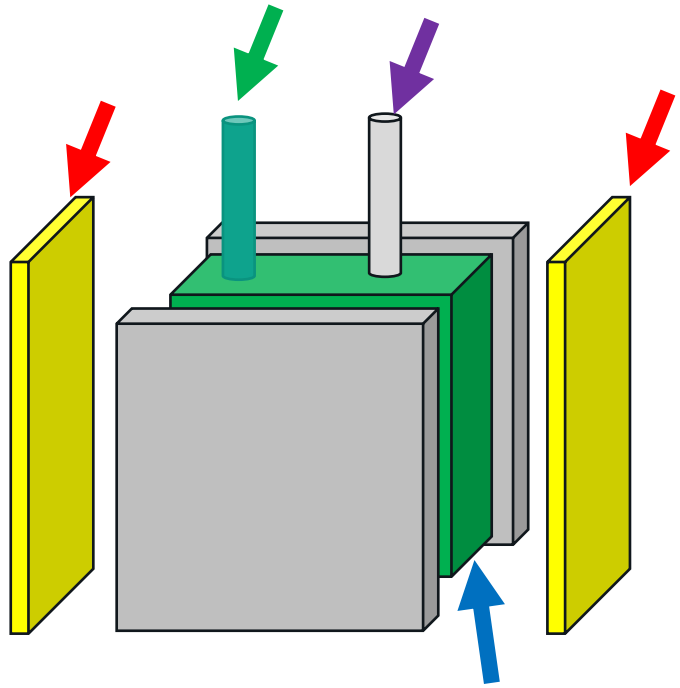
# Lumen employs mini-bioreactors for higher throughput analysis

1. LED panels are attached to the sides (red arrows), illumination pathlength is the same as the large reactors

2. Air and $CO_2$ lines are inserted at the top into the open plastic bag (green), pH probe is inserted as well (purple)

3. Culture is inserted in a bag (blue arrow) between two metal plates



**Small-Scale Reactors**

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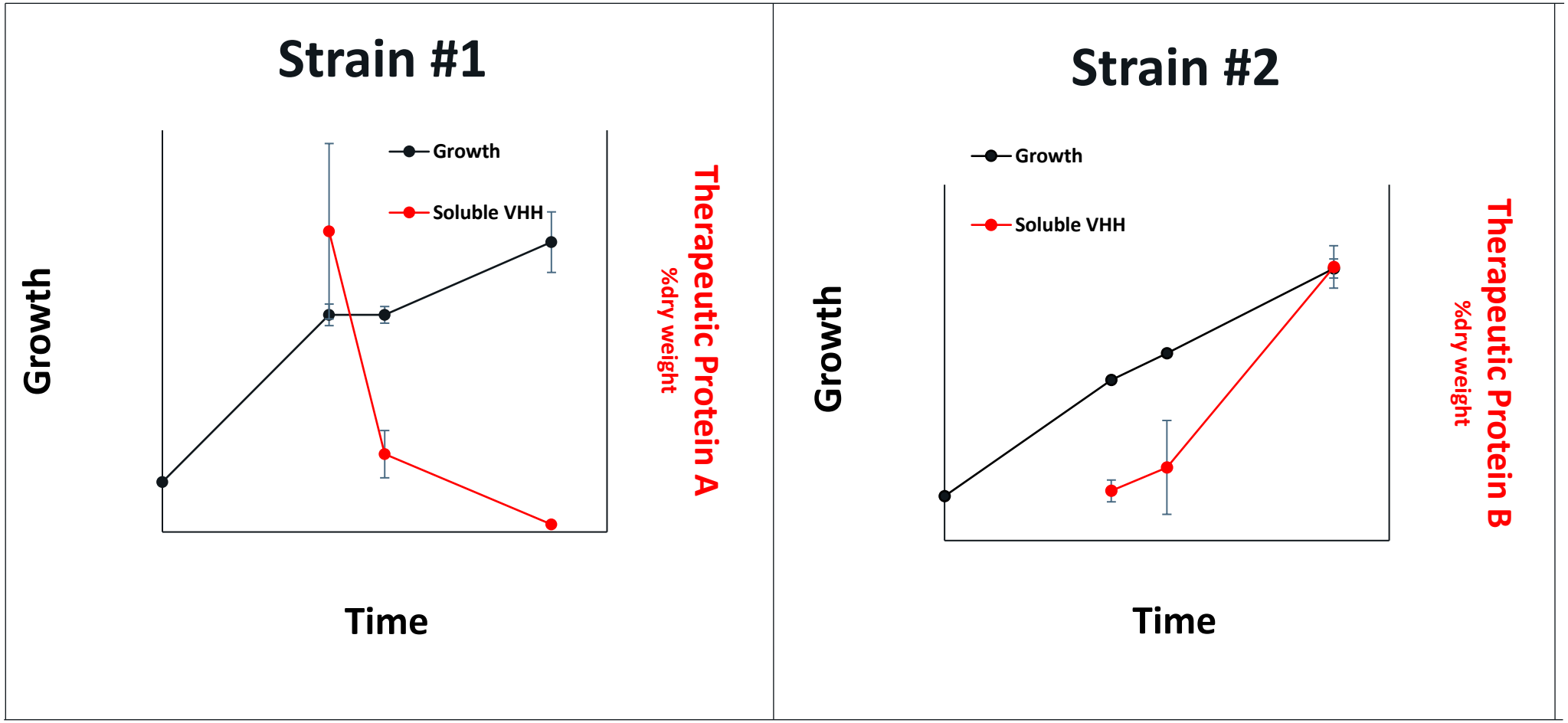
# Each strain needs a different set of parameters for optimization

