Engineering Bacteria for Drug Production:

Complexity and Synthetic Biology

Jay Keasling

University of California, Berkeley &

Lawrence Berkeley National Laboratory

Berkeley, CA 94720

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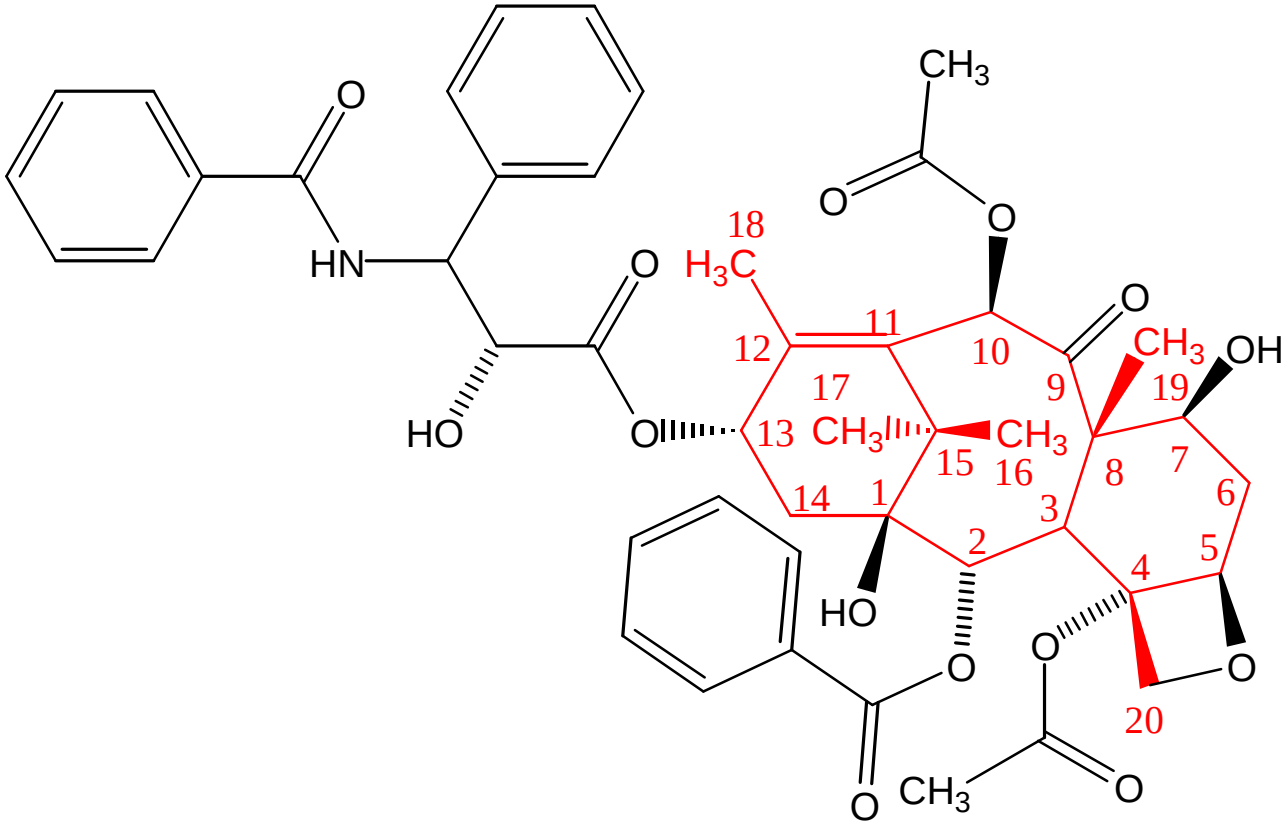
Taxol



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Total synthesis of taxol

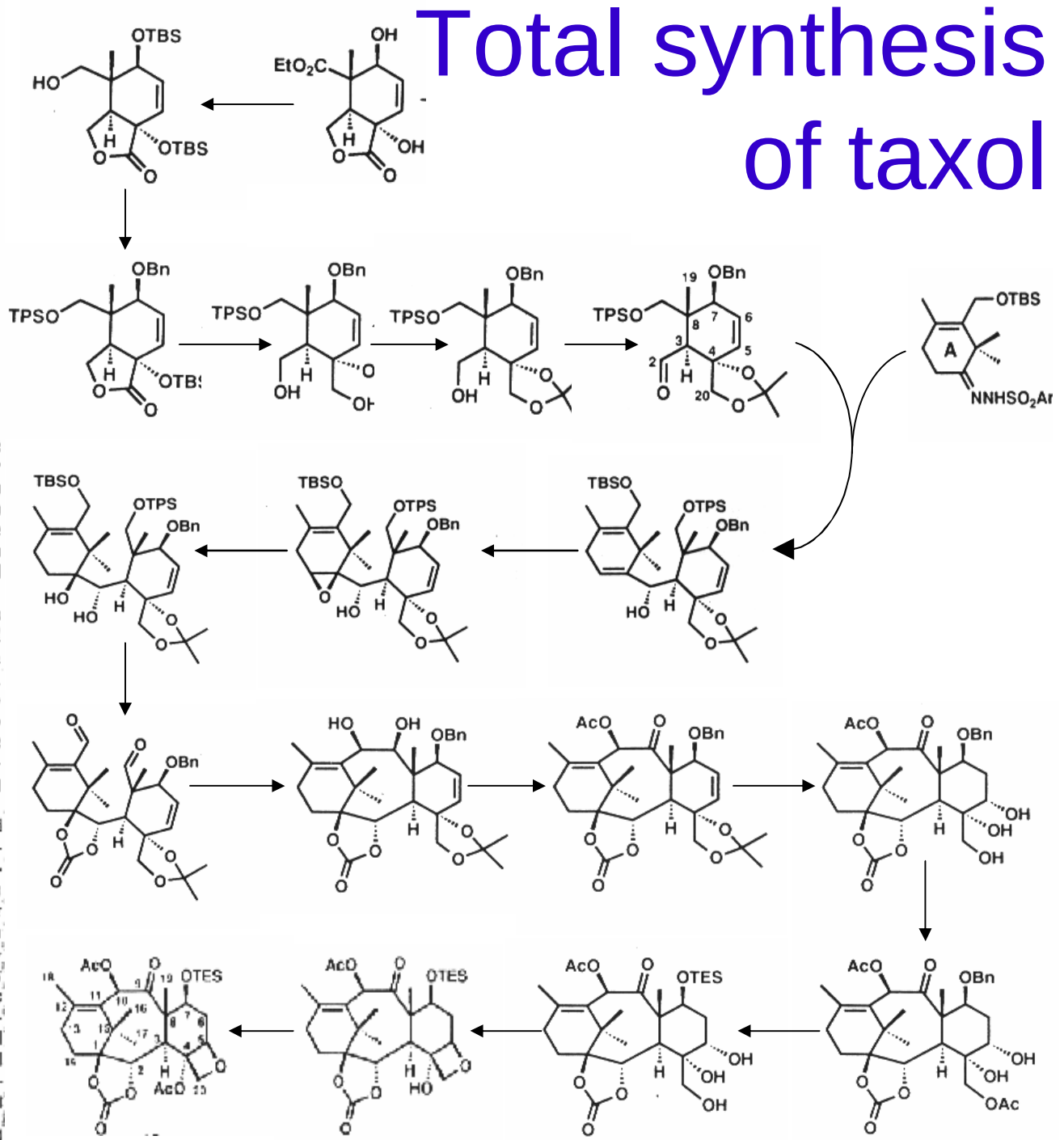
K. C. Nicolaou*†, Z. Yang*, J. J. Liu*, H. Ueno*, P. G. Nantermet*, R. K. Guy*, C. F. Claiborne*, J. Renaud*, E. A. Couladouros*, K. Paulvannan* & E. J. Sorensen*†

* Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037, USA
† Department of Chemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093, USA

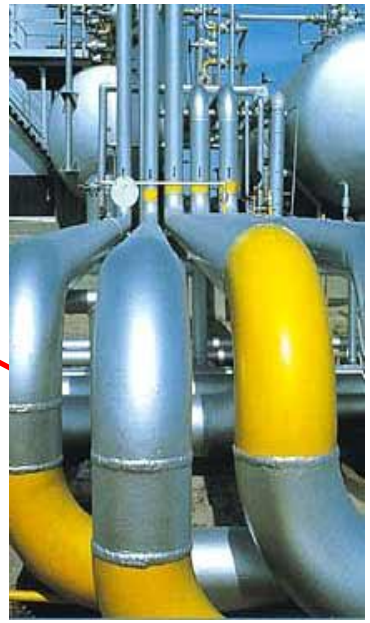
Taxol[1–4], a substance originally isolated from the Pacific yew tr (*Taxus brevifolia*) more than two decades ago, has recently bee approved for the clinical treatment of cancer patients. Hailed having provided one of the most significant advances in cance therapy[5], this molecule exerts its anticancer activity by inhibitin mitosis through enhancement of the polymerization of tubulin a consequent stabilization of microtubules[6]. The scarcity of tax and the ecological impact of harvesting it have prompted extensive searches for alternative sources including semisynthesis, cellular culture production and chemical synthesis[2,3]. The latter has been attempted for almost two decades, but these attempts have been thwarted by the magnitude of the synthetic challenge. Here we report the total synthesis of taxol by a convergent strategy, which opens a chemical pathway for the production of both the natural product itself and a variety of designed taxoids.

The strategy for the present synthesis of taxol (**1**, Fig. 1*a*) was based on a retrosynthetic analysis involving the bond disconnections[7] shown in Fig. 1*b*. Thus, in the synthetic direction the following key operations were proposed: (1) two fragments, representing precursors to rings A and C (see Fig. 1*a*), were to be coupled by a Shapiro reaction[8] and a McMurry coupling[9] to assemble the ABC ring skeleton; (2) instalment of the oxetane ring; (3) addition[10] of the various substituents around the peripheries of rings B and C; (4) oxygenation[11] at C-13; and (5) esterification to attach the side chain[12].

The previously reported intermediates **2** (ref. 12) (Fig. 2) and **8** (refs 7, 13) (Fig. 3) served as the starting points for the convergent synthesis of taxol reported here. Figure 2 presents the construction of the requisite C-ring aldehyde **7** from **2**. Protection of both hydroxyl groups in **2** with TBS groups (95%) (for abbreviations see figure legend) followed by selective reduction of the ester group with $LiAlH_4$ at 0 °C, furnished primary alcohol **3** (94% yield). Acid-catalysed deprotection of the secondary alcohol in **3** proceeded in a highly selective manner to give the corresponding diol (90% yield), which was then selectively protected with a TPS group at the primary position and a benzyl group at the secondary to afford compound **4** in 80% overall yield. The γ-lactone in **4** was then reductively opened with concomitant desilylation at the tertiary position using $LiAlH_4$ a



Building the factory from parts



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Designing and building parts: "Parts Engineering"

Mole balance $V = \frac{F_{Ao} X}{-r_A}$

Rate law $-r_A = kC_A$

$k = Ae^{-E/RT}$

Stoichiometry $C_A = C_{Ao}(1-X)$

Combine $V = \frac{C_{Ao} v_o X}{kC_A(1-X)} \Rightarrow t = \frac{1}{k}\frac{X}{(1-X)}$

Energy Balance $T = T_o - \frac{(-\Delta H_{rx})X}{C_{pA}}$

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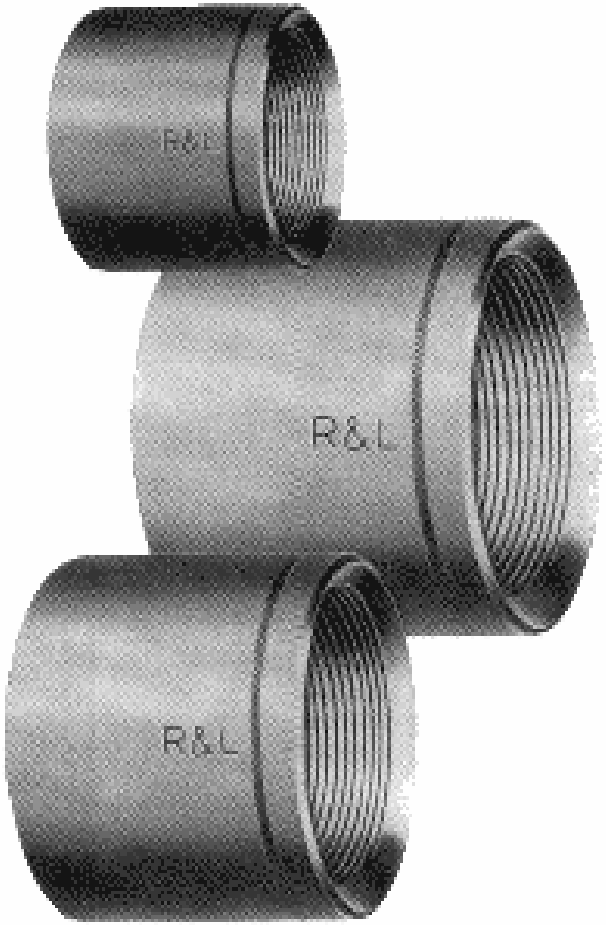
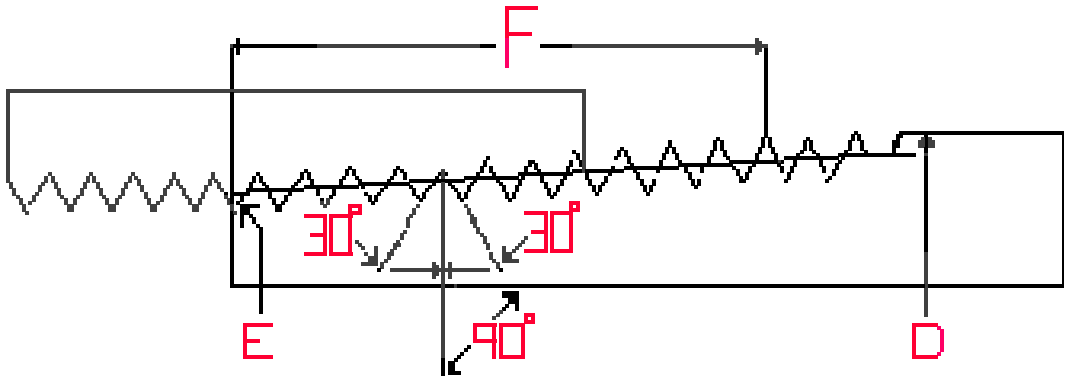
Using standard parts





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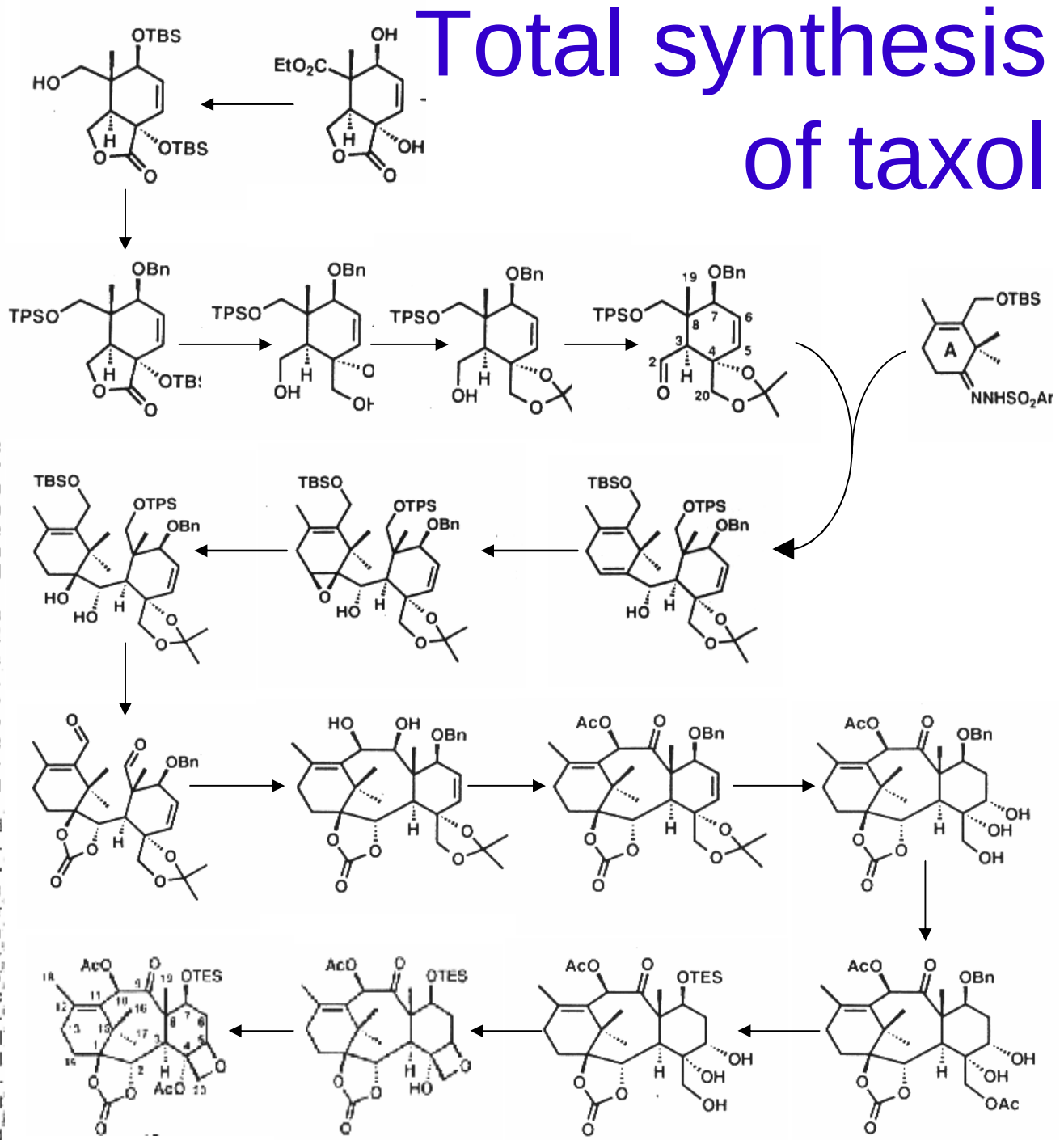
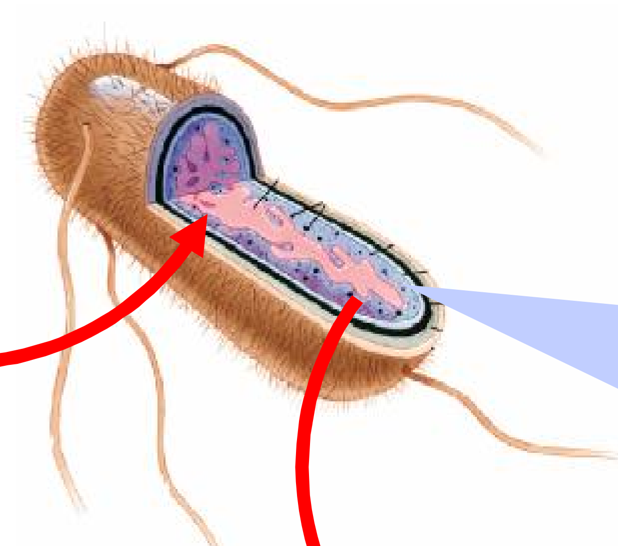
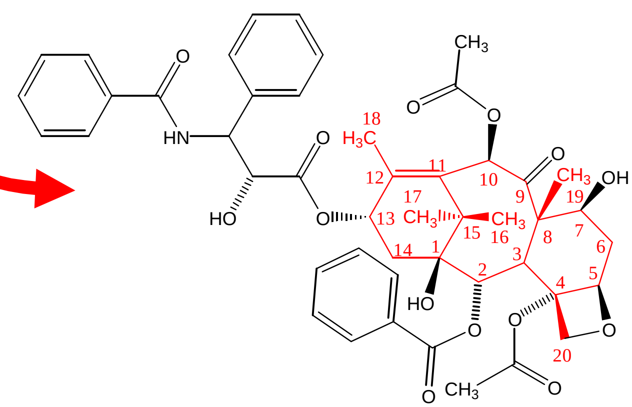


