https://github.com/AdrianMolecule/bioreactor

Bioprocessing Tools & Equipment

mit media lab

Biosummit 5.0 MIT- Media Lab

November 19, 2021

Adrian Filip (Ottawa Bioscience, Ottawa Canada)
David J. Castillo (Glyxon Biolabs, Mexico City, Mexico)
Adrian Jones (Val Des Monts, Québec, Canada)
Alexander Klenov (Pipette Jockey, Toronto, Canada, past contributor)
Alexander Yakovlev (Ottawa Bioscience, Ottawa, Canada)
Vladyslav Boyko (Utah, USA)
And many more volunteer collaborators…

OTTAWA BIO SCIENCE + GLYXON

http://specyal.com/diybio/bioreactor.html

https://www.glyxonbiolabs.com/Proteopresso

What is Bioprocessing? & Why we need tools for it?

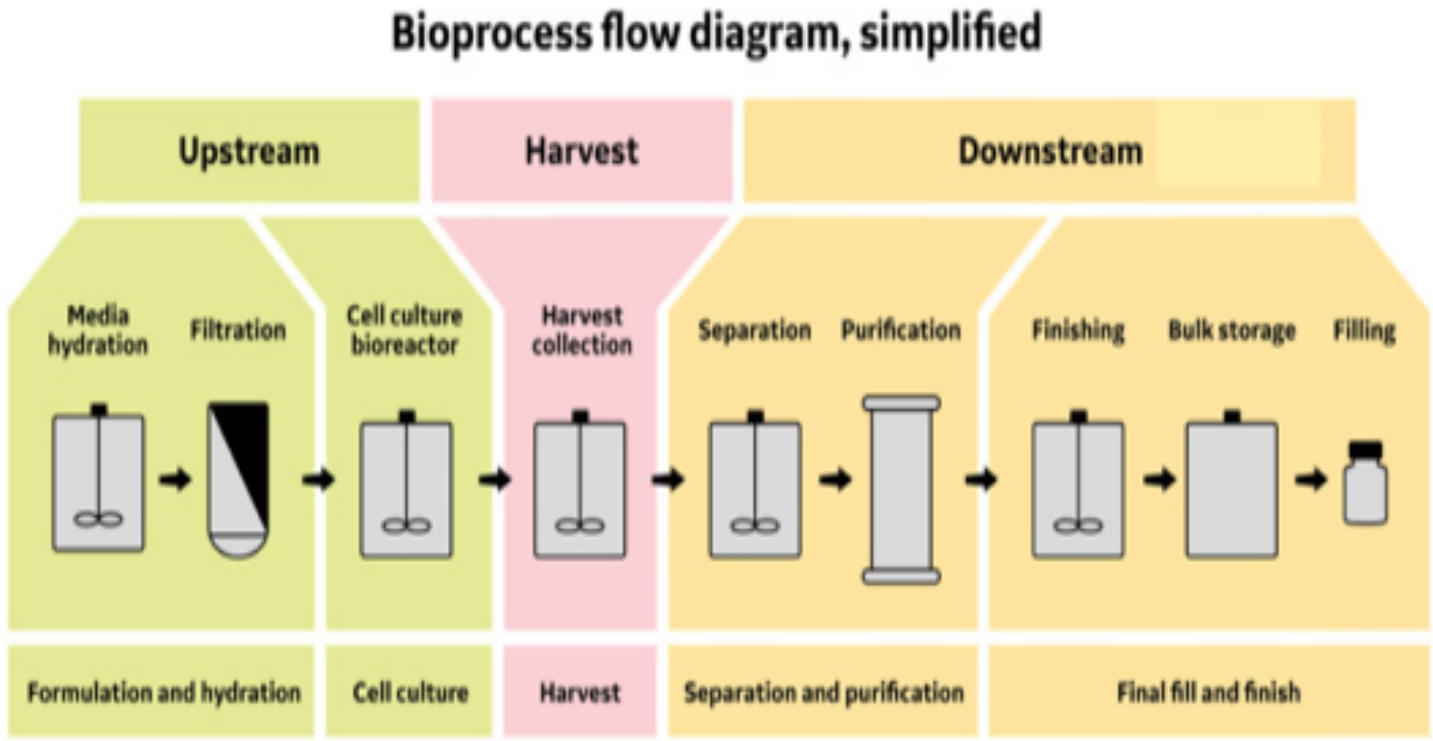
“The equipment may be divided in three categories: ***upstream***, ***downstream***, and ***support***. Upstream equipment deals with the ***growth*** of a host organism to ***produce a product.*** The product may be the ***organisms themselves,*** it may be ***held internal to the organism***, or it may be ***excreted into the growth medium***. ***Purification***, for example, ***filtration***, and ***chromatography*** of the resulting harvest from the upstream process is handled by ***downstream equipment***. Other pieces of equipment used in **biomanufacturing** such as incubators, utility carts, liquid mixers, holding tanks, bead mills and other cell disruptors can be defined as support equipment”*

Grow and produce

Full length organisms

Tissues
(Somatic lines)

Germinal lines
(spores, seeds,
reproduction cells, etc)



Separate and isolate:

Proteins, antibodies

Bacteria, plants and fungi extracts
e.g. :

Antibiotics

Hormones, growth factors

Secondary metabolites, pigments

BIOREACTOR 2020

PROTEOPRESSO 2021

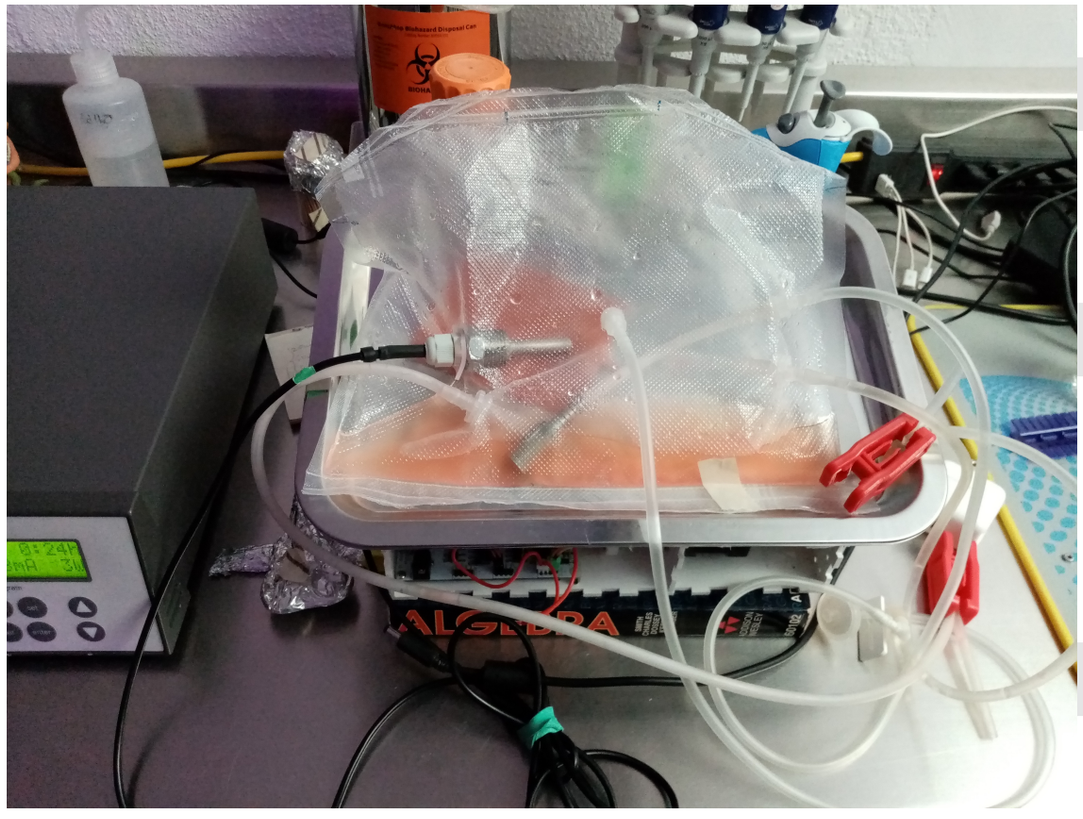
WE ARE HERE!!!!!!!!!

Bioprocessing is the set of techniques, tools and equipment that help us to grow, produce, extract, separate and purify biologically active biomaterials

*Kenneth P. Clapp, ... Eva K. Lindskog, in Biopharmaceutical Processing, 2018

Bioprocessing demand many expensive of stand-alone tools and machinery: incubators, growth temperature controlled rooms, bioreactors, chromatography columns, pumps etc .

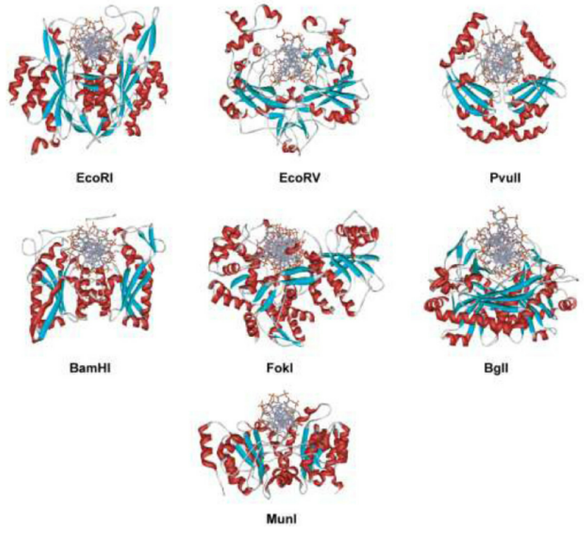
The HERE and NOW : <u>SIMPLIFIED</u> <u>INTEGRATION</u>



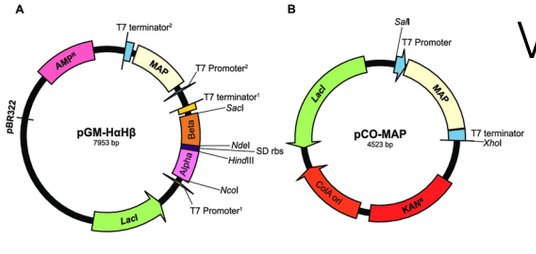
Insitu production of molecular reagents, tissues, biomaterials

This could overcome geographical barriers and limited or expensive supply chains.

