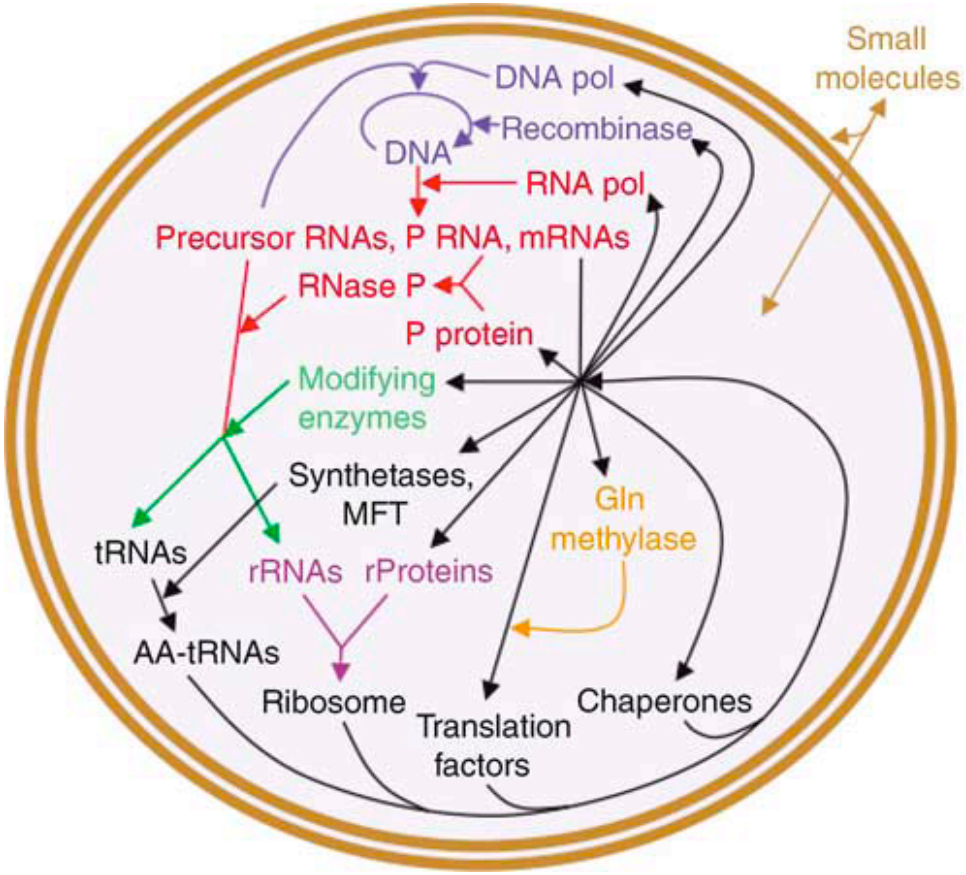
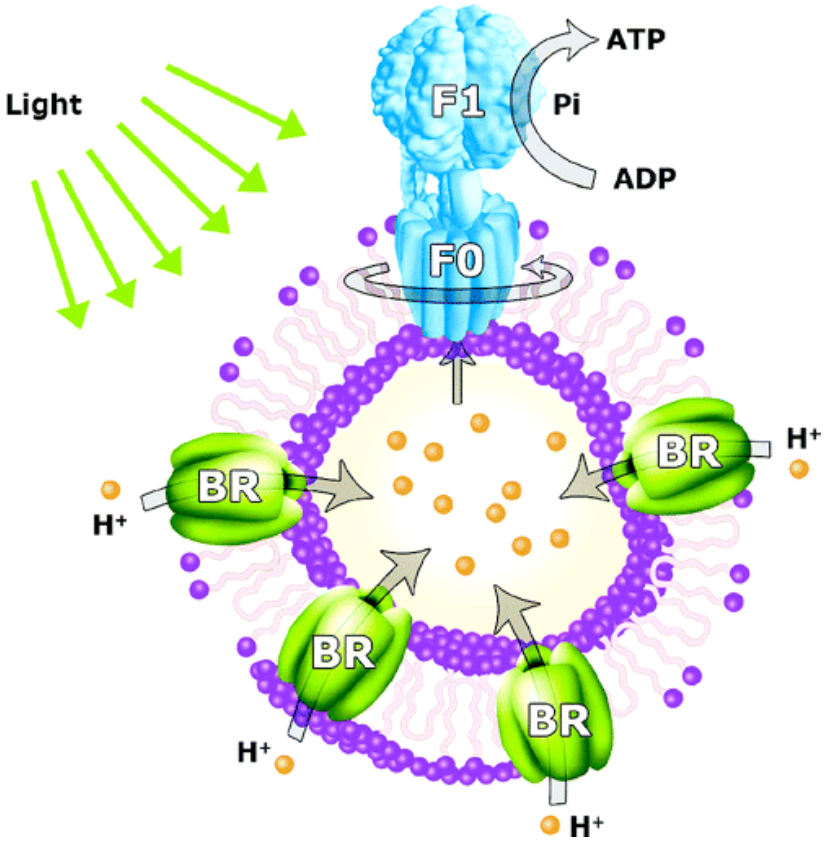
Lecture 25: Synthetic life



