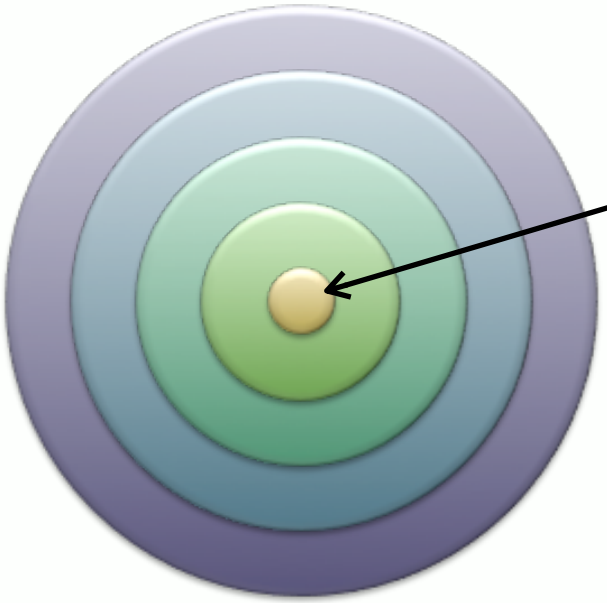
Concept



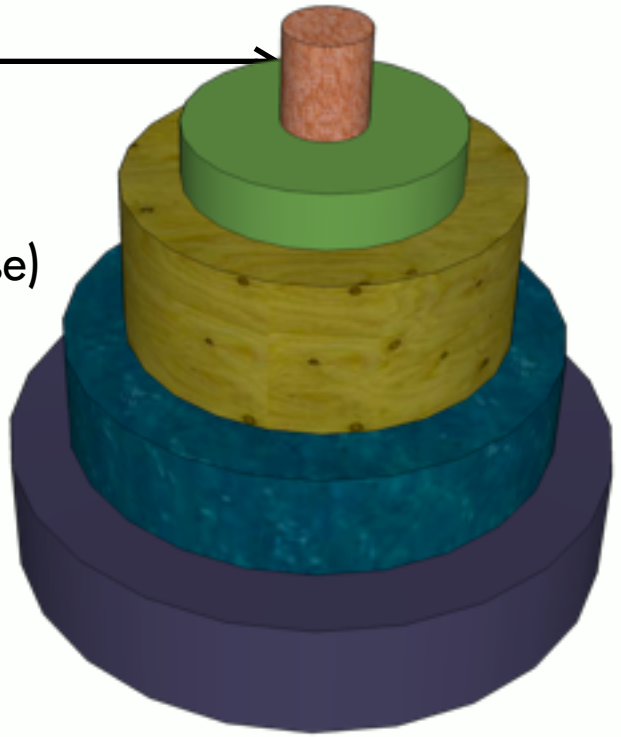
bound materials (metals)

fibers (silk, cotton, wool)

structural materials (cellulose)

crystals (silica, carbonate)

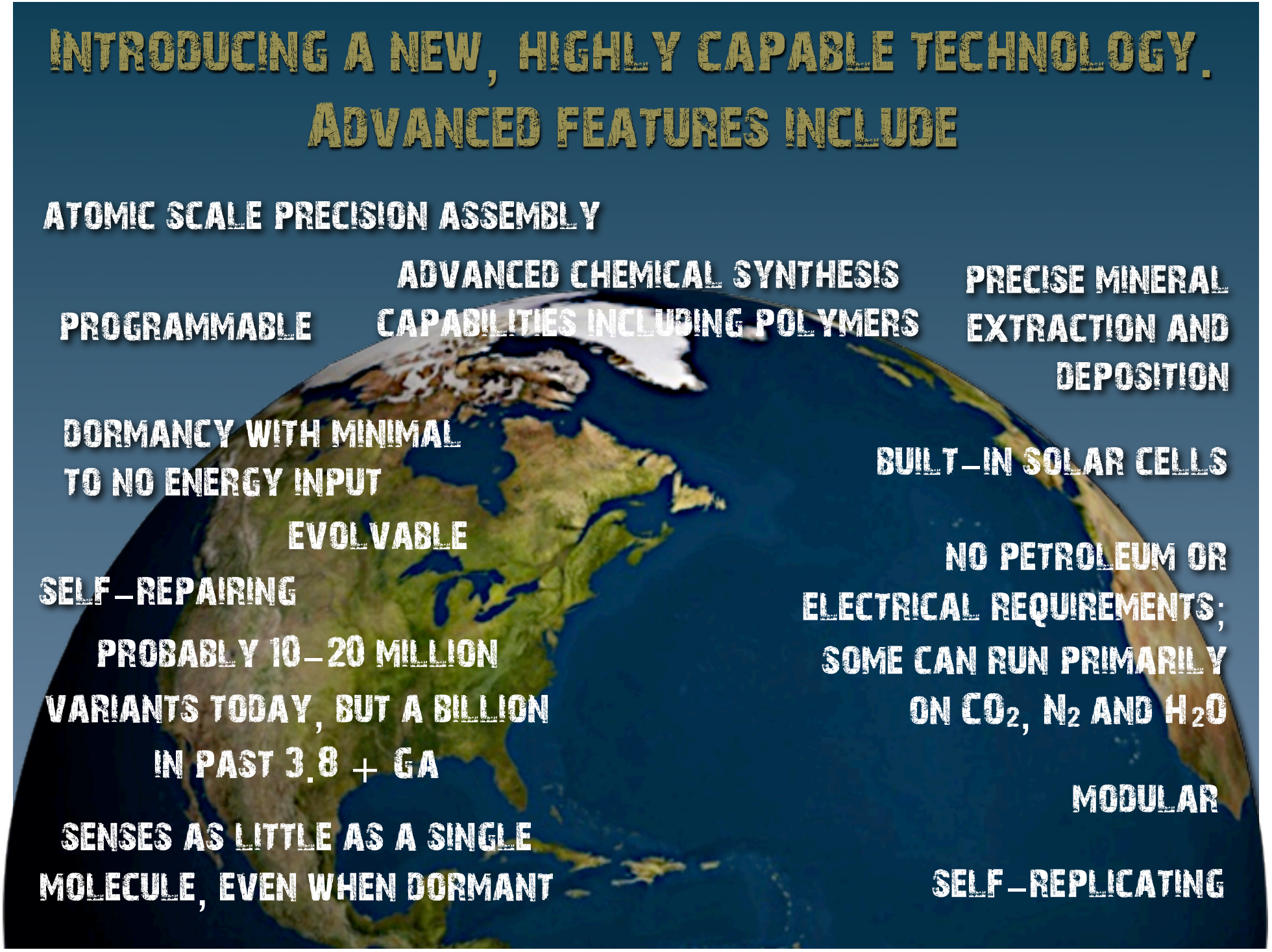
materials (latex)



Biomaterials out of thin air: in situ, on-demand printing of advanced biocomposites

Lynn Rothschild, Diana Gentry,Ashley Micks

Introducing a new, highly capable technology. Advanced features include



Atomic scale precision assembly

Advanced chemical synthesis capabilities including polymers

Programmable

Precise mineral extraction and deposition

Dormancy with minimal to no energy input

Built-in solar cells

Evolvable

Self-repairing

No petroleum or electrical requirements; some can run primarily on CO_2, N_2 and H_2O

Probably 10-20 million variants today, but a billion in past 3.8 + Ga

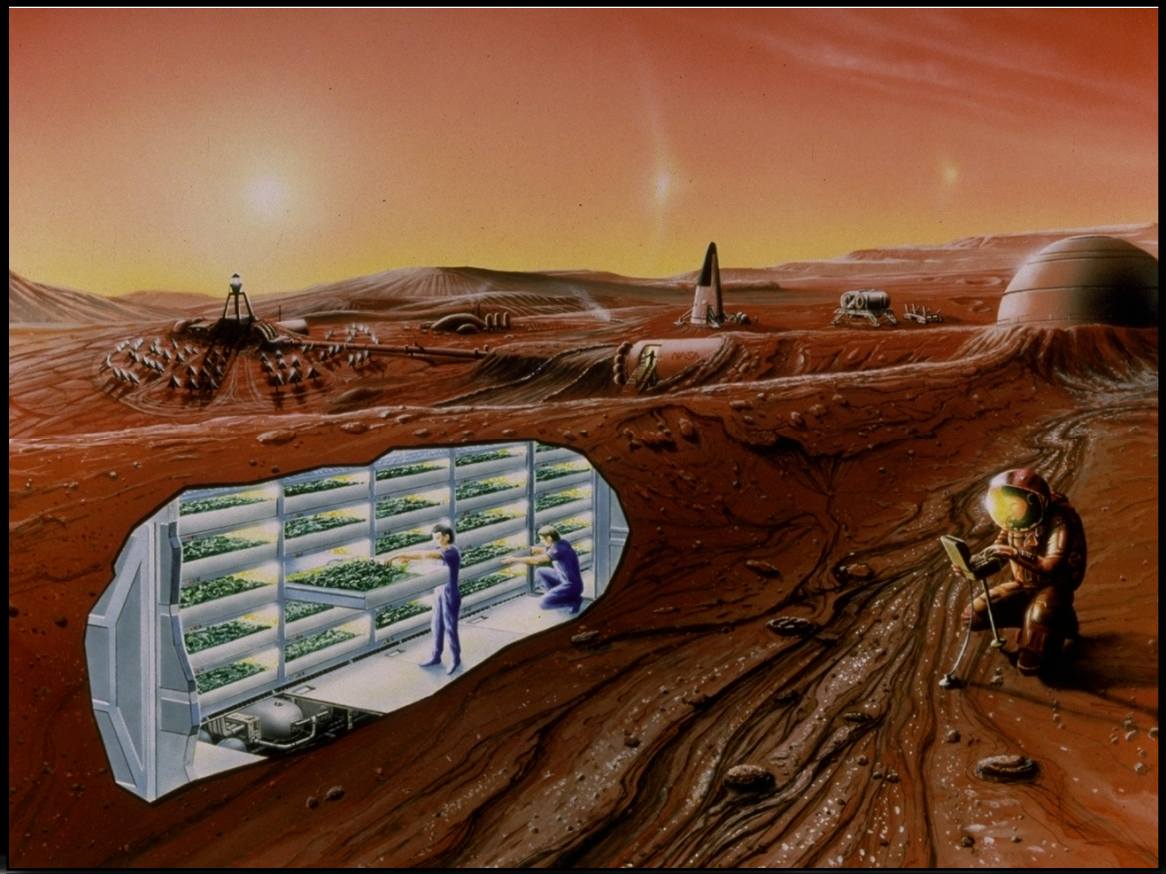
Modular

Senses as little as a single molecule, even when dormant

Self-replicating


going forth
from planet
earth

Needs for human settlement



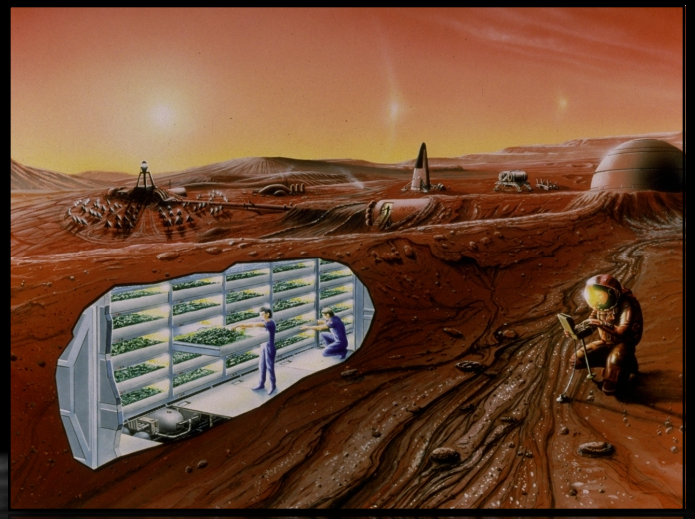
- Transportation
- Habitats
- Life support
  (food, oxygen, medicine, waste recycling, clothing, etc.)
- Power
- Heat
- Light
- Radiation protection