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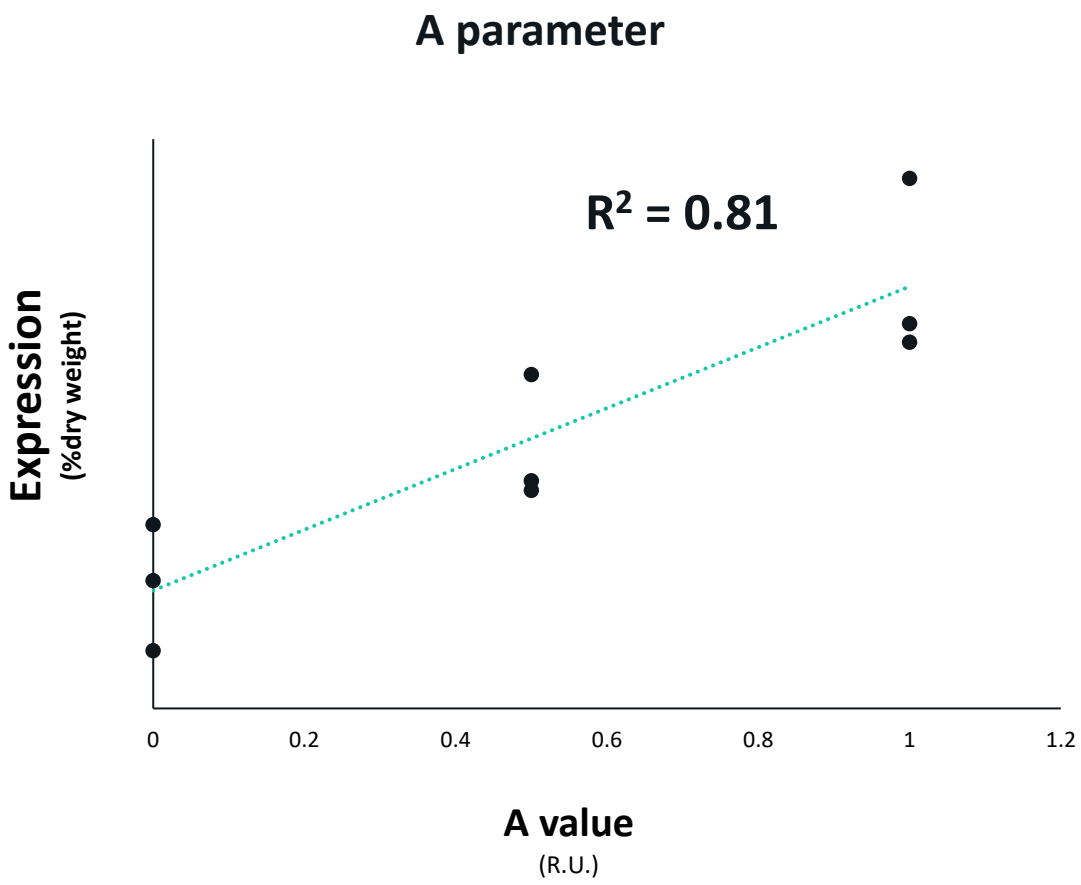
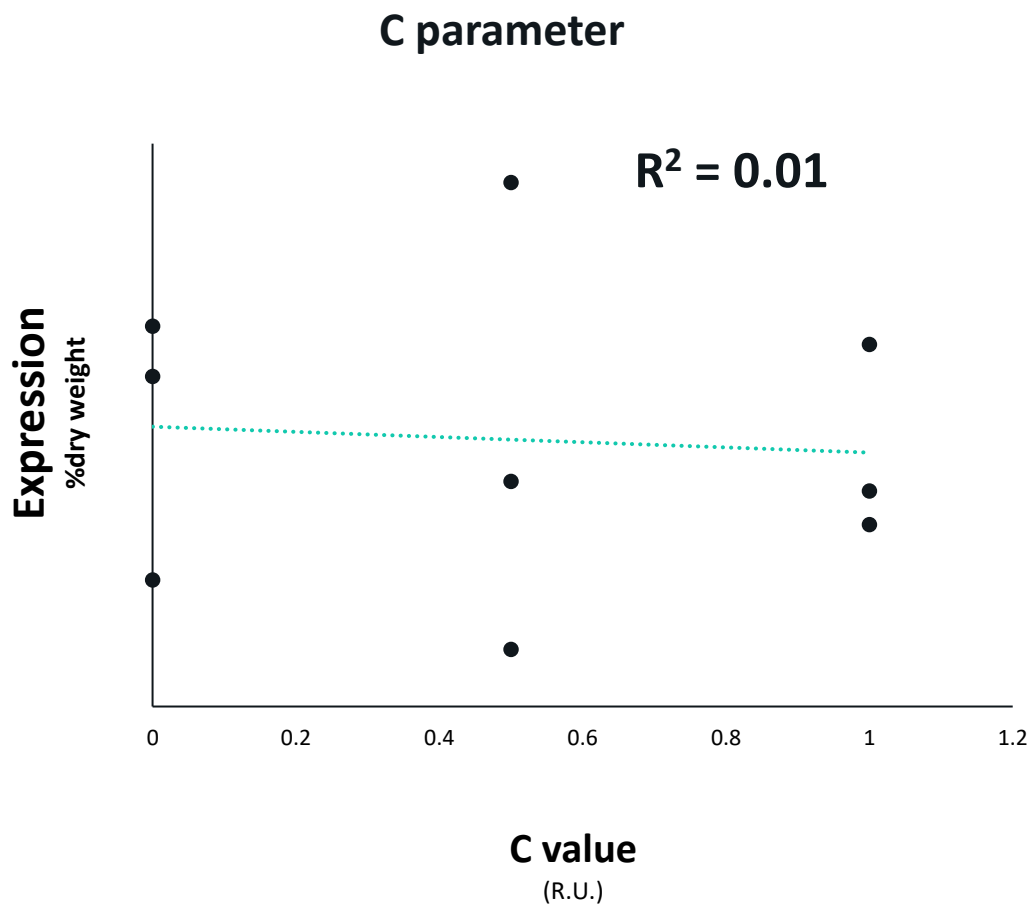
# Definitive screening design was used to optimize multiple parameters