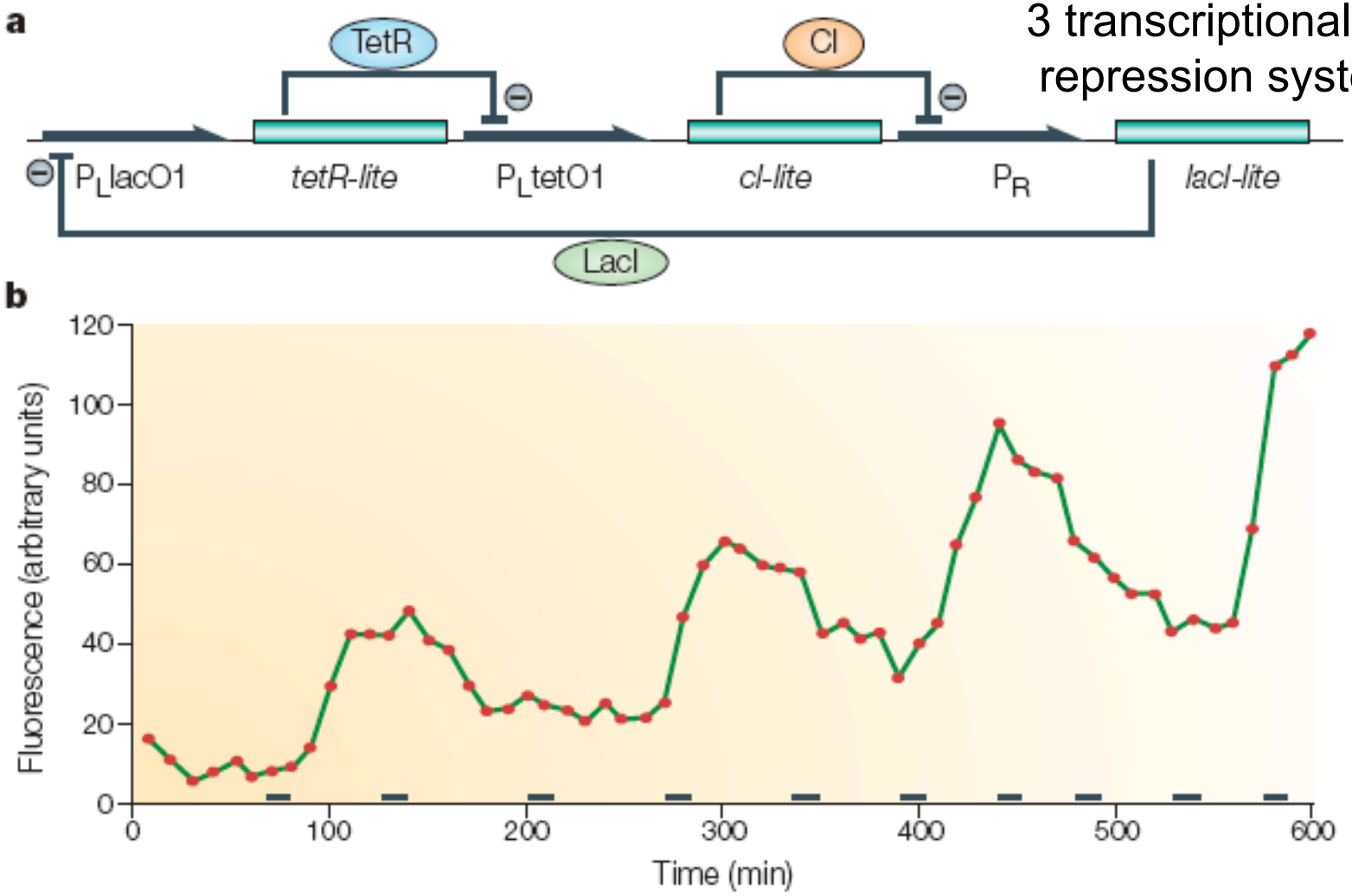


Synthetic biology highlights

- Minimal cell
- Synthetic cell
- Artificial cell (biomemetic chemistry)
- Death

Engineering genetic circuits

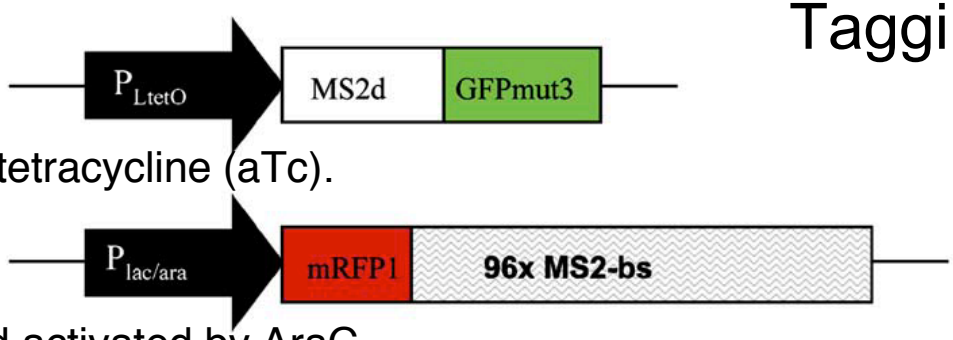
3 transcriptional repression systems



Repressilator: light oscillates as generations pass…

Gene transcription at single cell level

A



Tagging protein

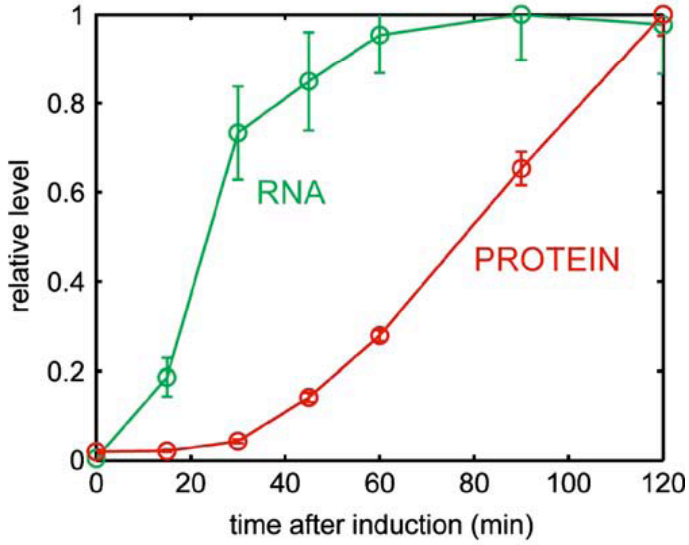
inducible by anhydrotetracycline (aTc).