Needs

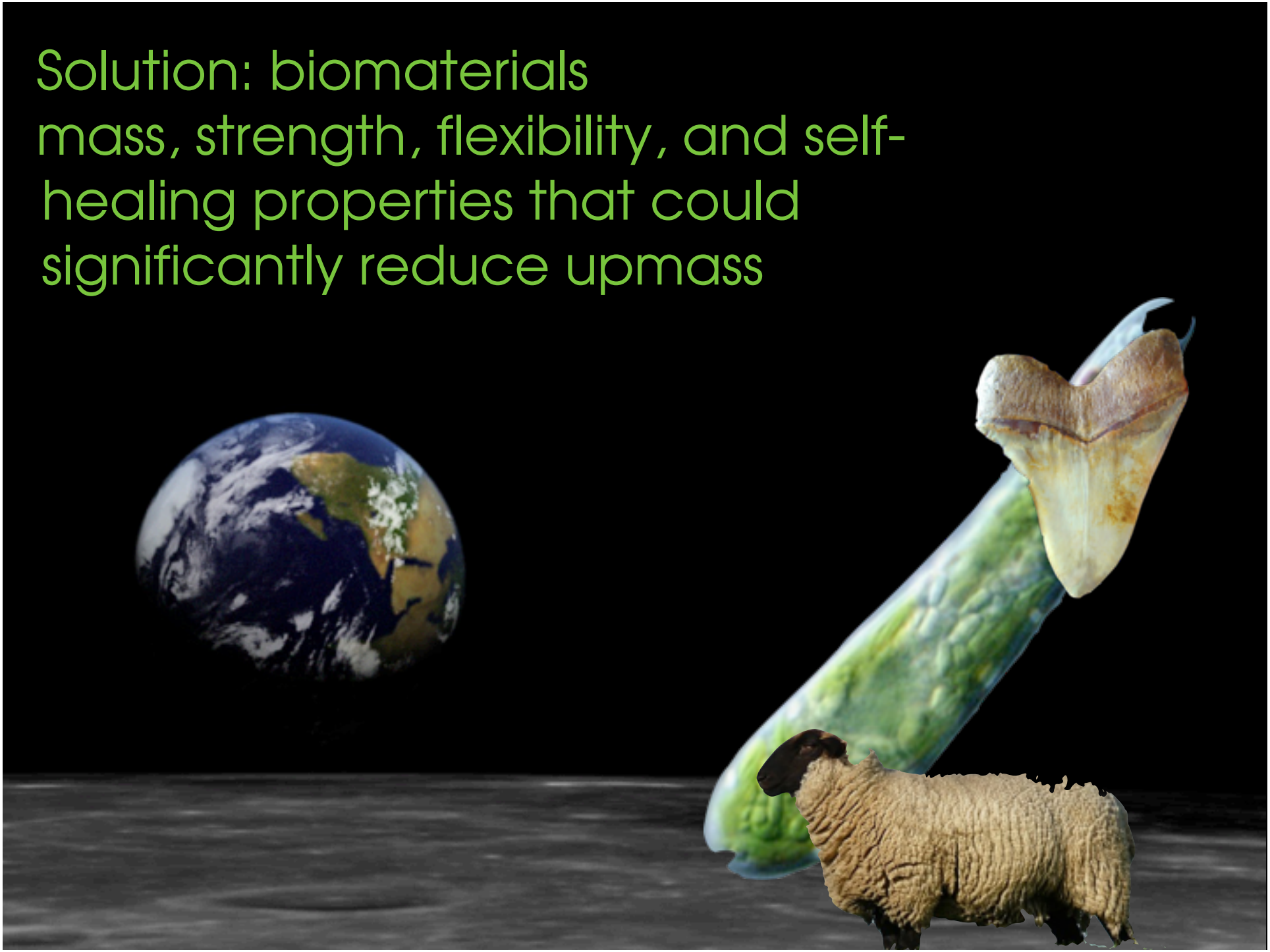
- Transportation
- Habitats
- Life support
  (food, oxygen, medicine, waste recycling, clothing, etc.)
- Power
- Heat
- Light
- Radiation protection

Challenges

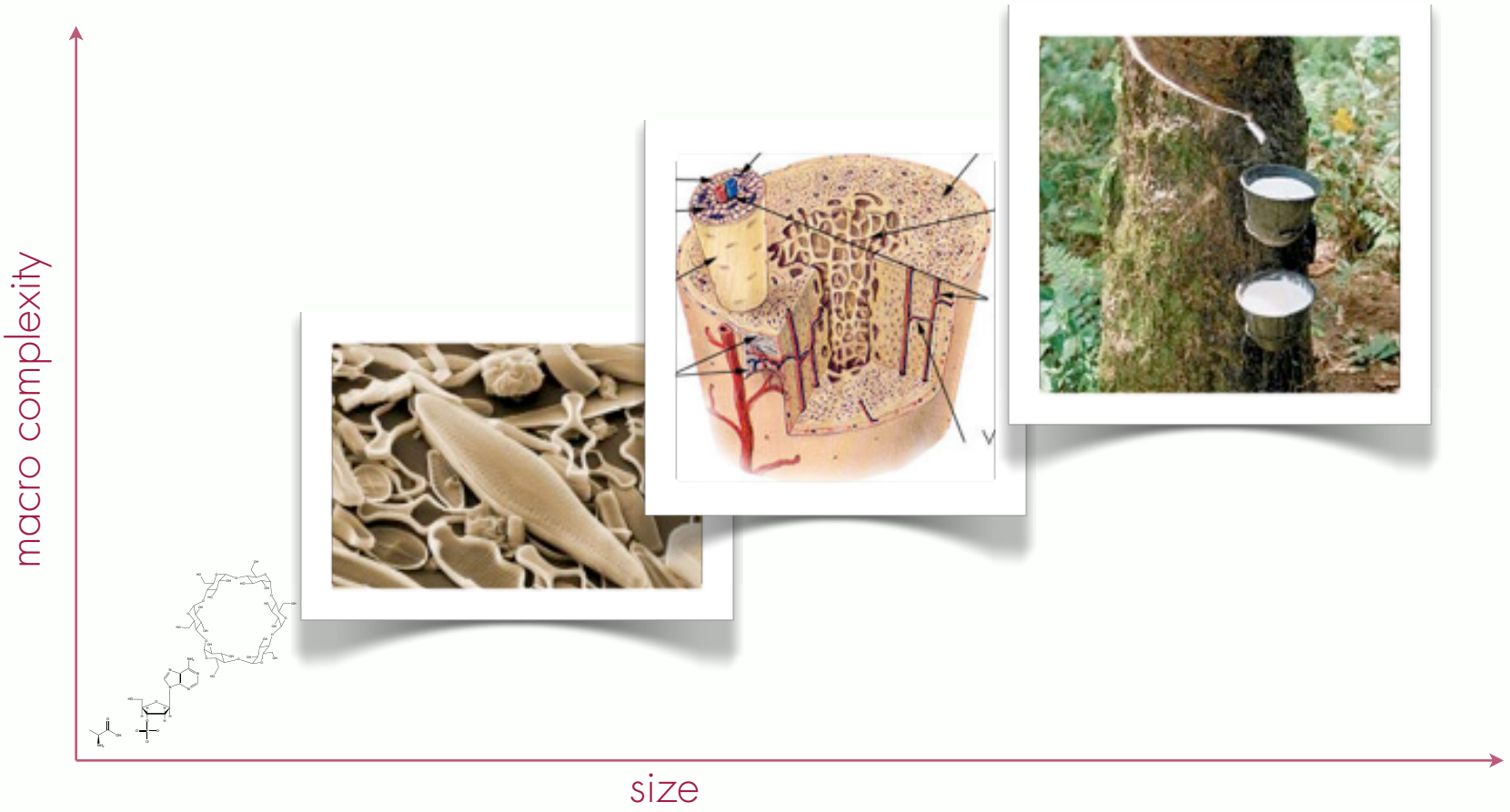
- Upmass
- Cost
- Storage
- Flexibility



Solution: biomaterials
mass, strength, flexibility, and self-healing properties that could significantly reduce upmass

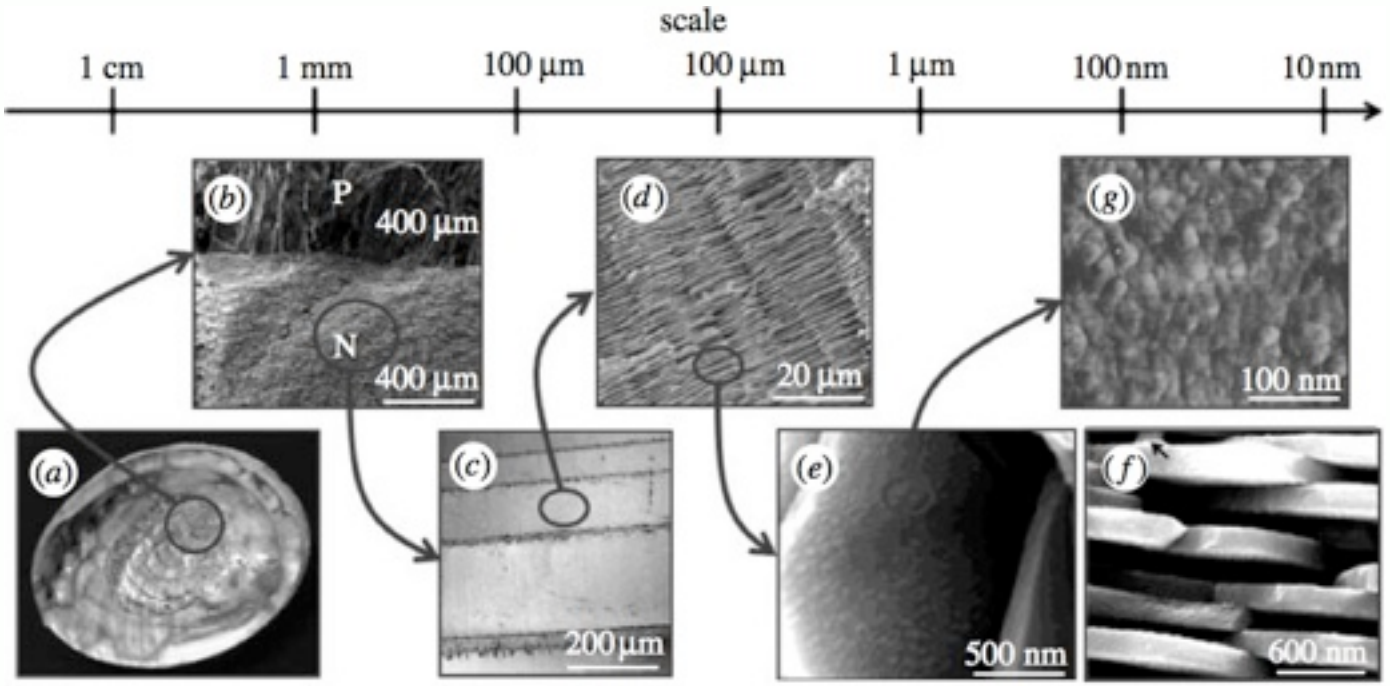


What is a biomaterial?

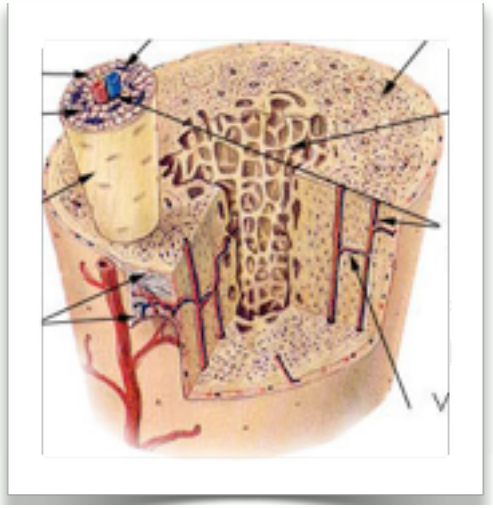


Biomaterials are natural materials produced by or incorporated into living systems, or human-made materials which mimic the same.

Biomaterials: Advantages



- ★ multiscale, hierarchical structures
- ★ unique mechanical properties
- ★ extreme functional customization



But there are problems with biomaterials

- Expensive, significant production infrastructure.
  Many biomaterials can only be produced as part of significant support ecosystem.
- Complex, non-predictable microstructure.
  The grain of wood, the porosity of bone, and so on are an integral part of the materials' desired mechanical properties, but are not deterministic when the material is naturally grown.
- Limited naturally-occurring compositions.
  Most biomaterials (unlike metal, plastic, etc.) cannot be easily combined or modified to produce new materials.