Tissue cultures

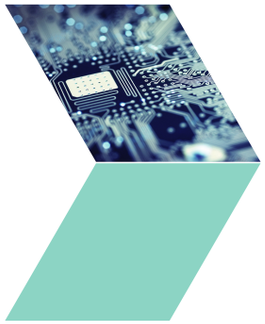


Modification enzymes



Vectors, plasmids, N.As.

ACHIEVEMETS



Phase 2

ELECTRONICS: PCB design

A

Chromatography system motherboard (Proteopresso)

NEW BOARD!

MICROCONTROLLER

EPS32 module

SENSORS

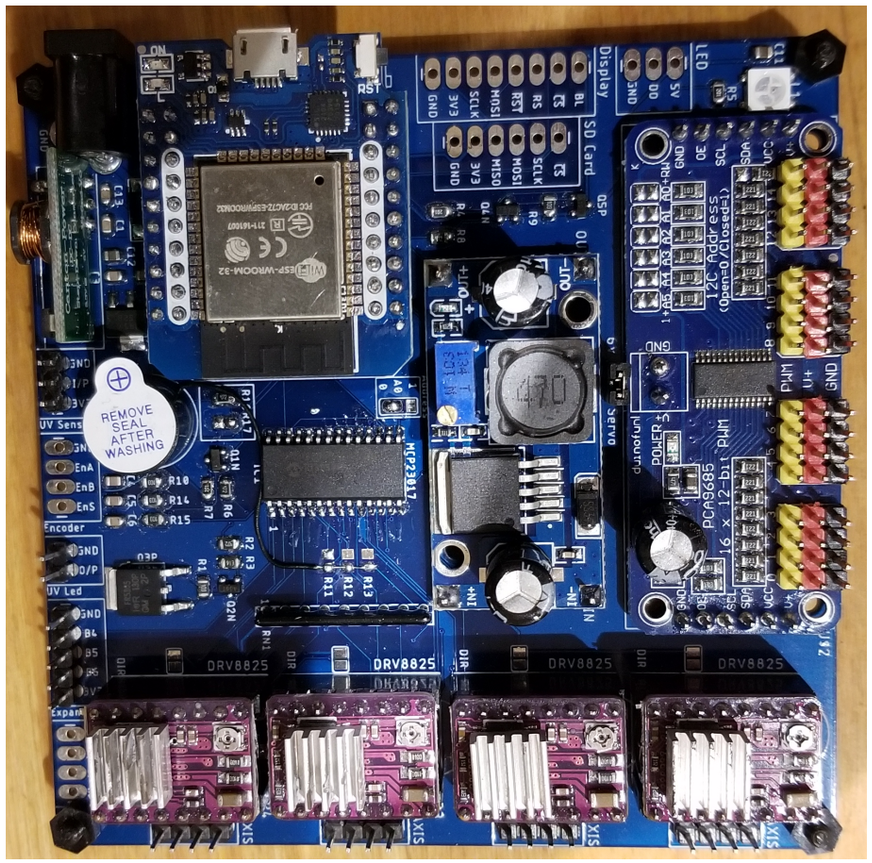
UV LED Light

UV photometer

Hydrostatic pressure sensor

Future features (early 2022):

12MP camera for object recognition



VALVES

16x Servo control board

6x valves mixer / gradient generator

Additional flow pass valves

4x NEMA17 drivers

To
-Fraction collector
-Liquid handling pipettor
-Cells and tissues 3D printer
-Plate holder

X Y Z

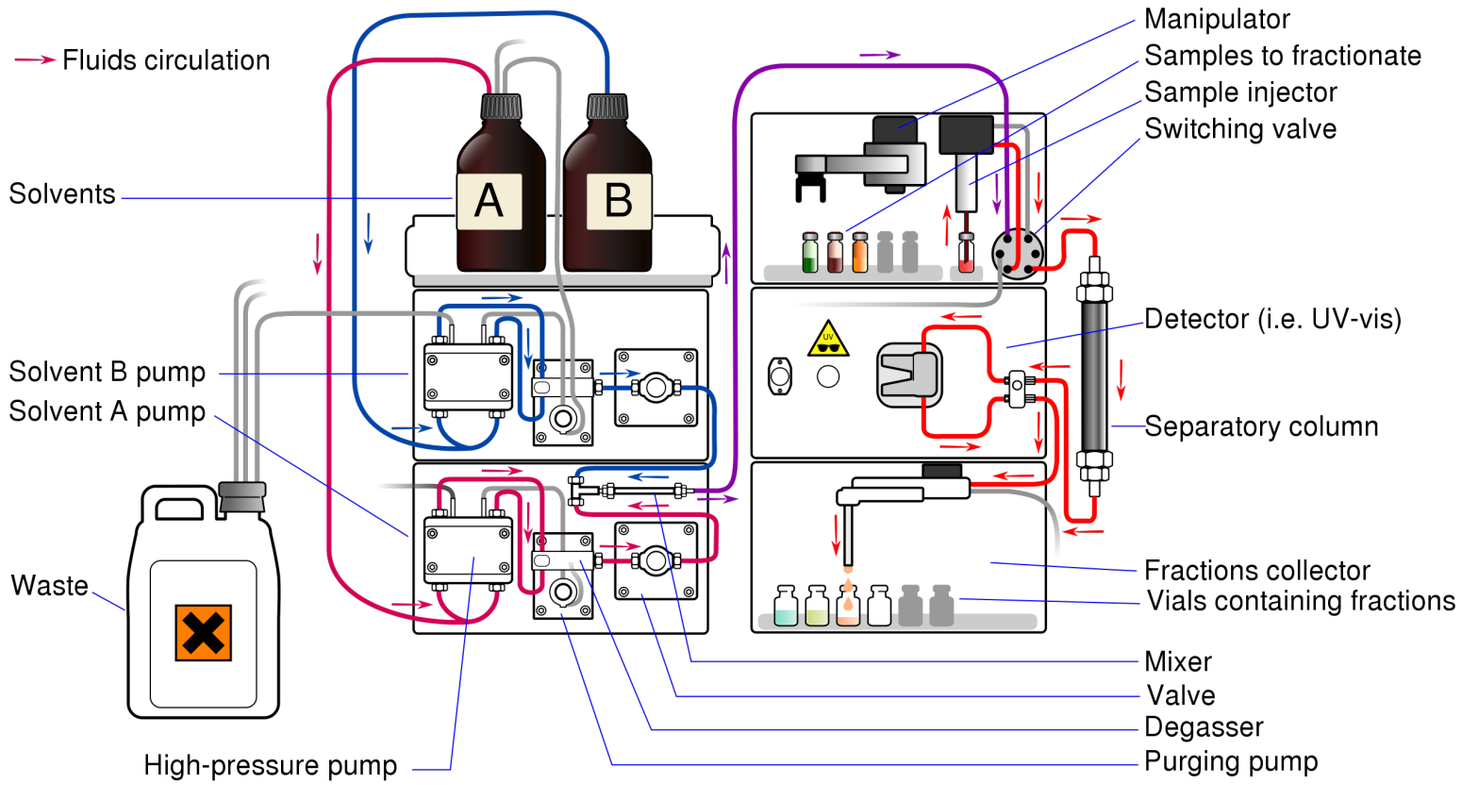
ROBOTIC ARM

P

PUMP

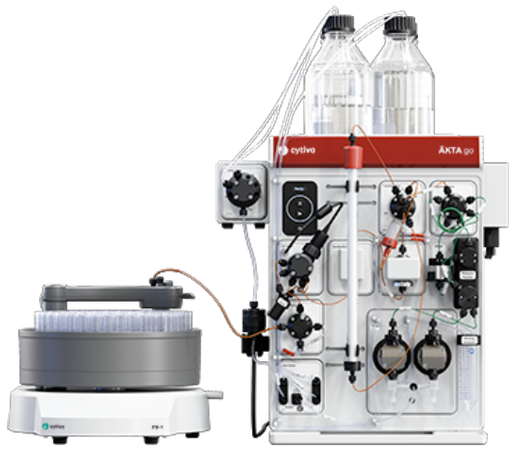
Variable volume Syringe pump / Cells or Materials Extruder

Anatomy of protein purification system



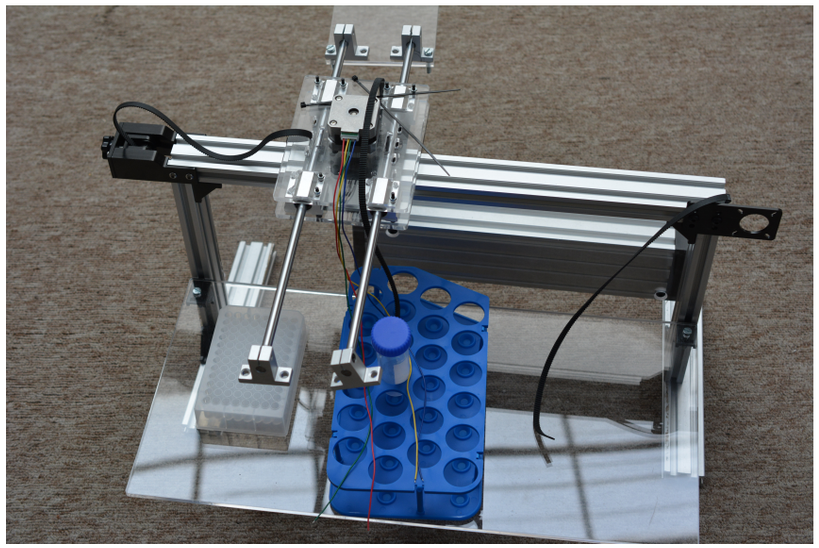
Complex, expensive machinery made simple

Commercial options



vs

Open Source Proteopresso



$$$$$$, around 20,000 USDlls or more…

$$, Bit more than 1,000 USDlls or less…

Proteopresso features (A little bit of a “Frankenstein”… in progress)

SCALABLE

Position selector valves array : 15 positions, 2 solvent mixer/ sample input, made with a simple disposable IV’s valves array and servo driven selector.

