Securing operational mastery
of living matter

USA plan for building cell(s)

Drew Endy
Stanford Bioengineering

Prepared for the US NSF
6 December 2018

Life goes as four regimes

1. Natural lineages

3. Edit w/ intention

2. Screen & breed

ATCG .... 0101 .... ATCG

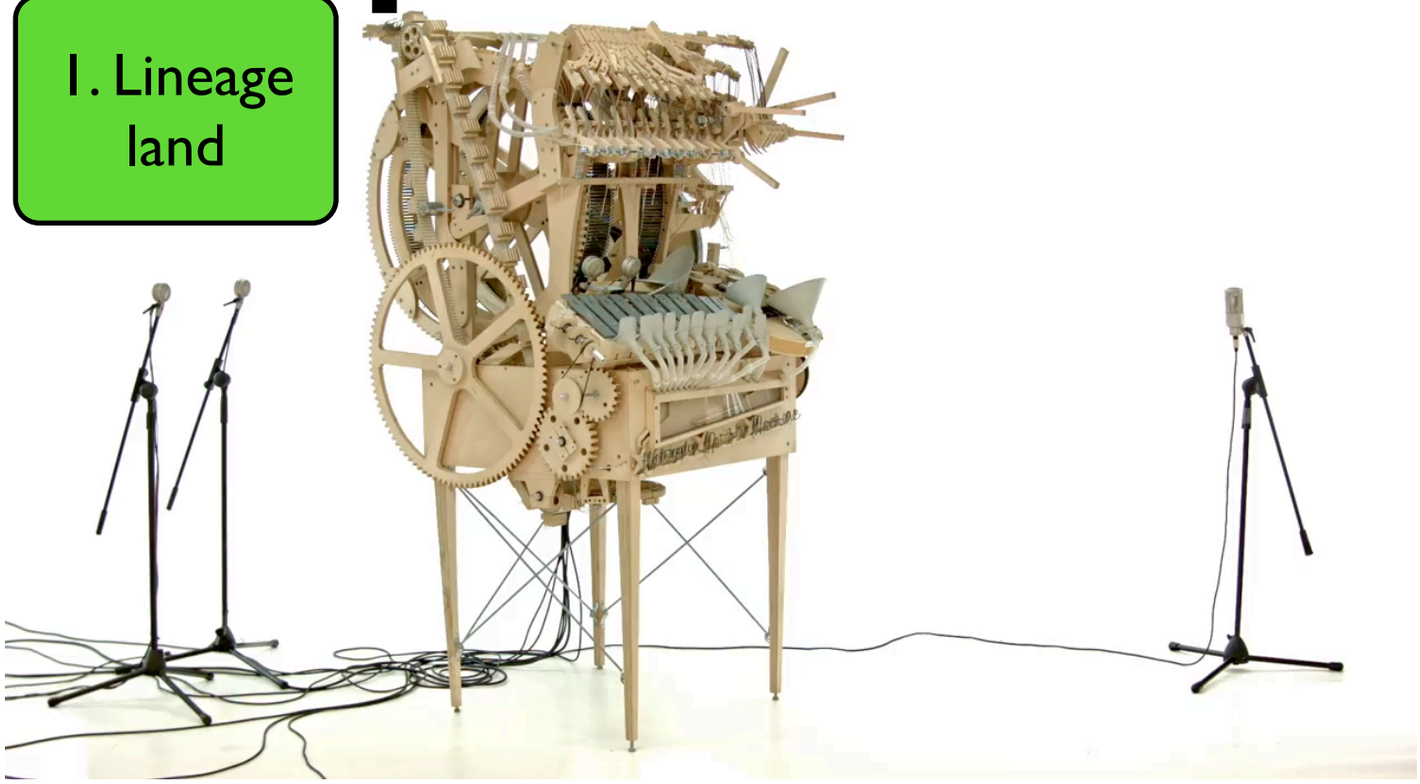
SEQUENCE

SYNTHESIZE

4. Synthetic & lineage agnostic

Regime 4 is the most different, powerful, and important

1. Lineage land

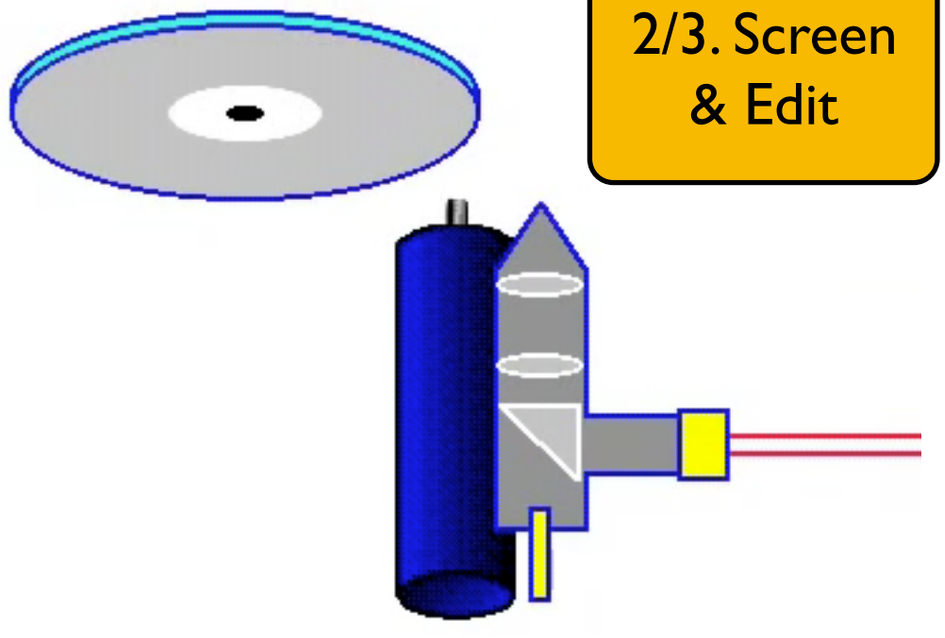


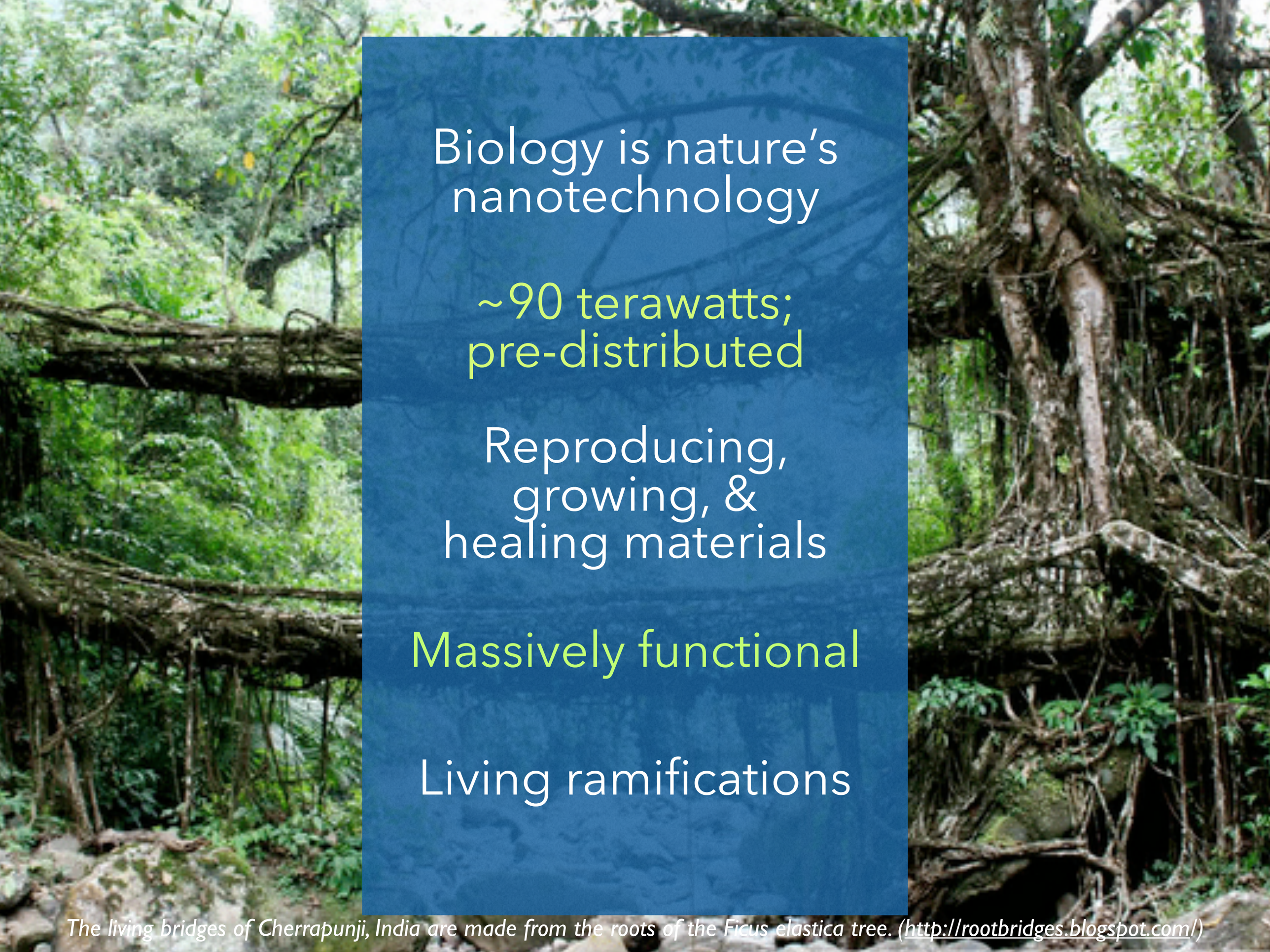
4. Lingeage agnostic

napster™

2/3. Screen & Edit

2/3. Screen & Edit





Biology is nature's nanotechnology

~90 terawatts; pre-distributed

Reproducing, growing, & healing materials

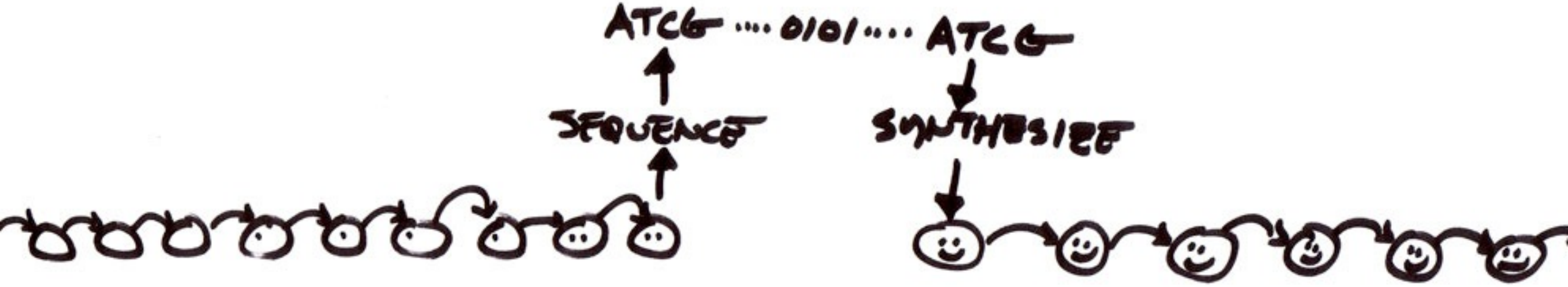
Massively functional

Living ramifications

The living bridges of Cherrapunji, India are made from the roots of the Ficus elastica tree. (http://rootbridges.blogspot.com/)

What *could* we do?

(1) biology + internet = bionet
(2) lineage-agnostic organism**s**

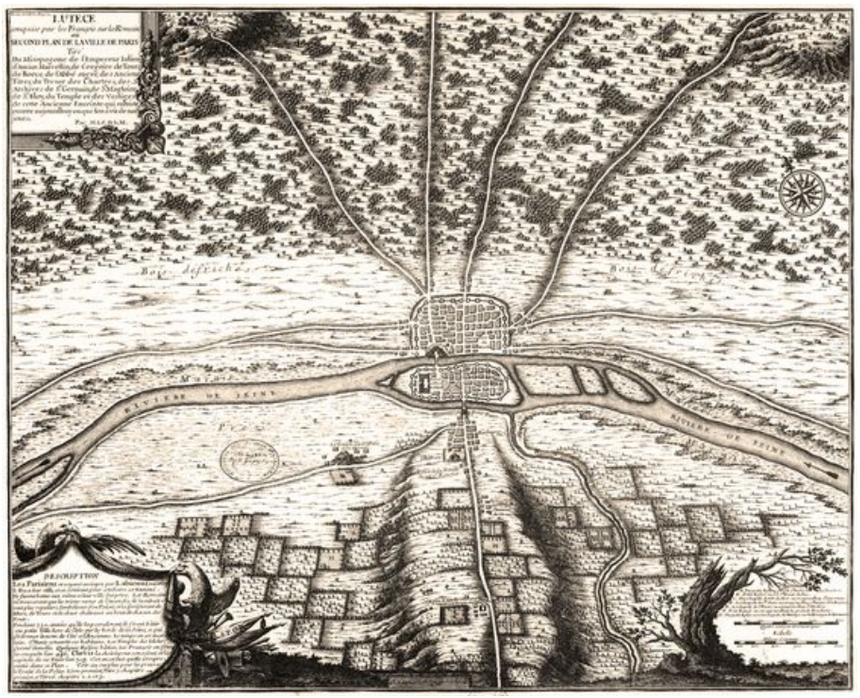


Why *should* we do it?

We can soon realize the practical capacities to support 10 billion people in partnership with nature. Mastery of living matter essential ingredient.

Energy,
Information,
& Matter mostly local
& <u>all</u> limiting…

Energy,
Information,
& Matter all networked
& <u>none</u> limiting…



Paris ~500AD

Paris ~2025AD

Why *must* we do it?

vices w[illegible] situation generates. A government adapted to such men would be one thing; but a very different one that for the Man of these states. Here every one may have land to labor for himself if he chuses; or, preferring the exercise of any other industry, may exact for it such compensation as not only to afford a comfortable subsistence, but wherewith to provide for a cessation from labor in old age. Every one, by his property, or by his satisfactory situation, is interested in the support of law and order. And such men may safely and advantageously reserve to themselves a wholsome controul over their public affairs, and a degree of freedom, which in the hands of the Canaille of the cities of Europe, would be instantly perverted to the demolition and destruction of every thing public and private. The history of the last 25. years of France, and of the last 40. years in America, nay of it's last 200. years, proves the truth of both parts of this observation.

But even in Europe a change has sensibly taken place in the mind of Man. Science had liberated the ideas of those who read and reflect, and the American example had kindled feelings of right in the people. An insurrection has consequently begun, of science, talents and courage against rank and birth, [illegible] contempt. It has failed in it's first ef- [illegible] instrument used for

Jefferson to Adams re: "natural aristocracy" October 1813

https://oaklandunderfoot.com/2016/12/23/old-concrete-in-the-west-bay/

https://www.kqed.org/science/466518/surprise-tree-rings-not-limited-to-trees-rocks-have-them-too

https://en.wikipedia.org/wiki/Aqueduct_of_Segovia