Building a Taxol factory inside a microbe





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Microbial production of drugs

Advantages

- Microbial fermentations are relatively simple to scale up
- Inexpensive starting materials can be used
- Production not affected by weather conditions
- Consistent supply
- Pure product can be made (free of other contaminating terpenes)

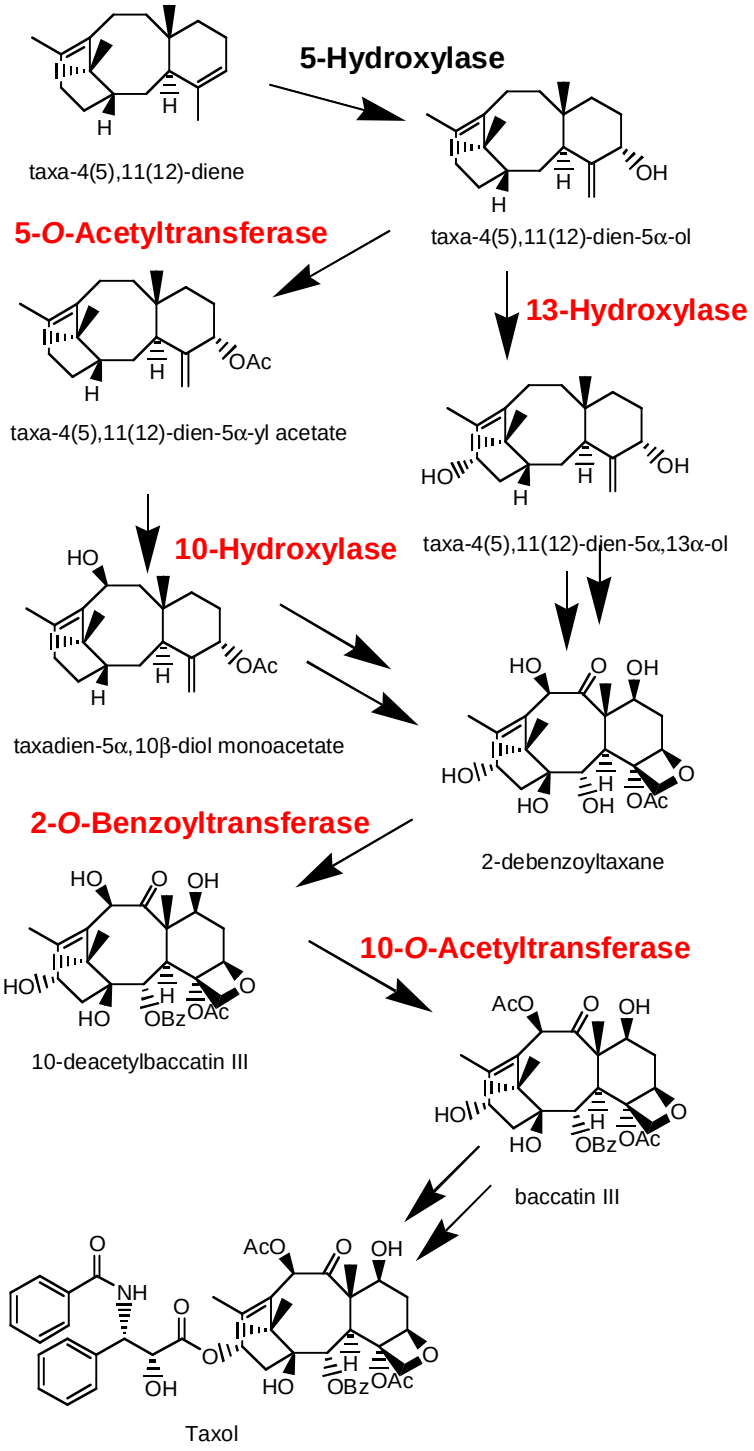
Microbial production of drugs

Challenges

- Need the genes for all of the enzymes in the pathway
- Not always simple to express in microbes the genes from very different organisms
- Need to balance metabolic pathways to optimize production
- Need a good “platform organism” with appropriate gene expression tools

Steps in creating a Taxol producer

Identify the biosynthesis pathways



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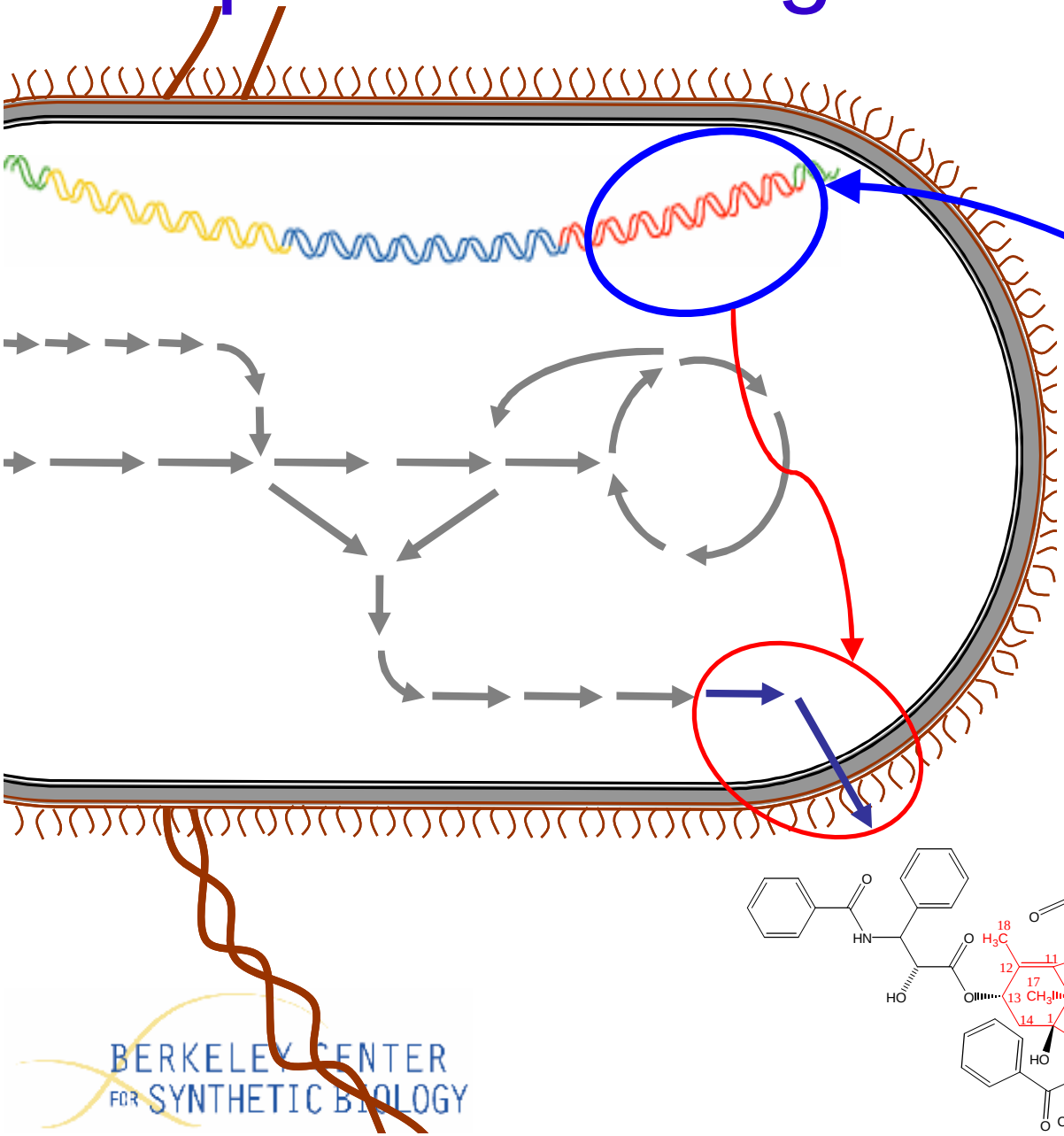
Steps in creating a Taxol producer

Clone the genes into our microbe of choice



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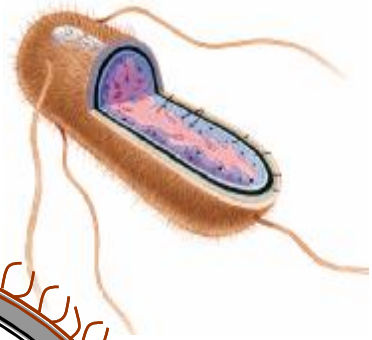
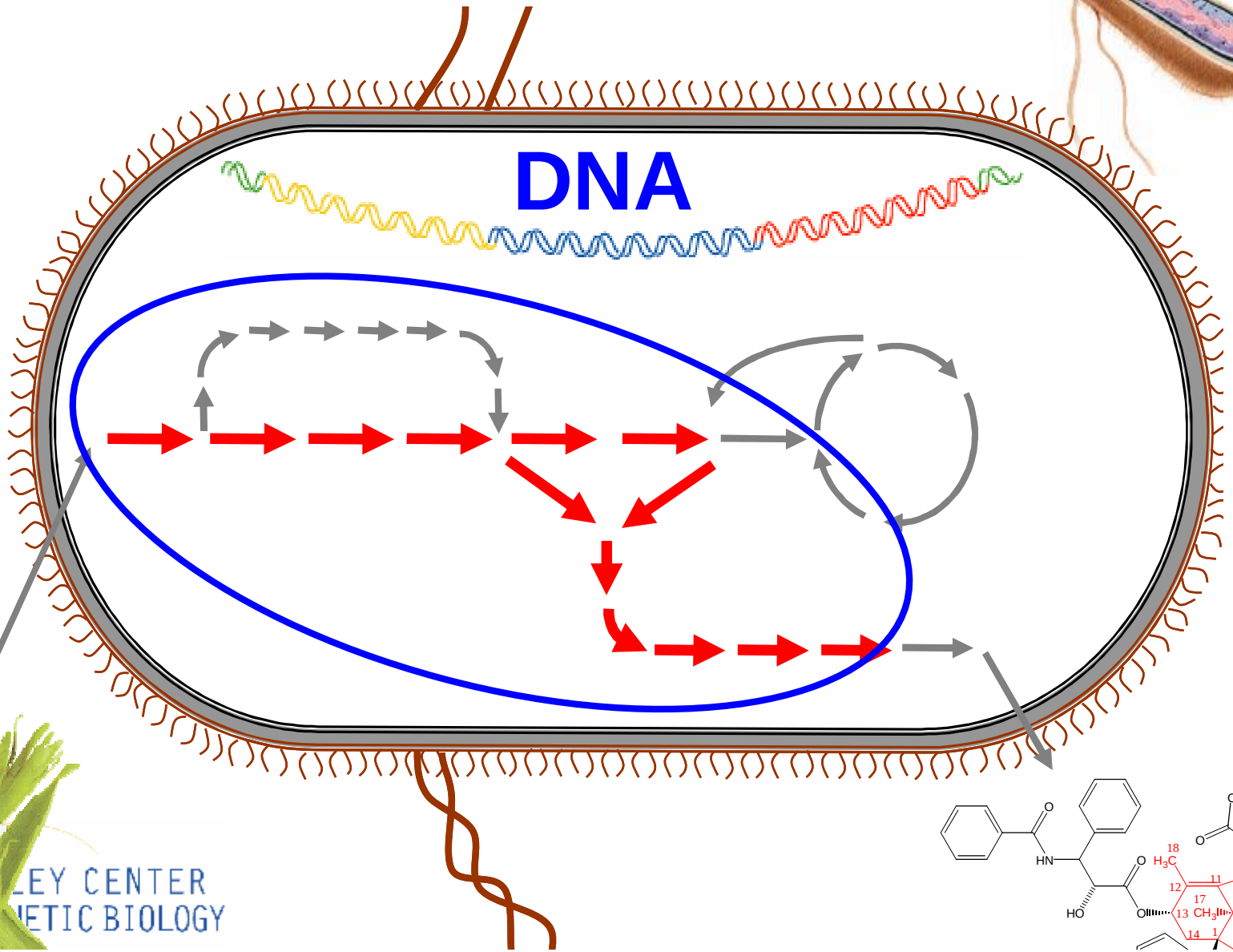
Steps in creating a Taxol producer

Enhance production of precursors





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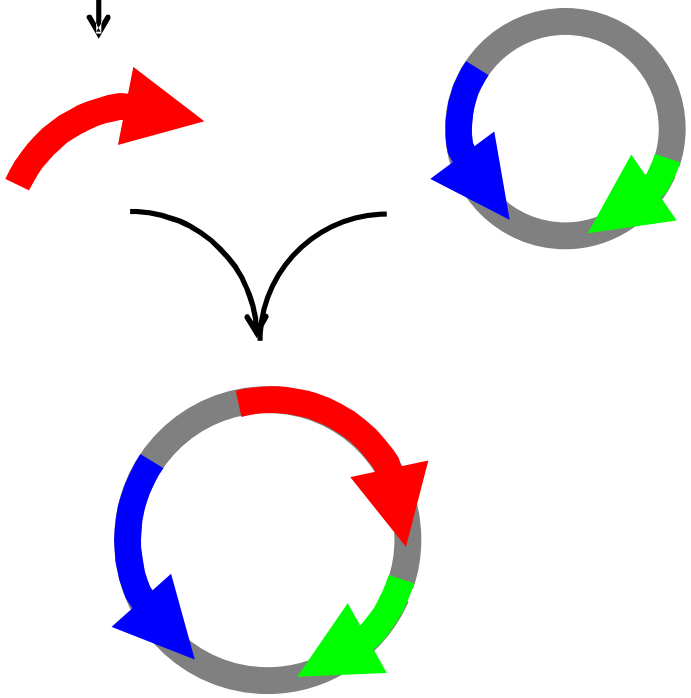
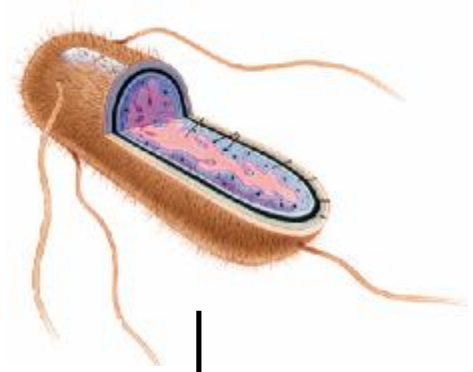
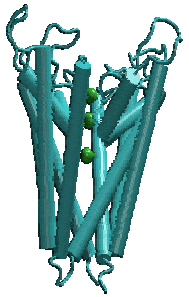
Basic Biotechnology

One Gene - One Protein







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Biological Unit Operations

- Natural biological parts and devices often have multiple interactions with other parts/devices in the cell
- Biological parts have no IEEE-equivalent standard for connections
- Many of the ‘good’ biological parts have been patented
  - royalties can be expensive
  - Open Source Biology

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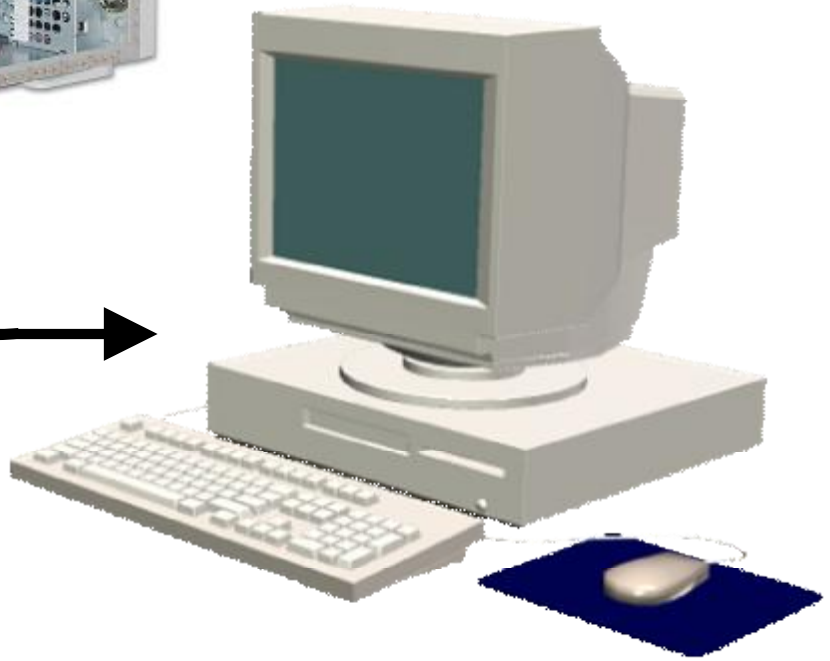
Computers are built from off-the-shelf devices

Devices

Chassis



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Abstraction

Systems

Devices

Parts

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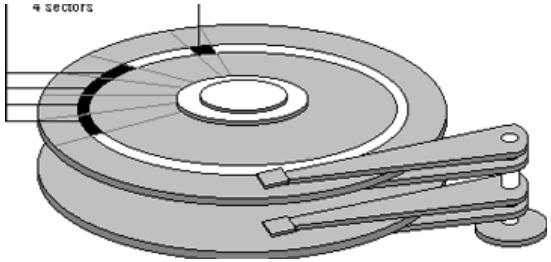
Compliments of Drew Endy, MIT