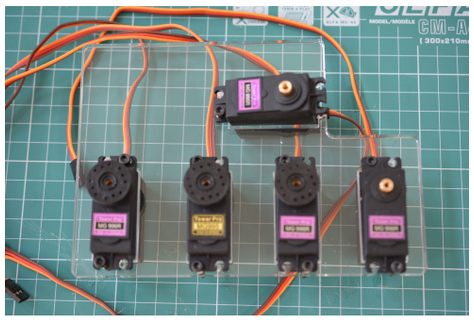
Heavy-duty Fraction collector : Adapted for micro plates and tubes up to 50ml (falcon tubes). Would be enhanced into a liquid handling robot (pipetting) or cells culture “3D printer”. Could fit most commercial pipettes, no need for expensive adaptors. It will incorporate a dual (Vis/IR) camera guided “Z” axis for repetitive, delicate, precision sampling, seeding, culturing or small objects manipulation.

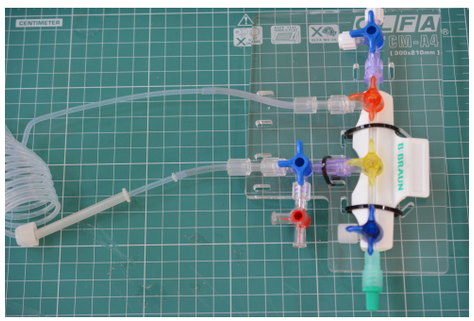
Dedicated UV spectrophotometric cell detector : For a quick protein quantitation, made on a CNC or laser cut micro-fluidic cell integrated with a graphic plotter software.

Syringe pump extruder and mixer/ gradient generator : Multi-volume adaptor for reusable glass or disposable plastic syringes. Incorporated pressure sensor will feedback chromatographic columns within functional range for sustaining an effective mobile phase flow.

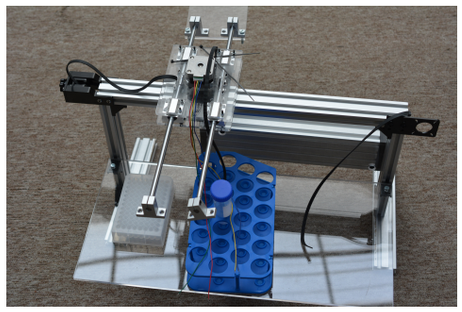
Friendly graphical interface : Simple, intuitive, EASY control on a “touch screen” app or through mobile devices and PCs.

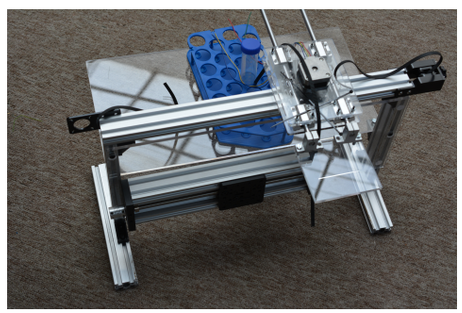
Servos valves array (mixer and gradient)

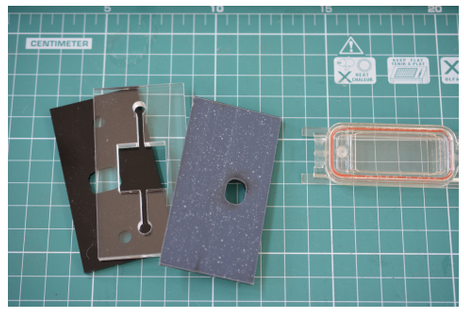




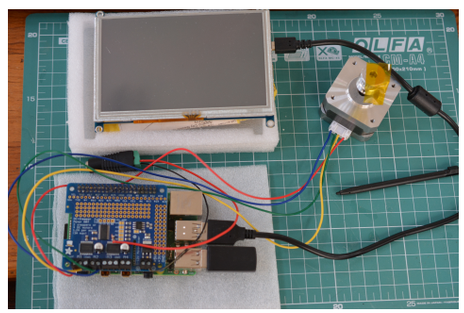
Fraction collector /syringe pump







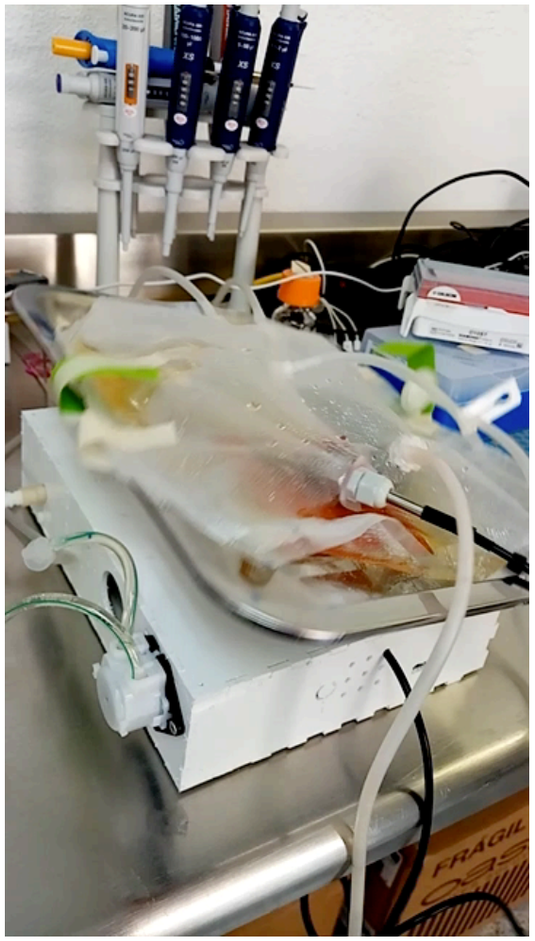
Cell flows (Vis/UV)



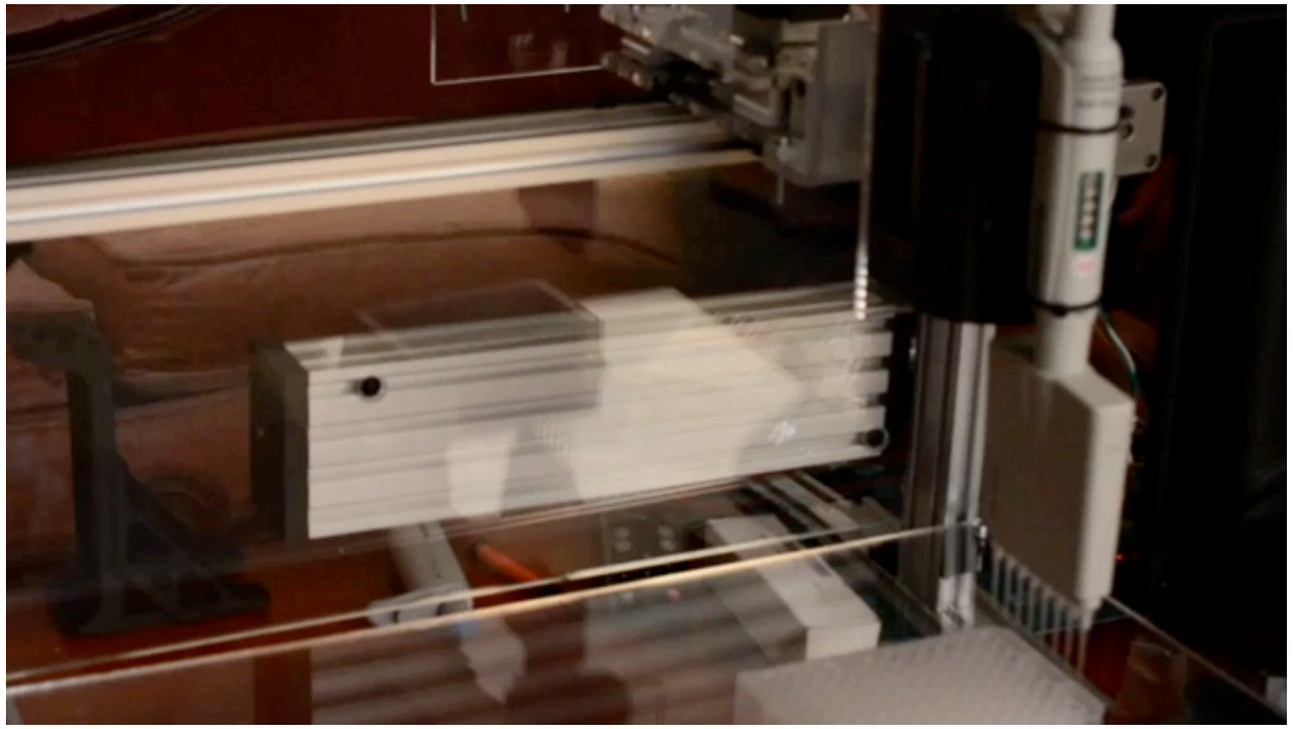
Touchscreen GUI

Phase 2

Test



Bioreactor in operation



Proteopresso fraction collector / liquid handling pipettor (2x speed)

Ni-NTA purification DNA pol Pfu Sso7d

Pfu sso7d 6xHis

HiTrap NiNTA GE

SDS-PAGE 12%

Future features

Proteopresso “a la carte” : User enabled and integrated online repository with protocols for diverse type of purification techniques, Integration with a dedicated software for “Select and run”unattended automation (IN PROGRESS)

Dedicated PCB : All mechanical and electronics integration into a single Open source motherboard. (COMPLETED)

Proteomics and genomics: Heated shaker in fraction collector for refolding experiments; printing head for nucleotides and proteins microarrays (Mid to late 2022)

Computer assisted Object recognition for manipulation of tissues, cultures and seeds. (IN PROGRESS Collab. SYNMIKRO-Marburg, Germany)

Light inducible OPEN plasmids : Over expression using Silica or cellulose tags (IN PROGRESS)

Mice antibodies against currently over expressed proteins for further analysis e.g. Wester-blot (IN PROGRESS-early 2022)

Two flavours: Proteopresso “Titan” (50cmx45cmx75cm) for high throughput applications and Proteopresso “Pico” (10cmx25cmx15cm) for the field (IN PROGRESS).