Solution: 3-D cellular printing

for earth:

- tissue engineering and medicine
- novel material design
- carbon sequestration

for space: reduce upmass while increasing mission flexibility: just-in-time manufacturing

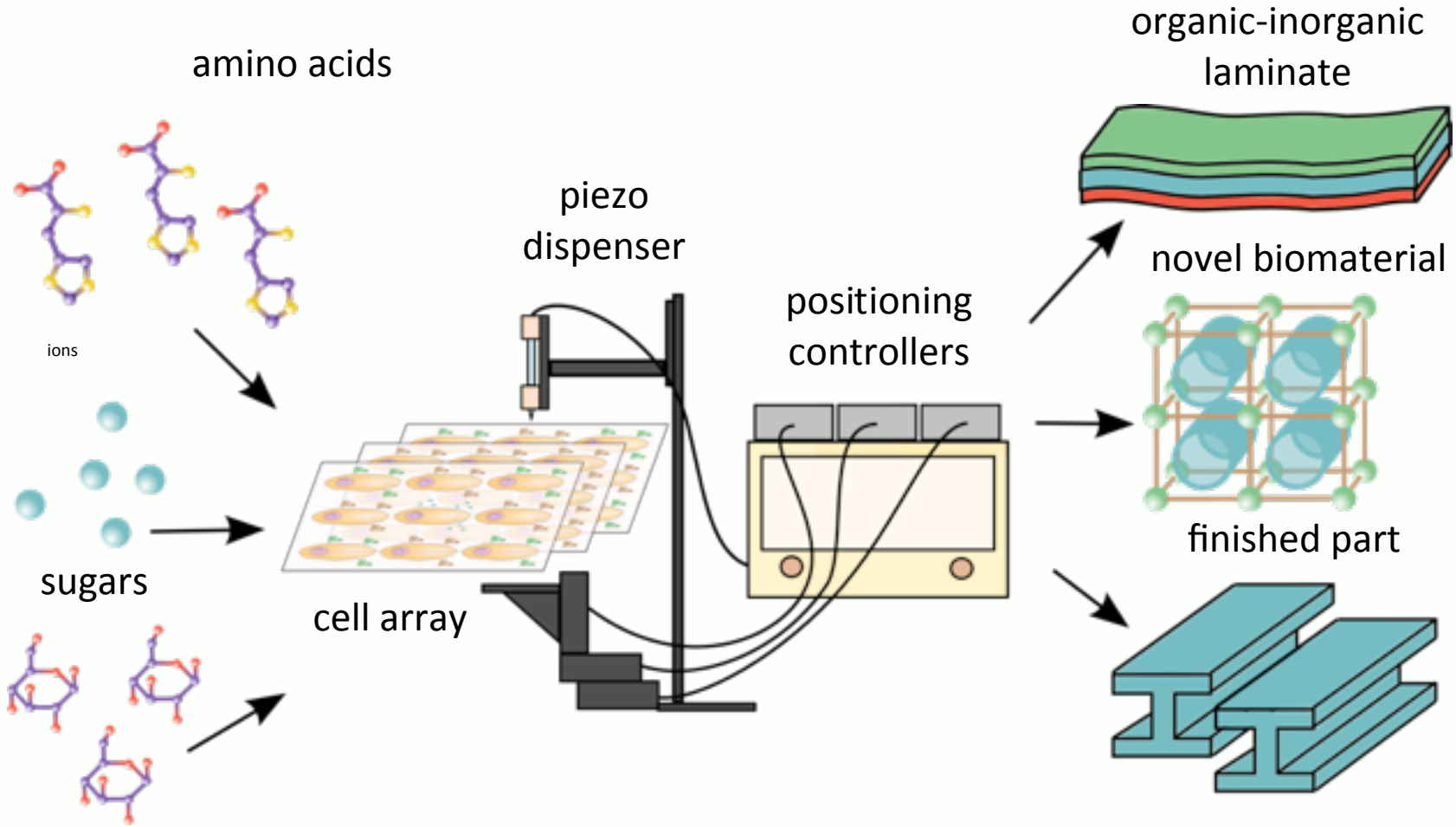
- food
- artificial organs
- advanced biocomposites for structures, filters, etc. etc.

WAIT! HAVEN'T WE HEARD THIS BEFORE?

NOPE.

- ★ Tissue engineering works with integrated living cells in larger functional groups and does not (usually) implement genetic engineering.
- ★ Traditional 3D printing uses the final material component(s) as feedstock, and is limited to (relatively) homogeneous materials.
- ★ Current synthetic biology for biomaterial production is largely limited to single, bulk, unstructured biomolecules (e.g., biofuels, pharmaceuticals).

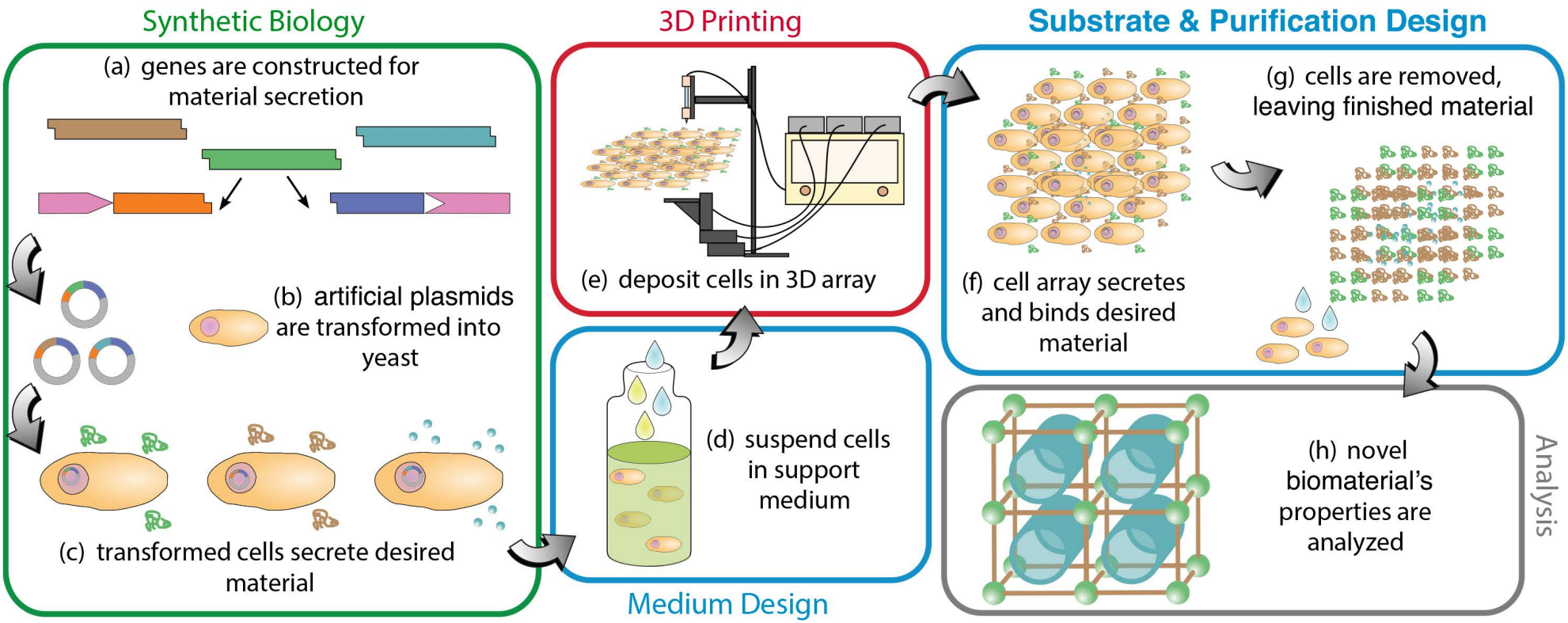
Unlocking Synthetic Biomaterials



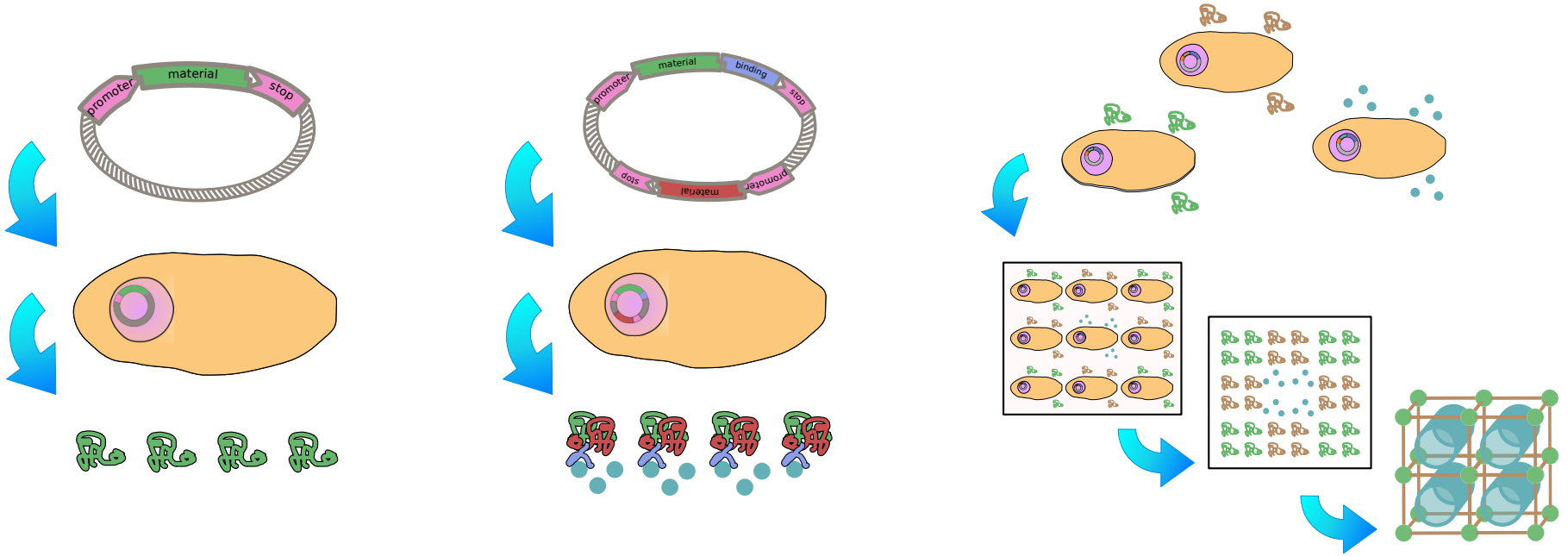
★ NIAC, 2013. Rothschild & Gentry

Goal: A Proof of Concept

To make a two-material grid demonstrating that al component parts of the technique are functional.