Abstraction

Systems

Devices



Parts

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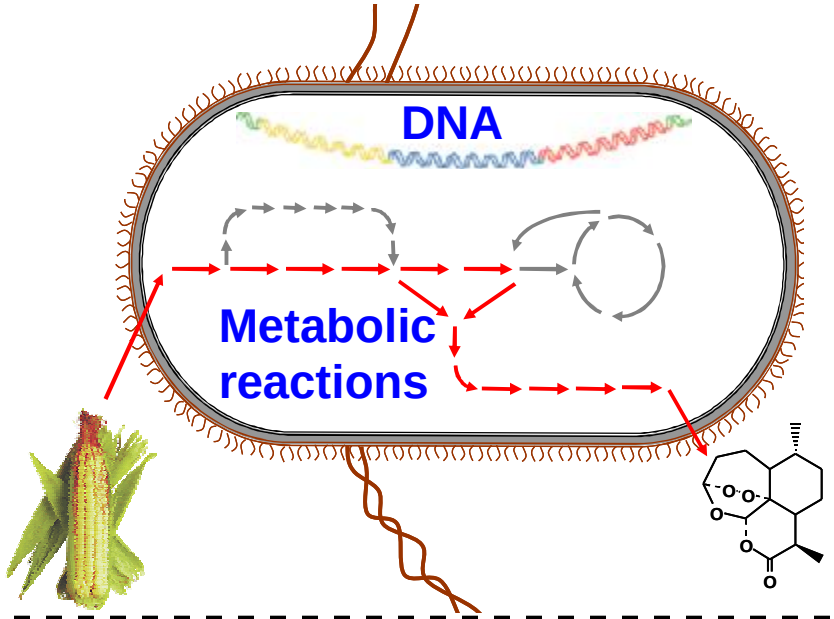
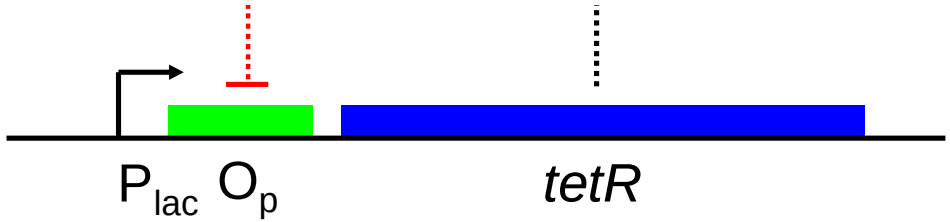
Abstraction

Systems



Devices



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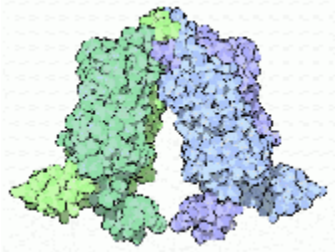

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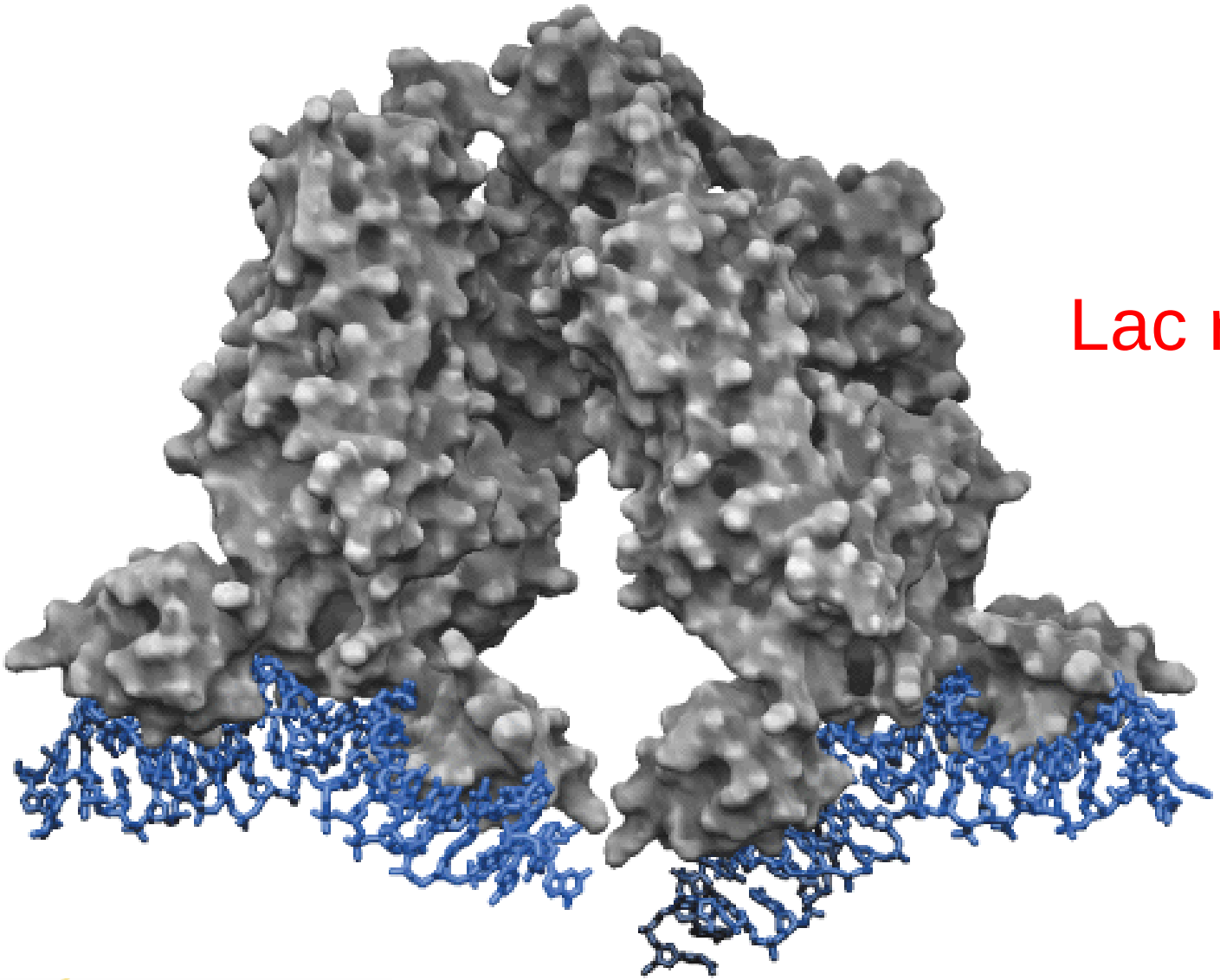
Parts



Lac repressor

DNA

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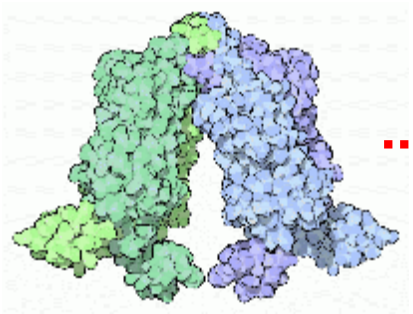
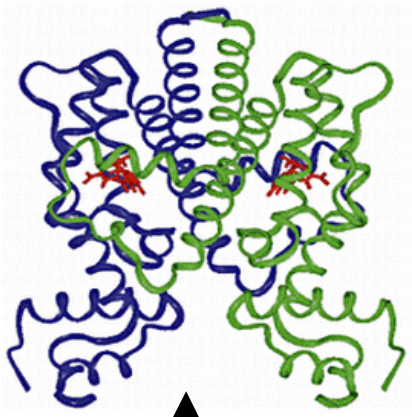
Devices: an inverter

Lac Repressor



Tet Repressor



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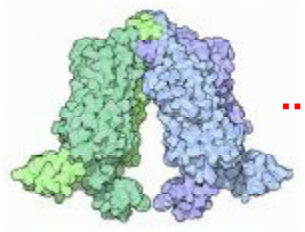
Devices: an inverter

Lac Repressor



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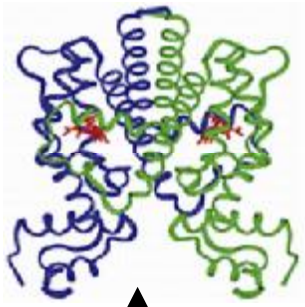

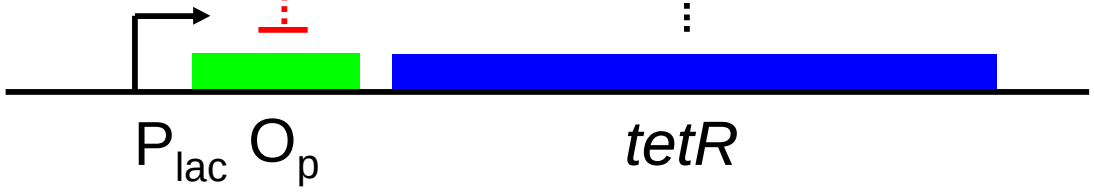

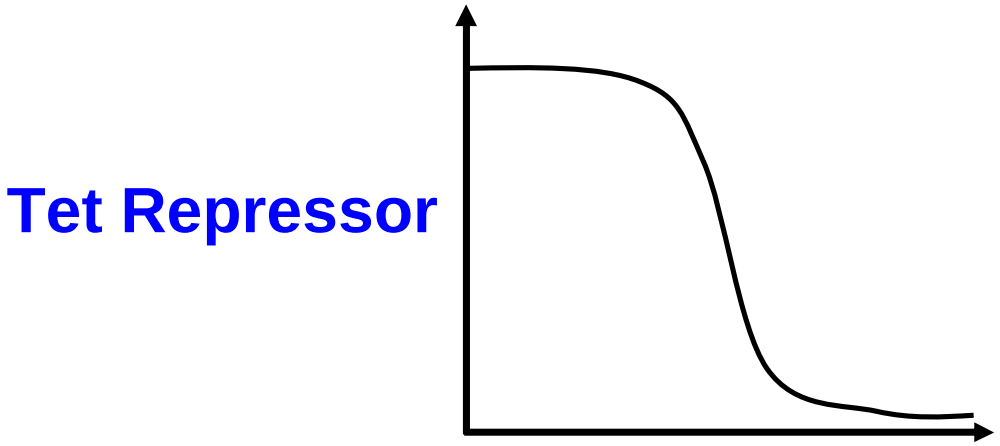

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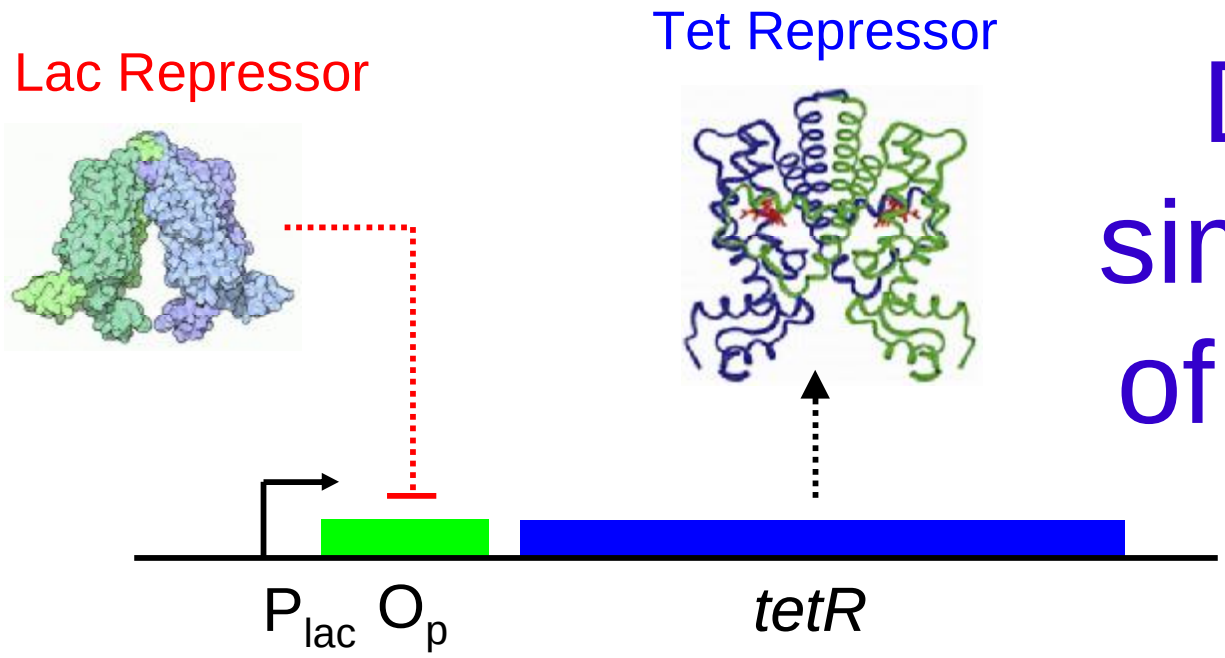
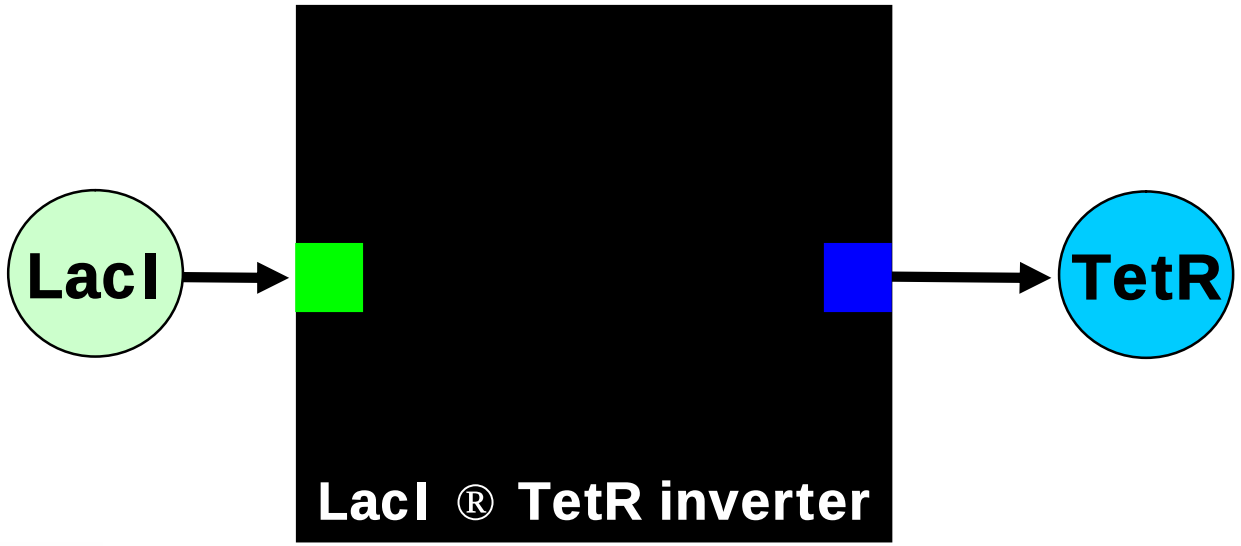
Devices: a simplified view of the inverter





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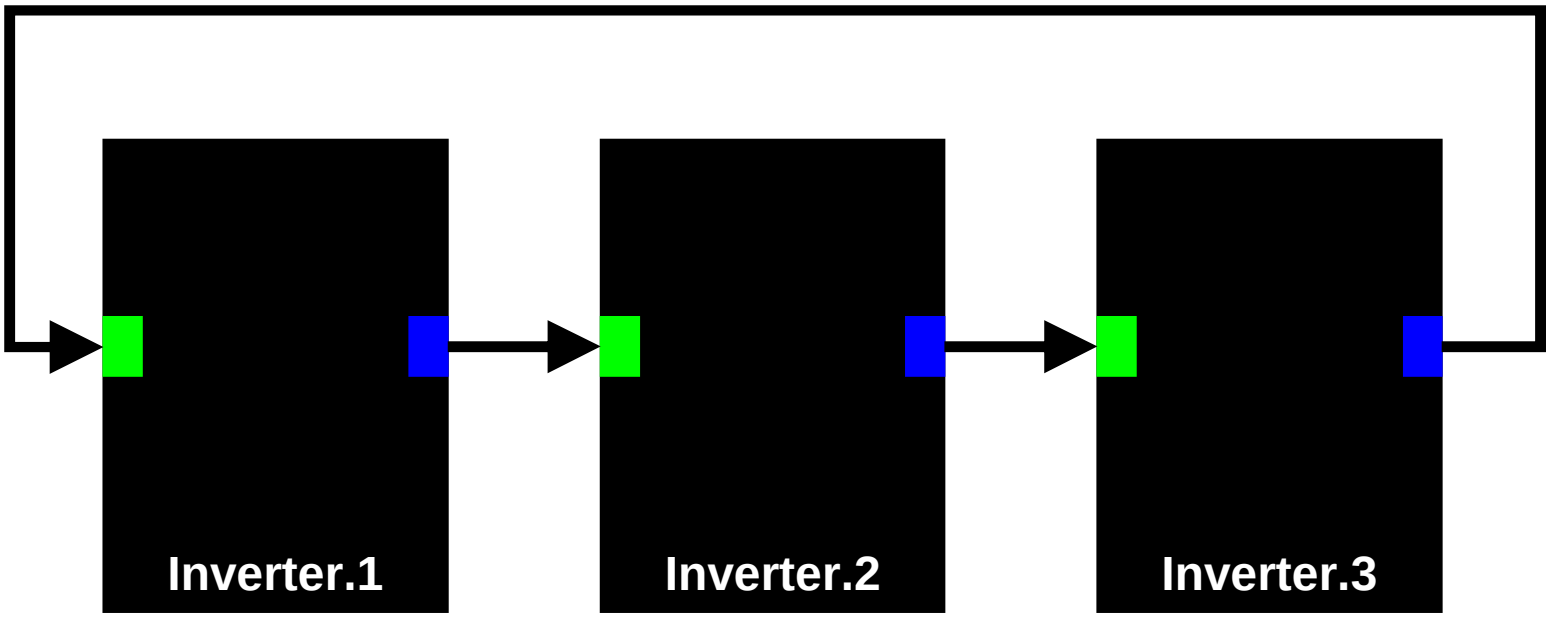
Systems: multiple inverters