Suite of new
light inducible vectors with diverse functional tags and reporters (Early 2022)

Light inducible plasmid and 6xHis binding domain

Bioreactor Project

First generation

pDawn-Pfu sso7d His_{6x}

Light inducible plasmid and silica binding domain

Proteopresso Project

His6x to Car-9 substitution

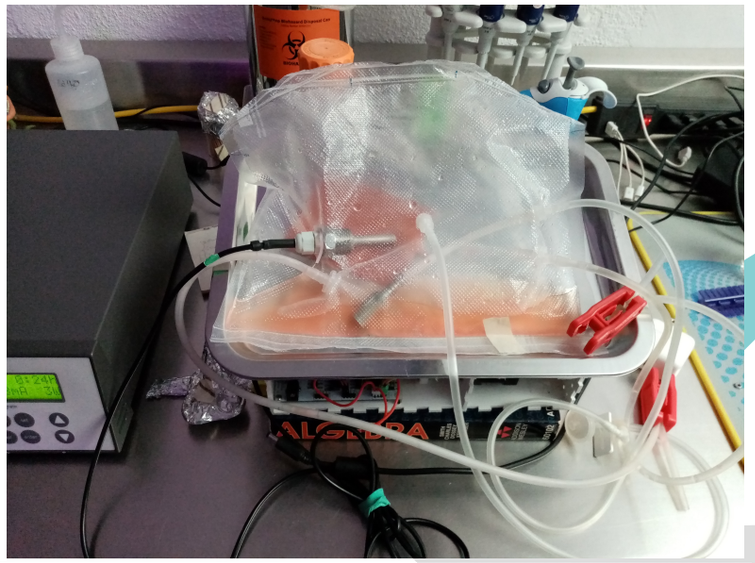
Second generation

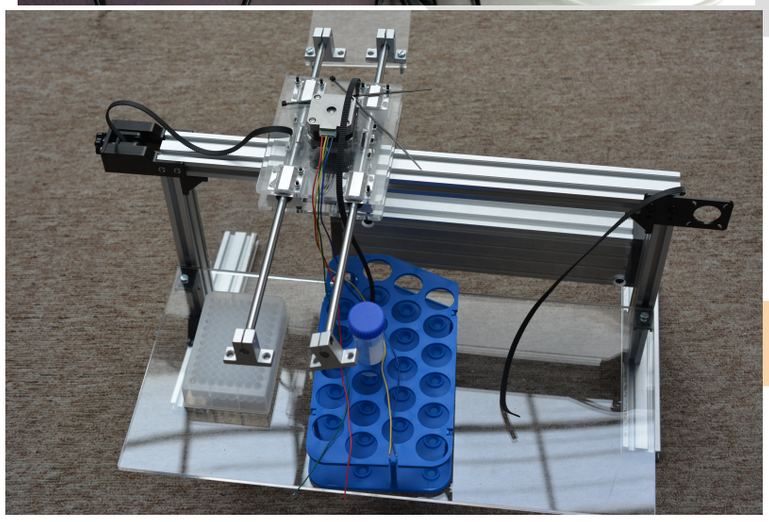
pDawn-Pfu sso7d Car9

Synergy

Bioreactor + **Proteopresso**

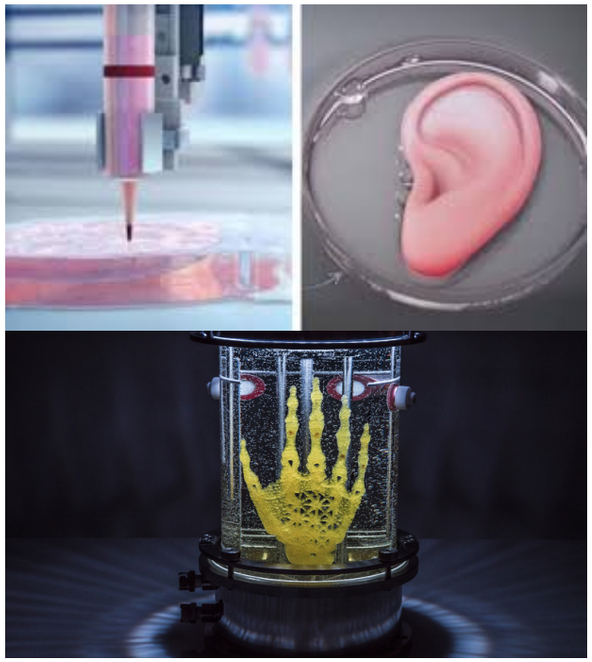
Long term applications of Bioprocessing automation





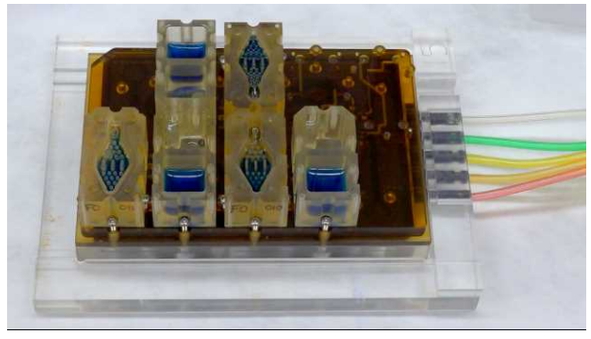
NEXT STEPS

Biofabrication, 3D printing of organs

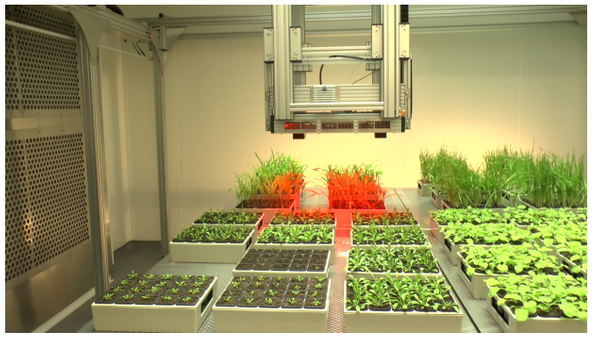


Biosynthesis of biocompatible scaffolds
(biopolymers, bioplastics, etc)

Organoids



Automated
Crop Phenotyping



Synergic duo:

Bioreactor = Facilitator,

Automated Chromoatography (Proteopresso) = accelerator

SUMMARY

- Three new strains controlled by an optogenetic switch that synthesize SARS Cov2 detection reagents. (2 DNApol, 1 Reverse transcriptase)
- Several versions of the rocker, one 3D printed and one laser cut, and one CNC prototypes. (1 liter vol. media)
- Several dedicated PCB capable of integrating multiple sensors and actuating heat, rocking, pumps and LED lights.
- Basic software to remotely control operating functions and adjustment of multiple variables (temperature, pH, rocking, optical density and several peristaltic pumps).
- Tested temperature control unit
- Tested pH control unit
- Tested growth flow cell for measuring optical density (uncalibrated)
- Tested fraction collector and syringe pump for chromatography system

IN PROGRESS

- UV/OD flow Cells
- Calibration of some sensors
- Finalized Optional sub-projects (Dissolved Oxygen monitoring)
- optimization for Mechanics and enhancement in software (GUI, wireless comms.)

Recombinant proteins for dummies

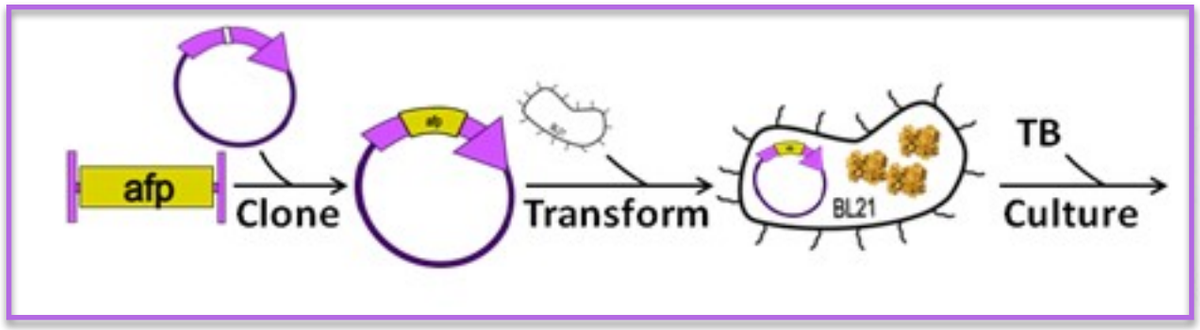
1hr Bioprocessing and Chromatography course…kinda.

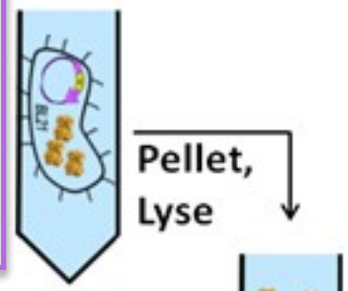
GENERAL PROTEIN PURIFICATION PROCESS (Bioprocessing)

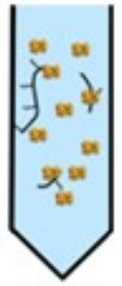
UPSTREAM
(Preparative steps)

Molecular biology techniques:

moclo, tags, FP's, affinity tags, terminators, promoters, etc.