Implied — not necessary to change the very molecules of life too much

Implied — we will enable the construction of artifacts unseen in nature

https://www.fosterandpartners.com/projects/millau-viaduct/

RESEARCH ARTICLE

SYNTHETIC BIOLOGY

Design and synthesis of a minimal bacterial genome

Clyde A. Hutchison III,[1]*† Ray-Yuan Chuang,[1]†‡ Vladimir N. Noskov,[1] Nacyra Assad-Garcia,[1] Thomas J. Deerinck,[2] Mark H. Ellisman,[2] John Gill,[3] Krishna Kannan,[3] Bogumil J. Karas,[1] Li Ma,[1] James F. Pelletier,[4]§ Zhi-Qing Qi,[3] R. Alexander Richter,[1] Elizabeth A. Strychalski,[4] Lijie Sun,[1]|| Yo Suzuki,[1] Billyana Tsvetanova,[3] Kim S. Wise,[1] Hamilton O. Smith,[1,3] John I. Glass,[1] Chuck Merryman,[1] Daniel G. Gibson,[1,3] J. Craig Venter[1,3]*

We used whole-genome design and complete chemical synthesis to minimize the 1079–kilobase pair synthetic genome of *Mycoplasma mycoides* JCVI-syn1.0. An initial design, based on collective knowledge of molecular biology combined with limited transposon mutagenesis data, failed to produce a viable cell. Improved transposon mutagenesis methods revealed a class of quasi-essential genes that are needed for robust growth, explaining the failure of our initial design. Three cycles of design, synthesis, and testing, with retention of quasi-essential genes, produced JCVI-syn3.0 (531 kilobase pairs, 473 genes), which has a genome smaller than that of any autonomously replicating cell found in nature. JCVI-syn3.0 retains almost all genes involved in the synthesis and processing of macromolecules. Unexpectedly, it also contains 149 genes with unknown biological functions. JCVI-syn3.0 is a versatile platform for investigating the core functions of life and for exploring whole-genome design.

EME

Global Transposon Mutagenesis and a Minimal Mycoplasma Genome

Clyde A. Hutchison III,[1,2*] Scott N. Peterson,[1*†] Steven R. Gill,[1] Robin T. Cline,[1] Owen White,[1] Claire M. Fraser,[1] Hamilton O. Smith,[1‡] J. Craig Venter[1‡§]

Mycoplasma genitalium with 517 genes has the smallest gene complement of any independently replicating cell so far identified. Global transposon mutagenesis was used to identify nonessential genes in an effort to learn whether the naturally occurring gene complement is a true minimal genome under laboratory growth conditions. The positions of 2209 transposon insertions in the completely sequenced genomes of *M. genitalium* and its close relative *M. pneumoniae* were determined by sequencing across the junction of the transposon and the genomic DNA. These junctions defined 1354 distinct sites of insertion that were not lethal. The analysis suggests that 265 to 350 of the 480 protein-coding genes of *M. genitalium* are essential under laboratory growth conditions, including about 100 genes of unknown function.

Essential genes of unknown function

Unknown functions that are essential

Minimal Cells—Real and Imagined

John I. Glass, Chuck Merryman, Kim S. Wise, Clyde A. Hutchison III, and Hamilton O. Smith

Synthetic Biology and Bioenergy Group, J. Craig Venter Institute, La Jolla, California 92037