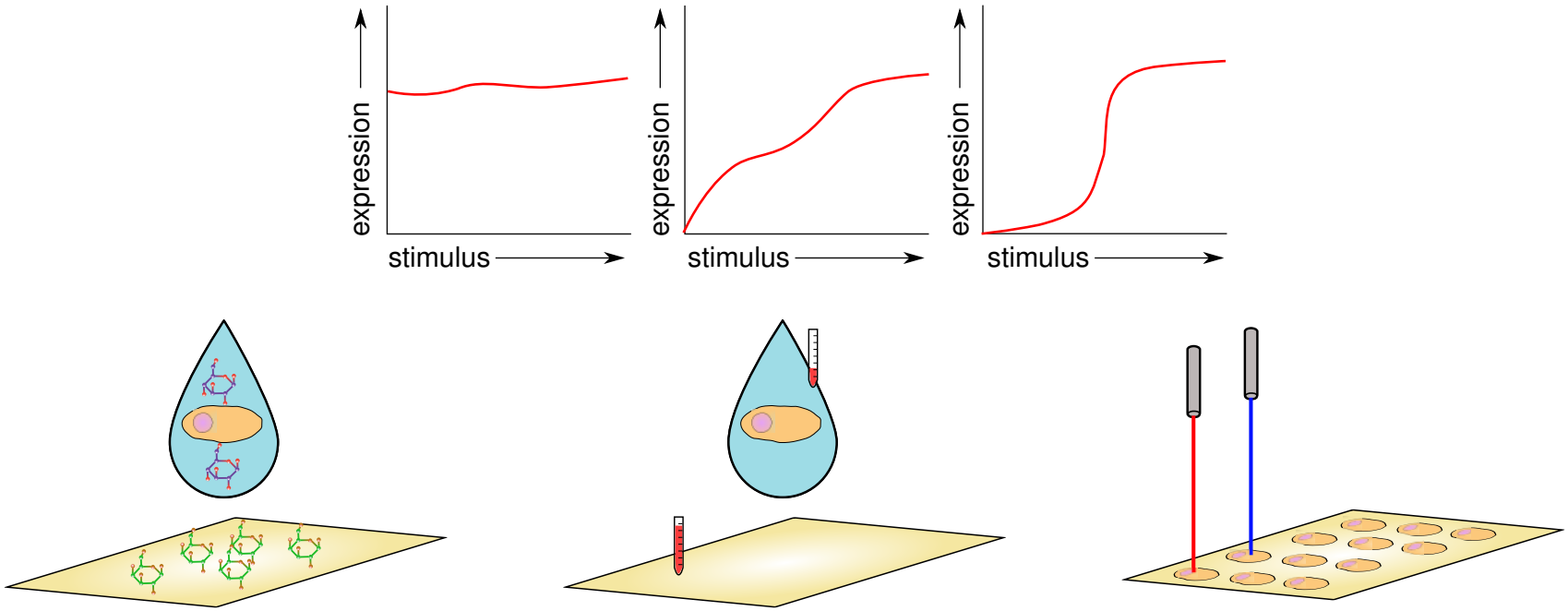


Material Selection



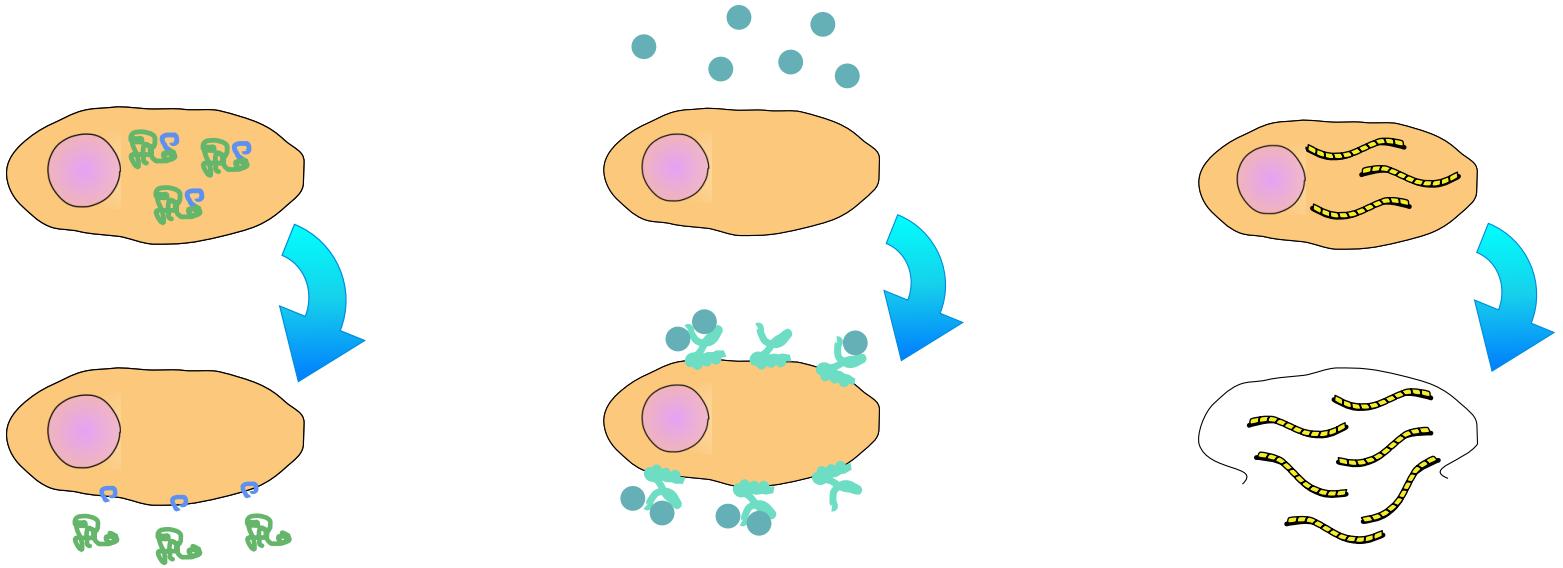
- Typical options:
  - any material a cell can make or move
  - few materials have established genetic parts
  - many are multi-gene or even multi-cell origin

Material Production Stimulus



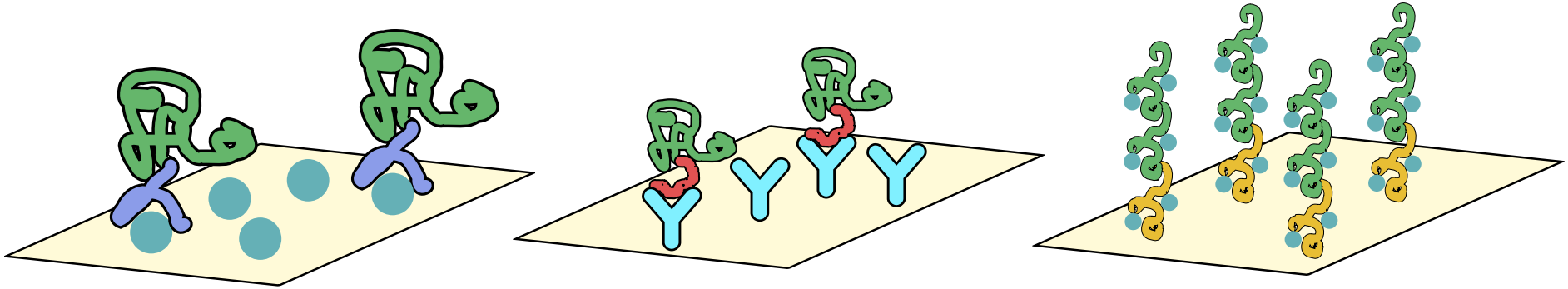
- Typical options:
  - chemical presence (fine internal structures)
  - thermal (flat form factors, gradients)
  - optical (layered materials, surface features)

Material Delivery Method



- Typical options:
  - secretion (proteins, some biominerals)
  - sequestration (metal ions, biominerals)
  - lysing (silk, cotton, fibers, bioplastics)

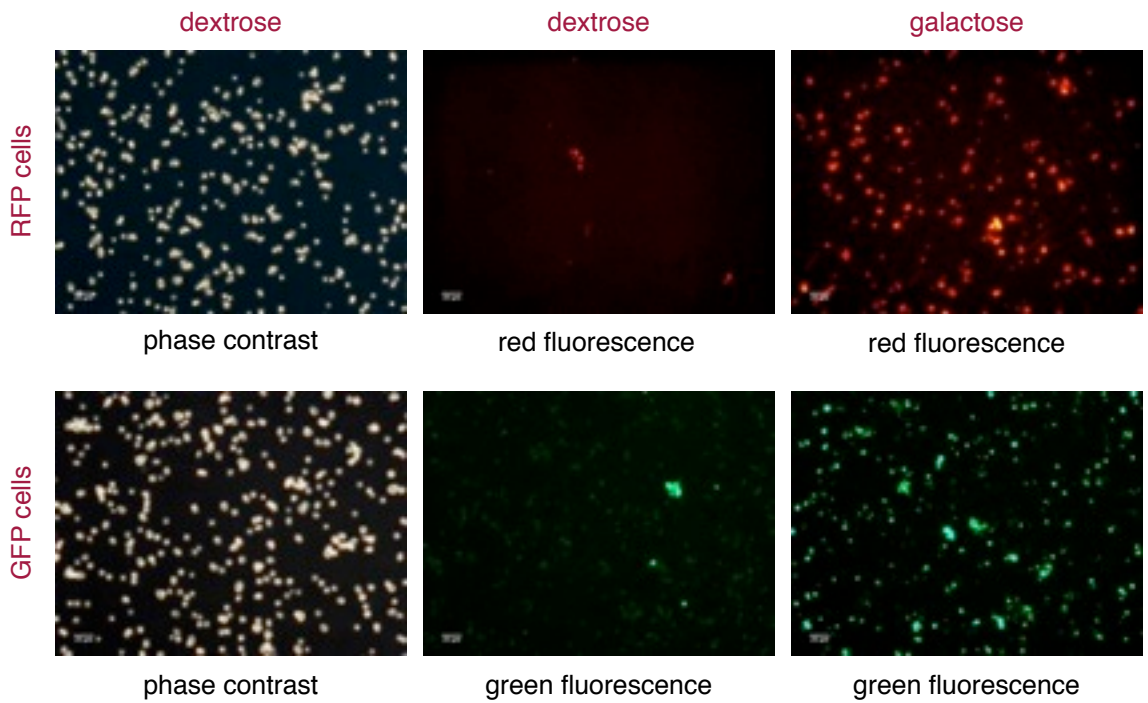
Material Binding & Washing



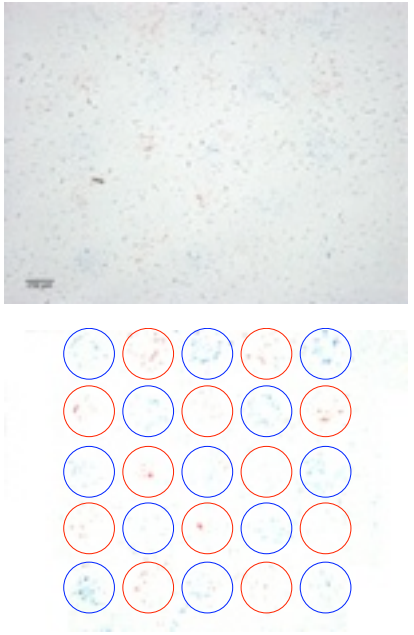
- Typical options:
  - polyhistidine tags (common)
  - antibody/epitope pairs (common)
  - self-assemblages (rare, but promising)

Proof of Concept Implementation (1)

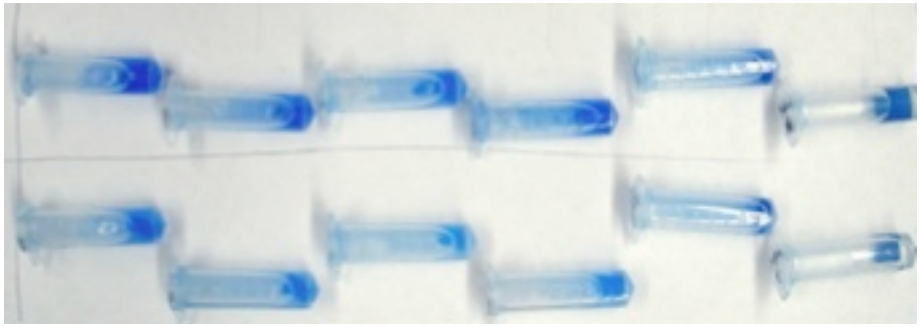
GFP/RFP fusion proteins on GAL1 promoter



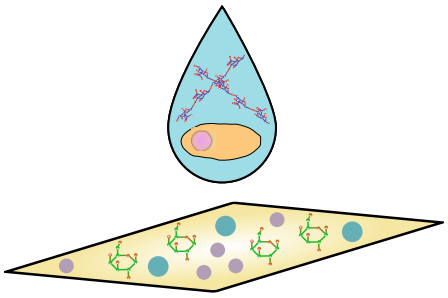
2-Material Grid Pattern



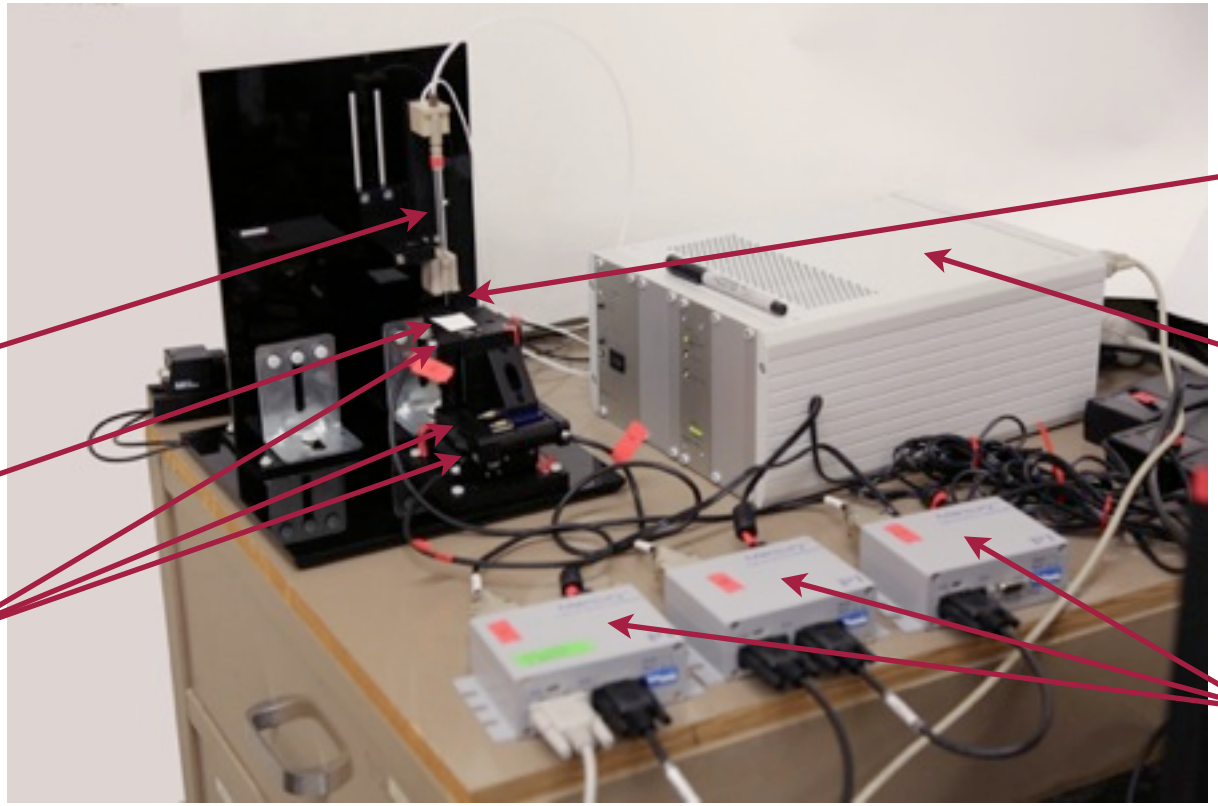
Polyhistidine/Ni^{+2} tag binding



Alginate/Galactose Substrate



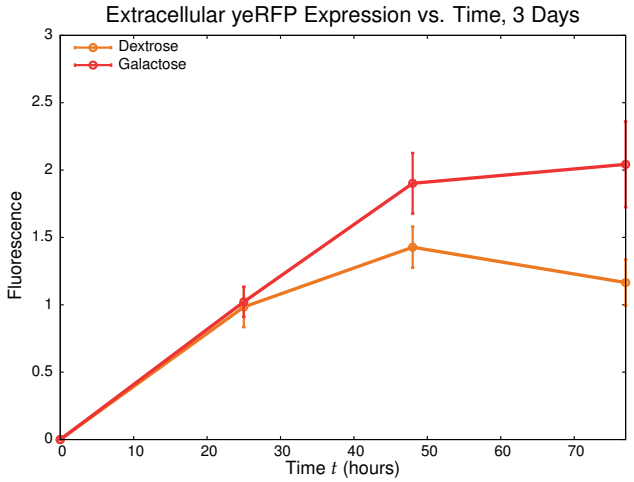
Proof of Concept Implementation (2)