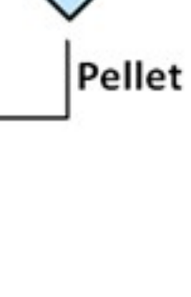


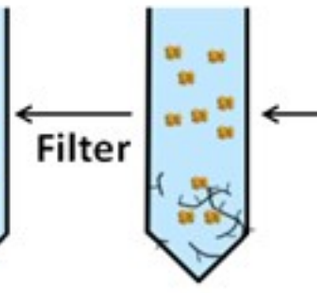


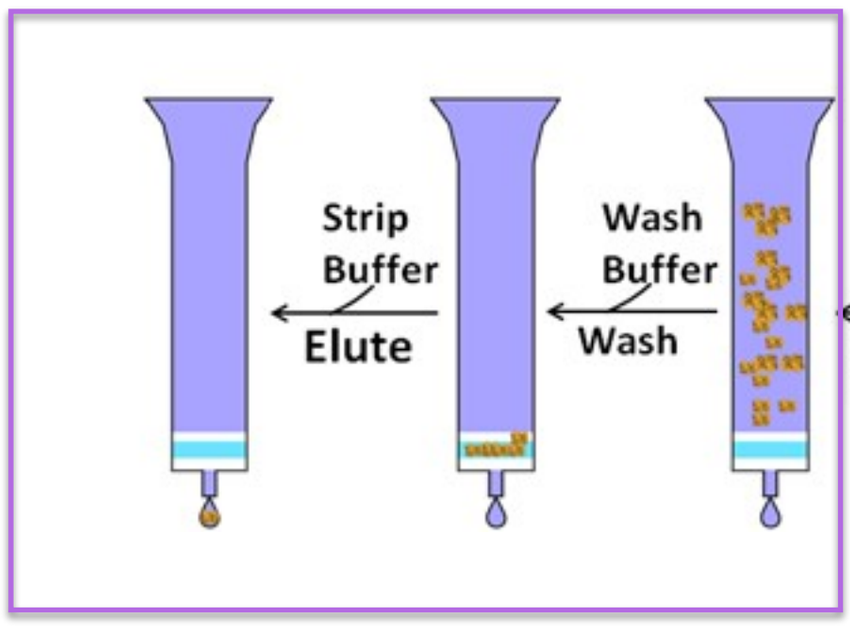
Bioreactor

EASY,
AFFORDABLE,
AUTOMATION

Means
no coding,
no messy cloning,
no clumsy purification,
no carpal tunnel syndrome… well, almost.







Ni-NTA Agarose, Sepharose, gel filtration, Silica, Antibodies affinity, etc.

DOWNSTREAM
(Recovery steps)

Proteopresso
(chromatography)

https://www.creative-biostructure.com/protein-purification_12.htm

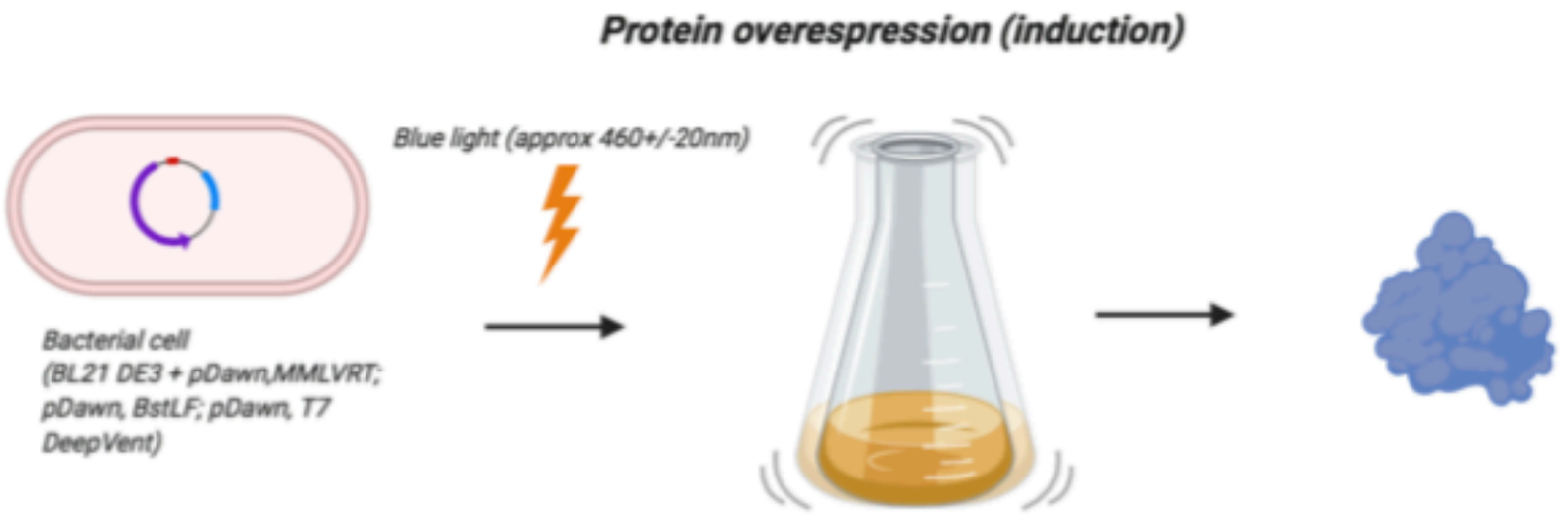
Q:Why do you need automated chromatography?

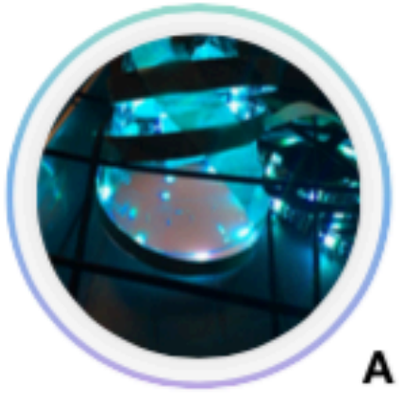
A: TOO MANY VARIABLES

growth cycle, antibiotic selection, cell culture monitoring, induction time, cell culture synchronization, lysis method, buffers composition, pH, salinity, temperature, oxidation-reduction potential, osmolarity, buffers strength, mobile phase speed, stationary phase selection, protein degradation, protein misfolding, protein aggregation, inclusion bodies…etc.

UPSTREAM

1: Reliable expression system (Bacterial strain, affinity tag, reporter, etc.)





A

B

Partial Results

GO

STANDARD PROCEDURE/
AVAILABLE REAGENTS &
EQUIPMENT

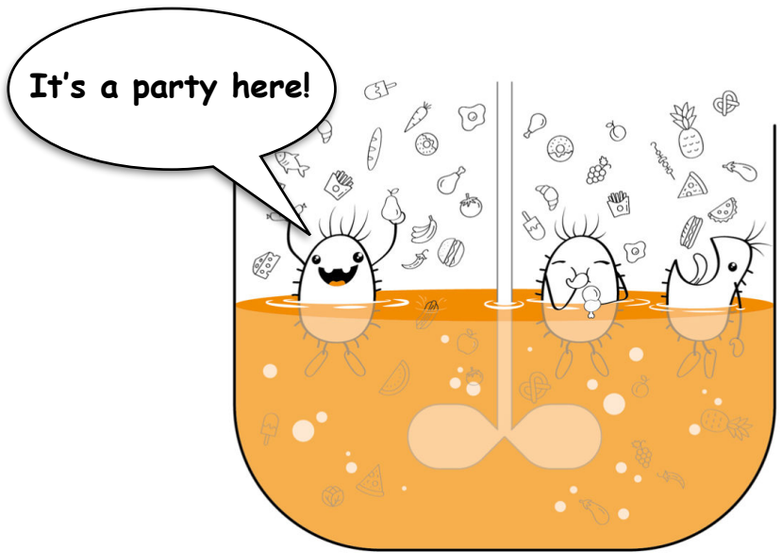
(A) 50ml LB culture inoculated with BL21(DE3);pDawn RFP, was induced by exposing it continuously to a home electronics cyan light LED array for 16hrs, at 220rpm RT. **(B)** After induction, Red fluorescent protein (reporter protein) can be seen by naked eye.

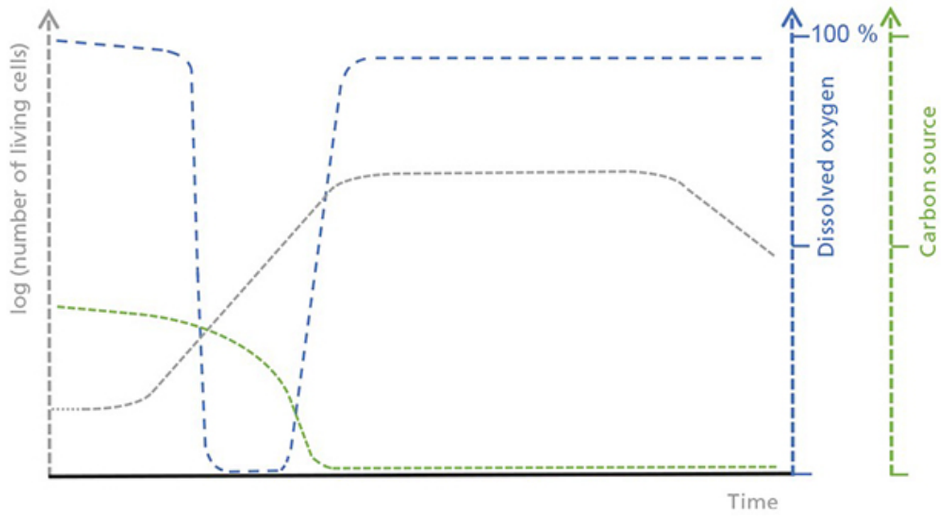
Where to start?

UPSTREAM

1: WHAT KIND OF CELLS YOUR WANT TO GROW?: KNOW YOUR BATCHES!

Batch: A “vicinity of cells”, within a “neighbourhood” or growth vessel.