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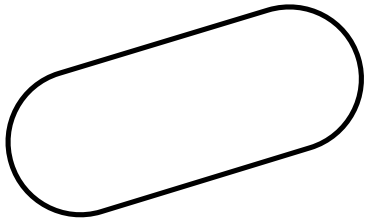
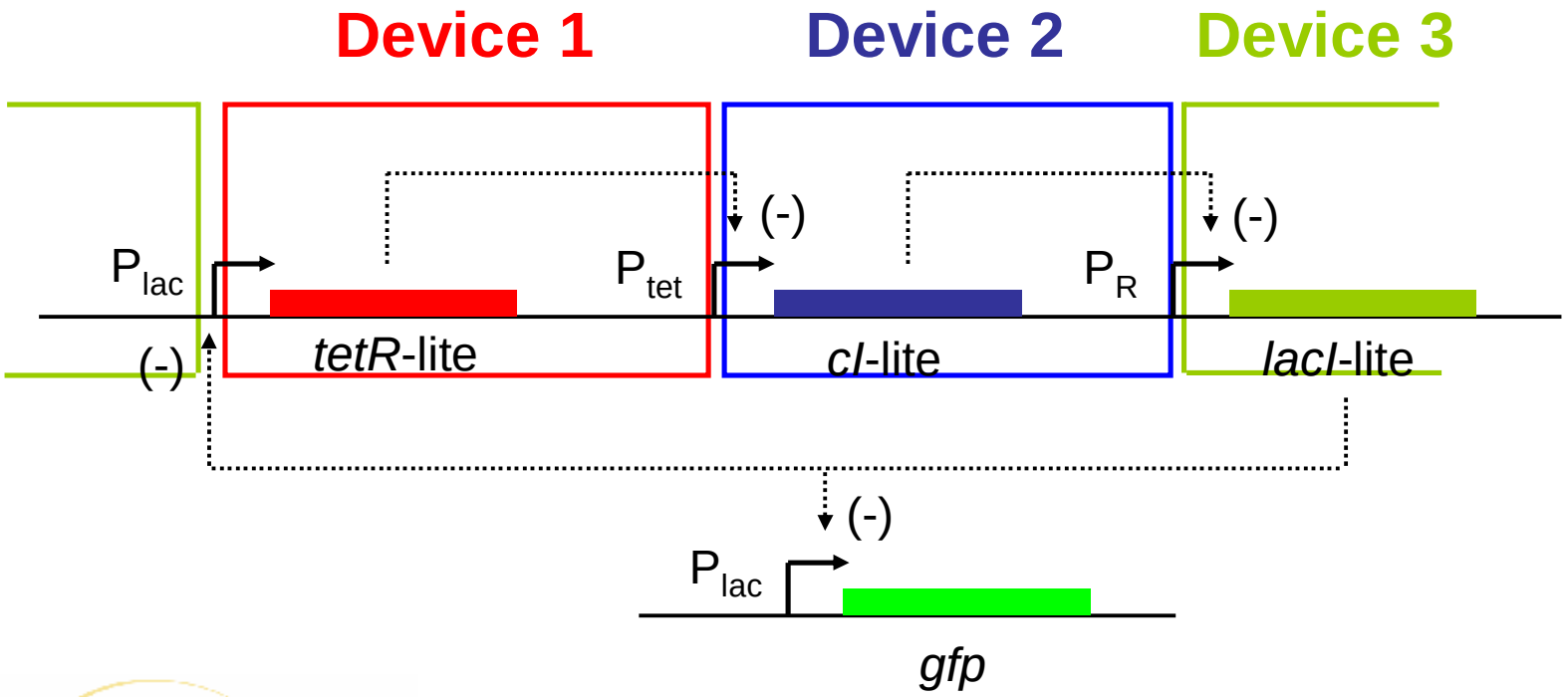
Building a bacterial blinker





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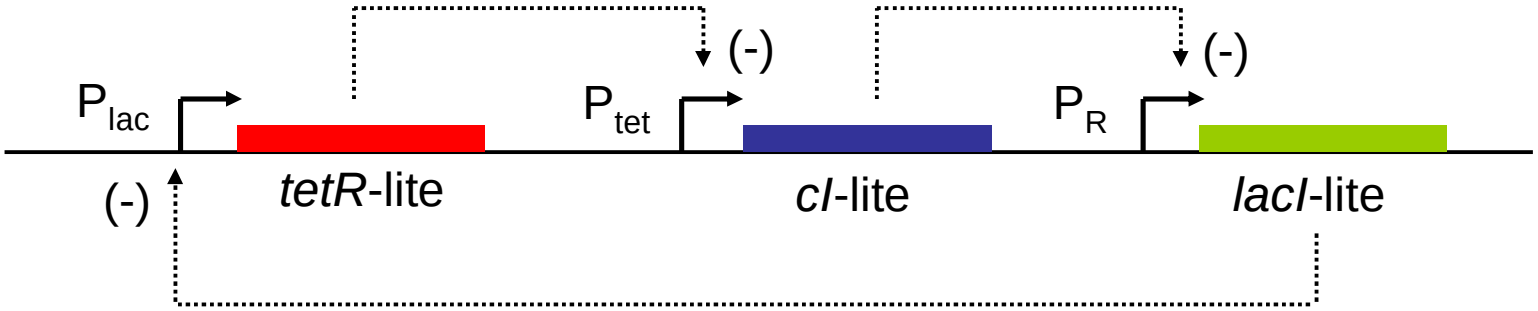
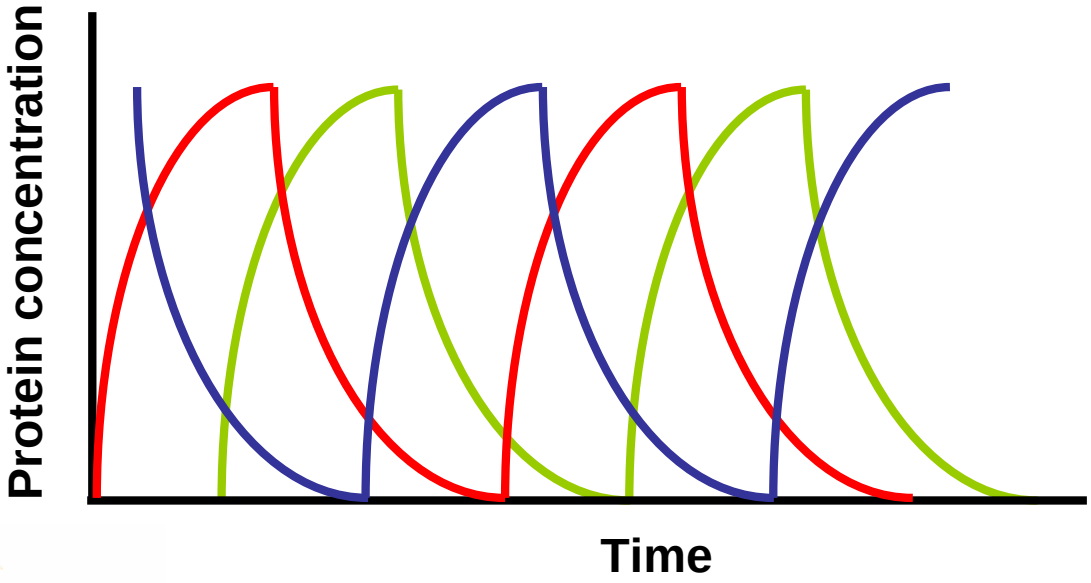
Elowitz & Leibler. 2000. *Nature* **403**:335-8

Building a bacterial blinker





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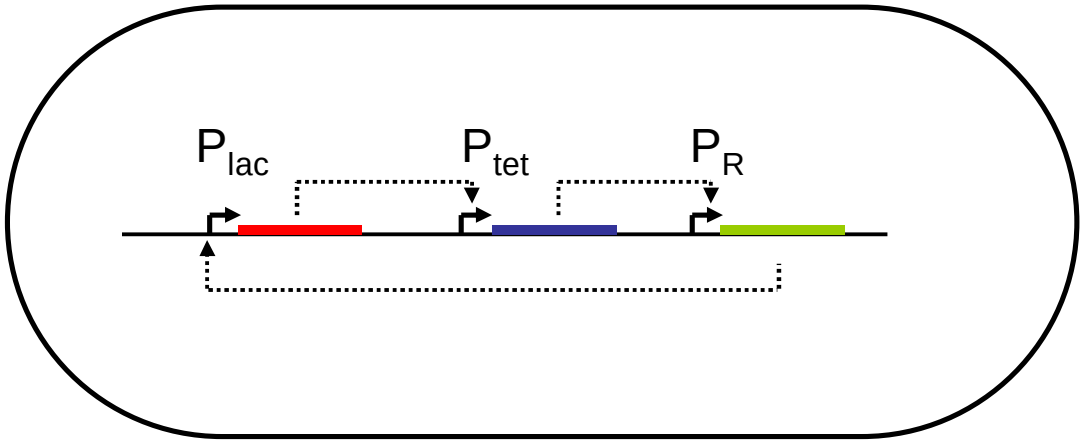

E. coli as the Chassis

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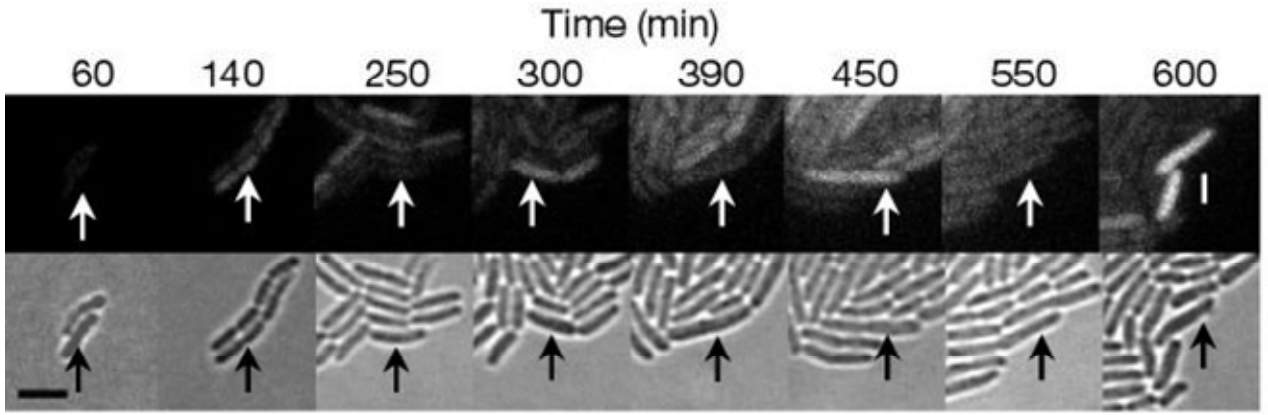

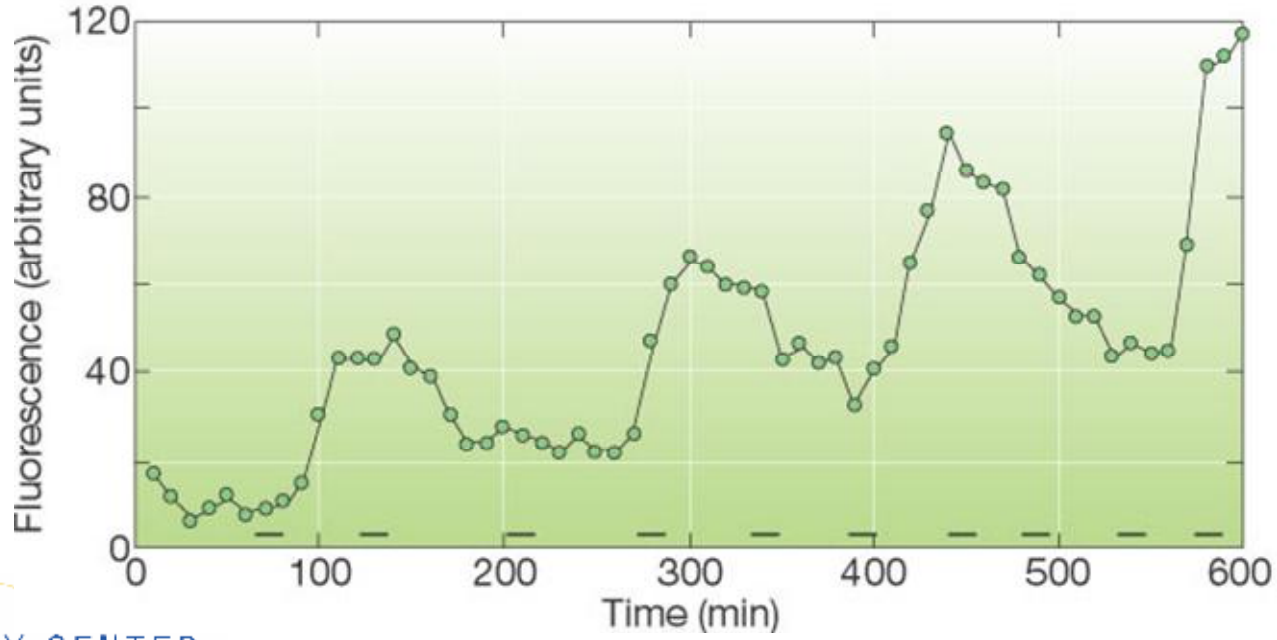

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Synthetic biology

- Design and synthesis of biological entities:
  - Enzymes (parts)
  - Genetic circuits (devices)
  - Entire cells (chasses)
- Enabled by the development of parts that can be assembled into larger, functioning devices
- Directly analogous to the design of integrated circuits
- Integrates systems and computational biology into design

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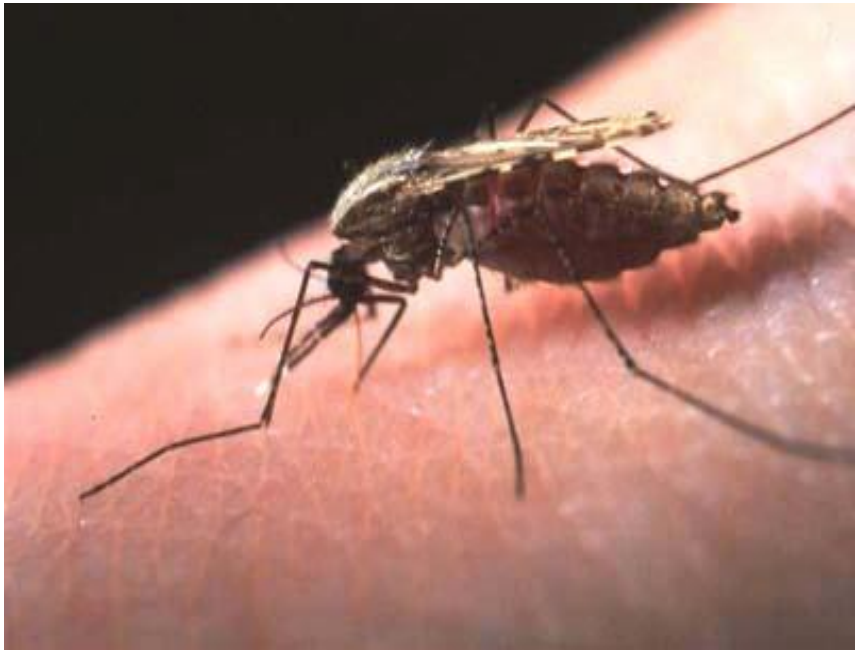
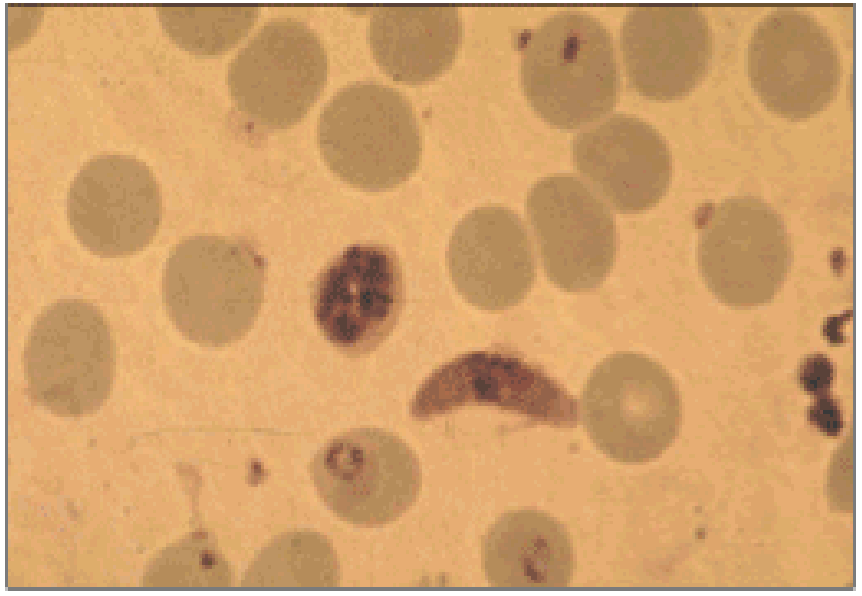
Case Study: Malaria

- Caused by *Plasmodium*, a single-cell protozoan
  - Transmitted by Anopheles mosquito
  - Destroys red blood cells





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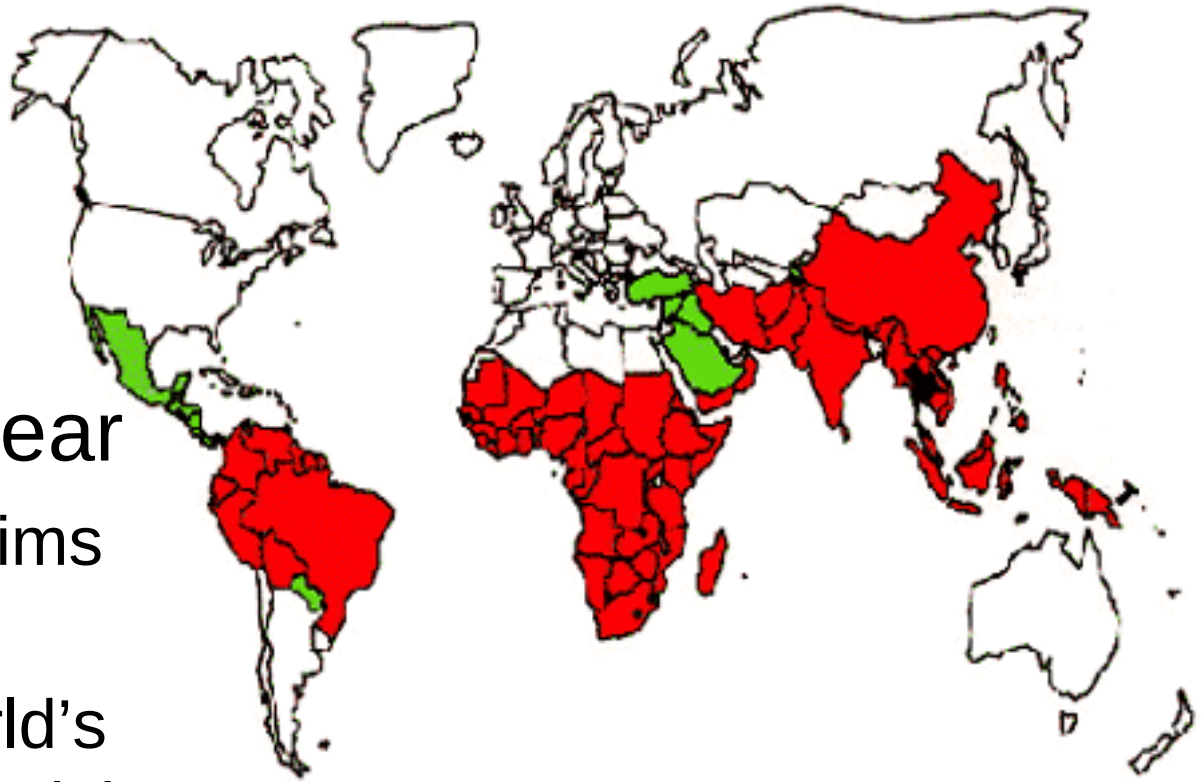
Malaria



- 1.5-2.7 million people die of malaria every year
  - 90% of the victims are children
  - 40% of the world's population is at risk
- Economists have proposed that malaria decreases the GDP of affected countries by as much as 50%.