Target mRNA

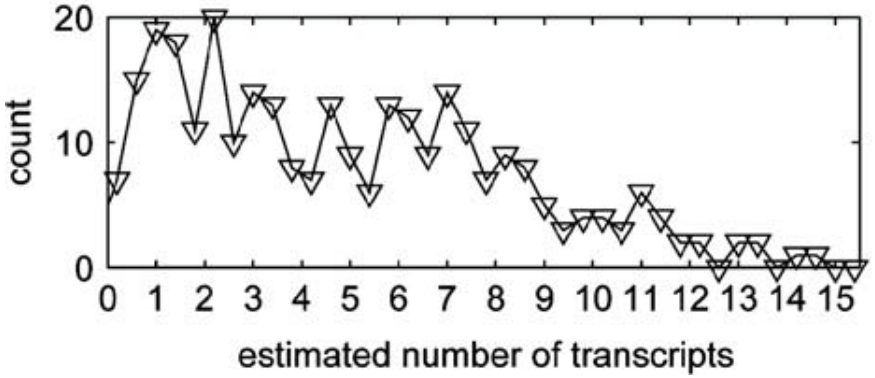
repressed by LacI and activated by AraC

B

C

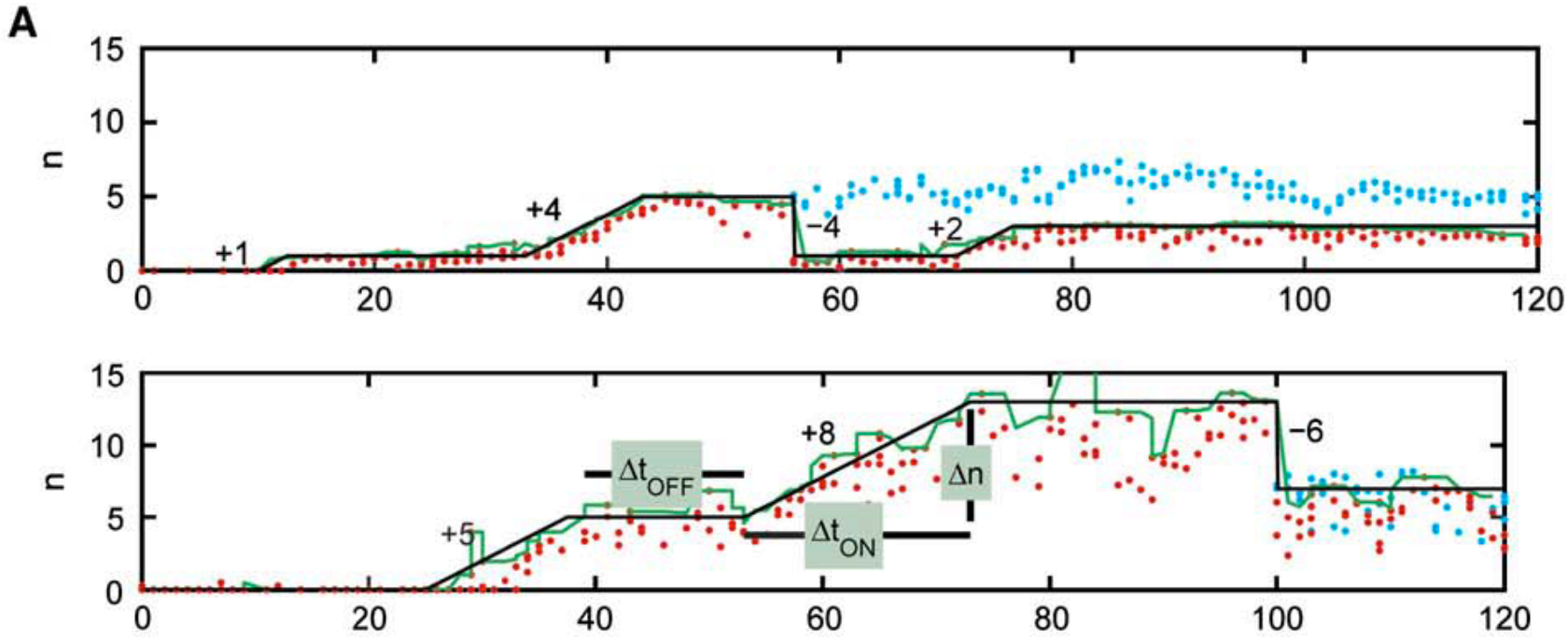