Correspondence: jglass@jcvi.org

EPILOGUE

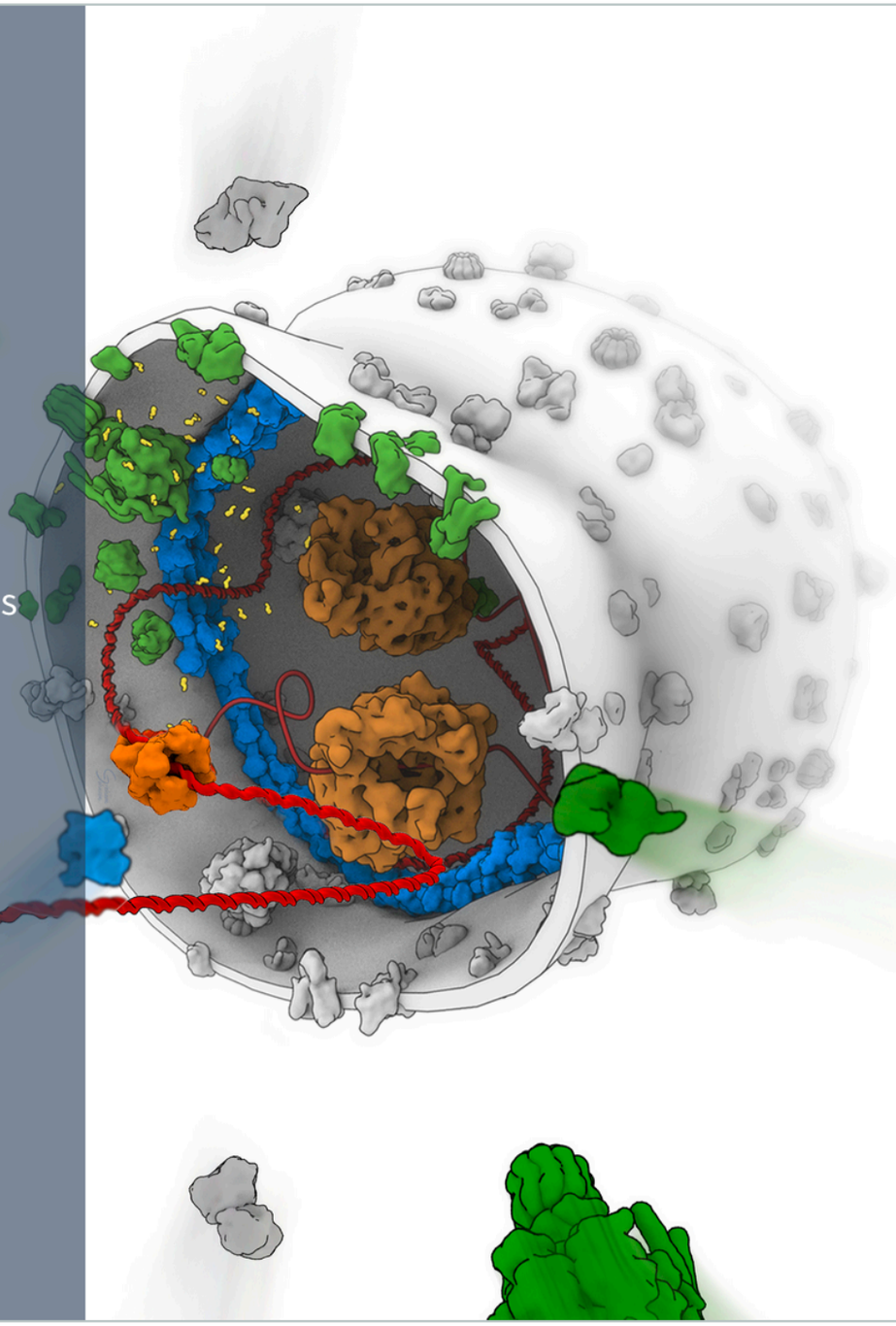
Recently, Bruce Alberts, former president of the United States National Academy of Sciences, wrote about the astonishing finding that 149 genes in the JCVI-Syn3.0 minimal cell were of unknown function. "Hundreds of talented young scientists should be leaping to fill this huge gap in understanding of fundamental biological mechanisms, perhaps earning several Nobel Prizes along the way. Over the long term, such results are certain to lead to powerful new approaches for improving human health and welfare" (Alberts 2016). Having a minimal bacterial cell has been a long-standing goal of cell biologists. No longer are we limited to working with imaginary minimal cells or naturally occurring or naturally occurring organisms with small genomes as surrogates. A minimal cell has now been constructed. Clearly, there is much about its biology that biologists do not understand. First principles of cellular life are waiting to be discovered.

The Synthetic Cell: a new frontier in science and technology

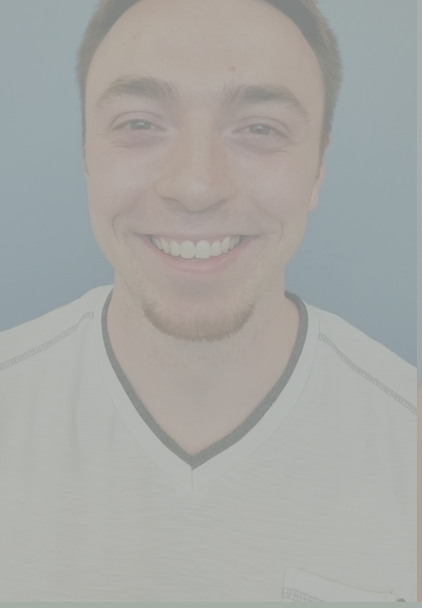
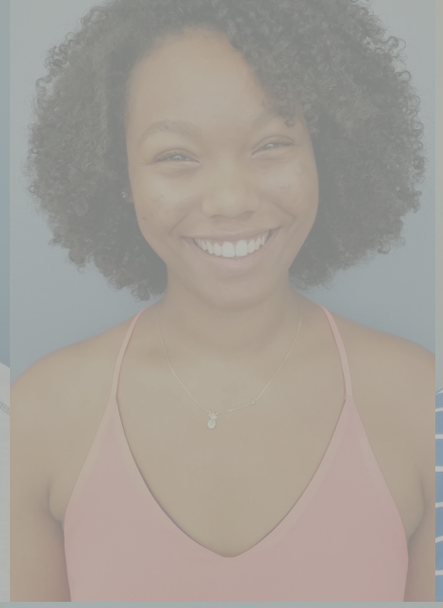
Can we build a living cell from lifeless components?

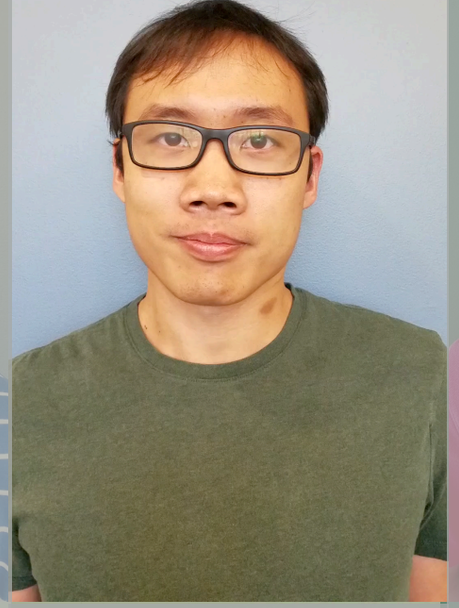
...and in doing so, understand how life works?

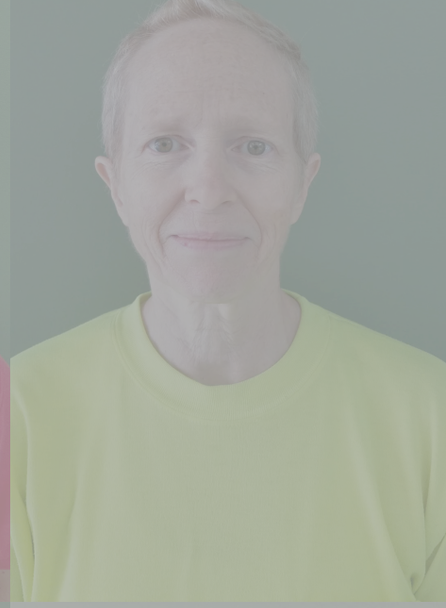
With this initiative we aim to address one of the grand scientific challenges of this century: building a synthetic cell from its molecular building blocks. Understanding the mechanisms of cellular life will bring vast intellectual, scientific and technological rewards.

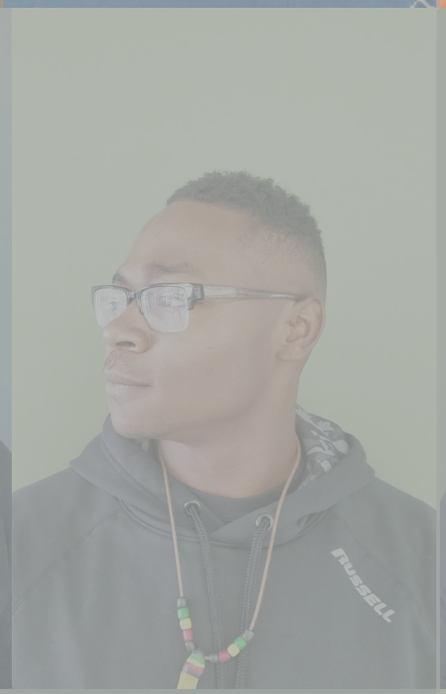


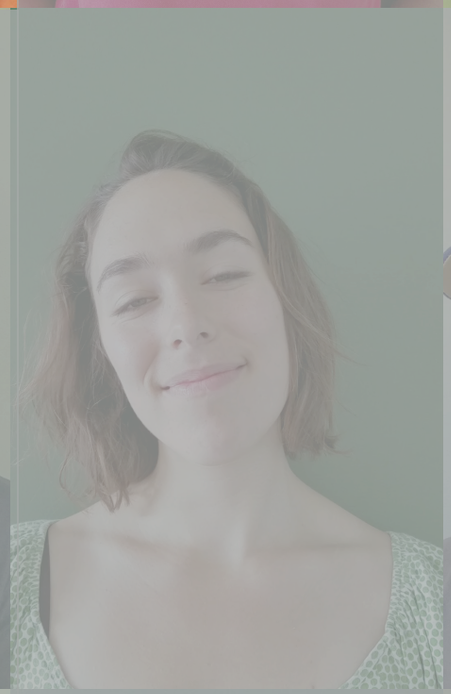
Building a synthetic cell is one of the grand scientific and intellectual challenges of the 21st century. While we have extensive knowledge about the molecular building blocks that form the basis of modern life, we currently do not understand how these building blocks collectively operate to define life. With BaSyC we propose to build a synthetic cell from the bottom-up, which arguably is the most fundamental approach towards