- After analyzing multiple runs, 4-6 parameters that affect expression were identified
- Definitive screening design, a design of experiment approach that optimizes these parameters

| A Parameter (A.U.) | B Parameters (A.U.) | C Parameter (A.U.) | D Parameter (A.U.) |
|---|---|---|---|
| 0 | 1 | 0.5 | 0 |
| 1 | 0 | 0.5 | 1 |
| 1 | 0.5 | 0 | 0 |
| 0 | 0.5 | 1 | 1 |
| 0 | 0 | 0 | 0.5 |
| 1 | 1 | 1 | 0.5 |
| 0.5 | 1 | 0 | 1 |
| 0.5 | 0 | 1 | 0 |
| 0.5 | 0.5 | 0.5 | 0.5 |

# Exploratory plots show first-order interactions

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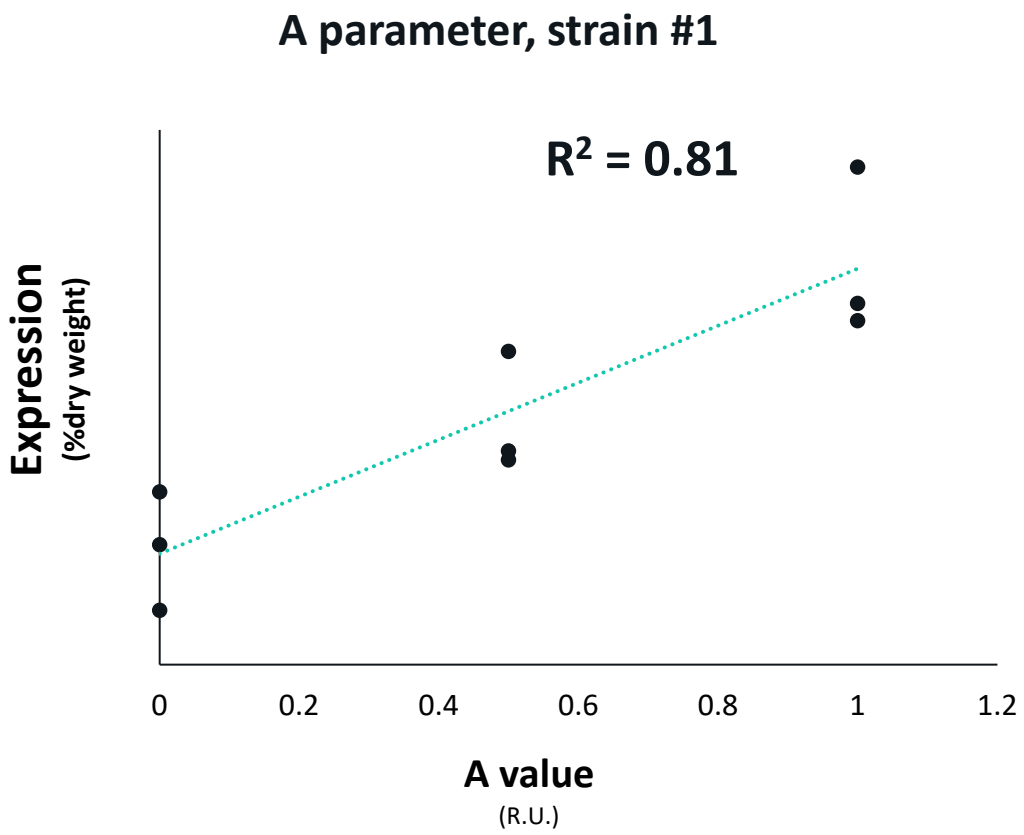
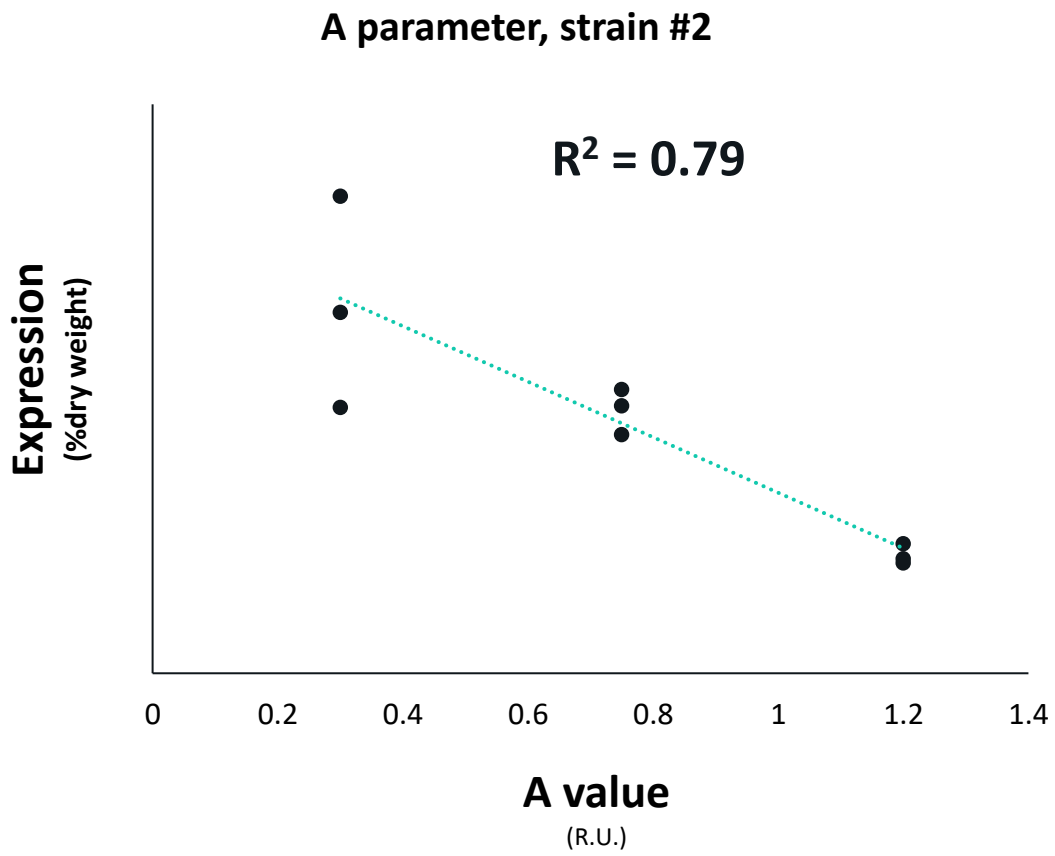
# Preliminary analysis shows strains respond in varied ways to growth conditions