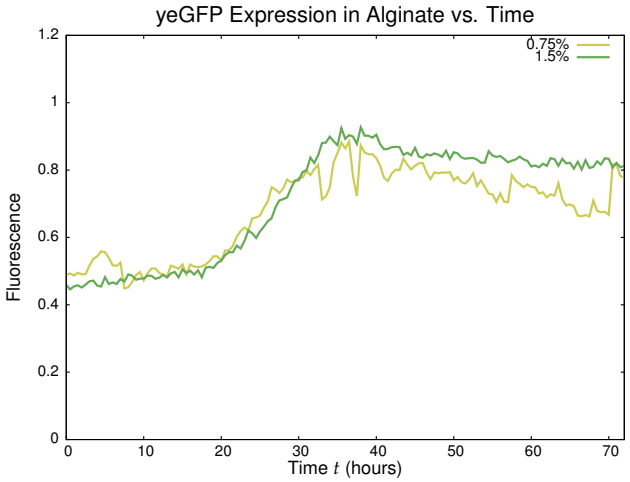
- piezo pressure dispenser with 3-axis platform
- COTS components with in-house software and hardware integration

Current Status (1)

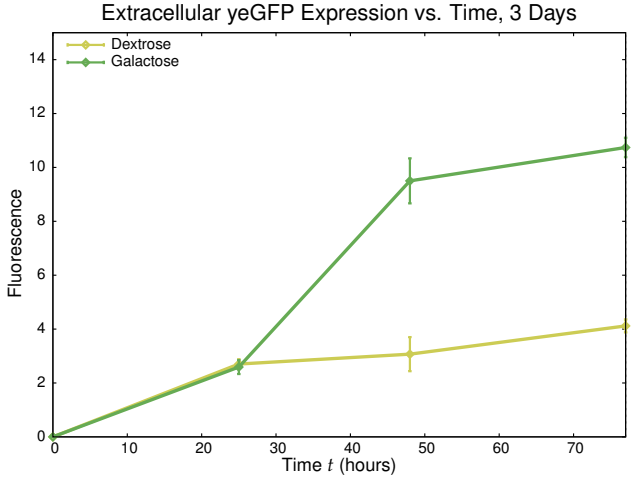
RFP secretion is strong



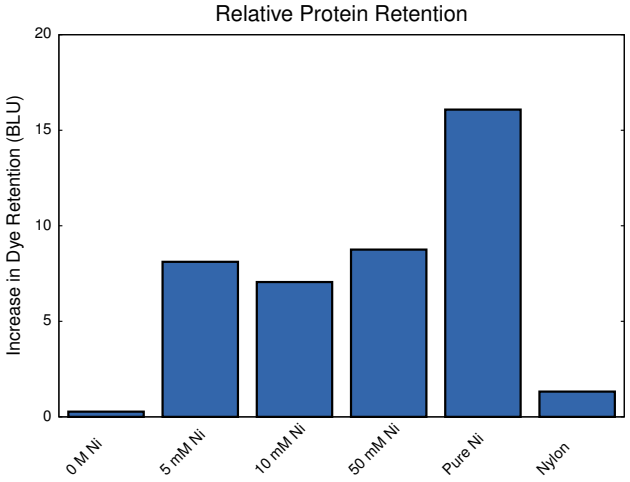
Post-printing expression is strong



GFP secretion is strong



Protein binding is detectable

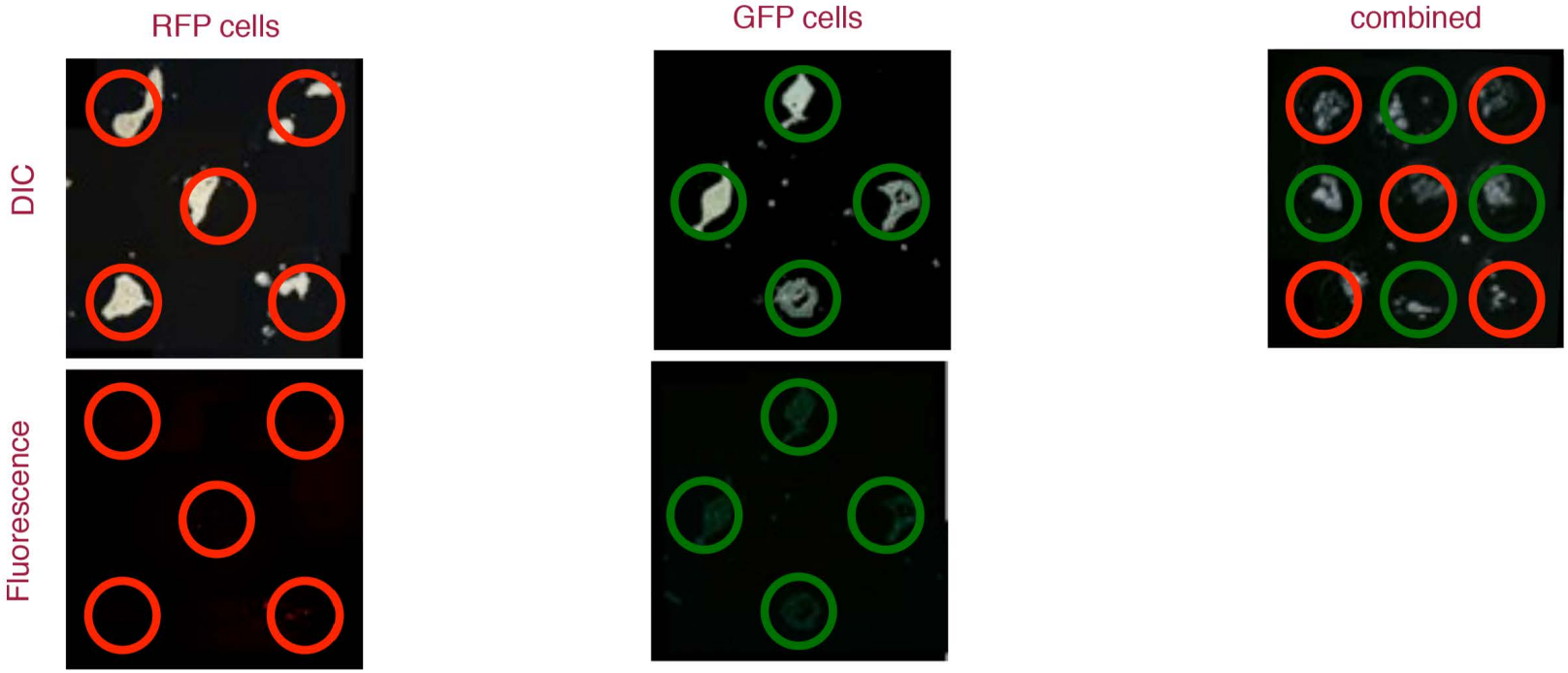


Current Status (2)

Printing parameters

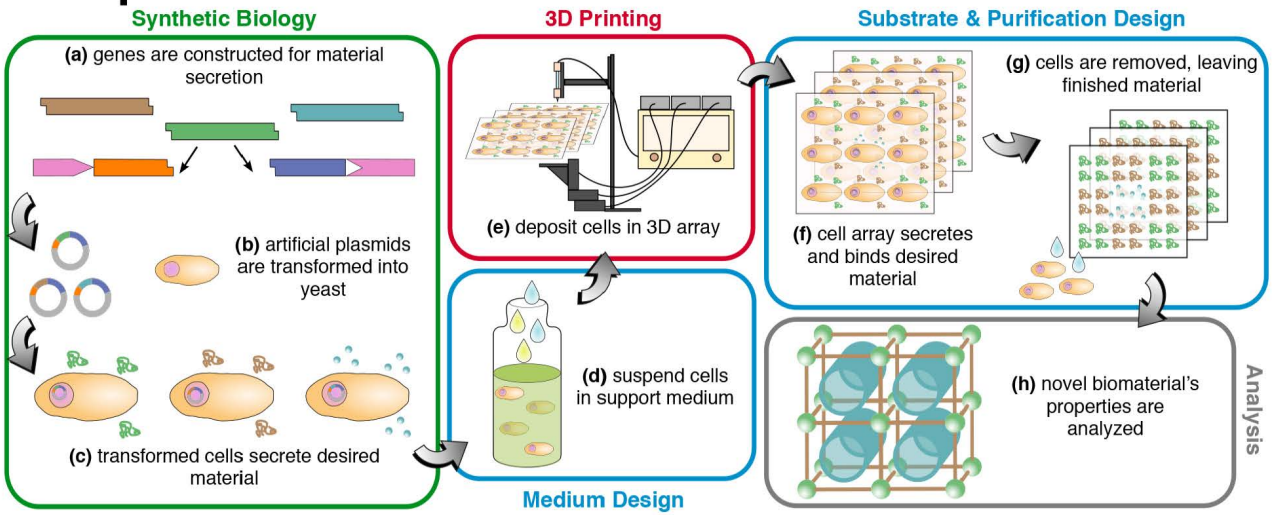
Metric	Minimum	Desired	Current
positioning precision	1 cell diameter $\sim$ 10 μm ✓	1 μm	2 μm
dispensing volume	$\leq$ 1000 $\frac{\text{cells}}{\text{“voxel”}}$ ✓	$\sim$ 1 $\frac{\text{cell}}{\text{“voxel”}}$	$\leq$ 10 $\frac{\text{cell}}{\text{“voxel”}}$
“voxel” size	enc. 1000 cells = 1 nL ✓	10 pL	20 pL
cell survival	50% ✓	90%	$\sim$ 50%
pattern completion	75%	95% ✓	$\geq$ 95%

Two-material printed grids



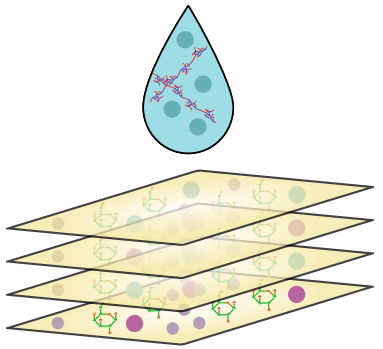
Phase I: Work in Progress

- All individual steps verified; end-to-end proof of concept is next

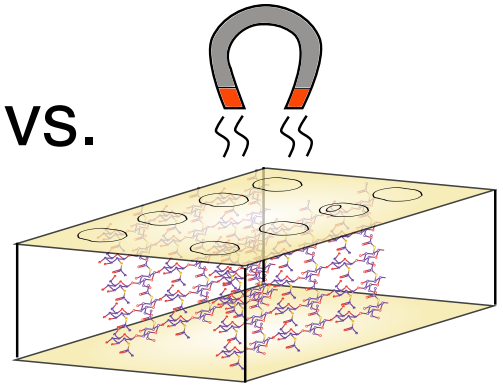


- Improve and quantify binding efficiency
- Move to better verification method
- Implement additional ‘base materials’
- Experiment with effects of layered deposition