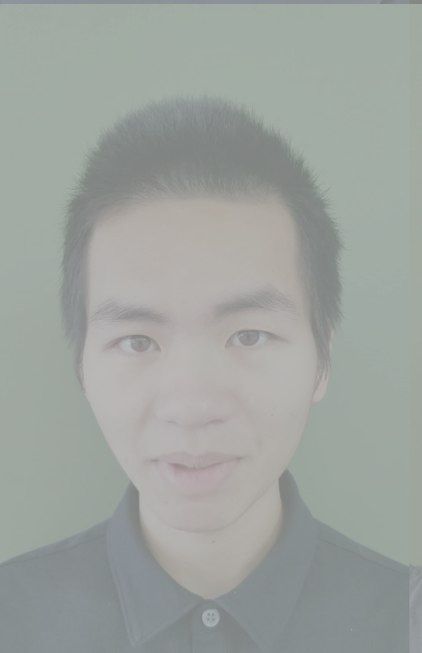



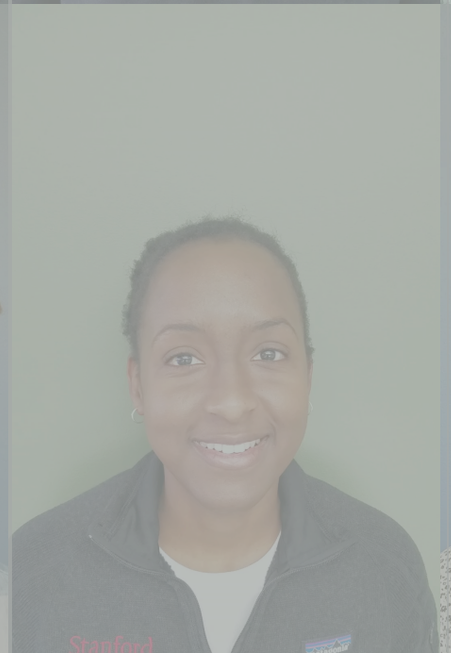






RUSSELL





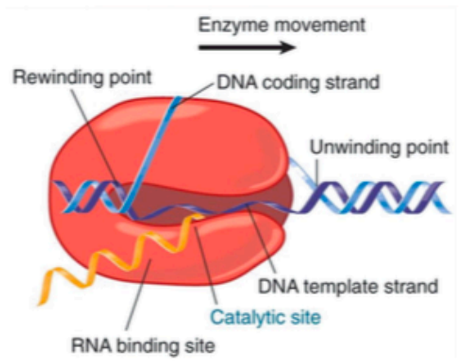


Stanford

Can we build a cell from lifeless components?

PURE (Protein Synthesis Using Recombinant Elements) is an in vitro TX-TL cell free system with all of its components **individually purified** from cells

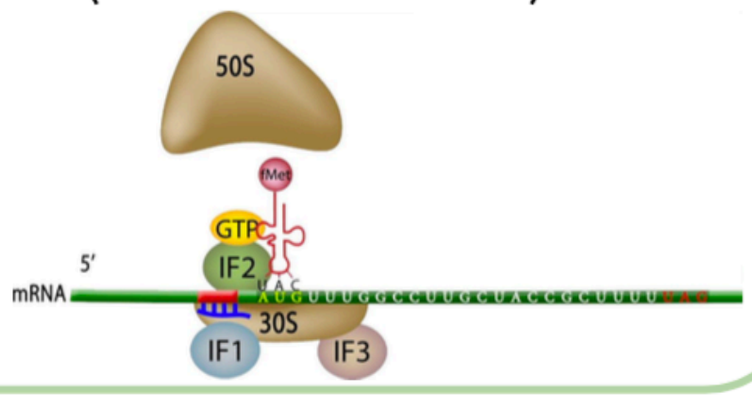
Transcription

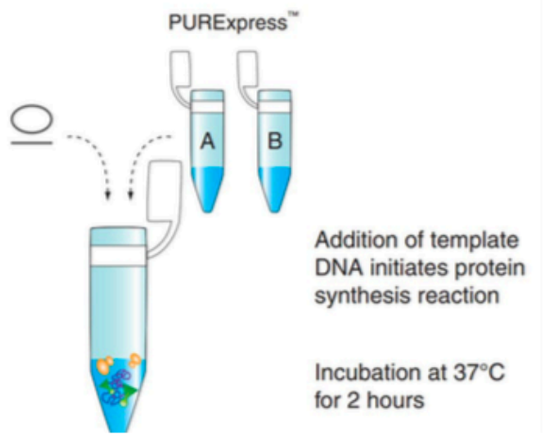
(DNA → mRNA)



Translation

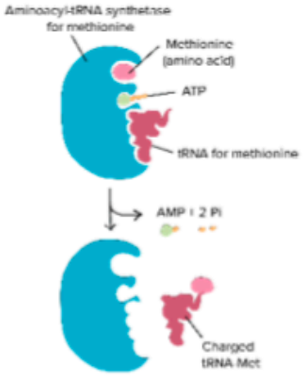
(mRNA → Protein)





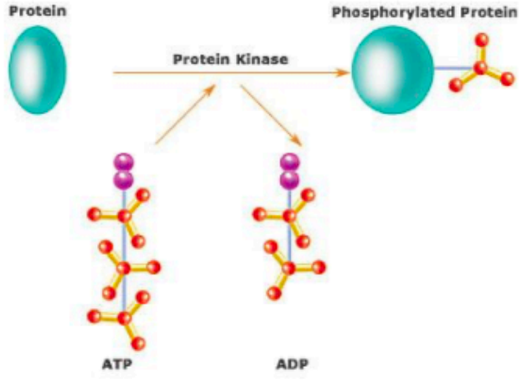
tRNA Aminoacylation

(AA + tRNA → AA-tRNA)

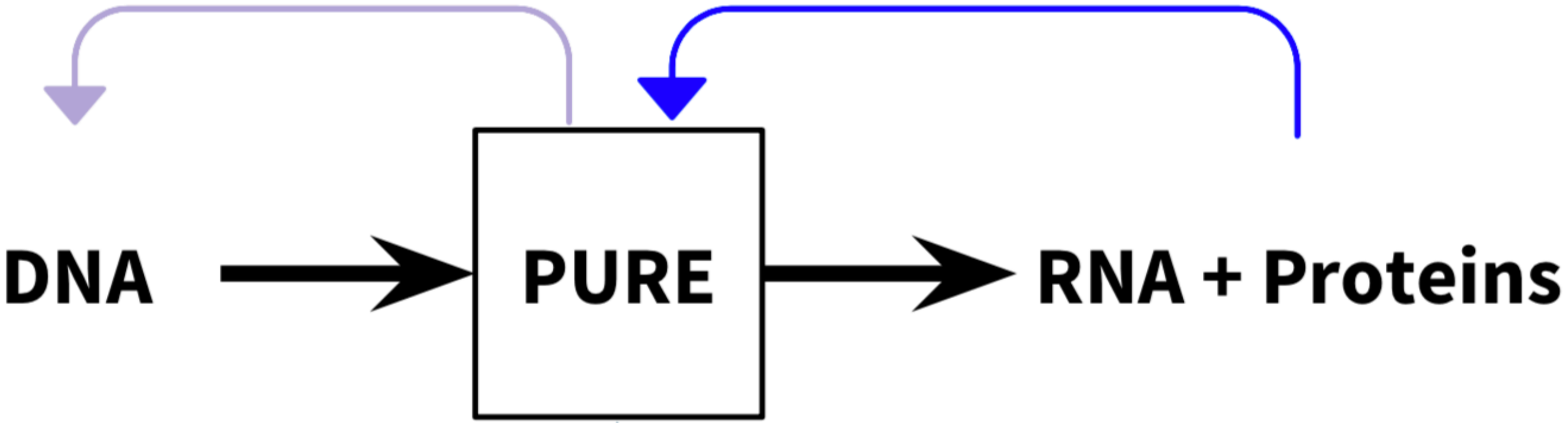


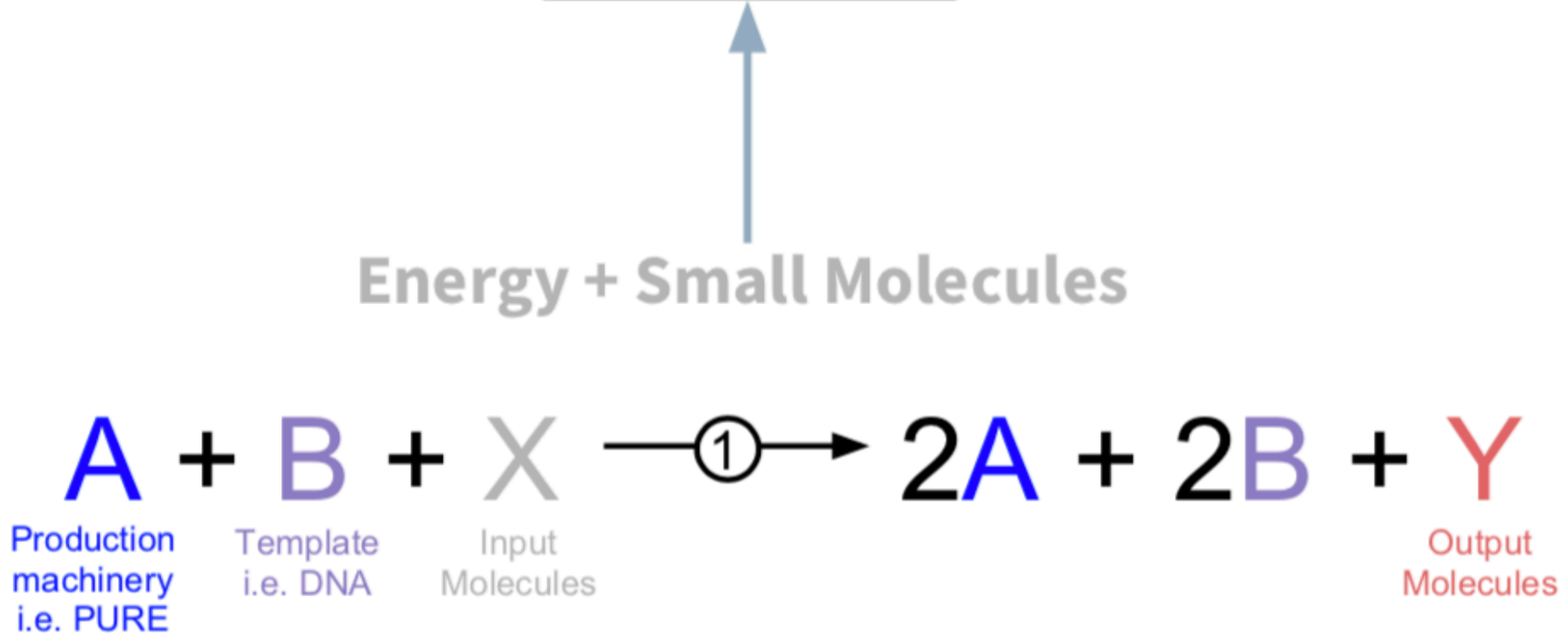
Energy

(Recycling rNTPs)



Can PURE beget PURE?