Which variables are critical that define the performance of a batch?:

Interdependent correlation

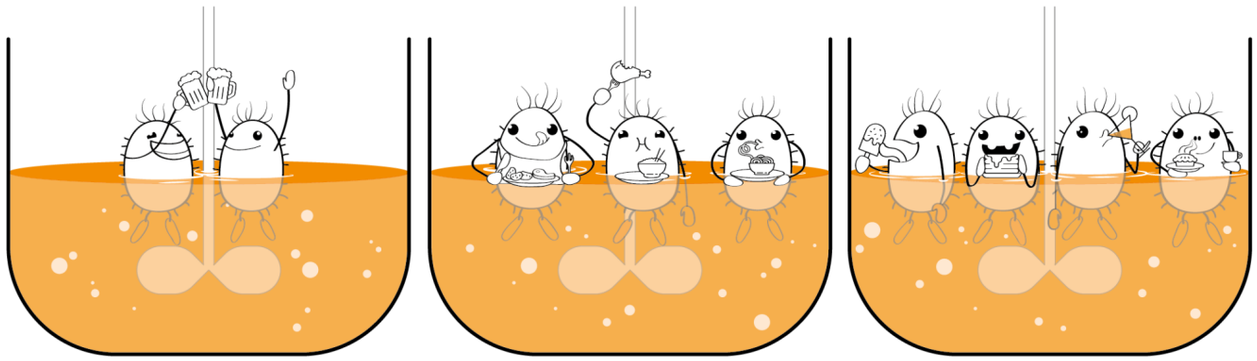
LIVING CELLS CONCENTRATION (growth), the demographics of your “bugs”
DISSOLVED GASES: From Breeze to “Toxygen”,
CARBON SOURCES : Feed them to live or live to feast and gorge sinfully

A Bioreactor is like a “chemical sim-city”

Batch Regimes

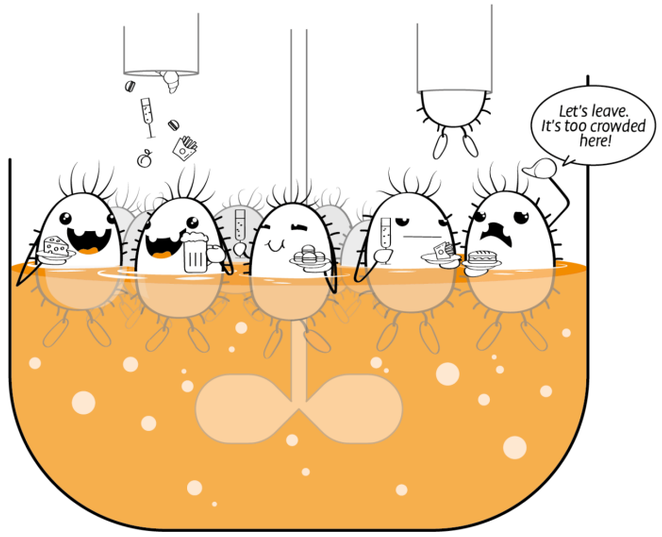
UPSTREAM
1: HOW DO YOU “FARM” YOUR CELLS?

Fed-Batch:

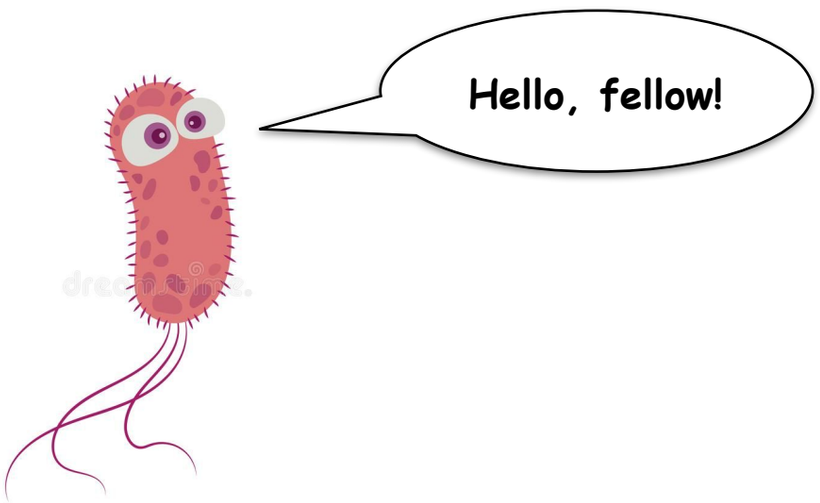


Sequential addition of nutrients (carbon source) activation by metabolic activation /repression

Continuous Batch:

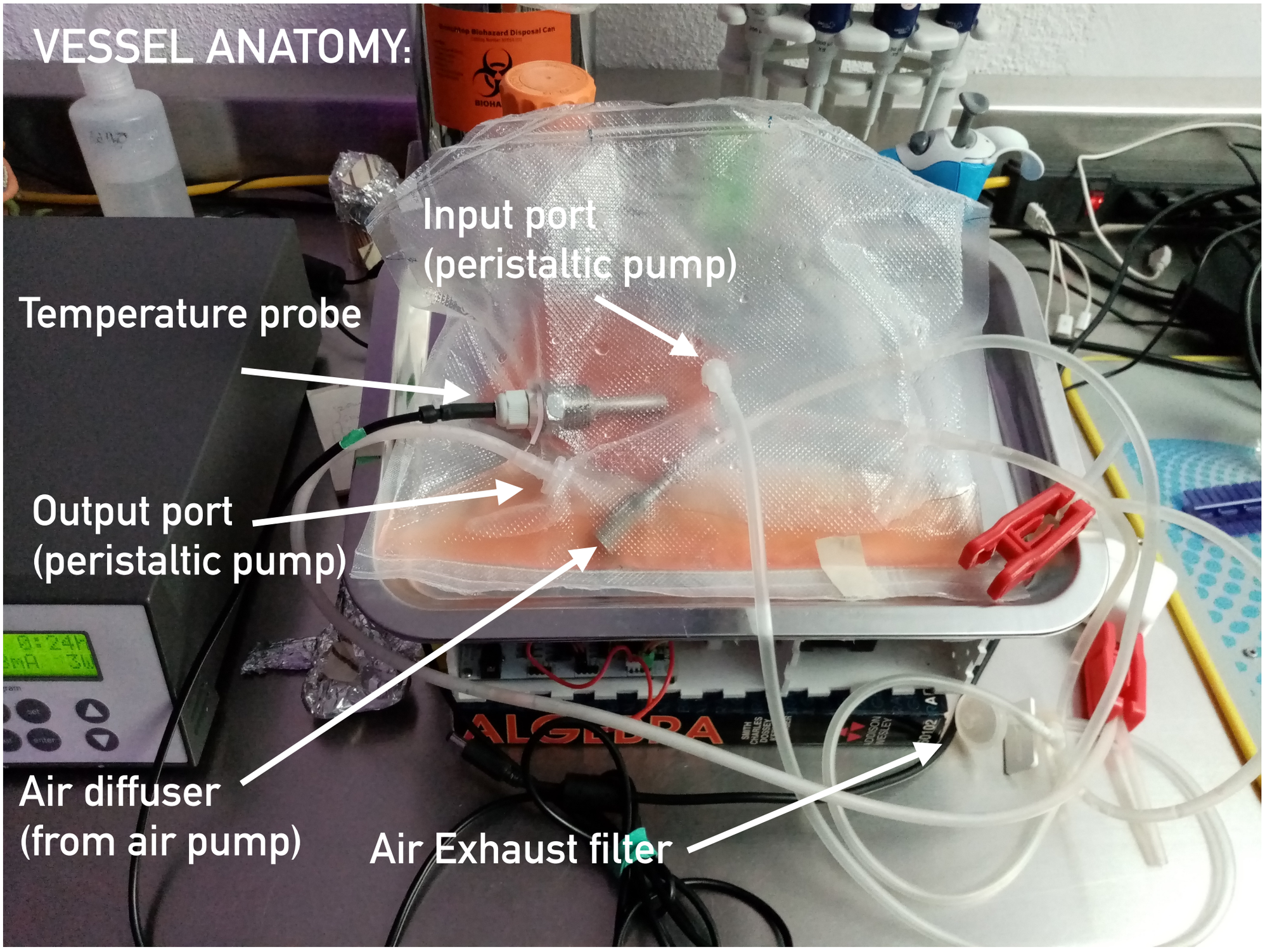


Recirculation of fresh media



E.coli BL21

UPSTREAM
2: GROWTH VESSEL FEATURES: “The landscape of your Batch”



UPSTREAM

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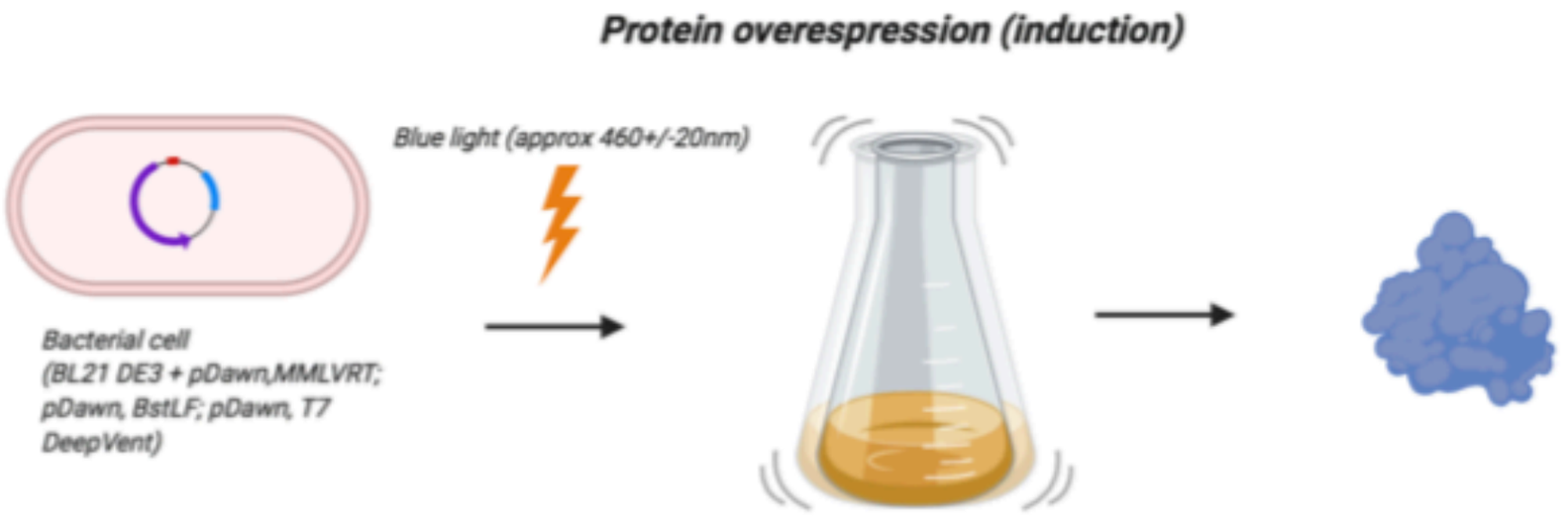