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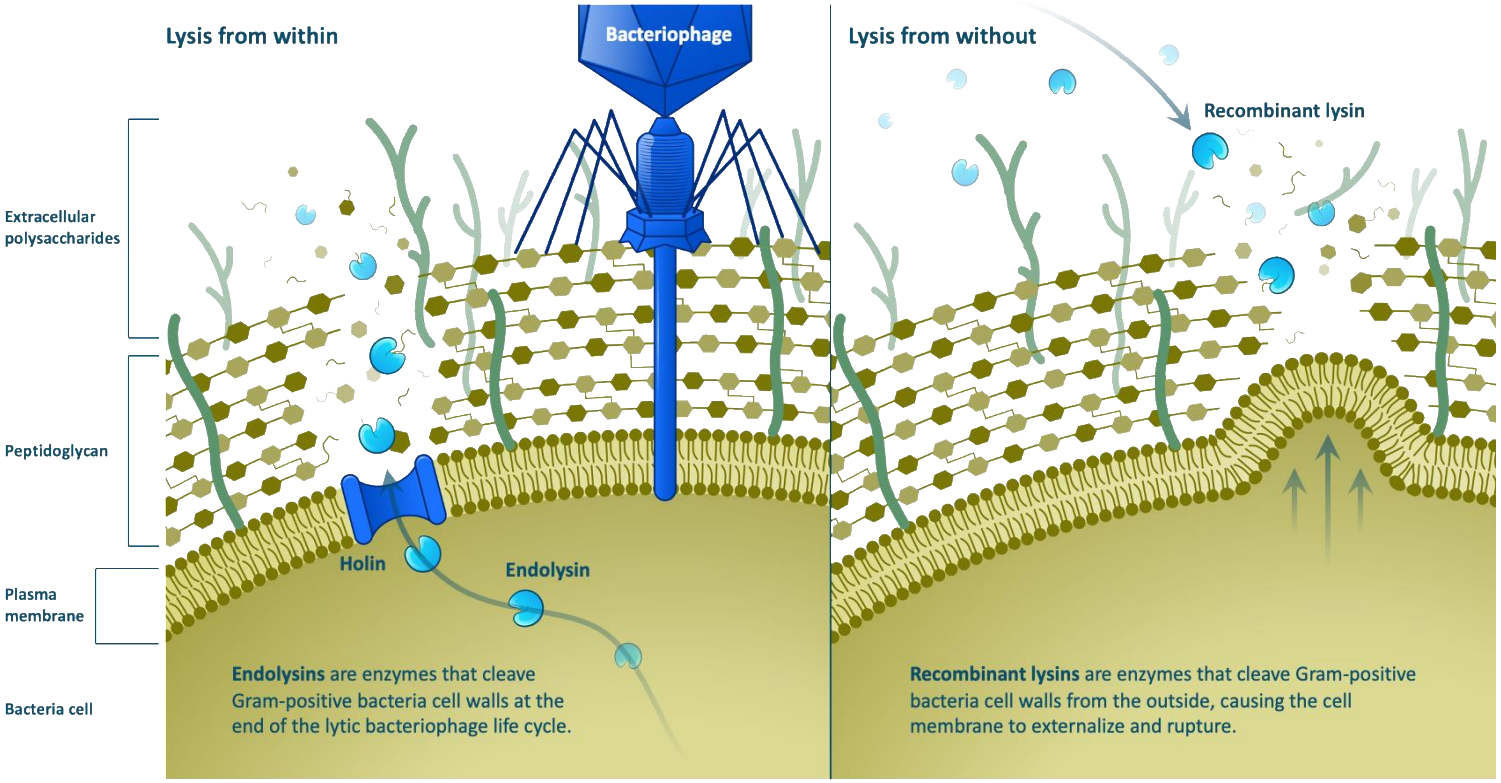
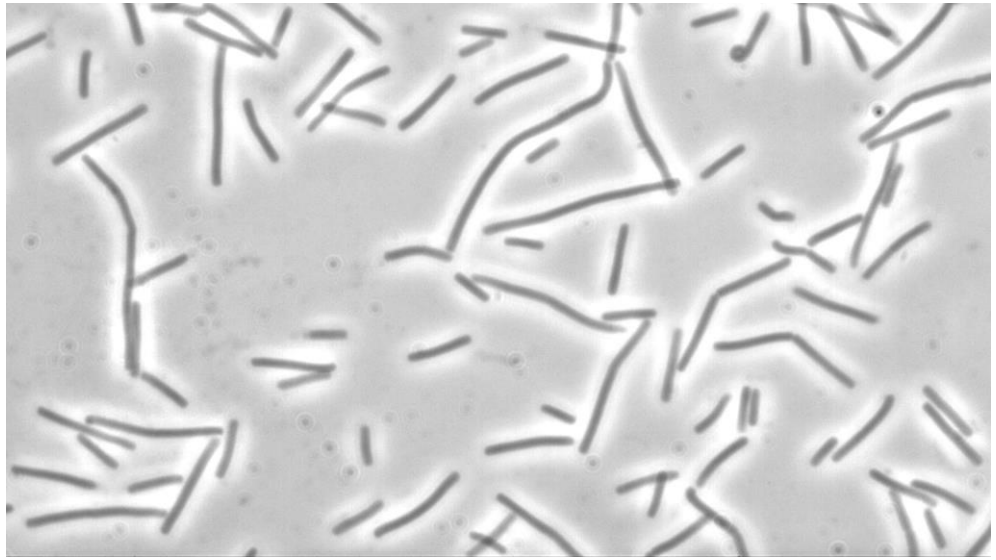
# Bacteriophage burst from cells using an enzyme called lysin