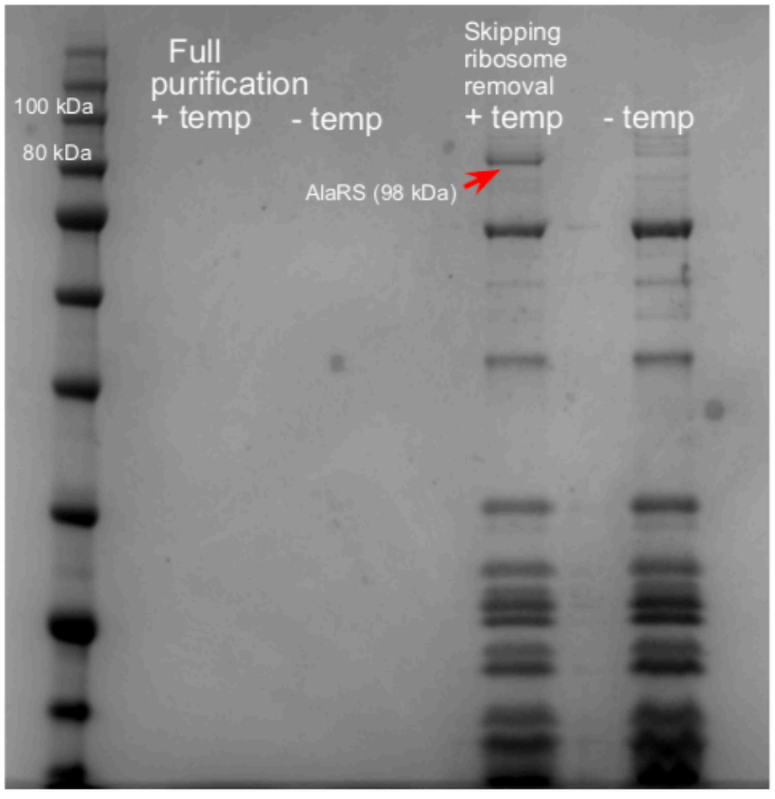




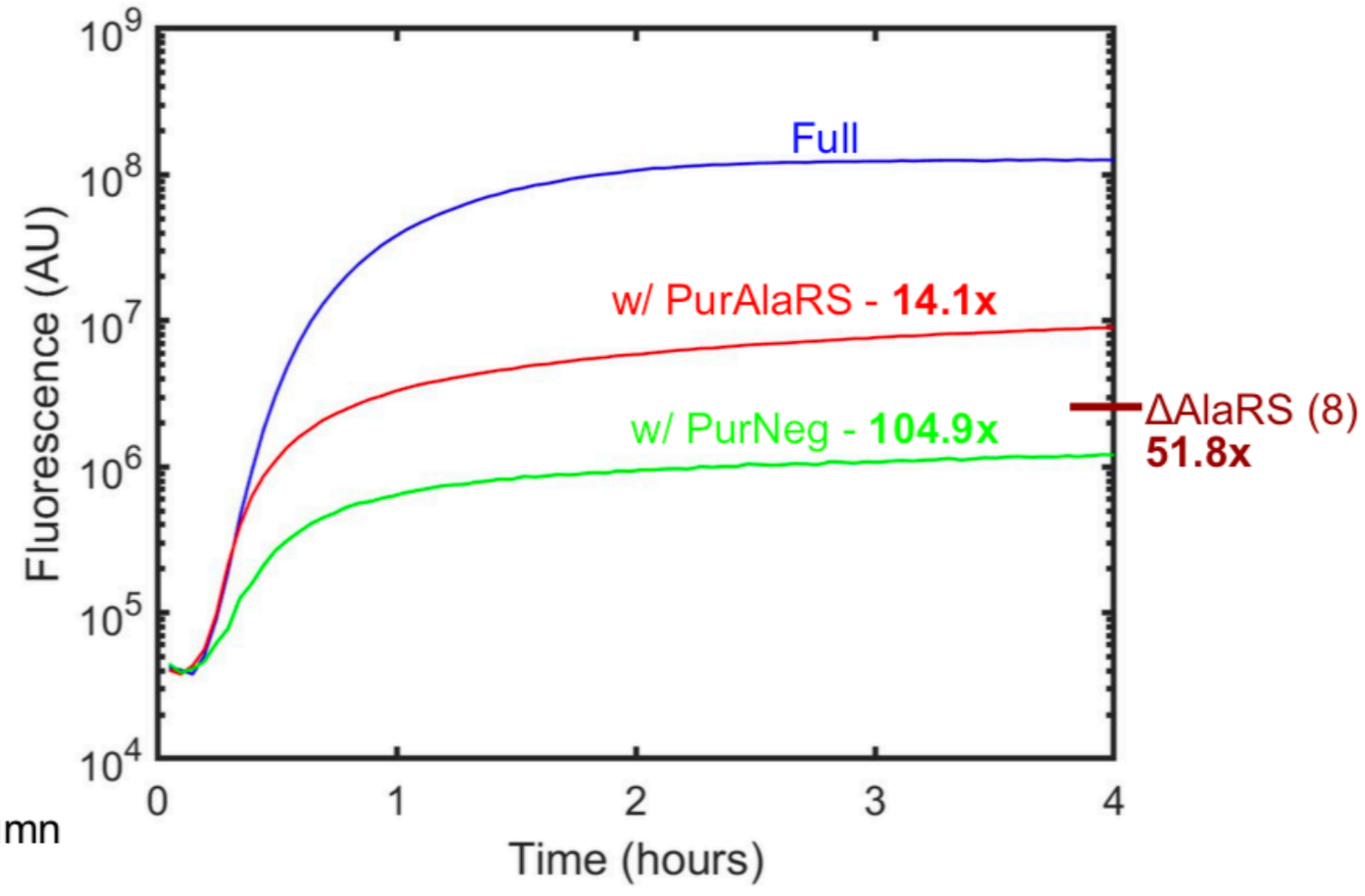
Eric Wei, unpublished

Qualitatively? looking good, baby steps
Quantitatively? ~30-fold too weak

AlaRS can be expressed in PURE and its inclusion increases expression – but not to level of positive control



AlaRS is too large and cannot flow through initial column



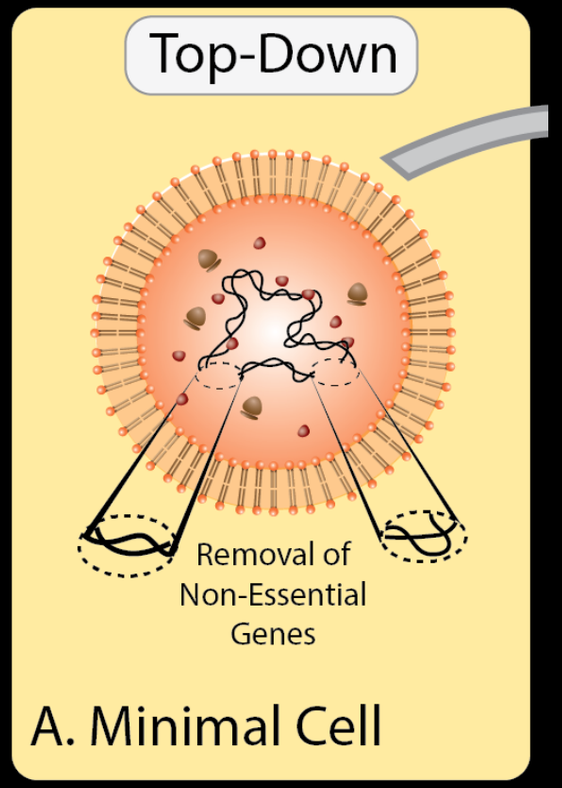
Possible explanations: Quantity of AlaRS added is too low; aminoacylation rate per AlaRS is decreased due to misfolding; inhibitory byproducts (PP_i) still left in mix

Next steps: Scale up PURE reactions to increase yield of AlaRS and increase buffer exchange time; **use strep purification process to provide cleaner prep**