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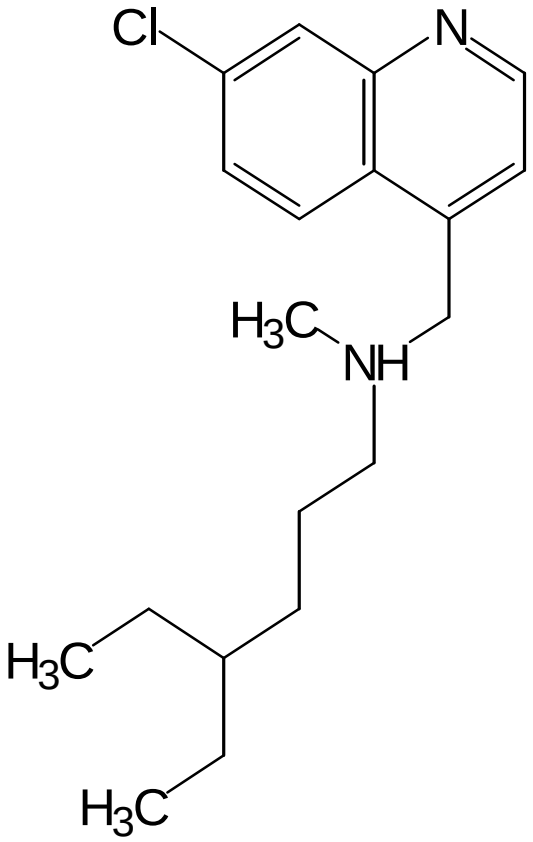
Chloroquine-based drugs

- Most widely-used drugs to treat malaria
- *Plasmodium* in South America and Southeast Asia is largely resistant to chloroquine



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A brief history of artemisinin

168 B.C. *Recipes For 52 Kinds Of Diseases* found in the Mawangdui Han Dynasty tomb

à Hemorrhoids

340 A.D. *Zhou Hou Bei Ji Fang (Handbook of Prescriptions for Emergency Treatments)*

à Fevers (malaria)

1972 Active ingredient (artemisinin) isolated

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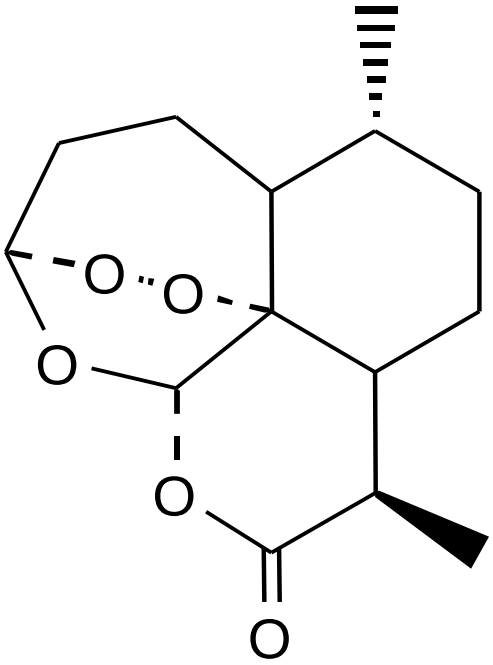
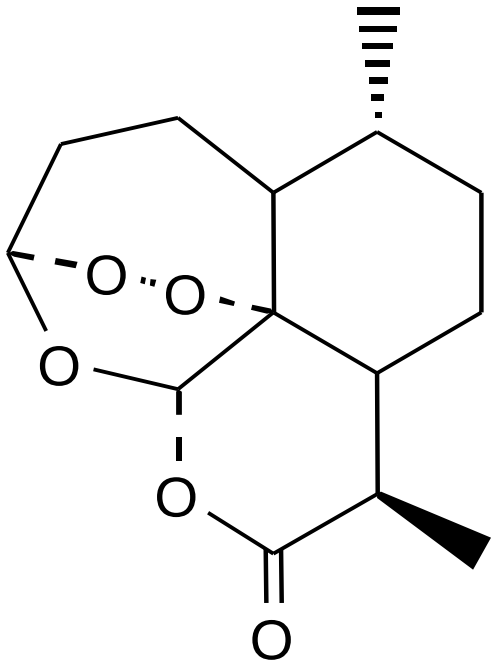


Artemisinin-based drugs

- The current cost is approximately $2.40.
  - Artemisinin adds $1.00-1.50 to the cost for drugs
  - Most developing countries spend less than $4/person/year on health care
- As many as 10-12 treatments are needed for each person annually
- World Health Organization estimates that 700 tons will be needed annually



Potential sources for artemisinin

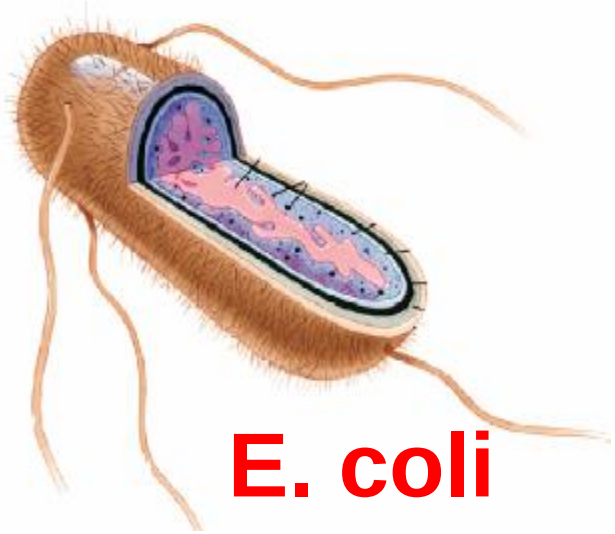
- Agriculture
  - Efforts are under way to plant *Artemisia annua* around the world
- Chemical synthesis
  - A synthesis route is known but it is too complicated for economical production
- Microbial
  - Need all of the genes from the plant

Goal

Reduce the cost of artemisinin-based anti-malarial drugs by an order of magnitude.

Approach

Engineer a microbe to produce artemisinin from an inexpensive, renewable resource.



E. coli

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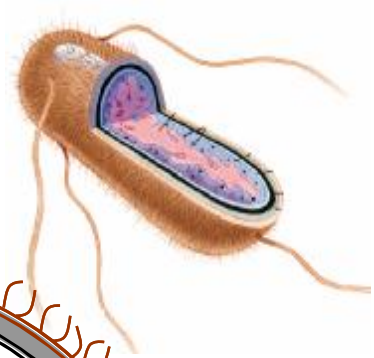
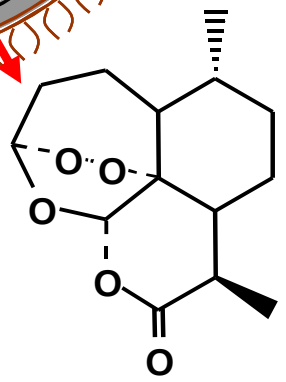
Production of artemisinin in a microbe



DNA

Metabolic reactions



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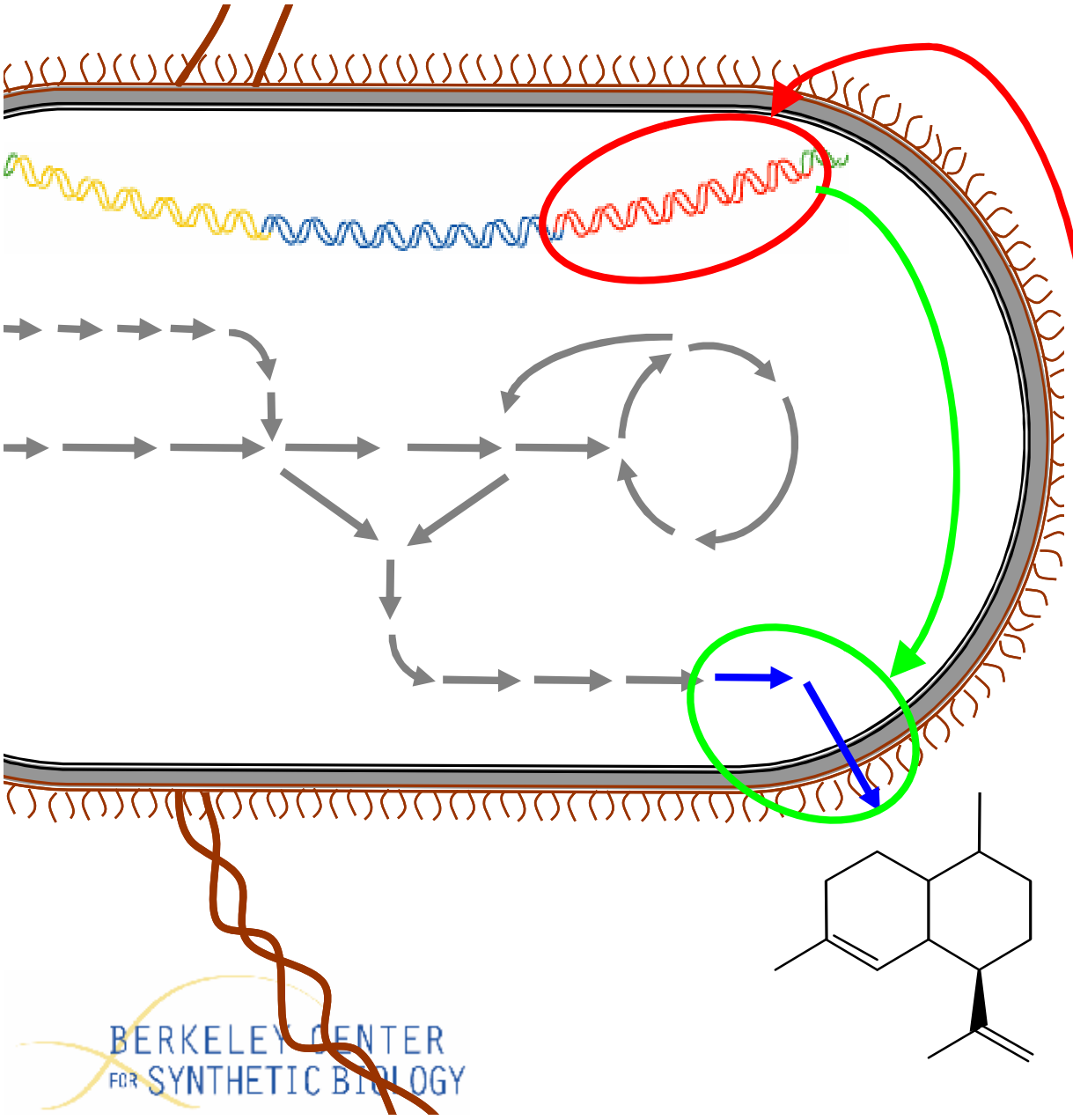
Transplanting artemisinin pathways



Clone the genes

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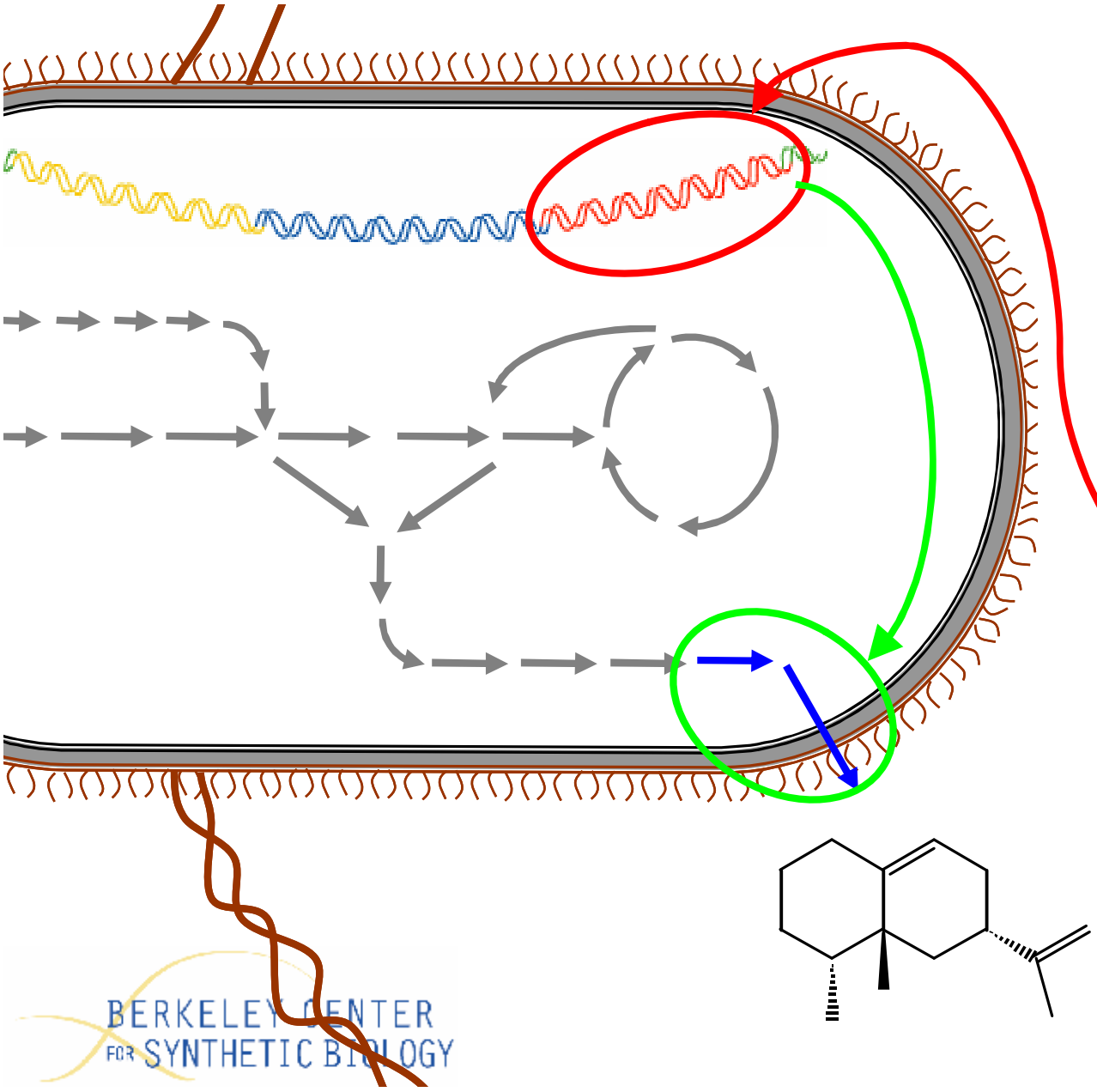
Production of a model isoprenoid in *E. coli*

Tobacco as an early model



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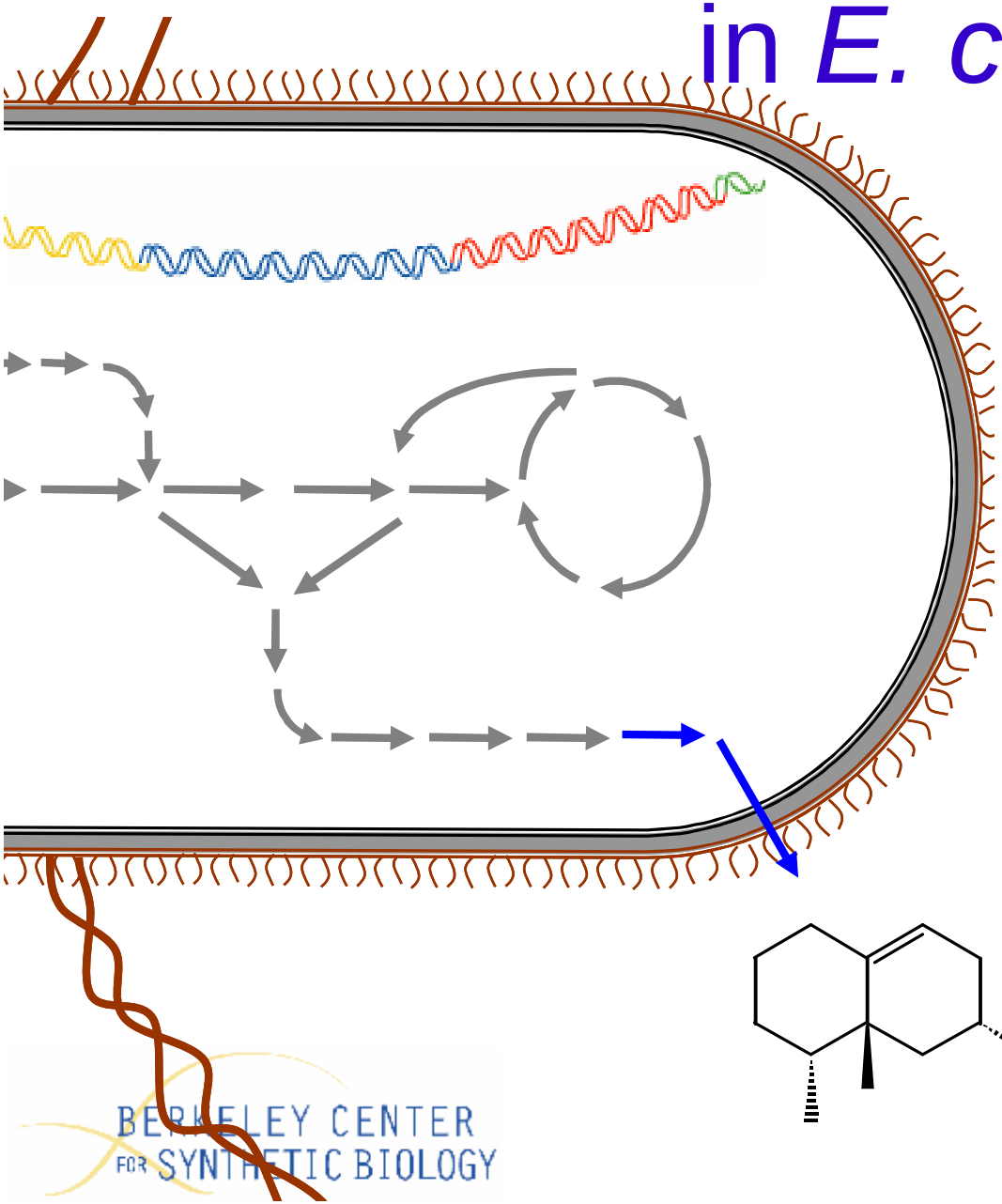
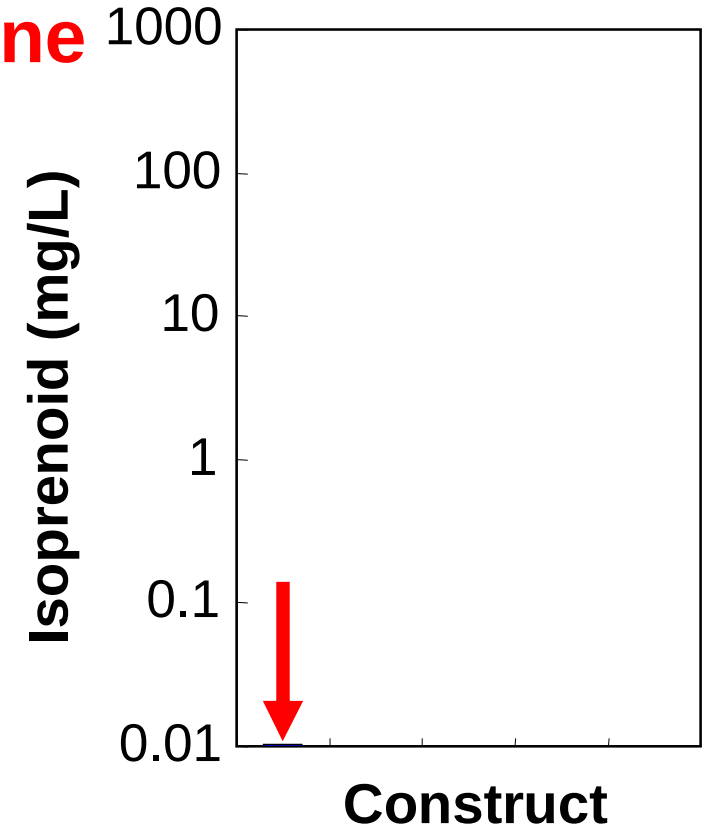
Production of a model isoprenoid in *E. coli*