Phase II: Potential Paths



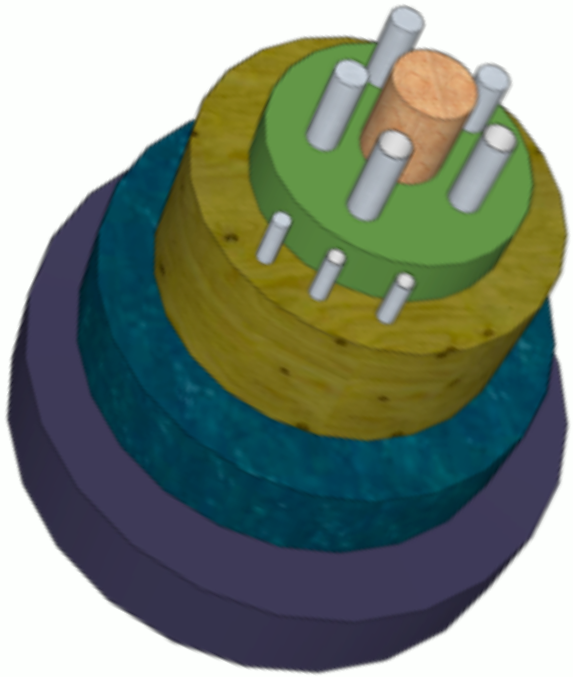
- 3D-print substrate/scaffold as well as cells for finer spatial control
- Implement optical control of expression
- 3D material grids: compare layered deposition, ‘wicking’, extrusion, etc. vs. different desired form factors



- Begin working with truly structural materials, including non-protein-based

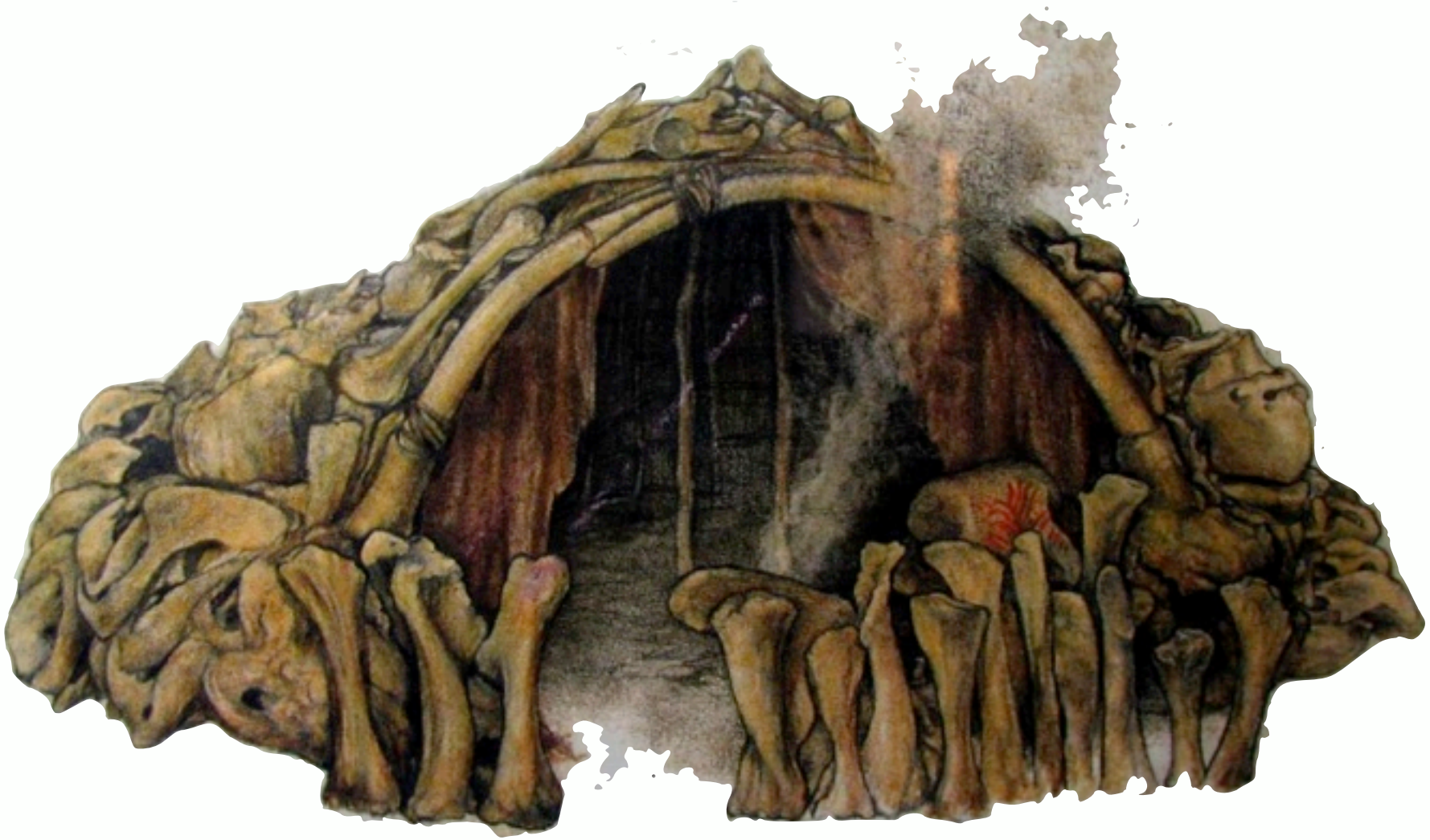
Where to go from here?

- Needed short-term improvements:
  - Improve dispensing parameter tuning
  - Explore alternate binding methods
  - Verify cell removal (pigmentation?)
- Next investigations:
  - Explore more supportive print substrates
  - Try a composite material
  - Try organic/inorganic materials Immediate
  - Implement additional, more structural material types
  - Add additional delivery methods
  - Add additional stimulus methods

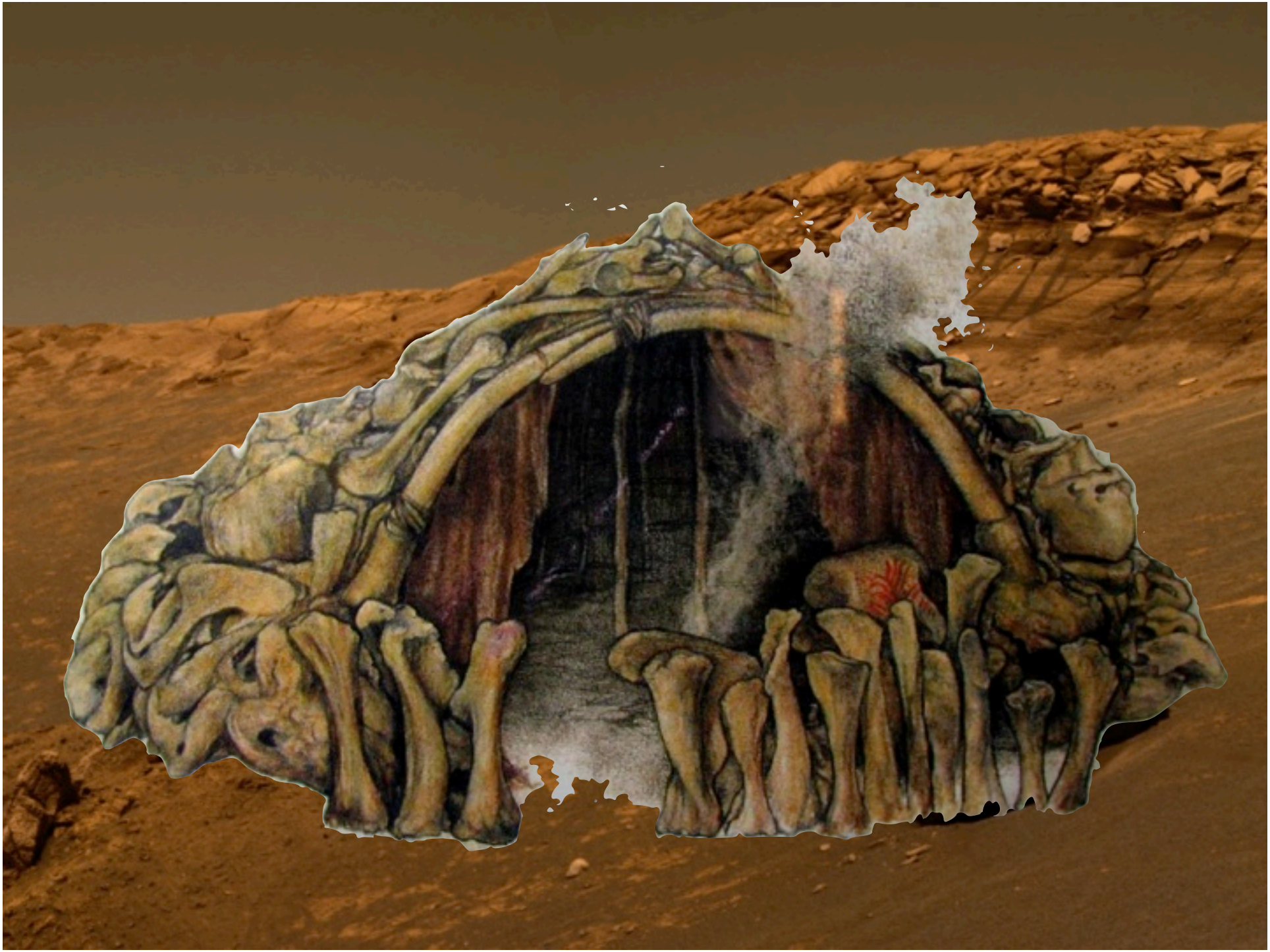


- Phase II
  - Use real production cells. Candidates include silk and cellulose production, metal binding, and organic/inorganic composite.
  - Explore alternate approach of two-laser activation of secretion

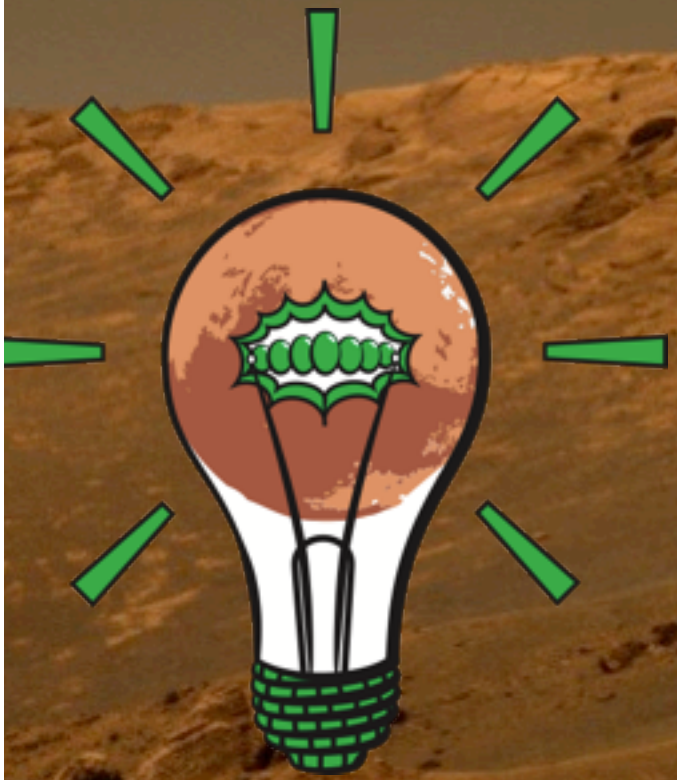
back to the future?