Single mRNA production



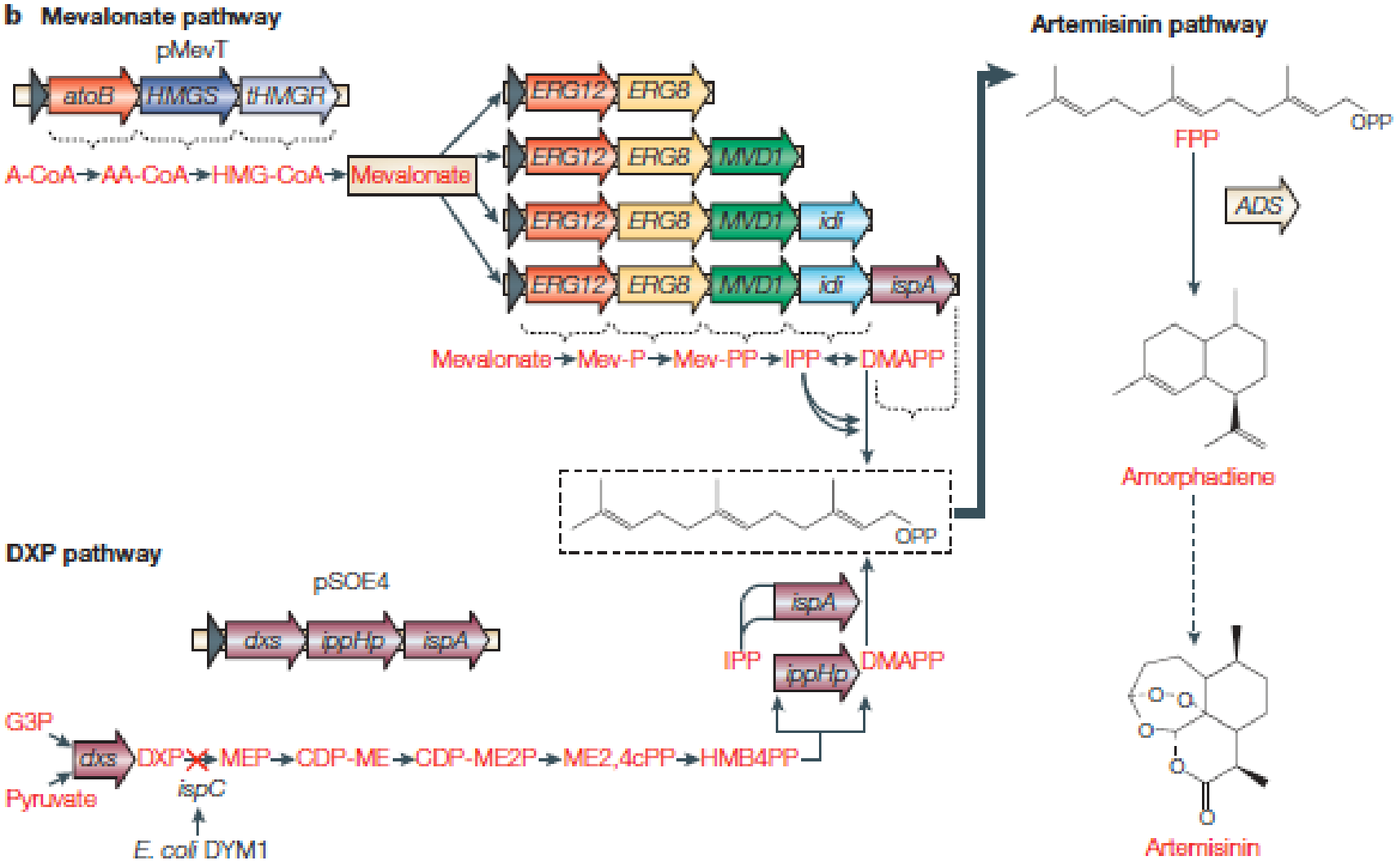
Variation of mRNA number across cells

Cell 123:1025 (I. Golding)