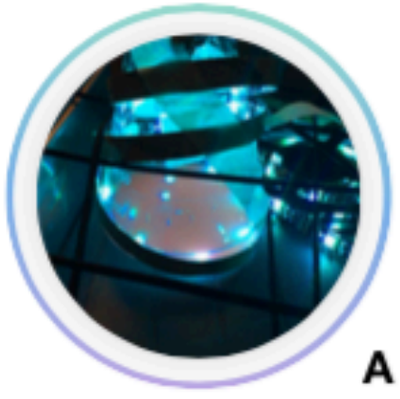

A

B

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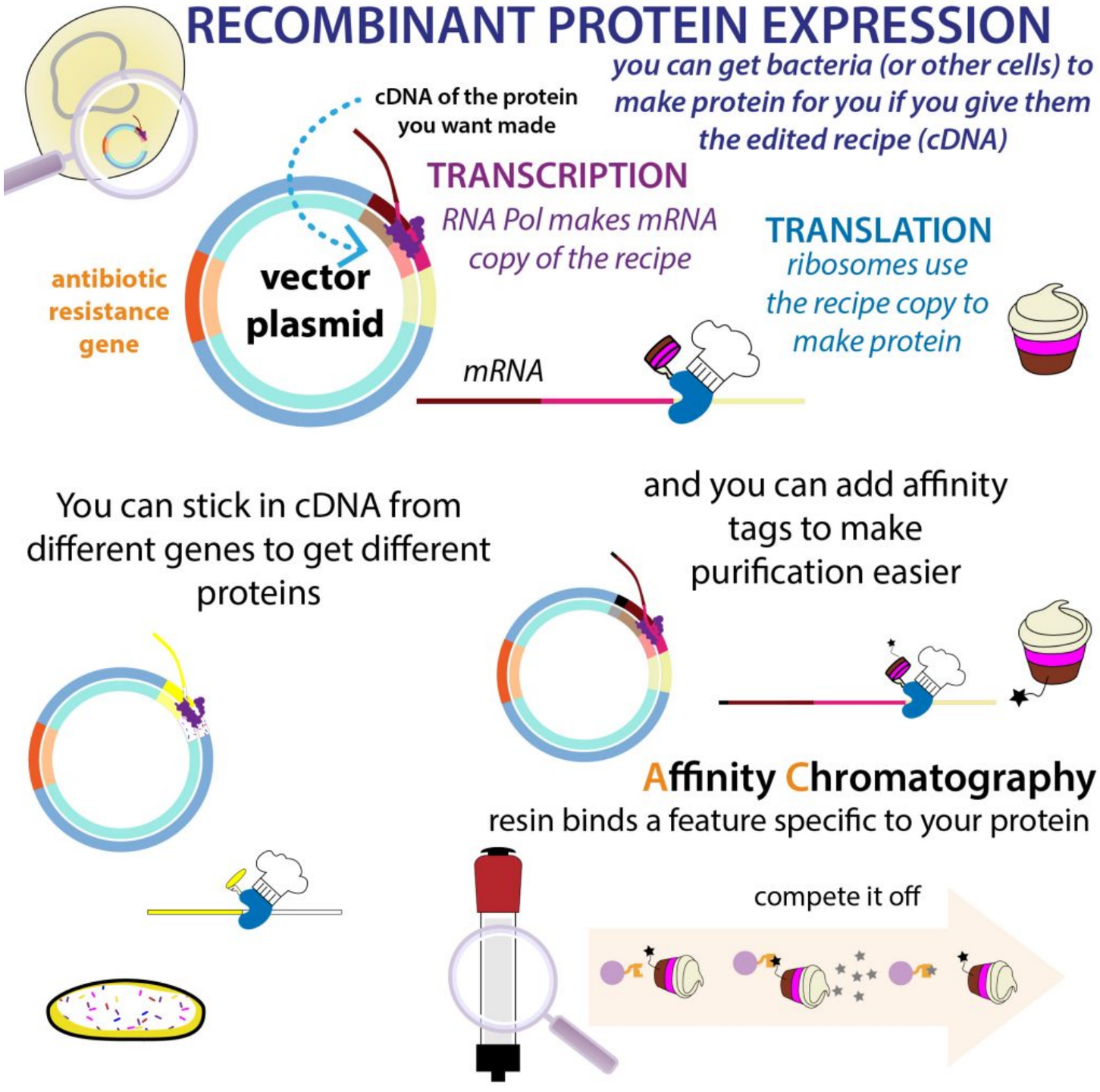
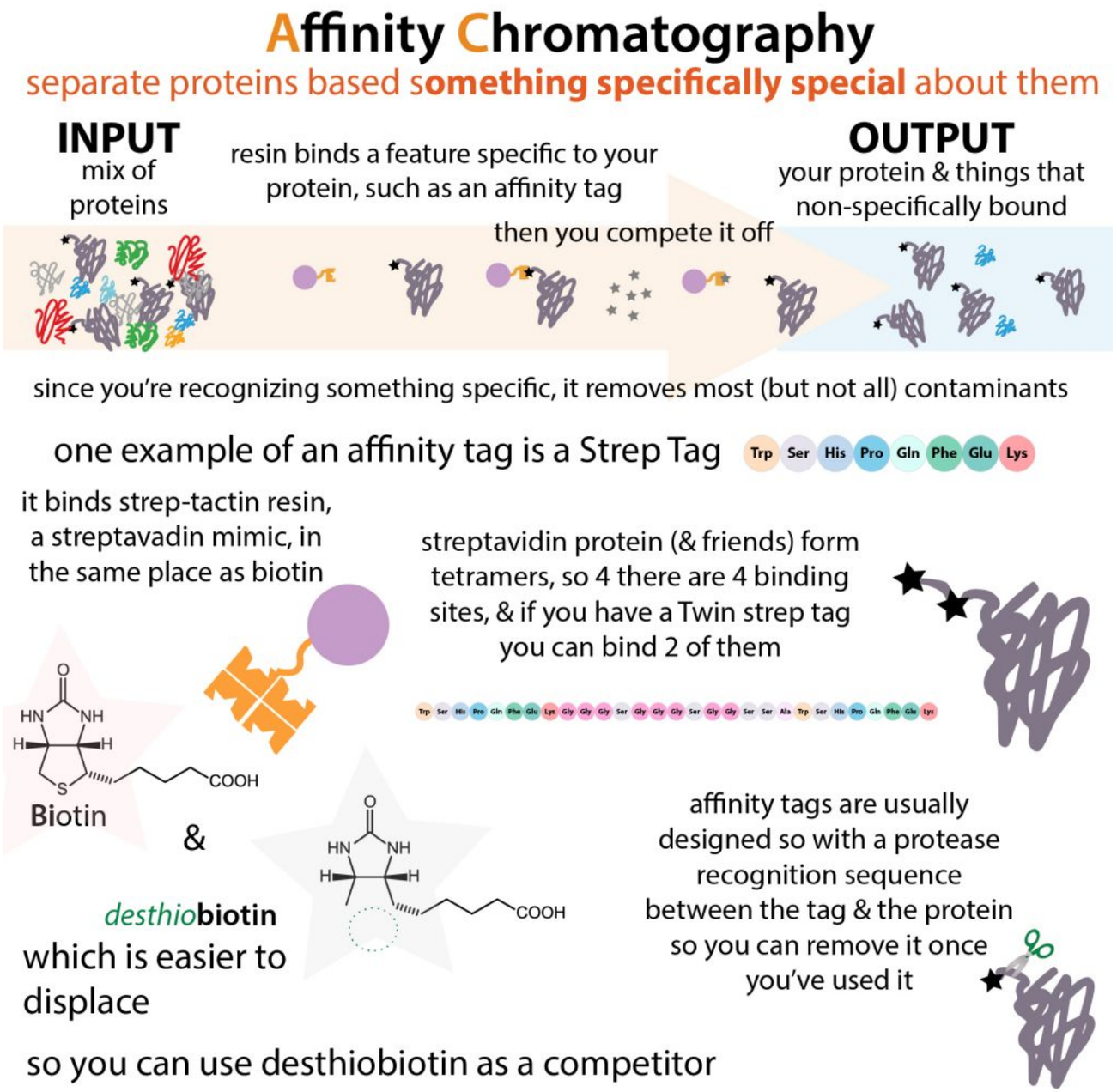
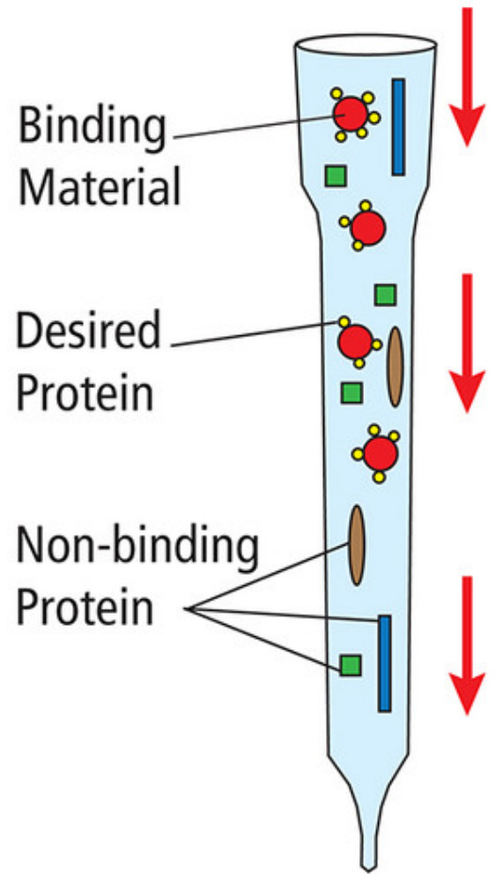