Very low production of isoprenoid resulted when using the native gene



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atcttgtgat catcccaaga caaaaccaga gaaaaagacc tgtctgtttt tttaagaagt
ctttatatta tttttttgt cggagaatct tataagcatg gcttcaggag gatcaaagtc
ggcagctttc atgcttctga tgctgaatct tggtctctat ttcgtcatca ccatcatcgc
ttcttgggct gttaatcacg gcatcgagag aactcgcgag tctggtaact aacaaagata
acaactgatt aagtaacaat taatccaacg ttagaaaatg tcatcatcaa tcttcttttt
gtggtatttt gcagcgtcga cactgtcact tccggcgaag atattcccga tatacttccc
ggtggggaac atggcgaccg gttttttcgt aatattcacg ttaatcgccg gcgtcgtcgg

Making a plant gene look like a microbial gene

atcttgtgat catcccaaga caaaaccaga gaaaaagacc tgtctgtttt tttaagaagt
ctttatatta tttttttgt cggagaatct tataagcatg gcttcaggag gatcaaagtc
ggcagctttc atgcttctga tgctgaatct tggtctctat ttcgtcatca ccatcatcgc
ttcttgggct gttaatcacg gcatcgagag aactcgcgag tctggtaact aacaaagata
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gtggtatttt gcagcgtcga cactgtcact tccggcgaag atattcccga tatacttccc
ggtggggaac atggcgaccg gttttttcgt aatattcacg ttaatcgccg gcgtcgtcgg

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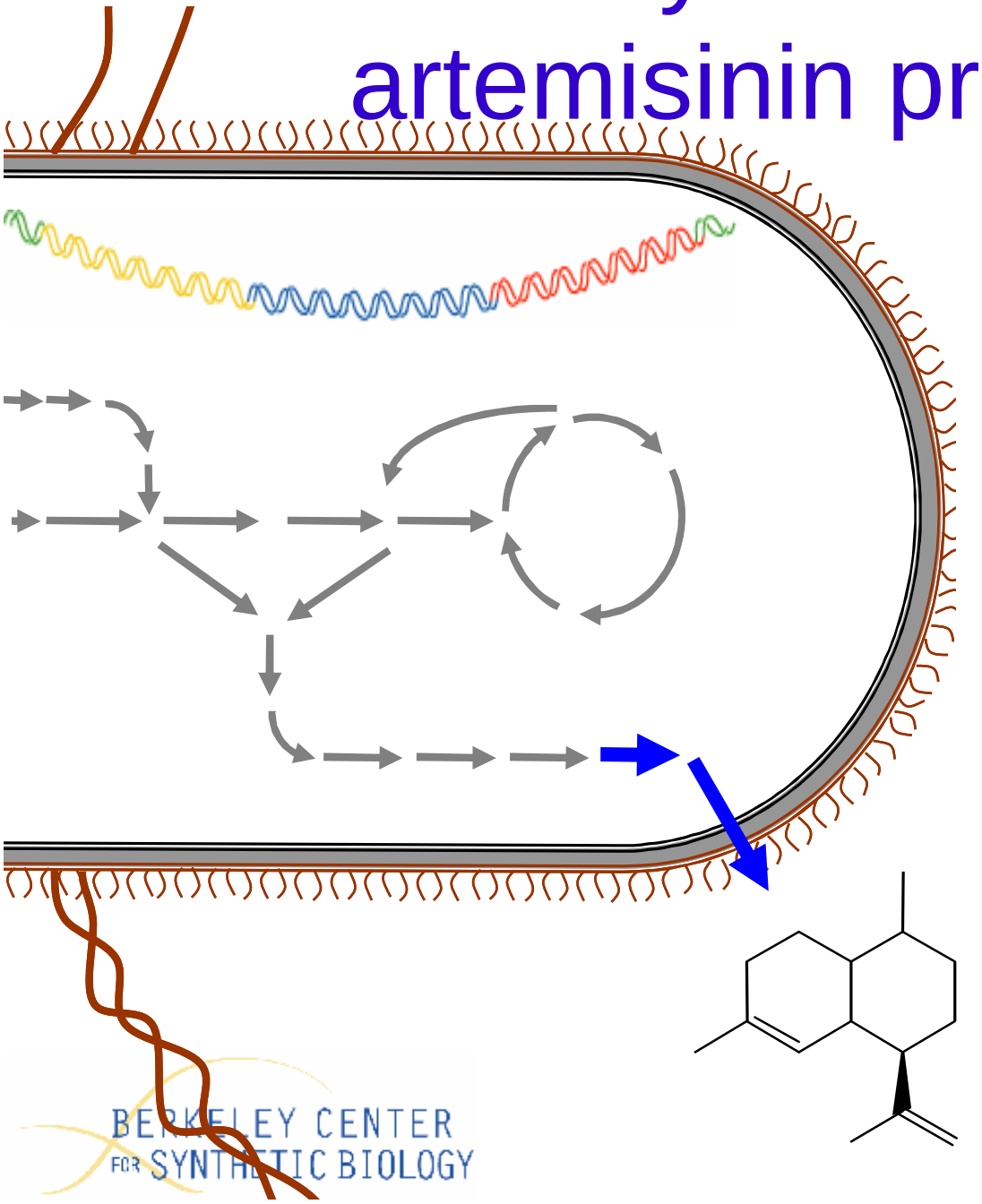
Gene resynthesis improves artemisinin production



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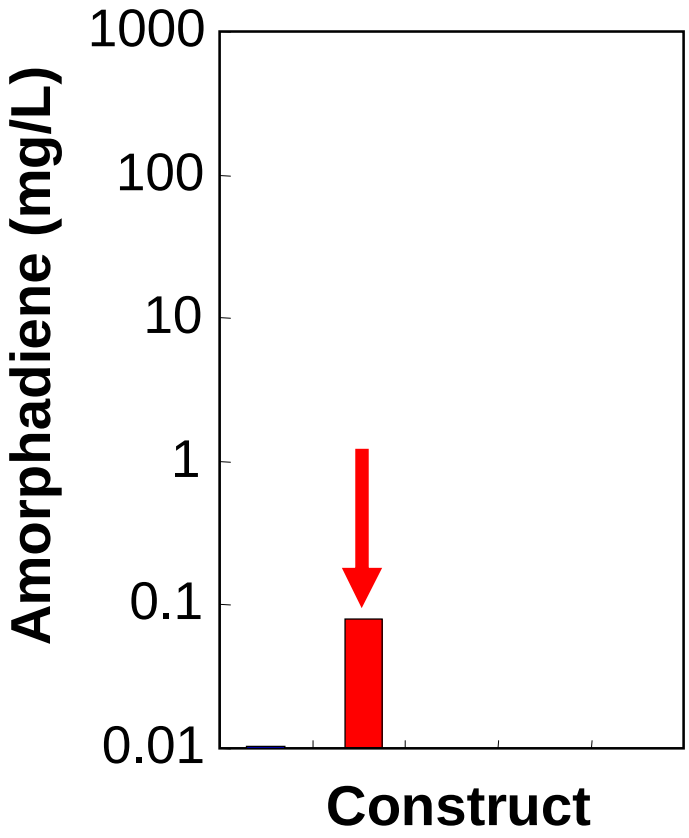
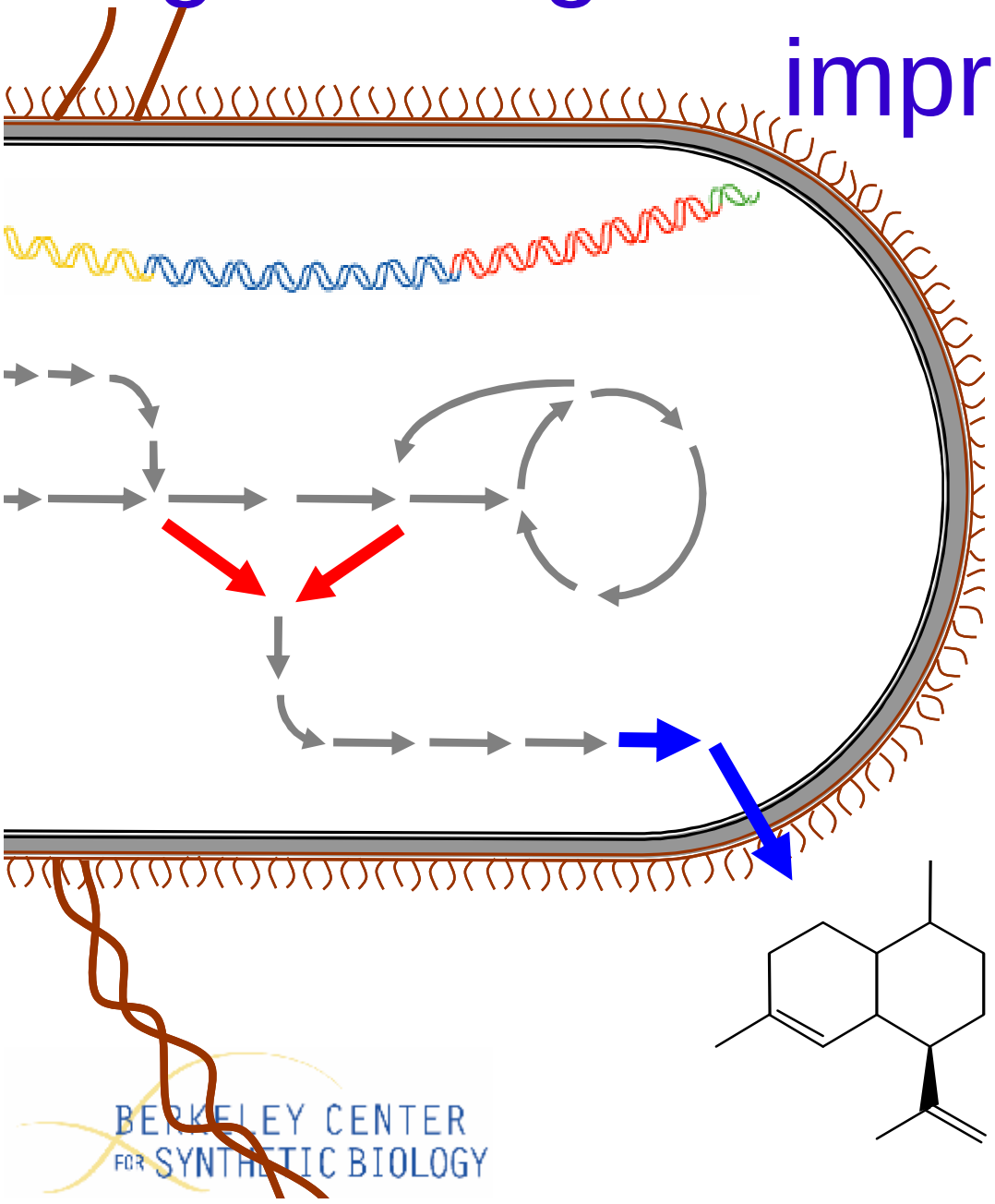
142-fold improved production!



Engineering *E. coli's* native pathway improves production



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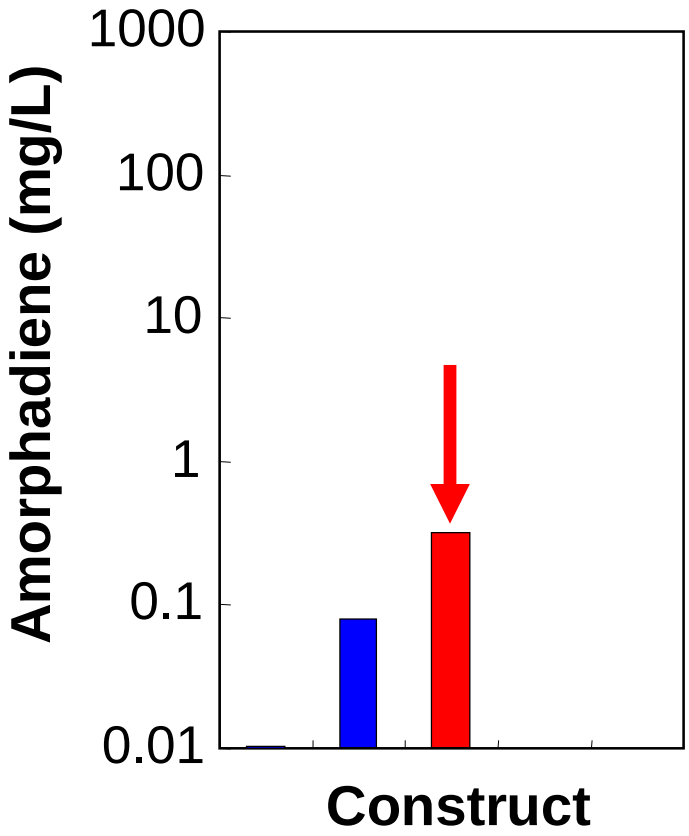
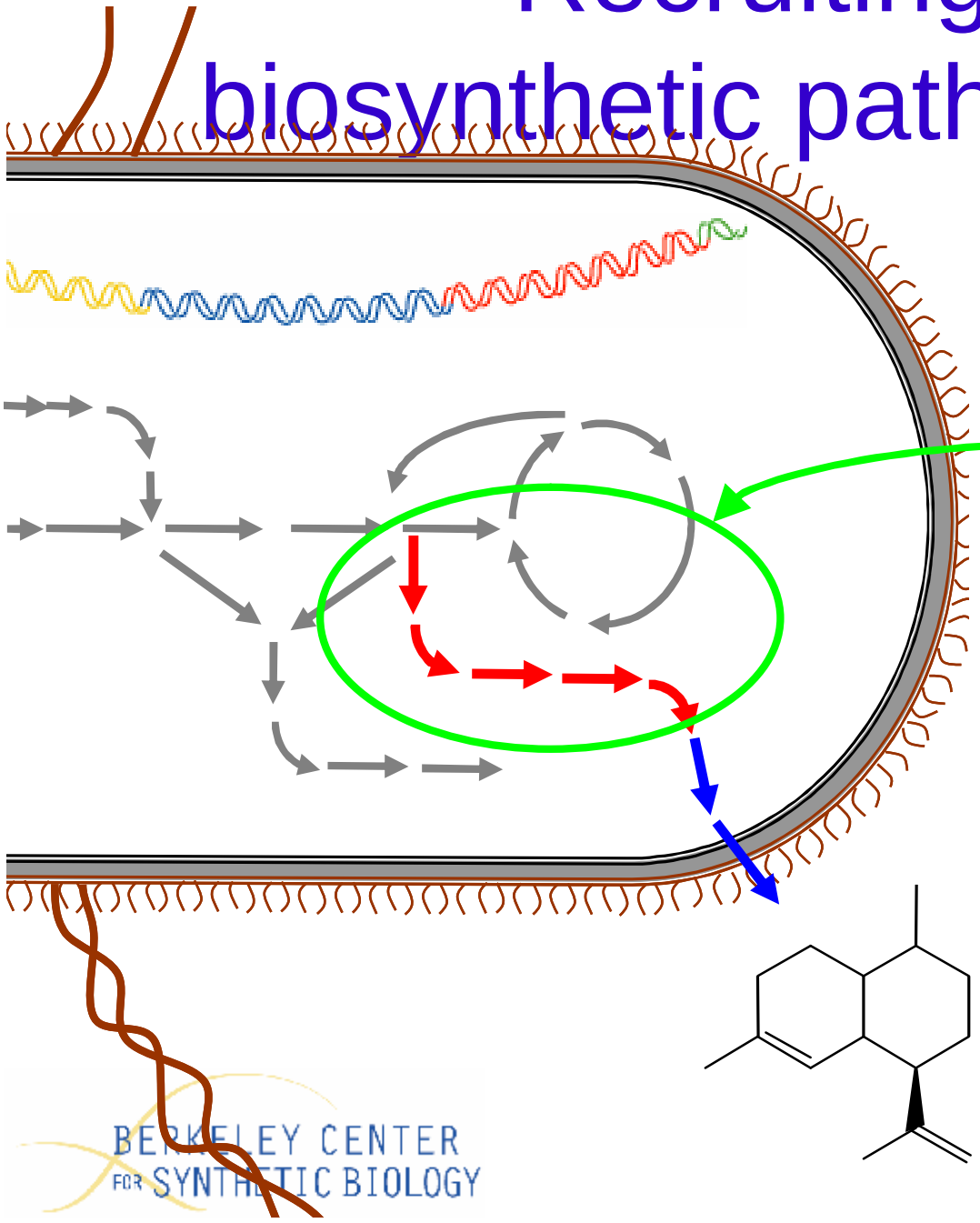
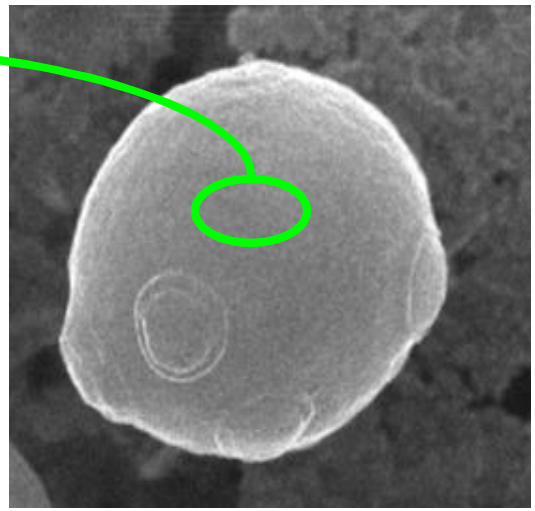
Three-fold improvement in production