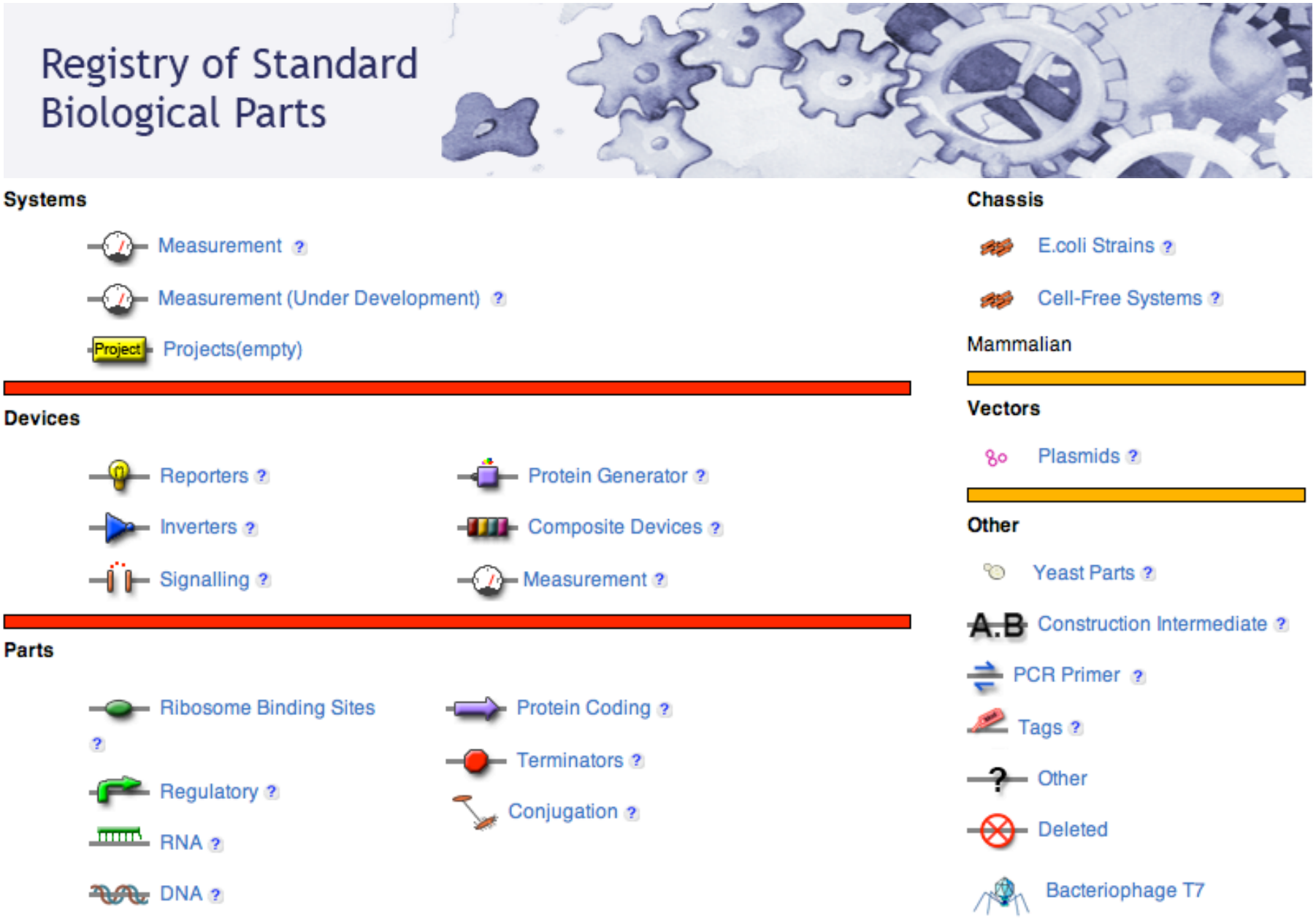


Transcription occurs in bursts!

Producing antimalarial drug with engineered bacteria



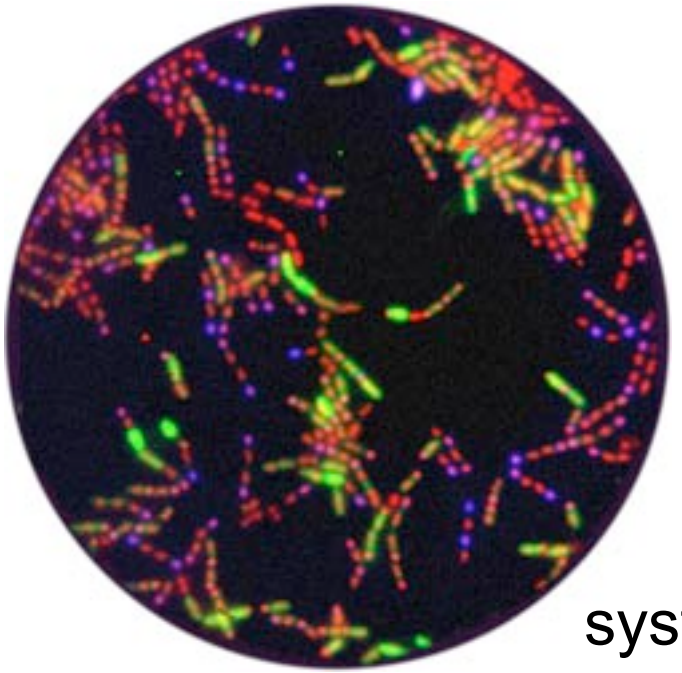
Nature Review Genetics 6:539



Library of standard DNA parts that encode basic biological functions (BioBricks)

http://parts.mit.edu/registry/index.php/Main_Page

Indispensable and essential genes



Bacillus subtilis: total 4,100 genes

192 are indispensable
Another 79 are essential

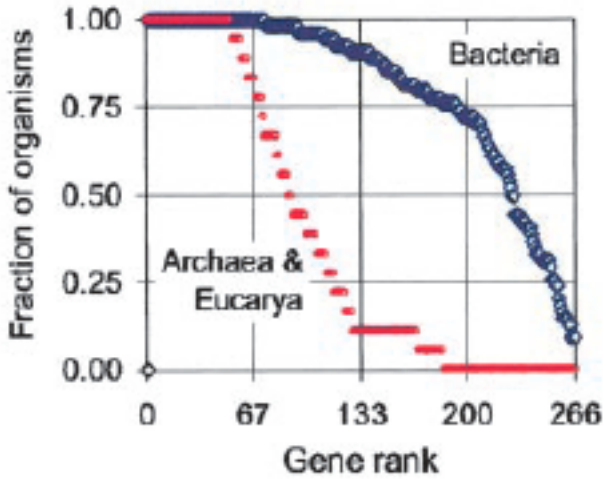
Grew thousands of B. subtilis cells and systematically inactivated one gene per cell

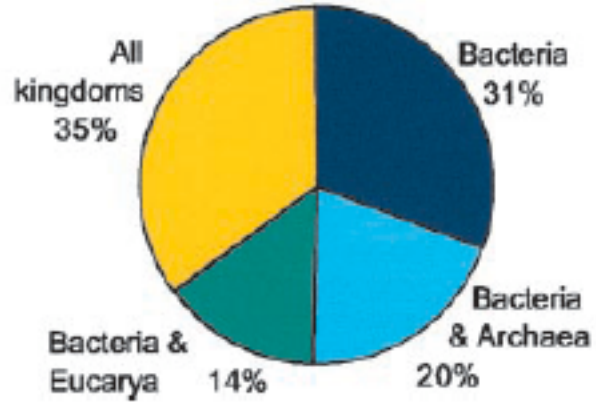
What is the smallest set of genes an organism needs to live in a particular Environment?