Eric Wei, unpublished

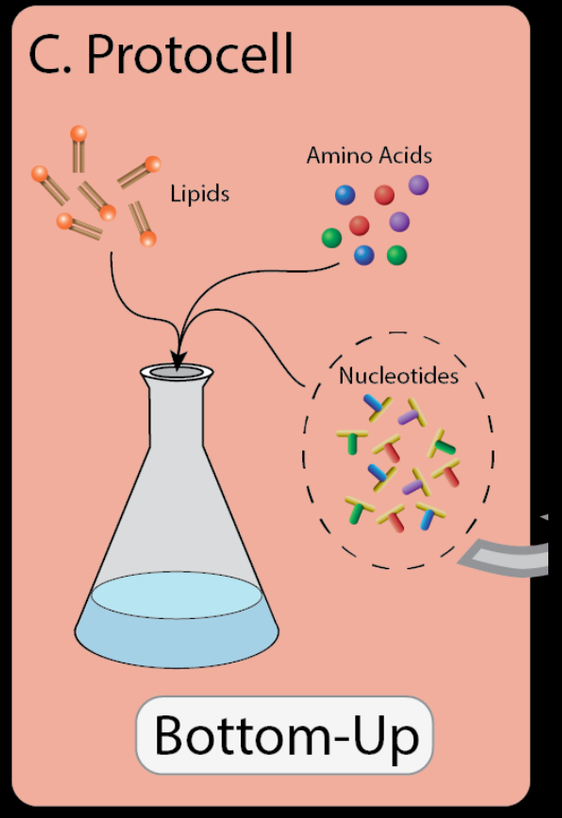
Essential genes of unknown function

"what we find in nature
we do not understand"
— D. Endy

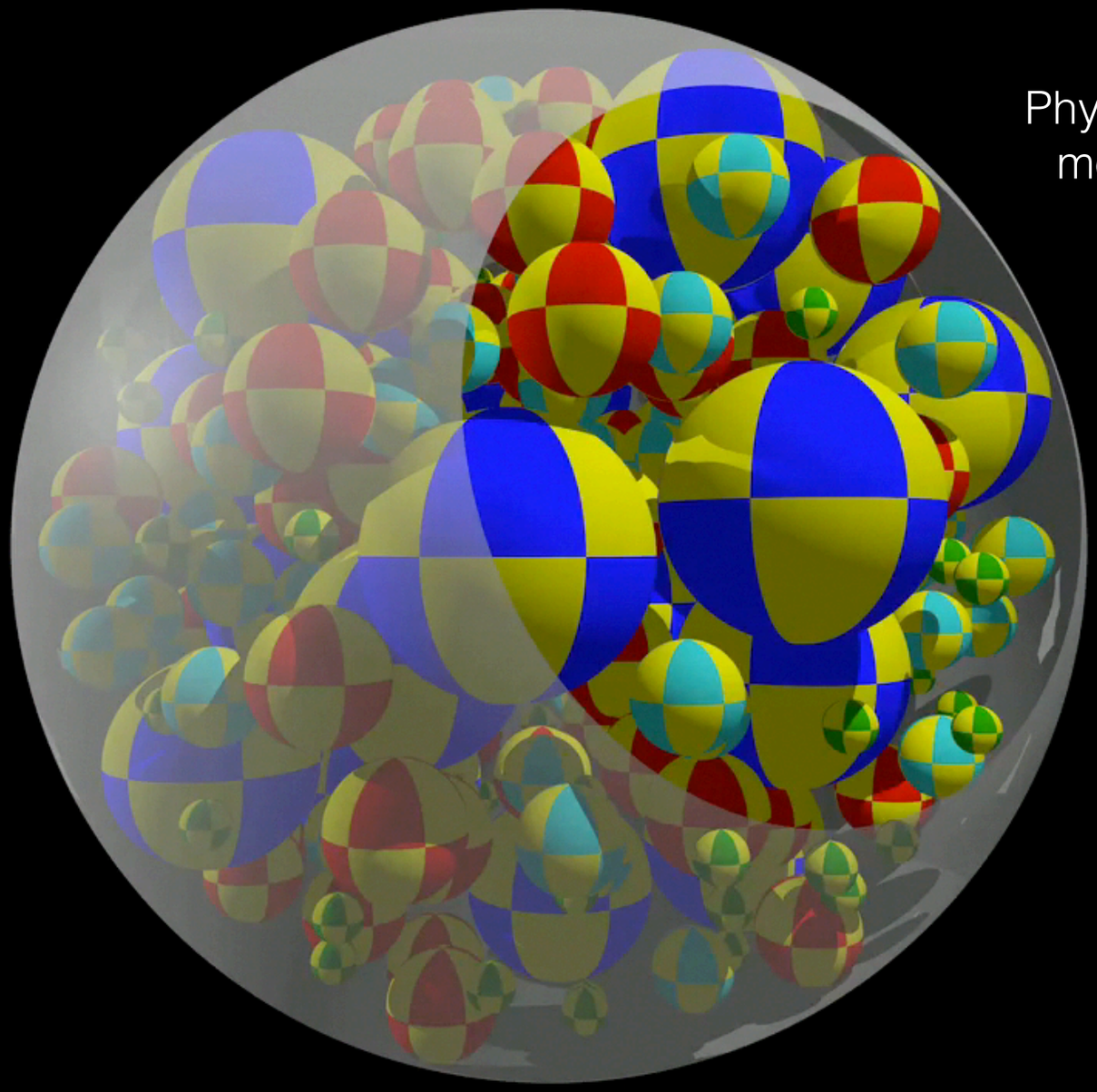


Unknown functions that are essential

"what I cannot create
I do not understand"
— R. Feynman



Essential physics of living systems



Aponte-Rivera, Gonzalez, & **Zia**
Phys Rev Fluids (2016); J. Fluid Mech (2018)

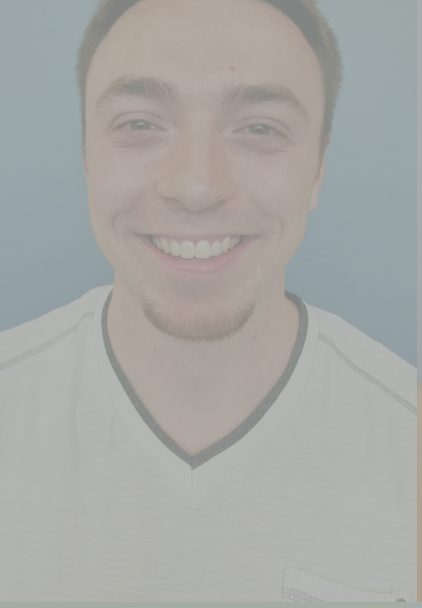
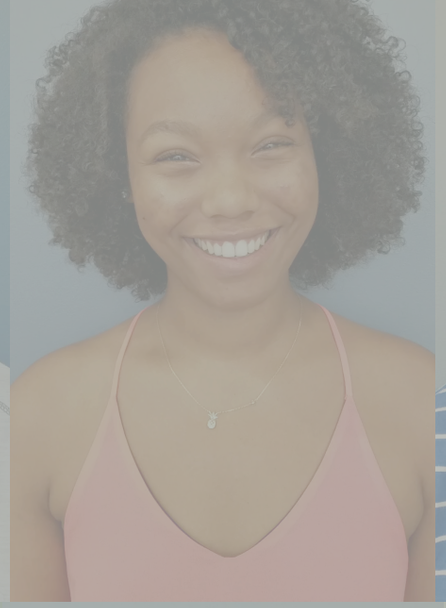
Physico-chemical forces w/ high fidelity from 5x size of water molecule up to whole-cell size for hours real-time evolution.

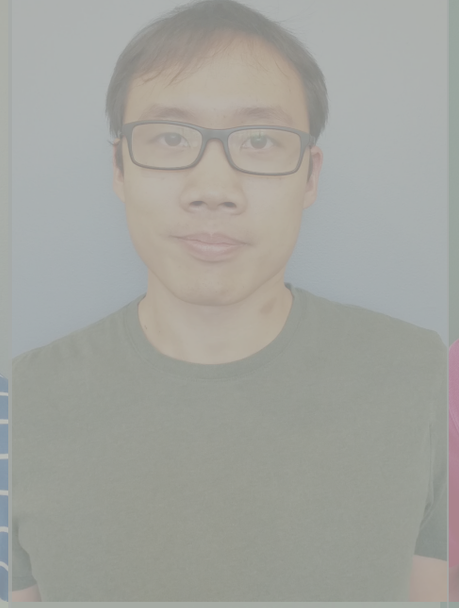
Detailed positions, trajectories, self-assembly, structure, osmotic pressure, diffusion, phase transitions.

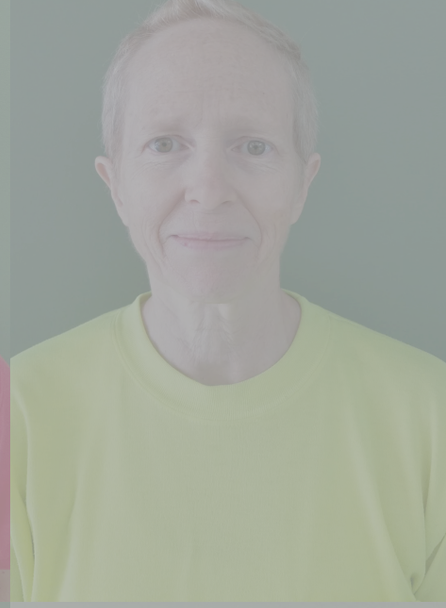
Stokesian dynamics simulation, many-body long-range hydrodynamic interactions, lubrication interactions, attractive forces, long-range repulsion, **(NOT ONLY)** Brownian motion