Recruiting the cholesterol biosynthetic pathway from yeast





Yeast

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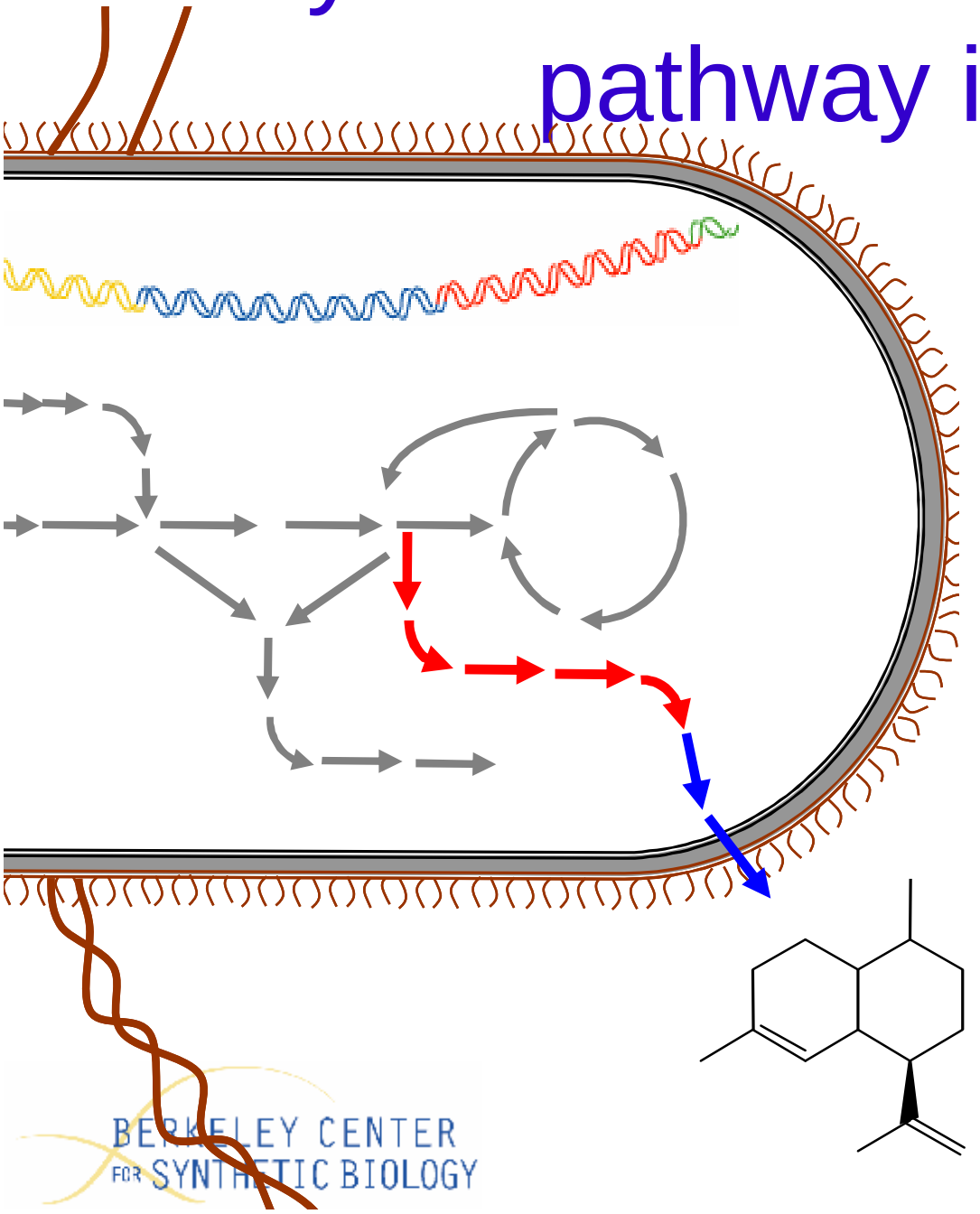
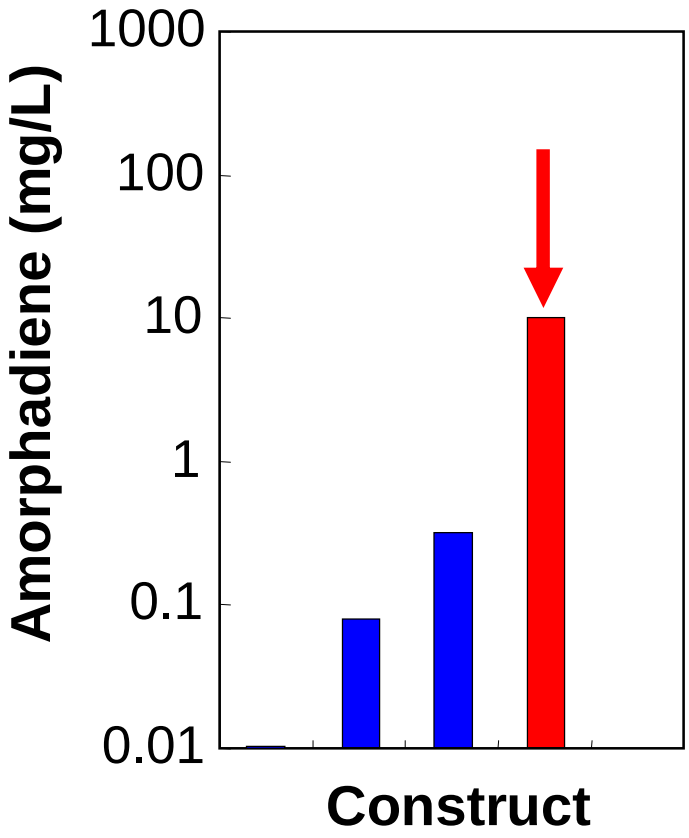
The yeast cholesterol biosynthetic pathway improves yields



~90-fold improvement in production



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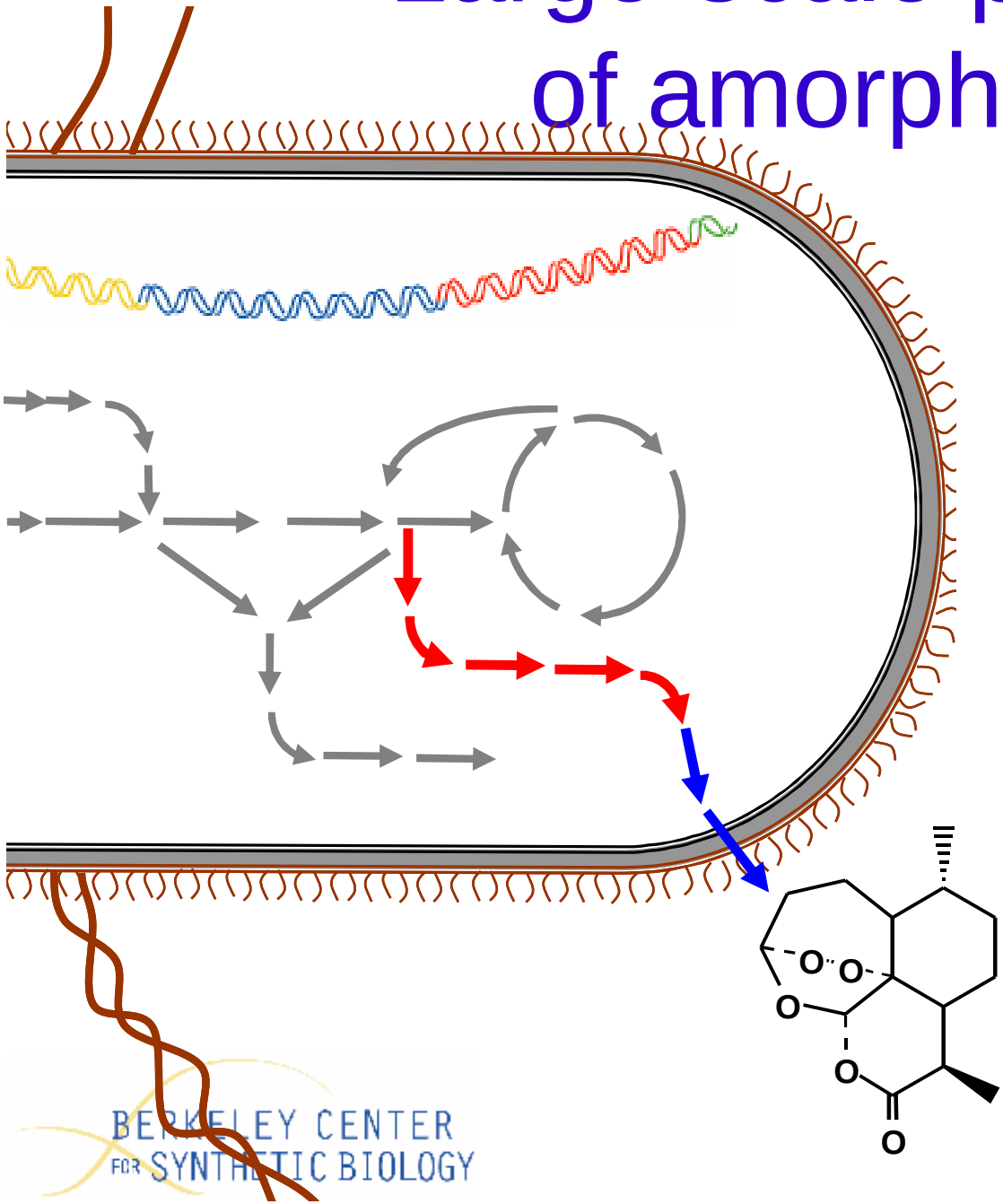
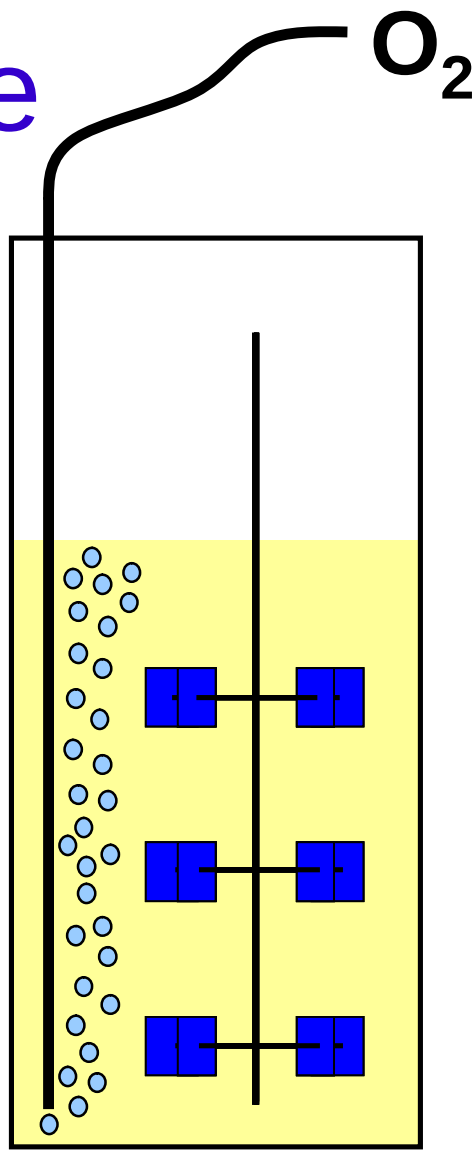
Large-scale production of amorphadiene





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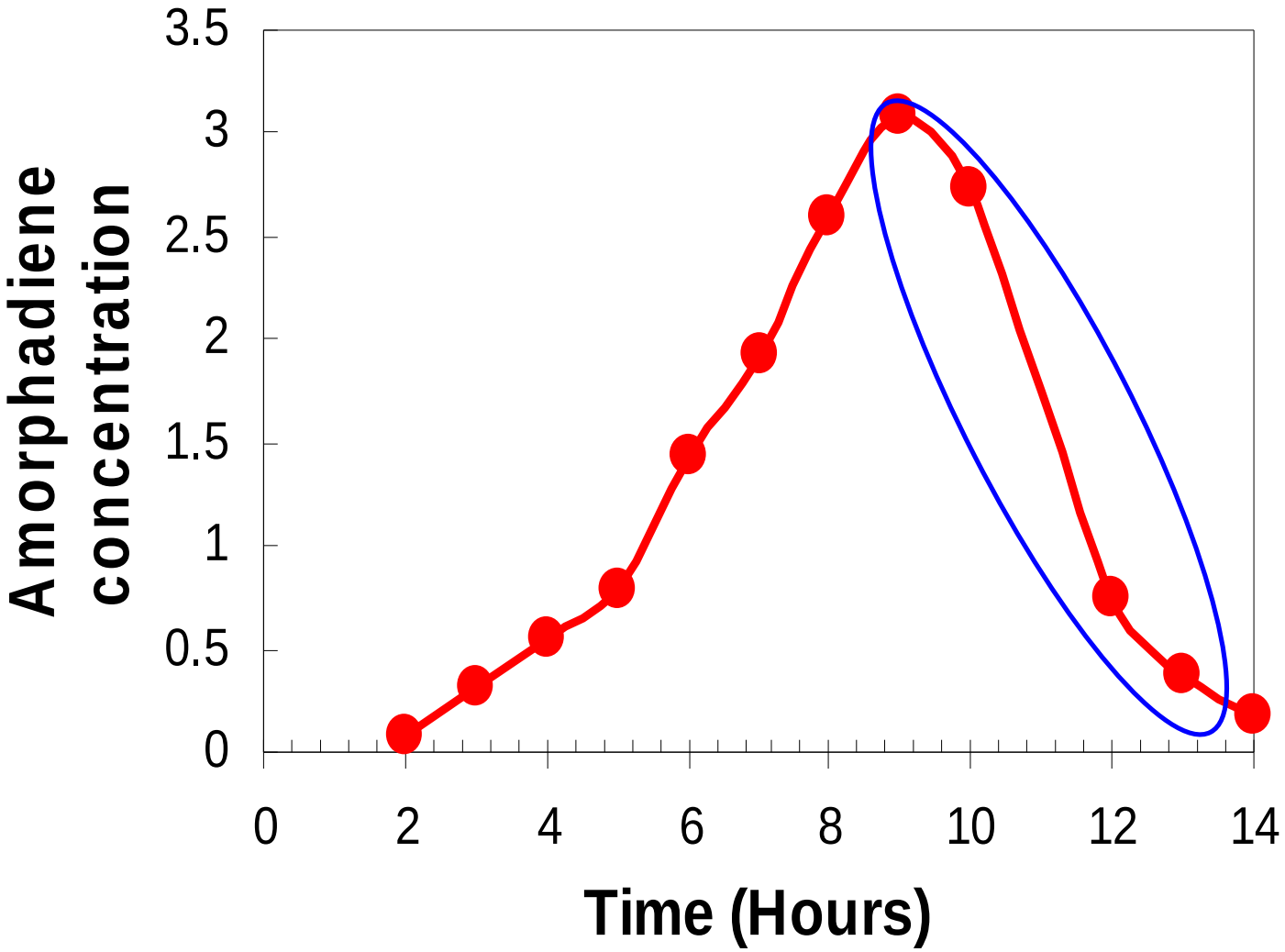
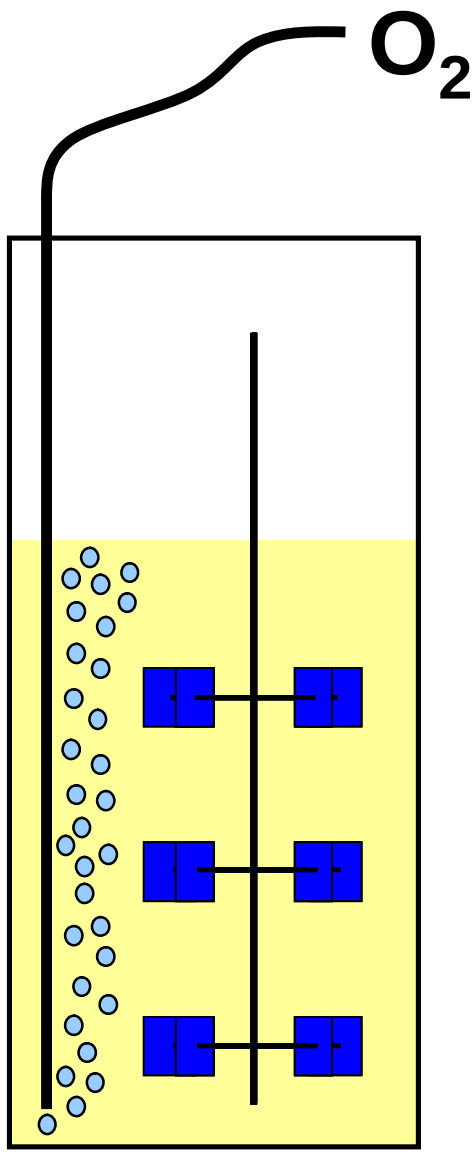
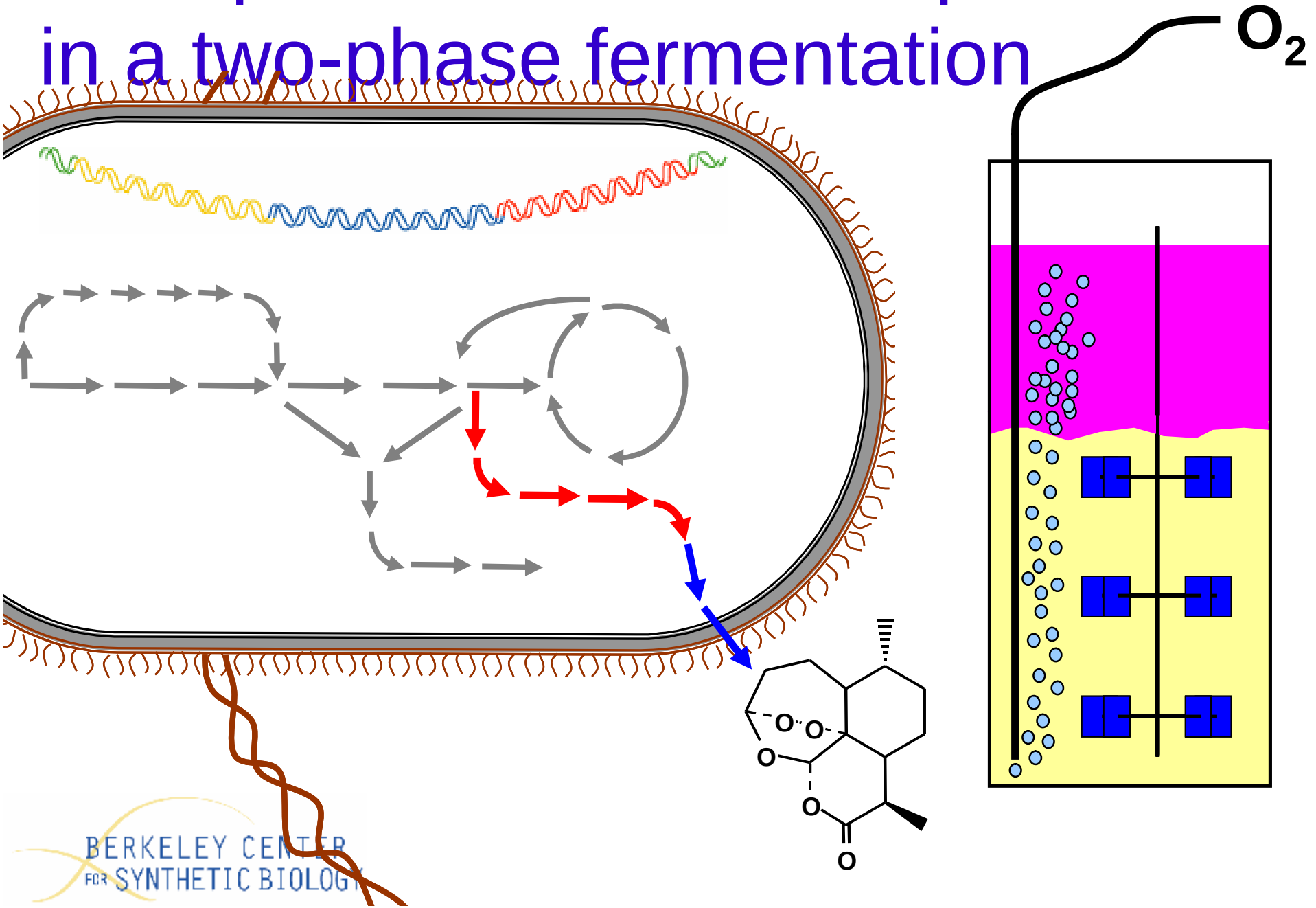
Amorphadiene is lost in large-scale production