PNAS 100(8):4678 (2003)

Table 2. *B. subtilis* essential genes

DNA metabolism	27
Basic replication machinery	16
Packaging and segregation	9
Methylation	2
RNA metabolism	14
Basic transcription machinery	4
RNA modification	6
Regulation	4
Protein synthesis	95
Ribosomal proteins	52
Aminoacyl-tRNA synthetases	24
Translation factors	10
Protein folding and modification	3
Protein translocation	6
Cell envelope	44
Membrane lipids	16
Cell wall	28
Cell shape and division	10
Glycolysis	8
Respiratory pathways	22
Isoprenoids	8
Menaquinone	8
Cytochrome biogenesis	3
Thioredoxin	3
Nucleotides	10
Cofactors	15
CoA	1
Folate	3
NAD	4
S-Adenosylmethionine	1
Iron–sulfur cluster	6
Other	15
Unknown	11
Total	271

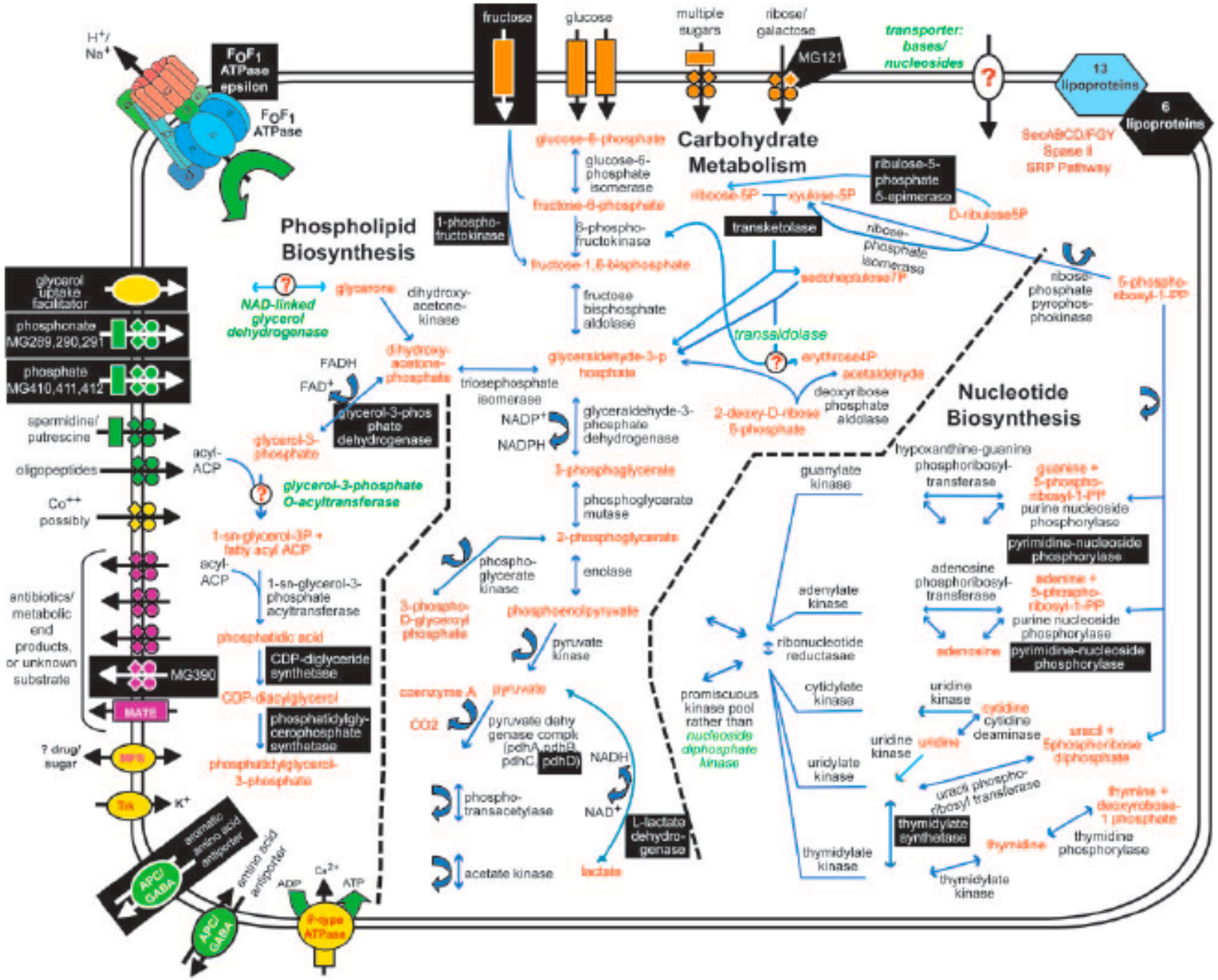




Minimal bacterial genome

- Patented (2007). Only 381 genes.
- The particular set of gene required to sustain free-living cell *(Mycoplasma laboratorium.)*
- 23% of genes of unknown function are essential
- Use: design bacteria for hydrogen gas production, conversion of cellulose into ethanol, or maybe straight 87 gasoline