## Real-time destruction of bacteria by a lysin



V. Fischetti, Rockefeller U.

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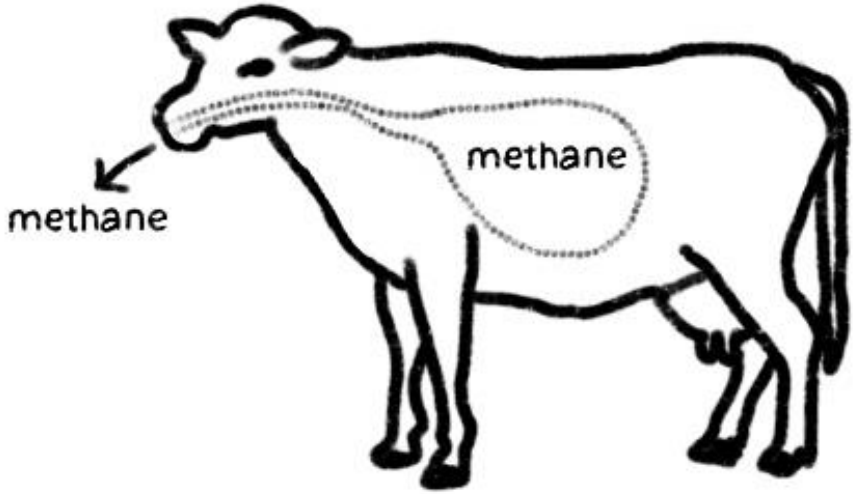
# Our climate change proposal…..

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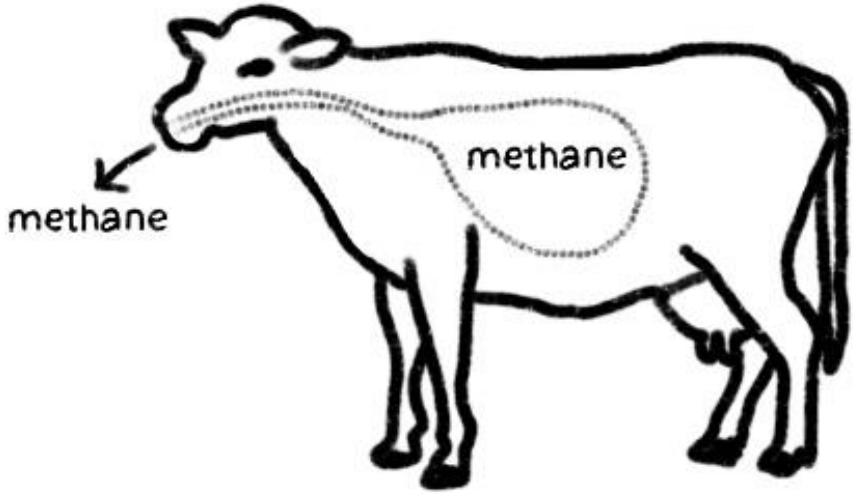
# According to the Environmental Defense Fund at least 25% of today's global warming is driven by methane from human actions, with the single largest source being livestock, which accounts for 31% of the global total

Due to its chemical structure methane is 80 times more potent than $CO_2$ at causing atmospheric warming, and it is much shorter lived than $CO_2$.

Therefore, reducing methane emissions would have an **immediate impact** on warming, while longer term solutions to reduce $CO_2$ emissions are negotiated.

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# There is just one major genus of rumen methanogens: Methanobrevibacter