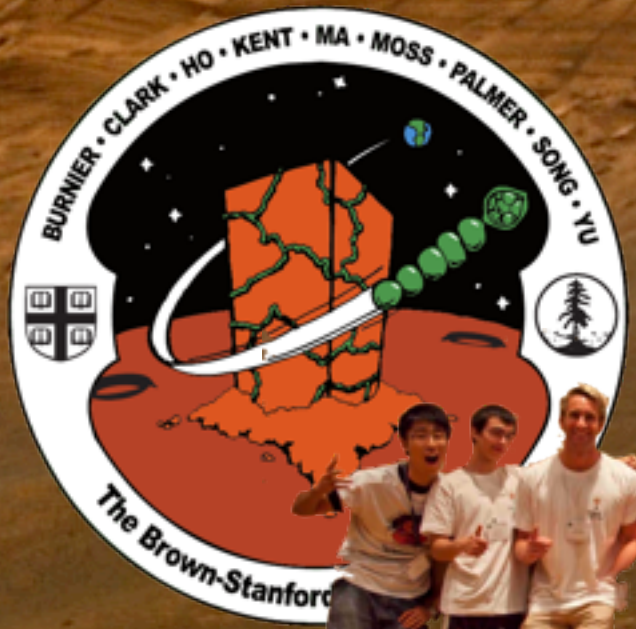


★ A dwelling in Mezhirich is made of mammoth bones partially supported by a wooden frame. Hides lining the hut serve as insulation. Source: Display, Dolní Věstonice Museum



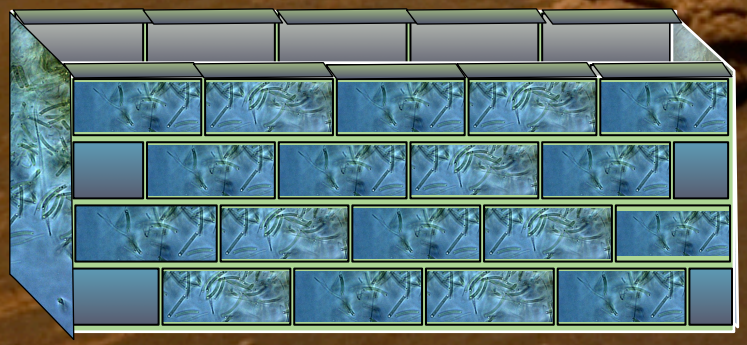
Synthetic Biology Applications for Space Exploration





Brown-Stanford iGEM 2011

RegoBrick



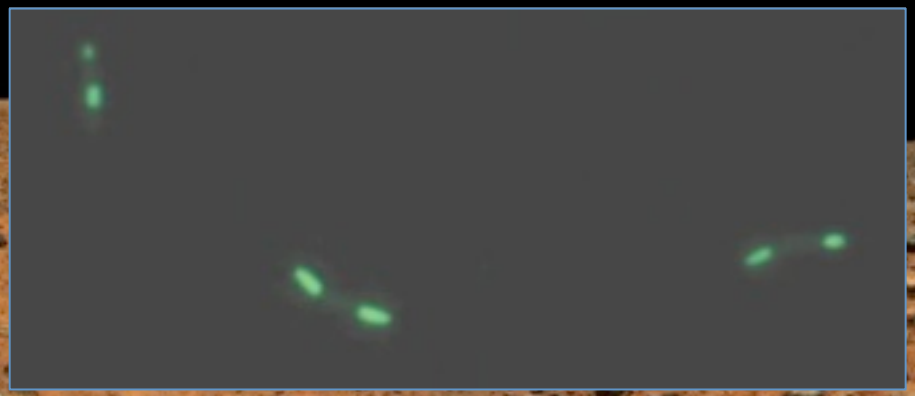
STANFORD-BROWN 2012

Stanford-Brown iGEM 2012

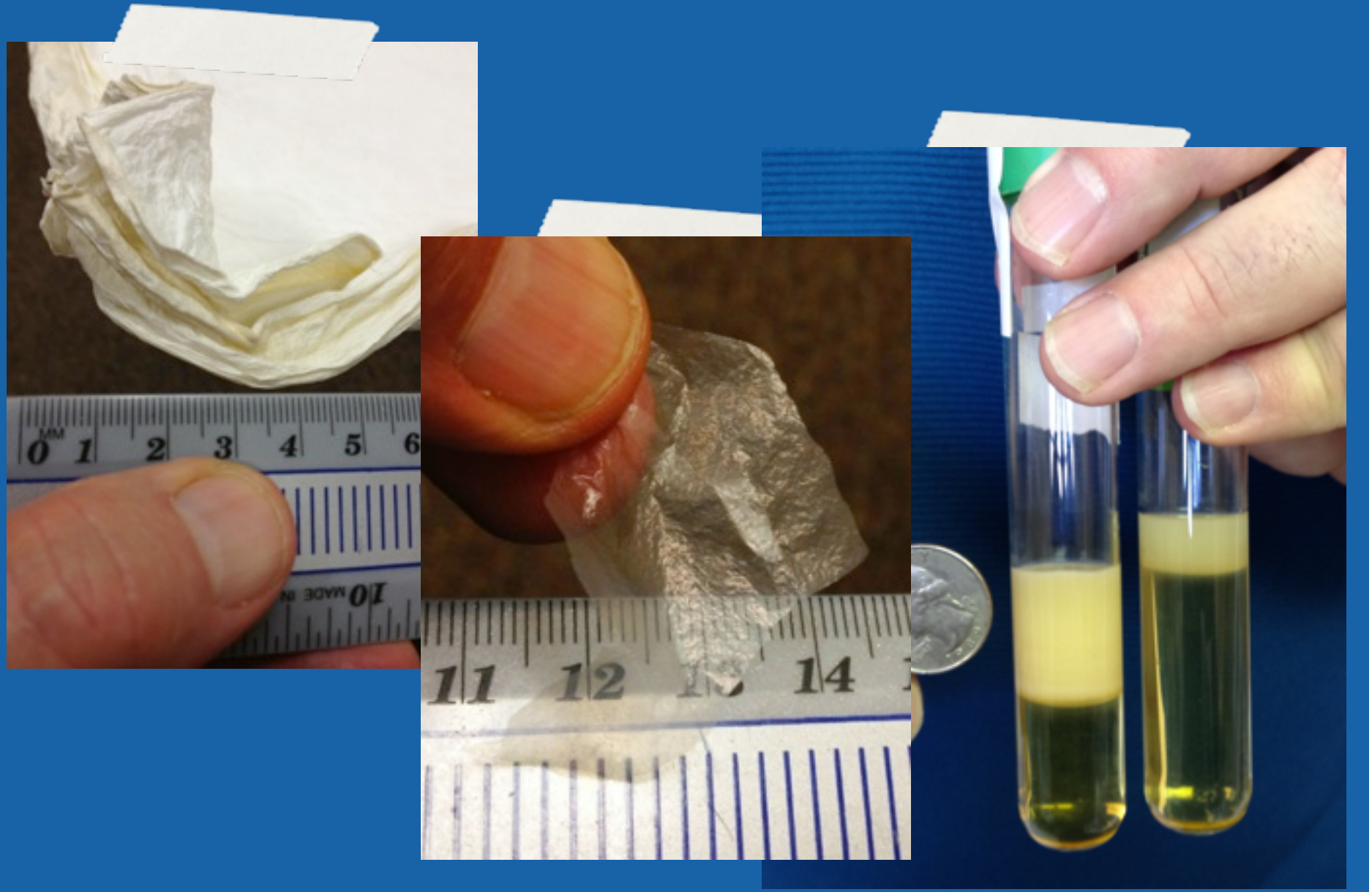
BIOMINING RESULTS

BB_J23100 BB_B0030 BB_B0015

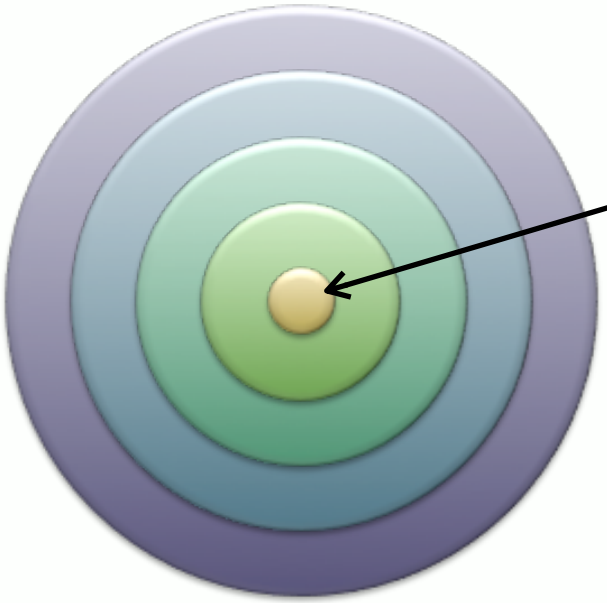
Promoter	RBS	FliC N-Terminus	GFP	Flic C-Terminus	Terminator



Microbial cellulose



Concept



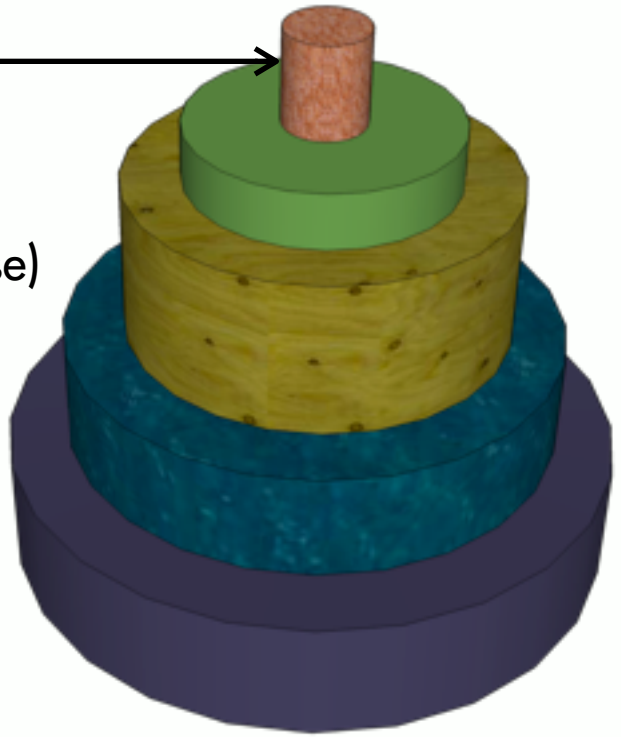
bound materials (metals)

fibers (silk, cotton, wool)

structural materials (cellulose)

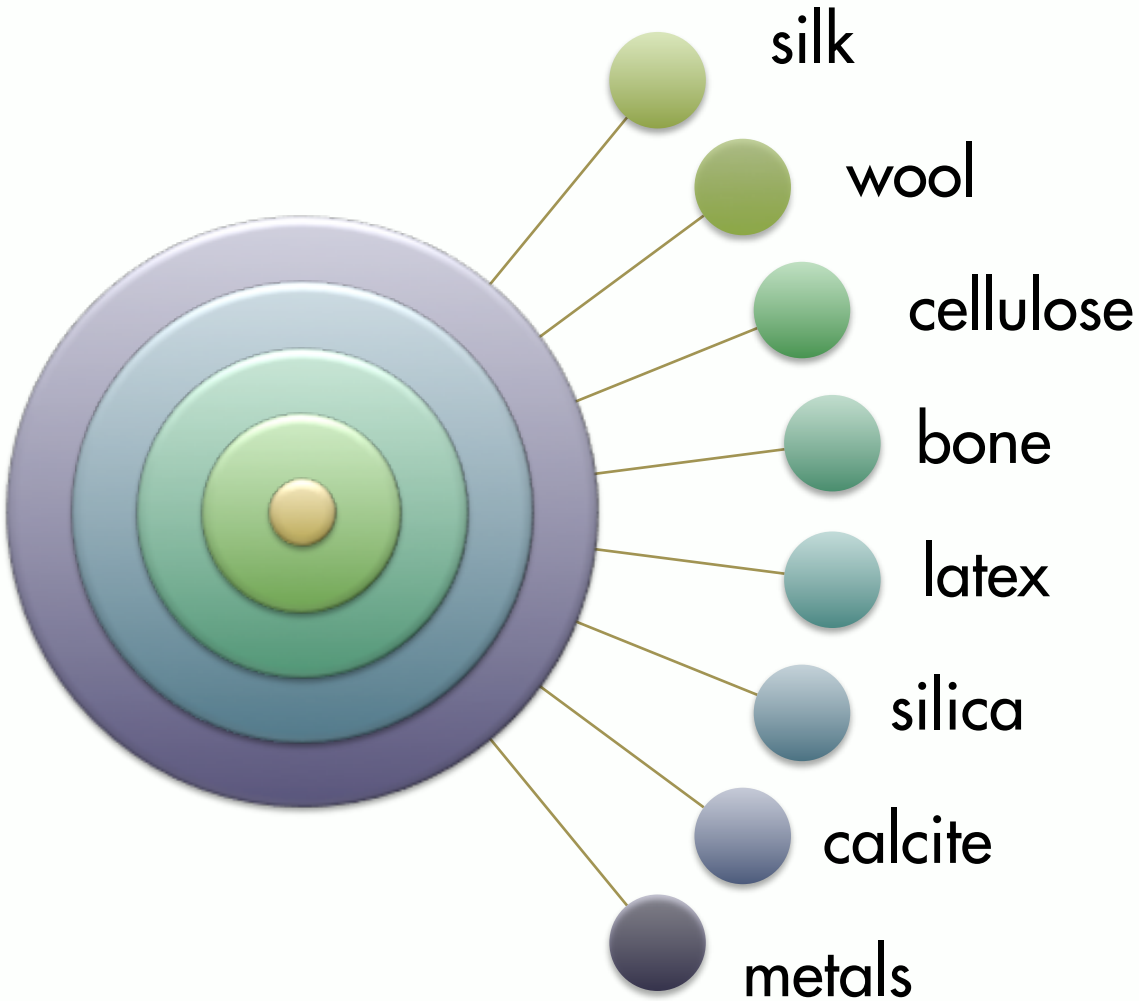
crystals (silica, carbonate)

materials (latex)