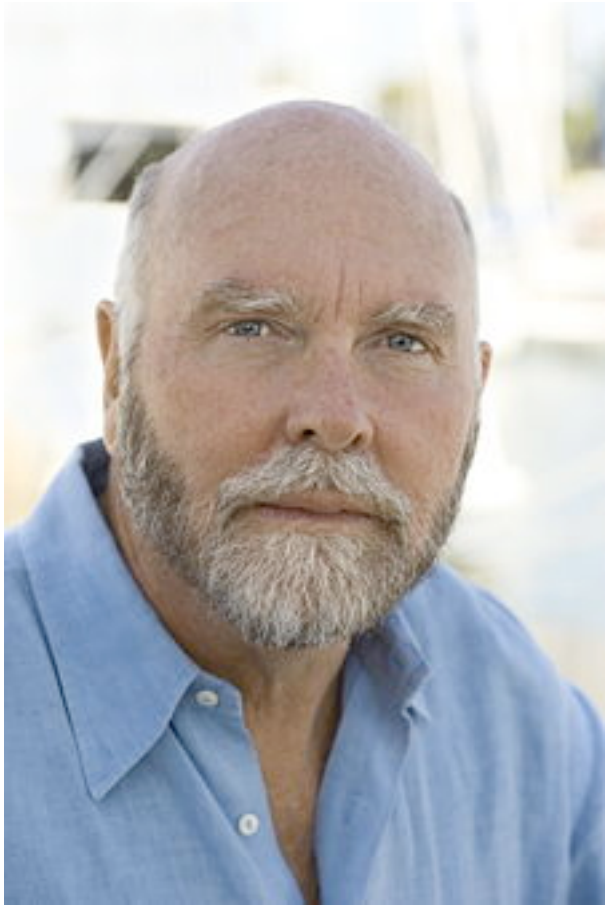
PNAS 103:425 (2005)


H+/ Na+
FoF1 ATPase epsilon
FoF1 ATPase
fructose
glucose
multiple sugars
ribose/ galactose
MG121
transporter: bases/ nucleosides
13 lipoproteins
6 lipoproteins
SecABCD/FGY Spase II SRP Pathway
Carbohydrate Metabolism
glucose-6-phosphate
glucose-6-phosphate isomerase
fructose-6-phosphate
1-phospho-fructokinase
6-phospho-fructokinase
fructose-1,6-bisphosphate
fructose bisphosphate aldolase
ribulose-5-phosphate 6-epimerase
ribose-5P
xylulose-5P
transketolase
D-ribulose5P
ribose-phosphate isomerase
sedoheptulose7P
ribose-phosphate pyrophos-phokinase
5-phospho-ribosyl-1-PP
transaldolase
erythrose4P
acetaldehyde
deoxyribose phosphate aldolase
2-deoxy-D-ribose 5-phosphate
glyceraldehyde-3-phosphate dehydrogenase
NADP+
NADPH
3-phosphoglycerate
phosphoglycerate mutase
2-phosphoglycerate
enolase
phosphoenolpyruvate
pyruvate kinase
pyruvate
coenzyme A
CO2
pyruvate dehy genase compk (pdhA,pdhB, pdhC pdhD)
NADH
NAD+
L-lactate dehydro-genase
lactate
phospho-transacetylase
acetate kinase
3-phospho-D-glycerol phosphate
phospho-glycerate kinase
Phospholipid Biosynthesis
NAD-linked glycerol dehydrogenase
glycerone
dihydroxy-acetone-kinase
dihydroxy-acetone-phosphate
triosephosphate isomerase
FADH
FAD+
glycerol-3-phosphate dehydrogenase
glycerol-3-phosphate
acyl-ACP
glycerol-3-phosphate O-acyltransferase
1-sn-glycerol-3P + fatty acyl ACP
acyl-ACP
1-sn-glycerol-3-phosphate acyltransferase
phosphatidic acid
CDP-diglyceride synthetase
CDP-diacylglycerol
phosphatidylglycero-phosphate synthetase
phosphatidylglycerol-3-phosphate
glycerol uptake facilitator
phosphonate MG289,290,291
phosphate MG410,411,412
spermidine/ putrescine
oligopeptides
Co++ possibly
antibiotics/ metabolic end products, or unknown substrate
MG390
MATE
? drug/ sugar
MFS
Trk
K+
aromatic amino acid antiporter
APC/ GABA
amino acid antiporter
APC/ GABA
ADP
Ca2+
ATP
P-type ATPase
Nucleotide Biosynthesis
guanylate kinase
adenylate kinase
ribonucleotide reductasae
cytidylate kinase
uridylate kinase
thymidylate kinase
promiscuous kinase pool rather than nucleoside diphosphate kinase
hypoxanthine-guanine phosphoribosyl-transferase
guanine + 5-phospho-ribosyl-1-PP
purine nucleoside phosphorylase
pyrimidine-nucleoside phosphorylase
adenosine phosphoribosyl-transferase
adenine + 5-phospho-ribosyl-1-PP
purine nucleoside phosphorylase
adenosine
pyrimidine-nucleoside phosphorylase
uridine kinase
cytidine
cytidine deaminase
uridine kinase
uridine
uracil + 5phosphoribose diphosphate
uracil phospho-ribosyl transferase
thymidylate synthetase
thymine + deoxyribose-1 phosphate
thymidine
thymidine phosphorylase
thymidylate kinase

Craig Venter's synthetic bacteria

1. Synthesize the genome you like
   (done) *Science* 319:1215
   6000 bases + enzymes + yeast

2. Replace natural genome with
   a synthetic one (entire genome)
   Science 317:632



3. Boot up (not done yet)

Synthetic minimal cell

Or how far can we push self-assembly?