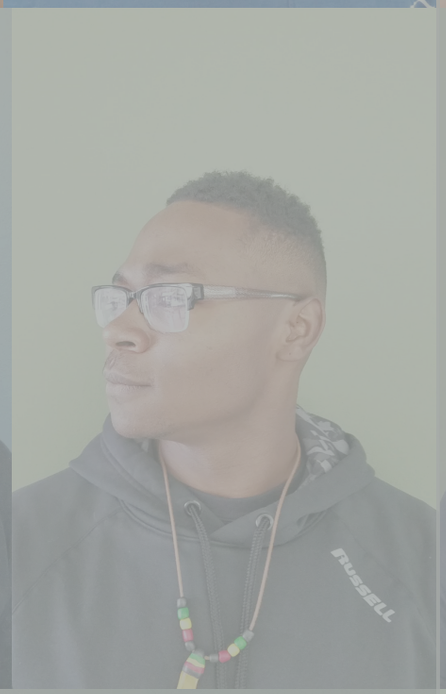
Roseanna Zia

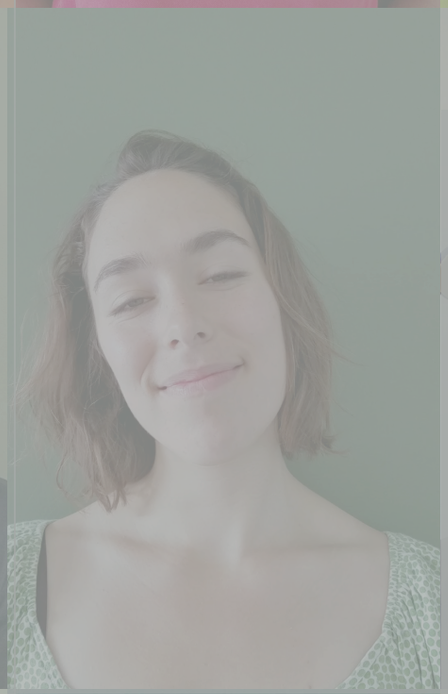
Emma Gonzalez
Alp Sunol

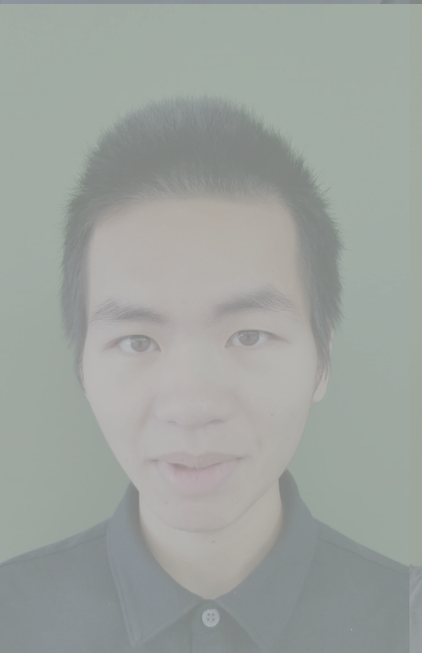



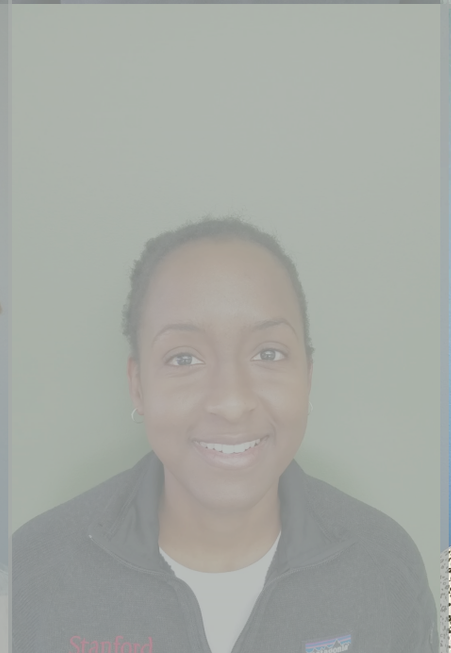




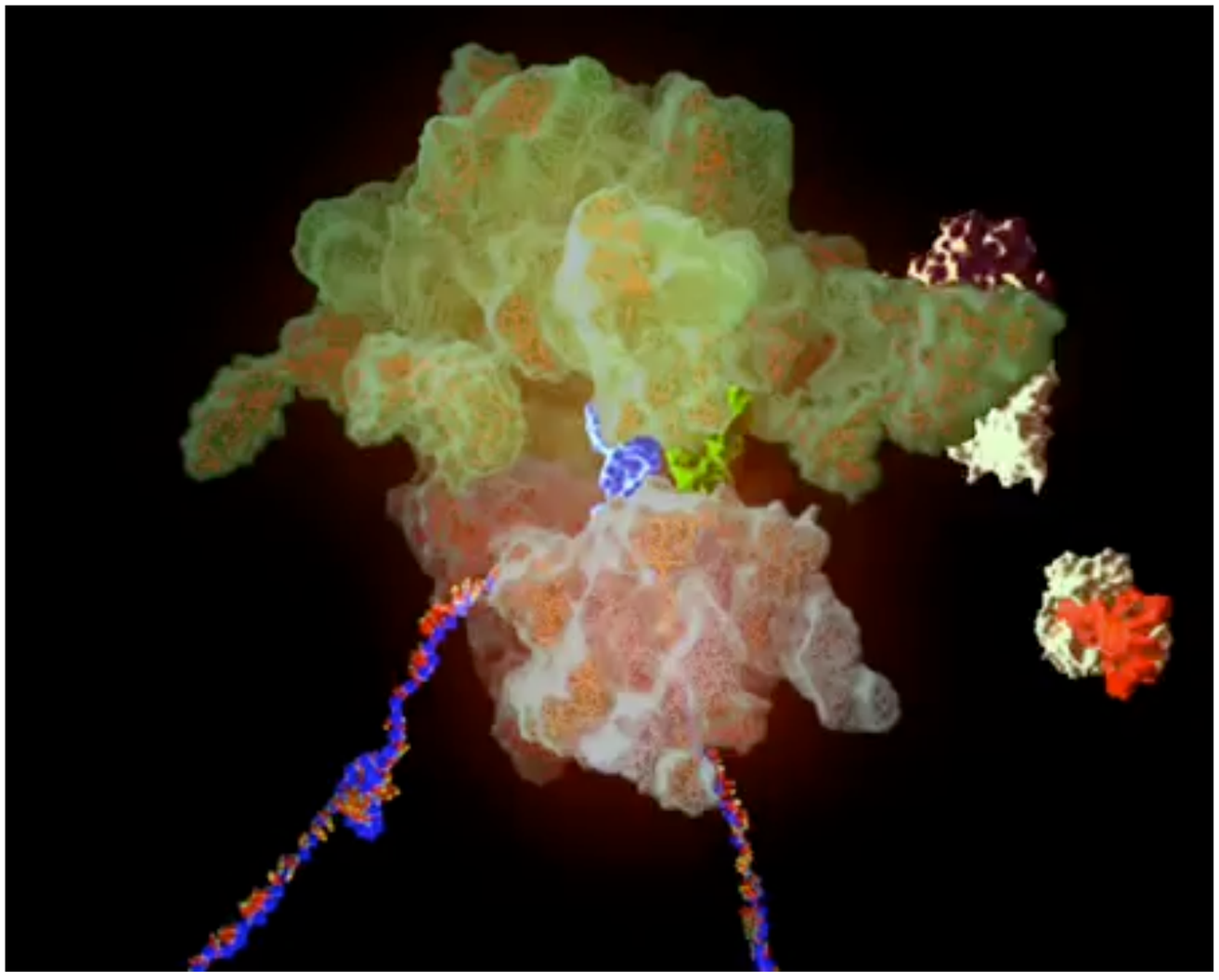







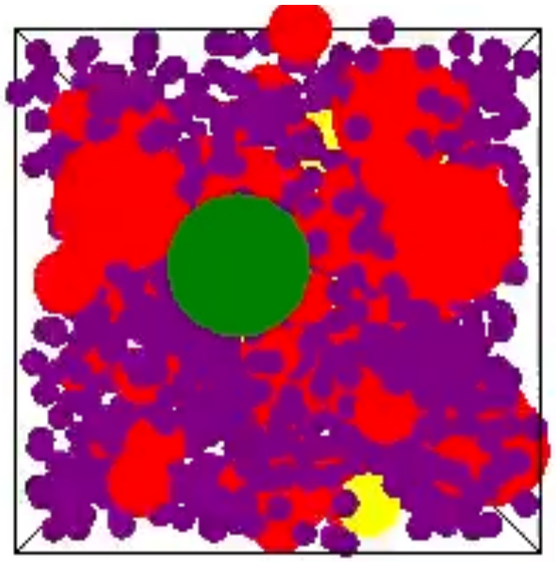



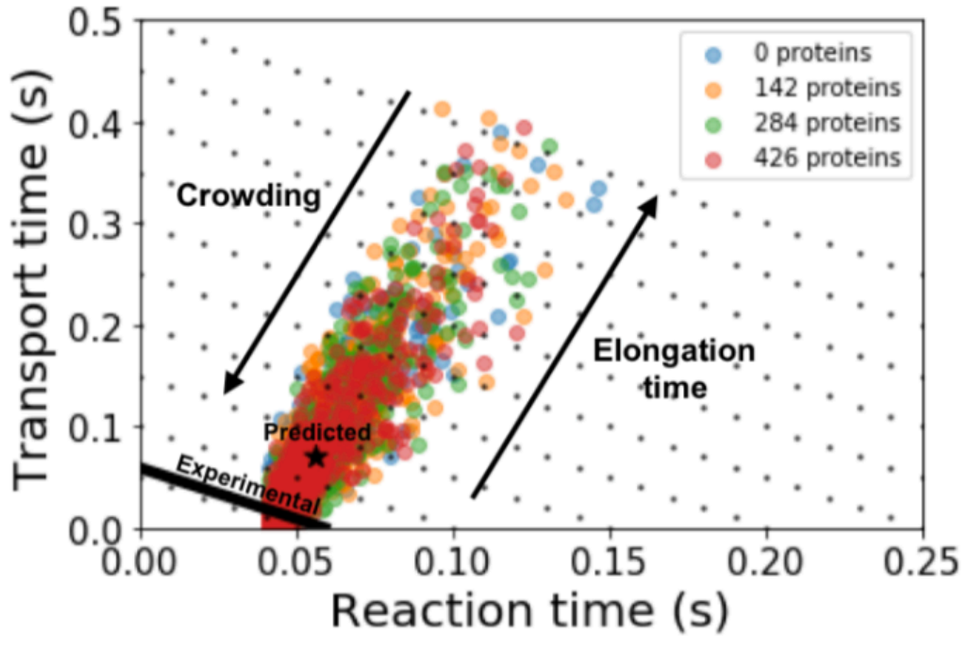
E.g., is Brownian diffusion alone sufficient to supply elongating ribosomes w/ charged tRNA?



https://youtu.be/q_n0lj3K_Ho

Ramakrishnan Group @ LMB





Maheshwari et al., unpublished

We are starting to be able to see...