*Methanobrevibacter ruminatum – 53%*

*Methanobrevibacter gottschalkii – 34%*

*All others < 10% each*

One lysin discovered by Lumen may eliminate >75% of all rumen methanogens

Lysin protein structure

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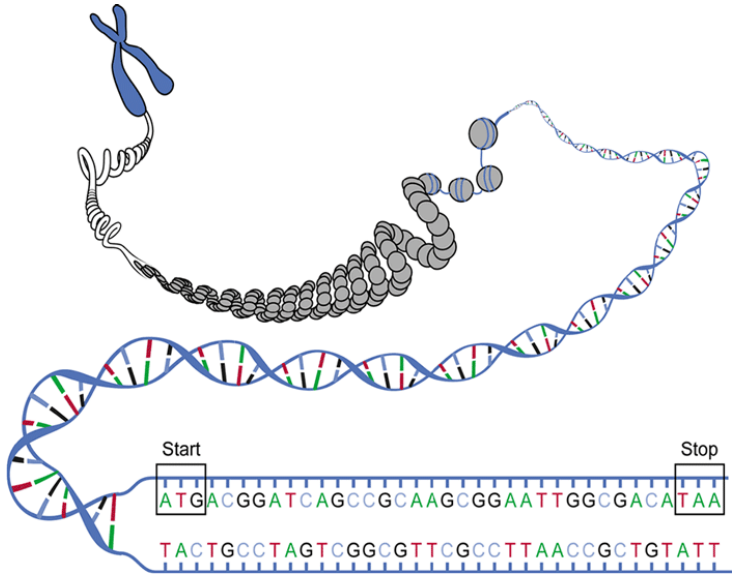
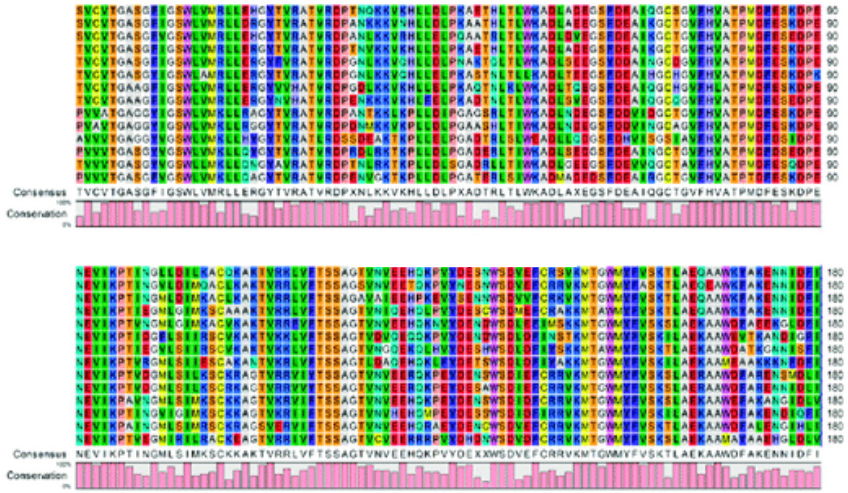
*Danielsson et al. (2017) Frontiers in Microbiol.* **8** *pp 1-15*

# Building spirulina containing a methanogen lysin

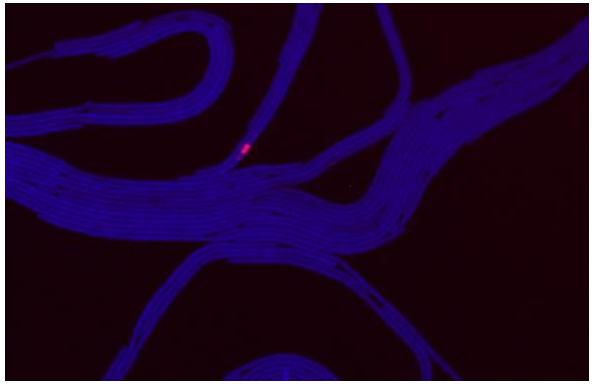
DNA sequences from the genomes of all known methanogen species

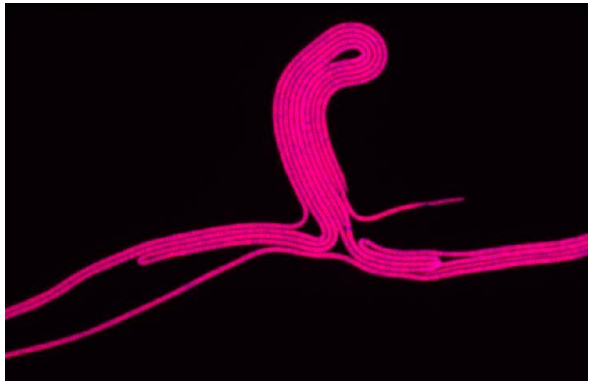



Protein sequence alignment using known lysins from other microbes was used to discover methanogen lysins