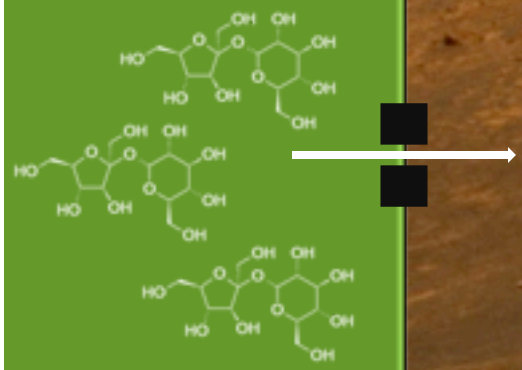
Biomaterials out of thin air: in situ, on-demand printing of advanced biocomposites

Lynn Rothschild, Diana Gentry,Ashley Micks


silk
wool
cellulose
bone
latex
silica
calcite
metals

Sucrose secretion

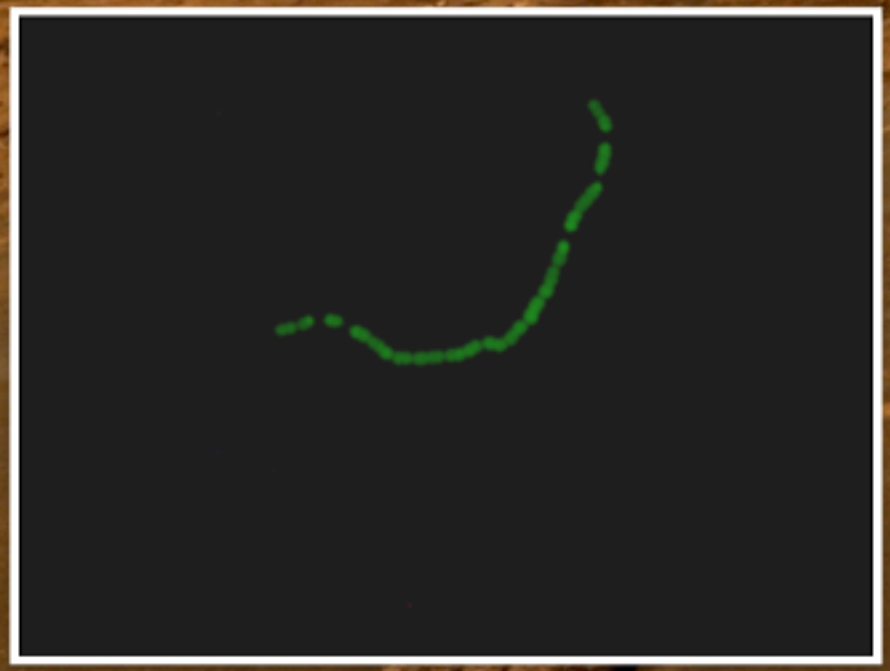
Salt induced sucrose production



- ★ We focused on sugar secretion
- ★ CscB sucrose permease
- ★ Why sucrose? not metabolized

Brown-Stanford iGEM

Results of triparental mating



Biomaterials out of thin air: in situ, on-demand printing of advanced biocomposites

Diana Gentry, Ashley Micks*, Lynn J. Rothschild, NASA Ames Research Center, *Stanford

the problem

Upmass is the single most significant limitation of our current space mission capability. Although biomaterials and biocomposites have mass, strength, flexibility, and self-healing properties that could significantly reduce upmass, their use is limited by the following drawbacks:

- Expensive, specific production. Many biomaterials can only be produced as part of significant support ecosystem.
- Inaccessible functional customization. The grain of wood, the porosity of bone, and so on are an integral part of the materials' desired mechanical properties, but are not deterministic when the material is naturally grown.
- Limited compositions. Most biomaterials (unlike metal, plastic, etc.) cannot be easily combined or modified to produce new materials.

This project builds on recent advances in:

- Synthetic biology. Libraries of standardized genetic parts which can be used for controlled cellular material production, delivery, and binding.
- 3D printing. Commercial off-the-shelf components which can be used to make of a pico- to nanoliter cell deposition system.
- Tissue engineering. Proven cell-compatible support hydrogels and scaffolds can be modified to bind the deposited biomaterials of interest.

the vision

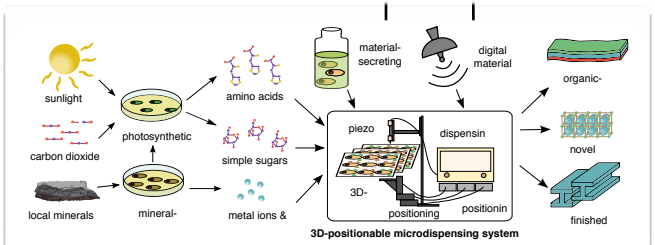


Figure 3 (above). This novel biomaterials manufacturing technique, when combined with other

this study

Objectives

- Feasibility and benefit analysis. Two mission contexts span the concept's scope (see below).
- Proof-of-concept demonstration. A simple grid of two proteins, fluorescent for easy detection, to validate the core technology concept.
- Proposed implementations for follow-on work. Avenues for future work on each core component (host cell, production control, material delivery, material binding, etc.).
- Complementary studies exploration. A survey of other emerging areas (in

Hardware set-up

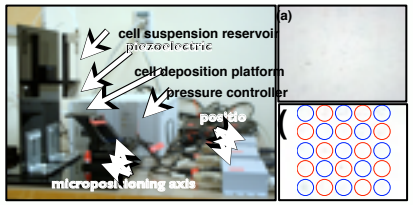


Figure 1 (left).
A test run of the hardware prototype using colored beads on nitrocelluse to simulate cells.
The same image cleaned up to show the original pattern overlaid with the results: 24 of 25 correct.
Figure 2 (right). The prototype cell-compatible 3D printing (microdeposition) system, fully assembled, that we will use for this study. The workspace is ~1 cm³, droplet size ~20

progress to date

Bioengineering Setup and Tests

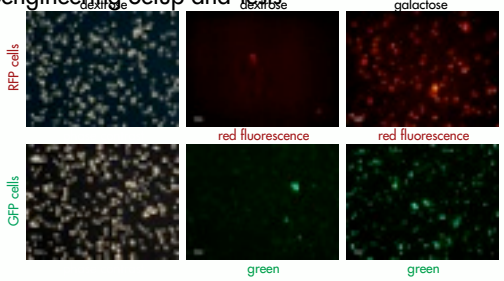


Figure 5. A demonstration of secretion control, using GFP and RFP in yeast

Cell Printing Setup and

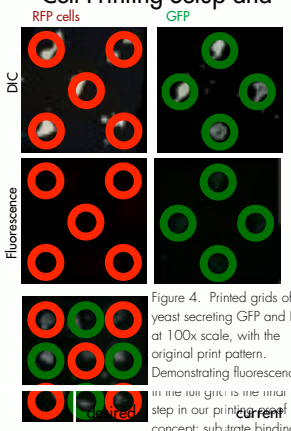


Figure 4. Printed grids of yeast secreting GFP and RFP, at 100x scale, with the original print pattern. Demonstrating fluorescence in the full grid is the final step in our printing proof of concept; substrate binding is the next challenge.

Current Performance

metric	minimum	current	
positioning precision	1 cell diameter (~10 µm)	1 µm	2 µm
dispensing volume	≤ 1000 cells/"voxel"	~ 1 cell/"voxel"	≤ 10 cells/"v
"voxel" size	enc. 1000 cells = 1 nL	10 pL	20 pL
cell survival	50%	90%	~50%
pattern completion	75%	95%	≥95%

potential impacts mission contexts

Potential Impacts

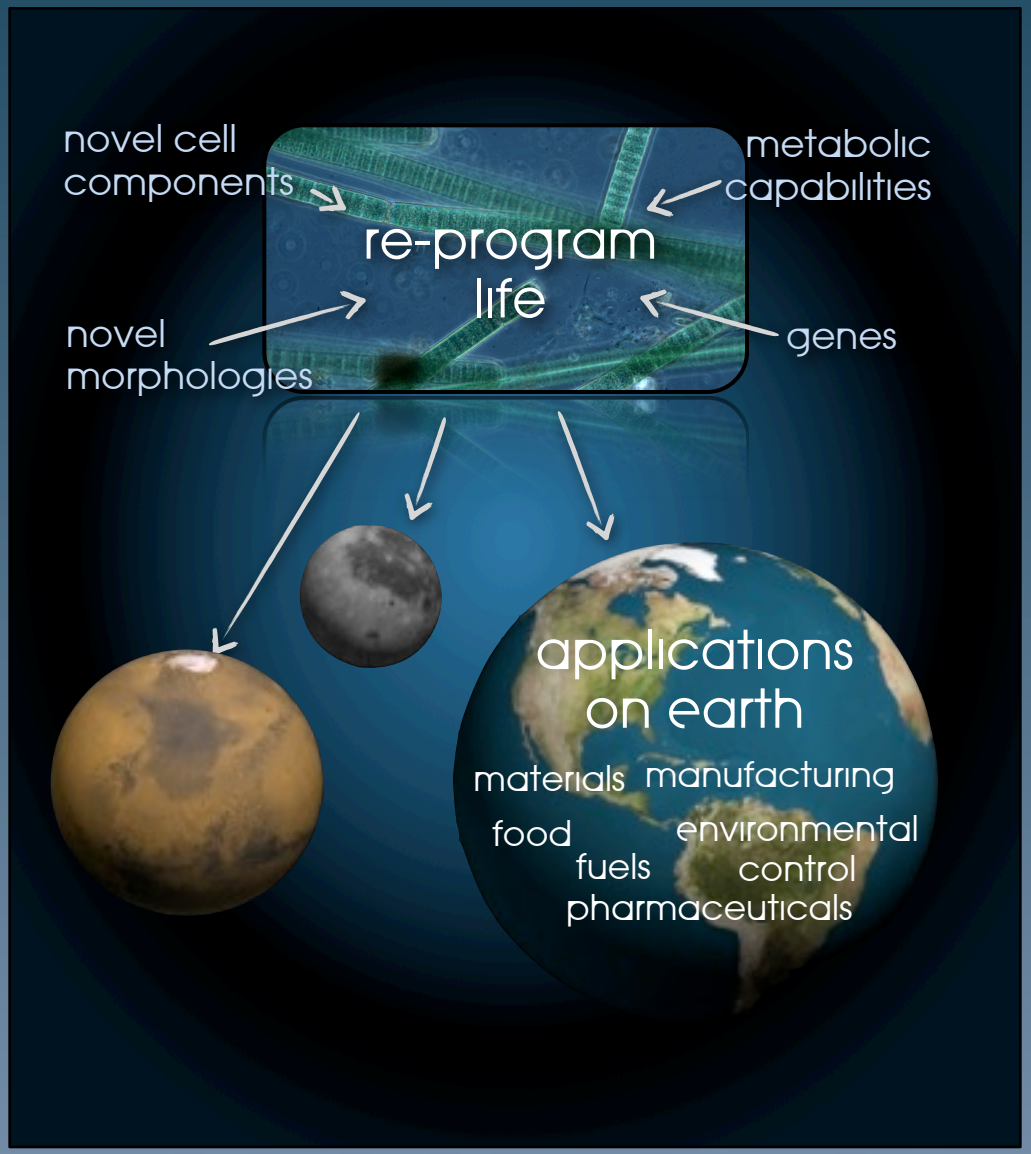
This application could dramatically expand manufacturing capabilities on Earth and in space:

- In situ resource utilization. A far greater range of materials and products will be available from the limited palette offered by in situ resource extraction techniques.
- Reduced equipment and material upmass for off-Earth habitats. Ready-to-use highly specialized construction materials (radiation hardened, compressive/tensile, light or dense) from an extremely low starting mass.
- Structured biomaterial production. New ready-to-use macro, micro, and molecular manufacturing techniques for traditional biomaterials such as wood, bone and shell.
- New and novel biocomposite creation. The ability to create completely novel material composites from any base material that cells can be engineered to produce.

Suggested Mission Contexts

- ISS part manufacturing. A 'minimal working example' making a finished biomaterial part aboard the International Space Station.
- A long-term Mars habitat. 'Cradle-to-grave' use at a hypothetical Mars habitat, covering everything from tools to construction materials.

Synthetic biology is....



- the design and construction of new biological functions and systems not found in nature
- an emerging, innovative, game-changing discipline