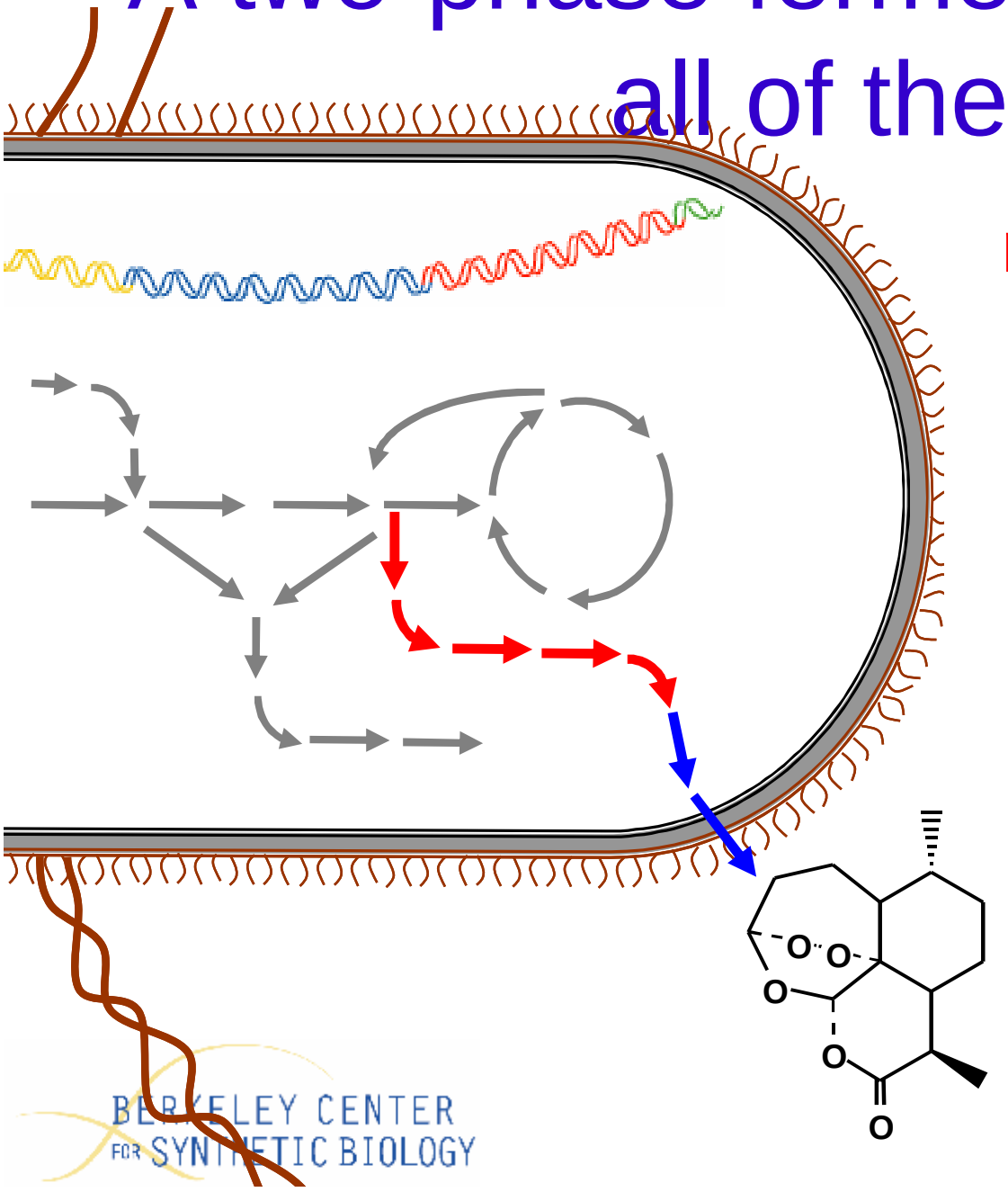


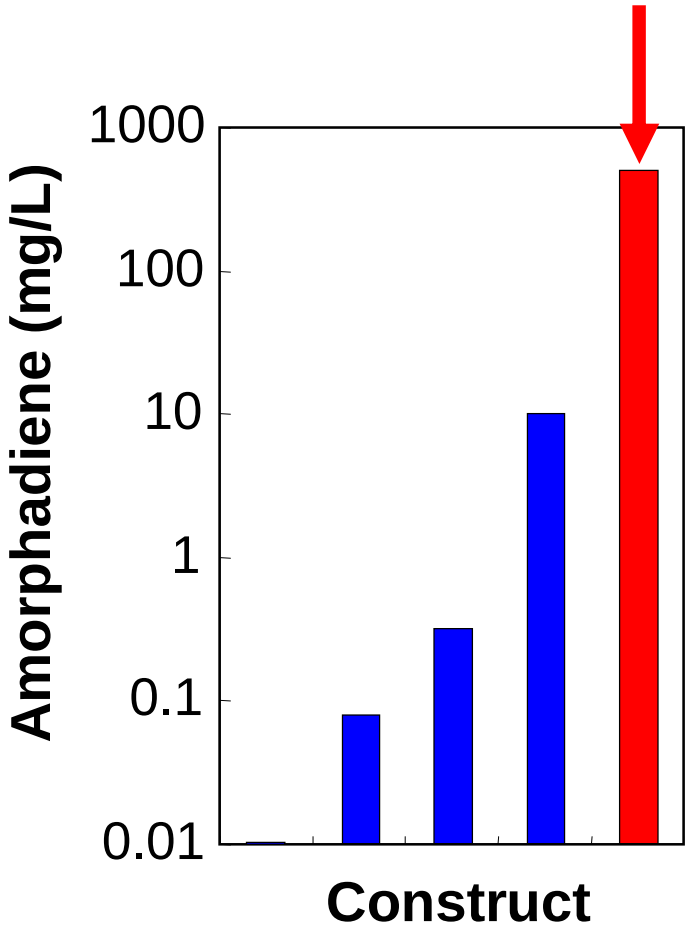
Amorphadiene can be captured in a two-phase fermentation



A two-phase fermentation collects all of the amorphadiene

… and improved production another 50 fold.





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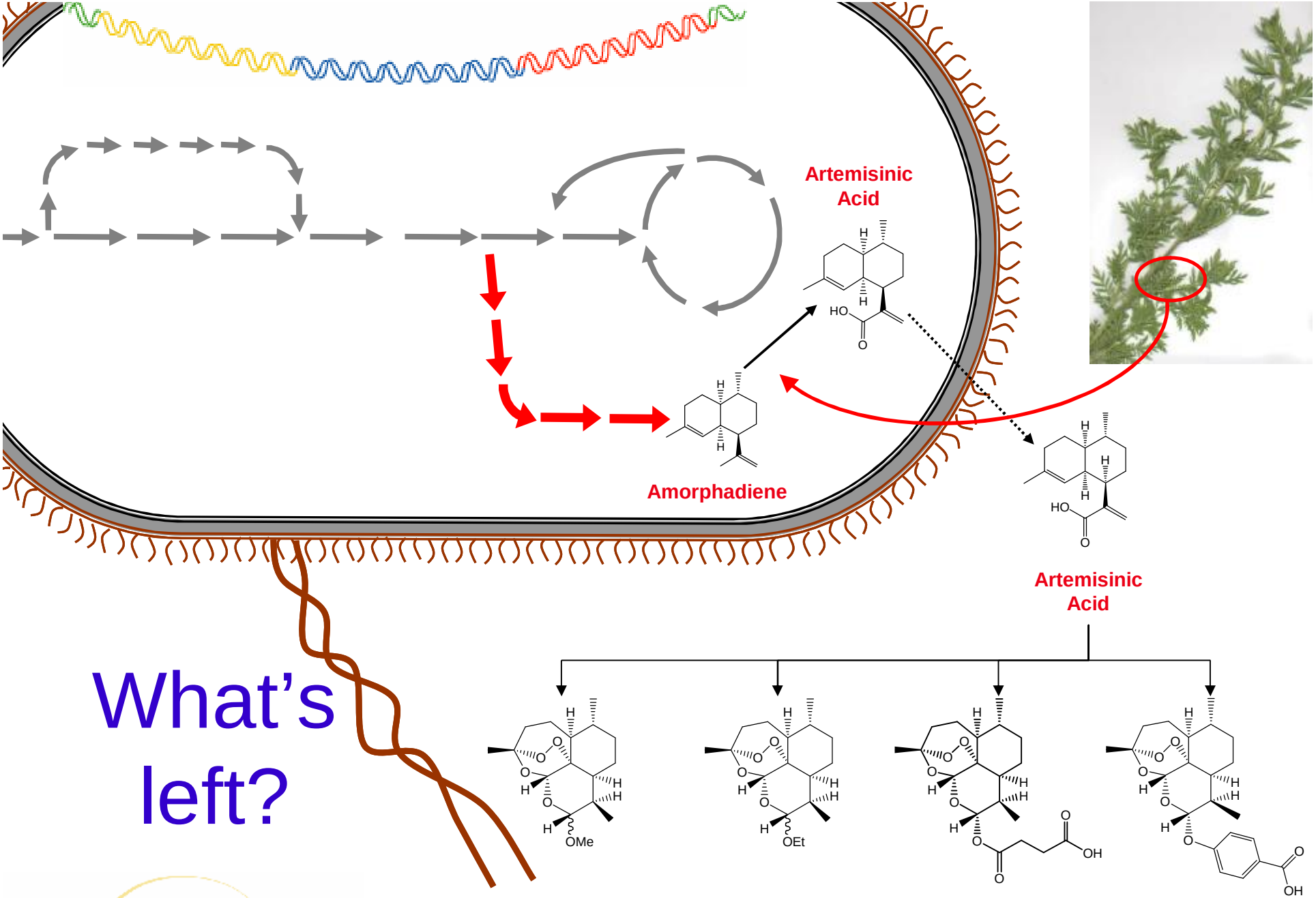
Artemisinic Acid
Amorphadiene
Artemisinic Acid
What's left?
Artemether
Arteether
Artesunate (Artesunic Acid)
Artelinate (Artelinic Acid)
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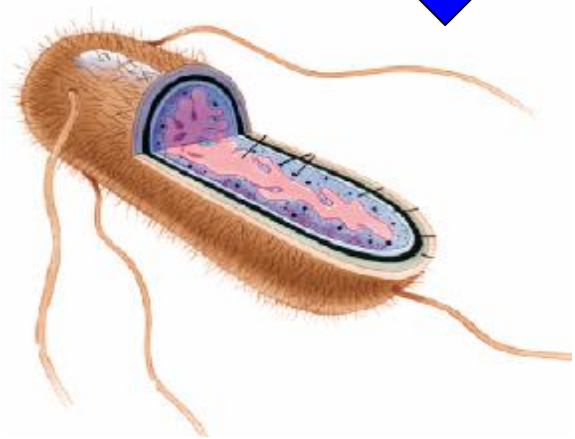
Research, Development & Delivery



BCSB
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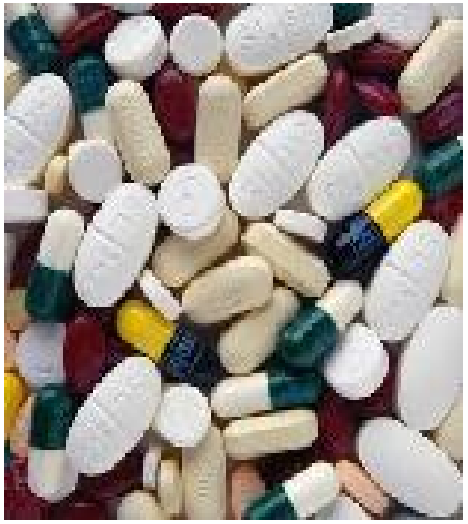
Amyris Biotechnologies



Institute for OneWorld Health

Intellectual property agreements

- The University of California, Berkeley granted Amyris Biotechnologies and OneWorld Health royalty-free exclusive licences to the necessary technology to produce artemisinin.
- The University of California, Berkeley granted Amyris and OWH up-front licenses to any technology developed for artemisinin production.

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Artemisinin costs

	Artesunate combination treatment
Current cost of drug	**$2.25-2.50**
Cost with new process	**$.21/.12**

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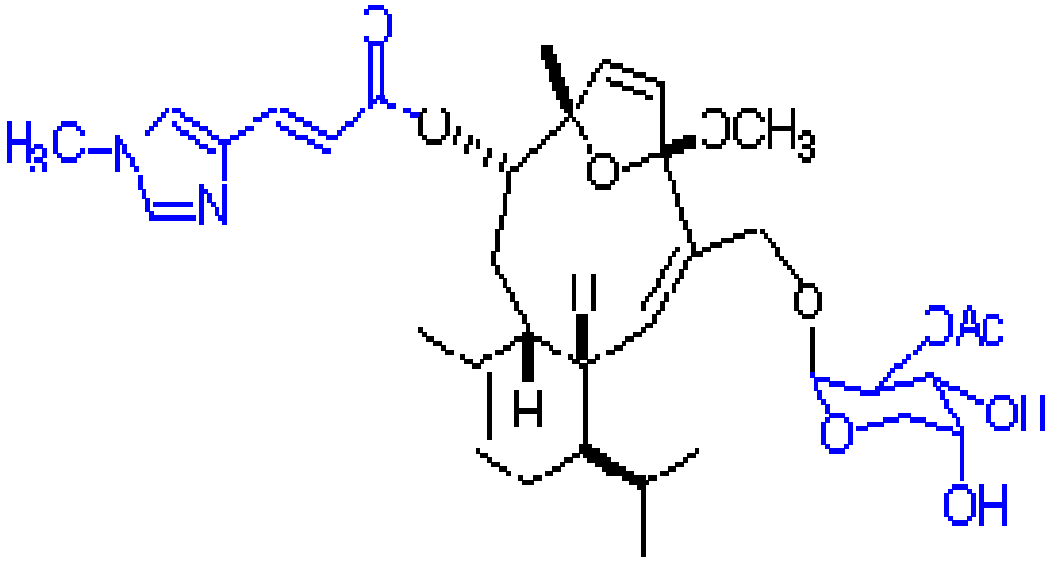
Eleutherobin: A potent anti-cancer drug

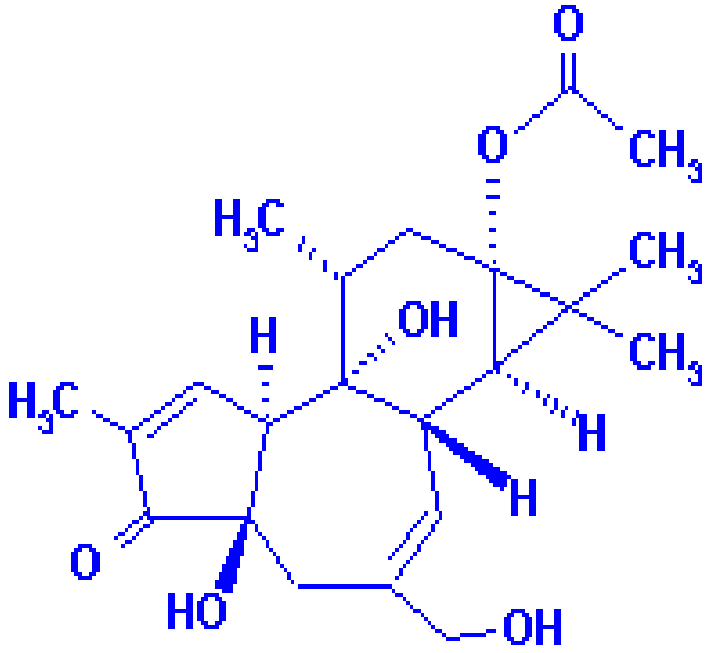


FOR SYNTHETIC BIOLOGY

Prostratin: an HIV therapy?

- Effective against hepatitis virus
- Isolated from the stems of the small Samoan tree *Homalanthus nutans*

- Inhibits human immunodeficiency virus type 1 (HIV-1) infection
- Up-regulates viral expression from latent proviruses



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UCB gets exclusive rights to genes

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Samoa and UCB share royalties

University of California, Berkeley
Vice Chancellor for Research
California Hall
Berkeley, CA 94720

Memorandum of Understanding between the Government of Samoa and the Regents of the University of California, Berkeley for Disposition of Future Revenue from Licensing of Prostratin Gene Sequences, an Anti-Viral Molecule

I. Preamble

This Memorandum of Understanding, effective as of the date of signing, is undertaken by the government of Samoa ("Samoa"), a sovereign nation, and The Regents of the University of California, Berkeley acting through its Office of Technology Licensing at the University of California, Berkeley at 2150 Shattuck Ave., Suite 510, Berkeley, CA 94720-1620 ("UC Berkeley").

Signed and agreed to the 13th day of August, 2004.

For the Government of Samoa

Its Prime Minister, Aiono Tuilaepa Sailele Malielegaoi

Date 12/8/04

For the Regents of the University of California, Berkeley

Its Vice Chancellor for Research, Beth Burnside, Ph.D.,

Date 8/17/04

Summary

- Synthetic biology offers a way to reduce complexity in biological systems
- A synthetic biology approach is important for building large, complex networks to produce chemicals
- Synthetic biology can be used to reduce the costs of drugs for the Developing World.

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