Briana Bibel, 2020 (The Bumbling Biochemist, Blog)

DOWNSTREAM
2: Reliable recovery system



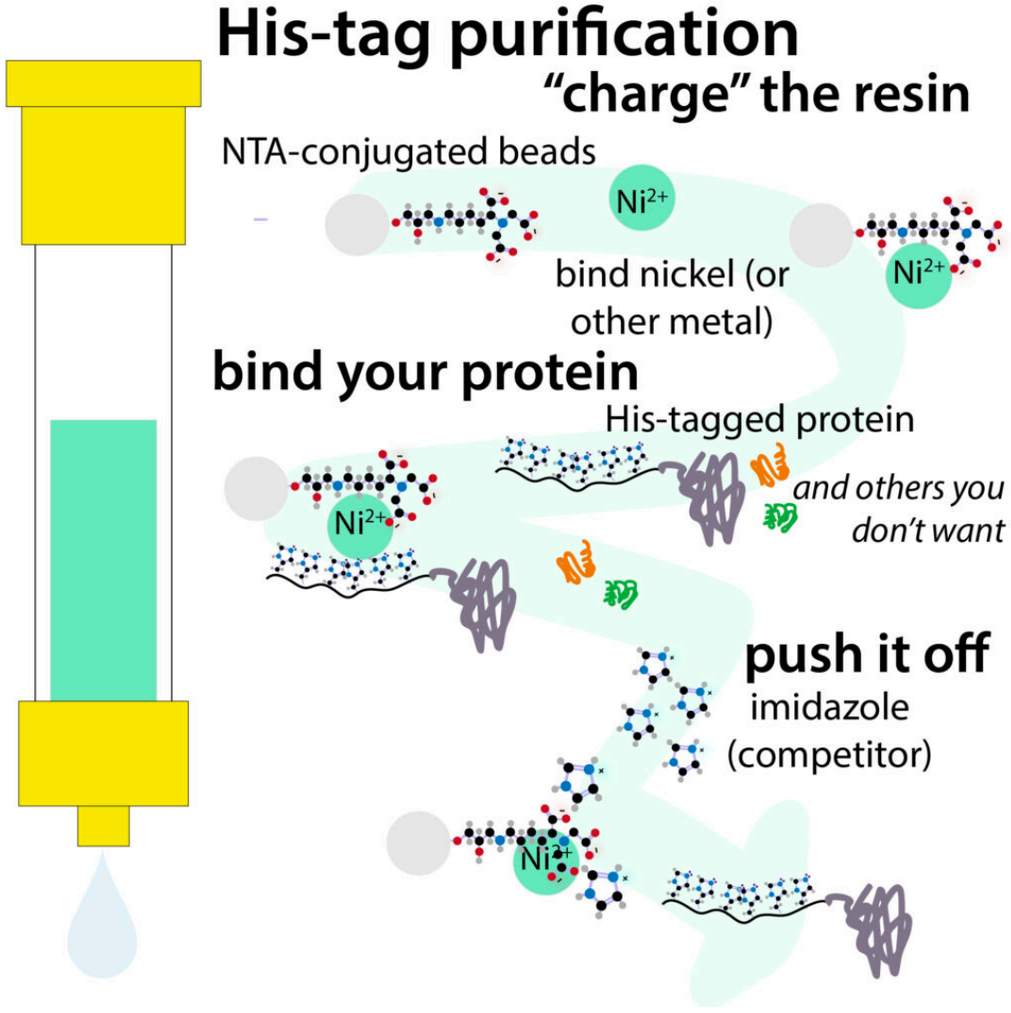


Briana Bibel, 2020 (The Bumbling Biochemist, Blog)

Edvotek 2020.

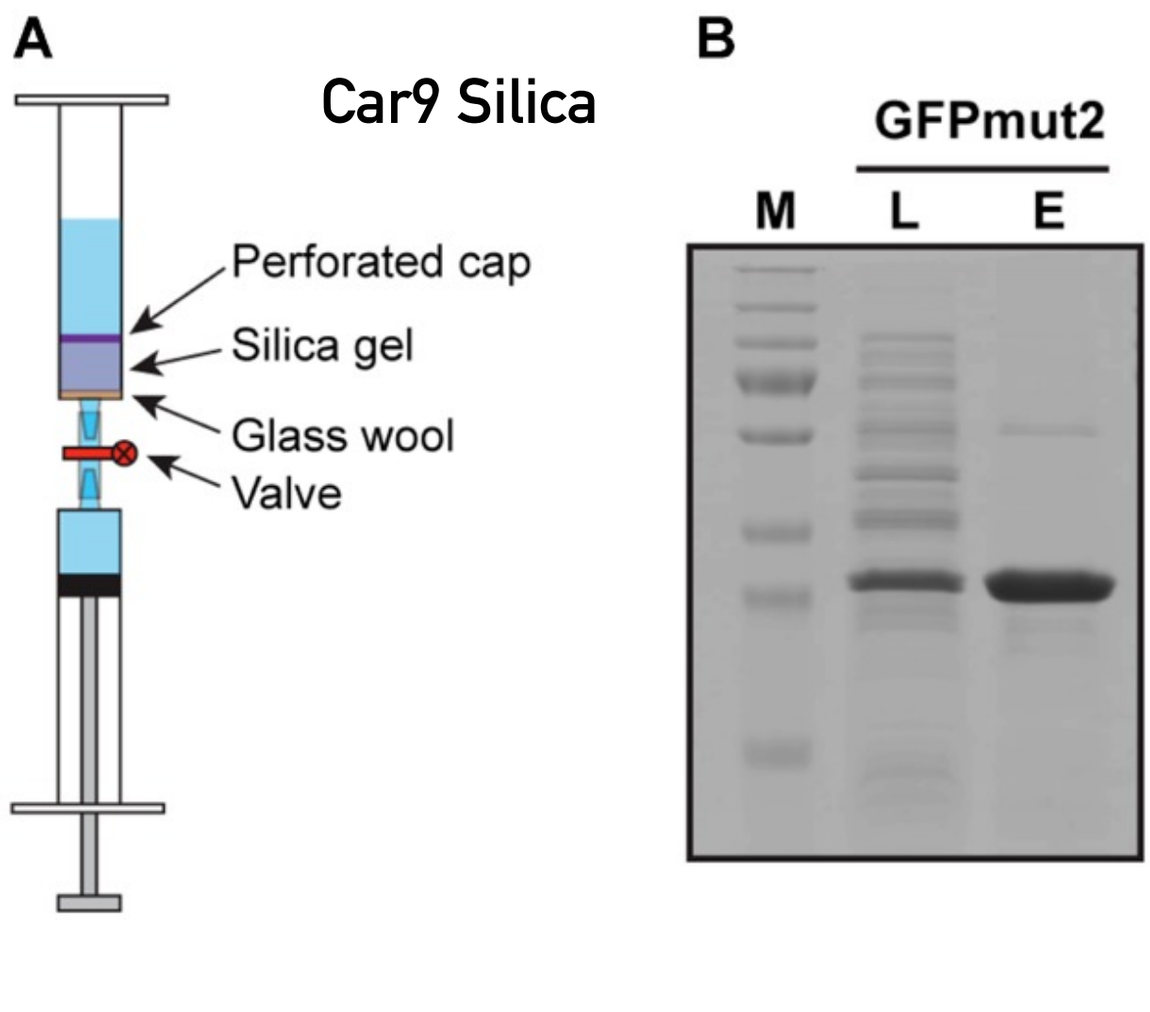
AFFINITY CHROMATOGRAPHY (PRACTICAL) PRINCIPLES

METAL ($$$$$$)



Briana Bibel, 2020 (The Bumbling Biochemist, Blog)

SILICA ($)



Coyle and Baneyx, 2014

AFFINITY CHROMATOGRAPHY (MANUAL) DEMO

https://www.sigmaaldrich.com/MX/en/technical-documents/protocol/protein-biology/protein-purification/instructions-for-affinity-purification-using-hitrap-columns

Thank you!

http://specyal.com/diybio/bioreactor.html

GLYXON + OTTAWA BIO SCIENCE

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Vladyslav Boyko
Nicolas Crudele
Adrian Jones
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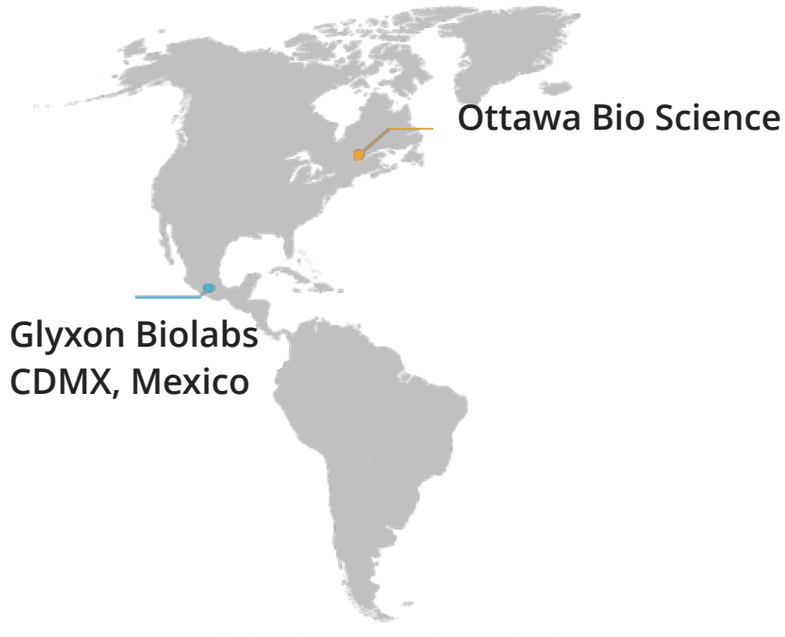
Charlotte Vanhoutte

and many more volunteers...



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Special acknowledgment to :

FREE GENES

PLEASE JOIN!