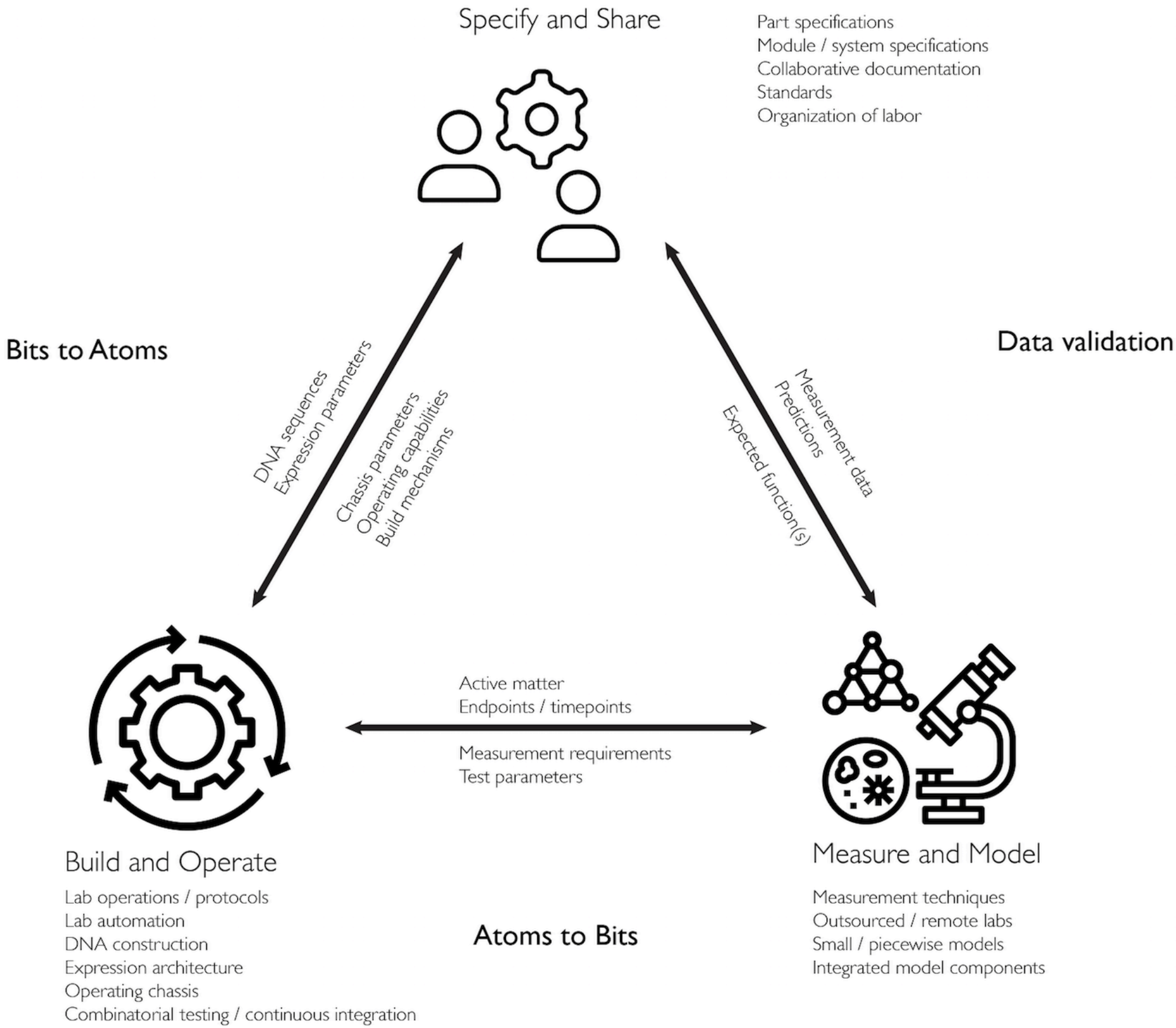
https://med.stanford.edu/news/all-news/2018/05/slac-stanford-open-facility-for-cryogenic-electron-microscopy.html

https://youtu.be/pbGRZZwX9Q8

https://jensenlab.caltech.edu/movies/

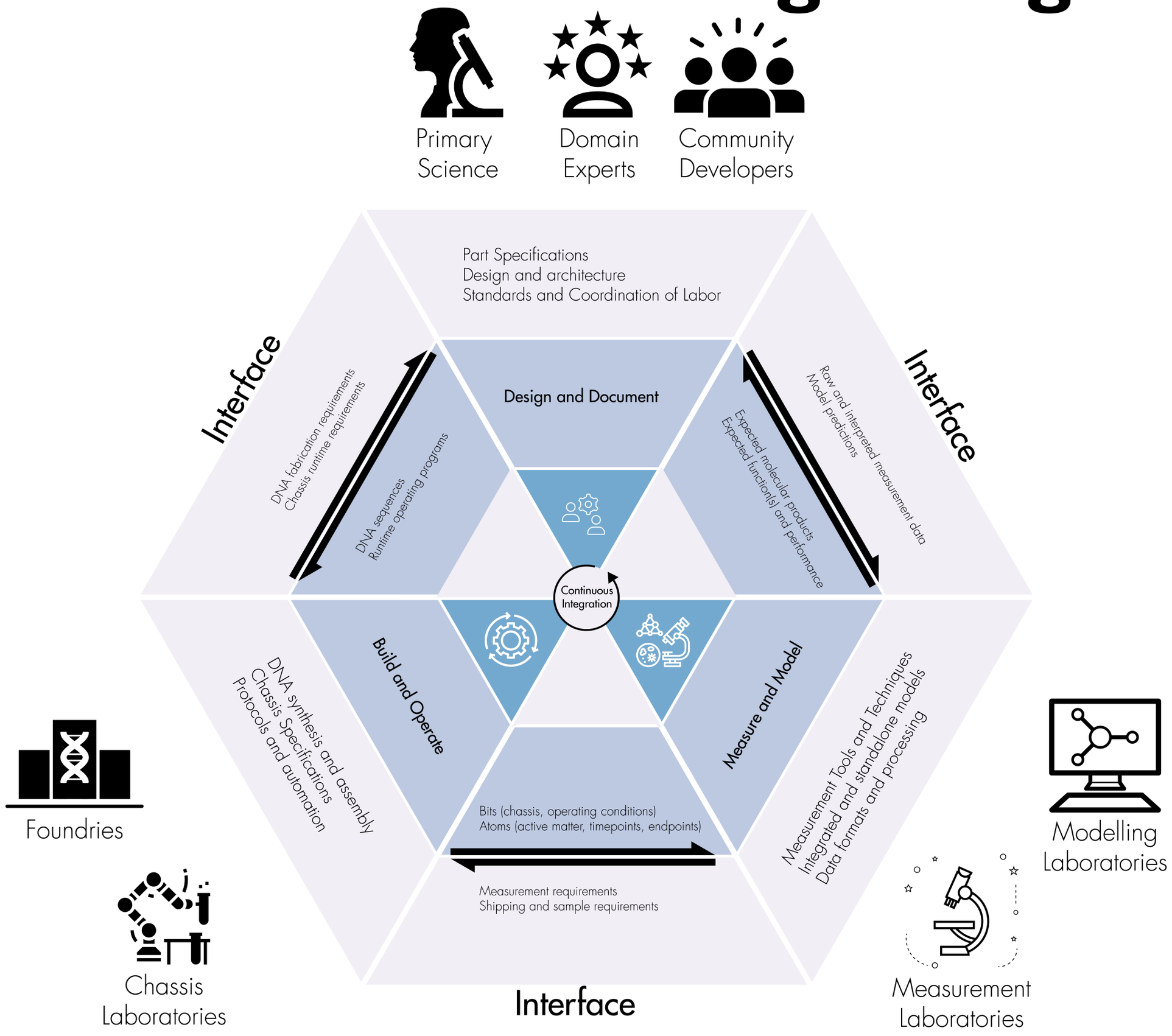

RUSSELL
Stanford

We now know how to get organized



Jackson-Smith, unpublished

We now know how to get organized



Jackson-Smith, unpublished

buildacell.io

Search

BUILD-A-CELL

ABOUT WORKSHOPS RESOURCES TEAM CONTACT

BUILD-A-CELL

Open collaboration supporting the science and engineering of building synthetic cells

WHAT IS BUILD-A-CELL?

Building cells is hard. But, if we could better work together, then we should be able to build many cells for many purposes. Build-A-Cell is an open community supporting people working together to build a diversity of synthetic cells.

JOIN THE ADVENTURE

Join our Slack channel by getting an invite. We can't wait to see you

To receive email updates fill out this google form.

Have feedback, interested in helping out? Drop us a line!

atg@buildacell.io

TWITTER GITHUB

#containers

☆ | 36 | 0 | Add a topic

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@murrayrm I made Sunday, August 19th requested.

Monday, August 20th

enge0213 5:10 AM
Left comments in github

murrayrm 7:00 AM
Thanks! I'll try to make an update based on the comments there and we'll see if we can finalize something for testing.

Friday, August 24th

murrayrm 12:07 AM
Protocol #1 Testers (@Neha Kamat, @Zoila / @Jan Gregrowicz @Milena Popovic @akshaym / @endy): how are things going?

Zoila 12:09 AM
We are still waiting for the syringes to come in before we begin.
👍 1

Neha Kamat 12:25 AM
I made some films today- aiming to try the rest tomorrow!

Milena Popovic 7:32 PM
@Zoila @Neha Kamat @Jan Gregrowicz @akshaym We have been standardizing the classic Yomo protocol while we wait for the supplies. Can you post your imaging protocols along with uploaded images, which type of microscope, filters, etc.

Jan Gregrowicz 9:59 PM
@Milena Popovic @Zoila I think we have not done any quantitative imaging yet in our lab. For the settings, many things will depend on a microscope used, so I think everybody will have to make a standard curve for HPTS. Tomorrow I will send details about the settings that we use, but I guess some things will be adjusted when we finally use HPTS.

Message #containers

Thread

#cellfree

acjs Aug 6th at 11:26 PM
Could we/should we do (or has someone done) a head-to-head cell free extract benchmark of, for instance, TXTL produced by different labs and commercial providers doing the same job?

5 replies

endy 21 days ago
If not then might be a great candidate for an interlab ala kelly et al 2008 jbe

wpoole 21 days ago
I think this is a great idea - once we get a standardized extract protocol we can all make a batch of extract and test a couple of genetic circuits. It might also be worth considering pooling the extracts together and doing proteomics or mRNA-seq on the cell cultures or some other assays to try to assess the differences between them (although proteomics are probably outside of my skill set)

murrayrm 21 days ago
Pretty sure that there is some existing work on comparing different types of extracts; we should ask Vincent or Mike Jewett.

murrayrm 21 days ago
Also, be careful about what you mean by "doing the same job": if all you want is protein expression, most things will work with different yields. But if you want repression, activation and other core

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4th USA "build-a-cell" workshop
8 February 2019
JCVI @ La Jolla, CA

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We choose to build the cells
and do the other things,

Gaps in understanding, tools, and portfolio
can all now be addressed directly,

~10 years, ~1.25 billion dollars,
unapologetically organized,

Inaction guarantees ceding future of
life; success enables flourishing.