Methanogen lysin gene engineered into the spirulina genome





Spirulina containing a methanogen lysin gene

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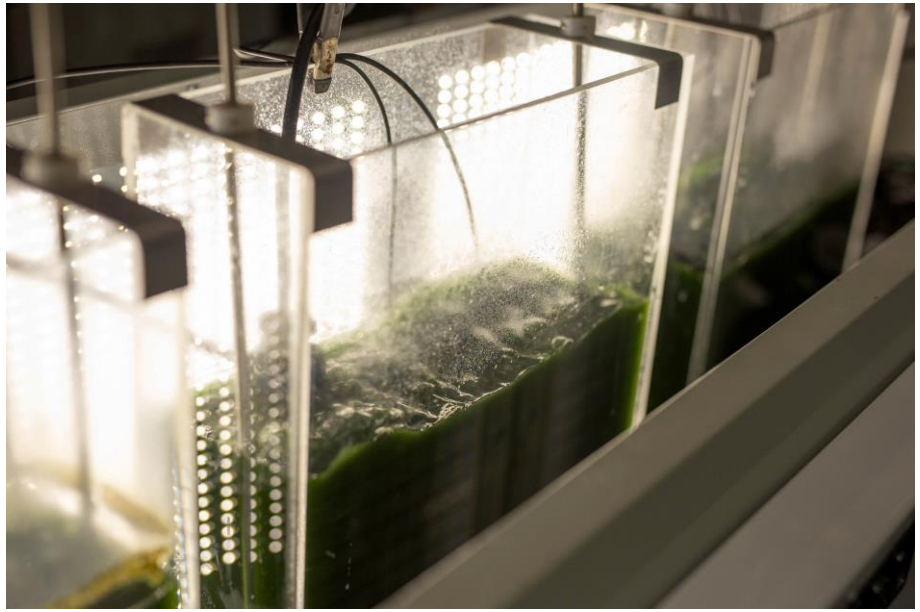
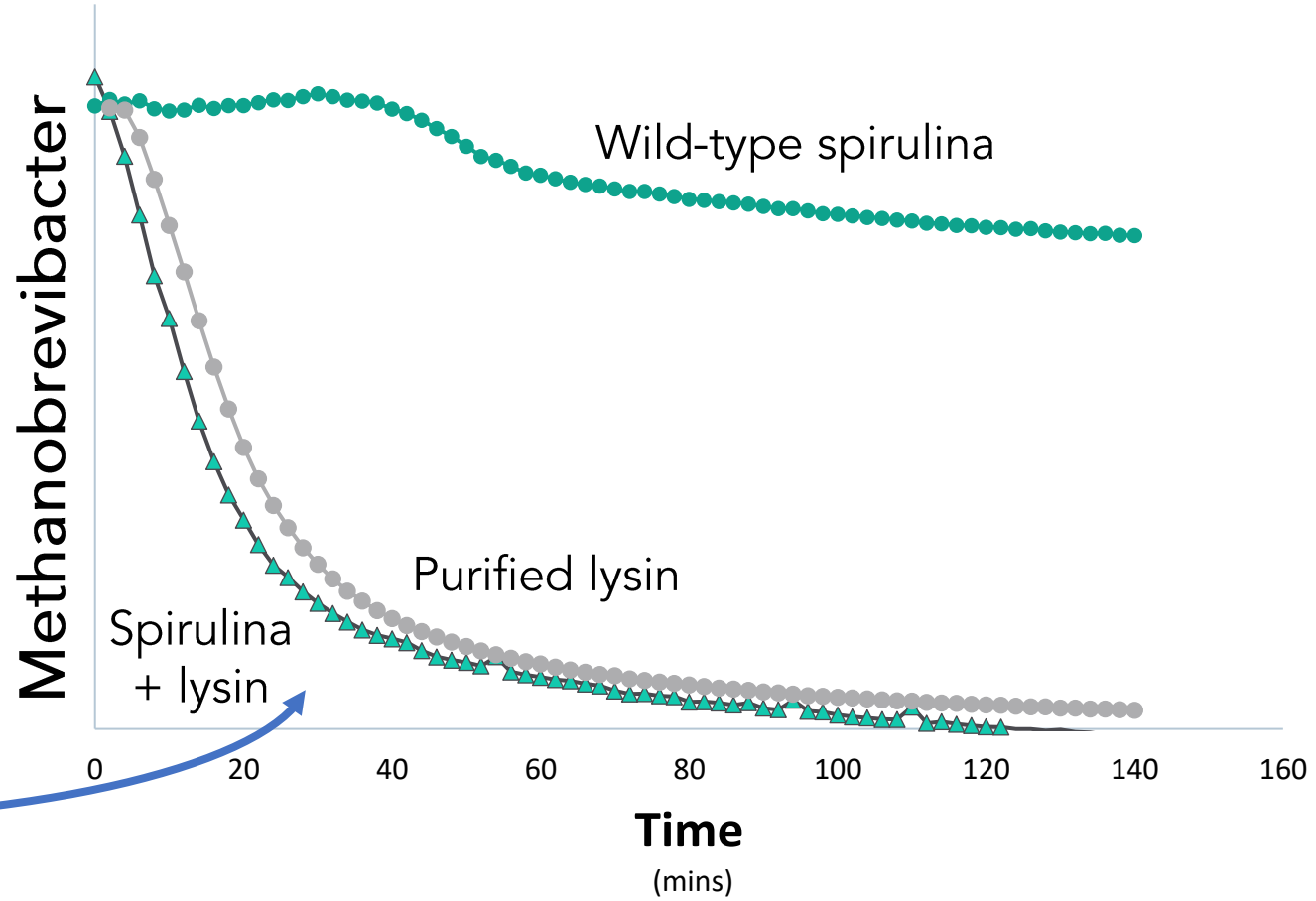
# A prototype spirulina strain expressing a methanogen lysin



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