Ribosomes, viruses can be assemble *in vitro*, but not cells!

Cell: self-replication, membrane-encapsulated collection of biomolecules

George Church

“Life cannot be understood by studying its parts, it should be put together from its parts”

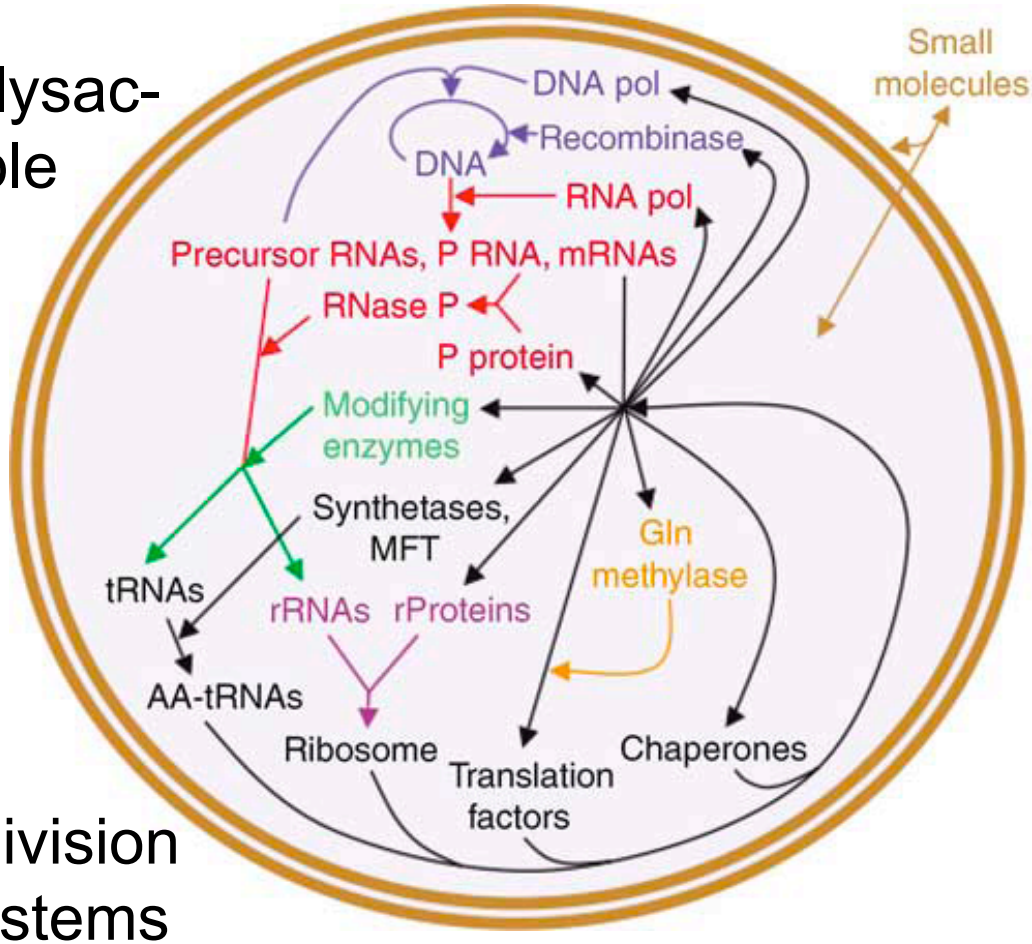
The simplest cell would be very complex. Let’s substitute complex subsystems with simplified (synthetic) analogs

Molecular Systems Biology (2006)

Approach

"Building machine from mysteriois parts will only create a mysterious machine"

Membrane proteins and polysac-charides may be dispensable



Subsystems:

- Genome replication
- Transciption
- RNA processing
- Translation
- Post-translation
- Compartments and division
- Integrating the subsystems

Minimum self-replicating system

Escherichia coli	Mycoplasma	3D structure
Coliphage f29 DNA polymerase	+	+
Coliphage P1 Cre recombinase	–	+
>Coliphage Lox/Cre recombinase site	–	+
Coliphage T7 RNA polymerase	Analog	+
>Coliphage T7 RNA polymerase initiation site	Analog	+
>Coliphage T7 RNA polymerase class II termination site	Analog	+
Lucerne viral hammerhead RNA	–	+
RNase P RNA	+	+
RNase P protein	+	+
>RNase P site/RNA primer for DNA polymerase	+	+
Small subunit 16S ribosomal RNA	+	+
All 21 small subunit ribosomal proteins (1–21)	+ except 1, 21	+
Large subunit 5S ribosomal RNA	+	+
Large subunit 23S ribosomal RNA	+	+
Large subunit 23S rRNA G2445 > m2G methylase: unidentified	**Unknown**	–
Large subunit 23S rRNA U2449 > dihydroU synthetase: unidentified	**Unknown**	–
Large subunit 23S rRNA U2457 > pseudoU synthetase	**Unknown**	–
Large subunit 23S rRNA C2498 > Cm methylase: unidentified	**Unknown**	–
Large subunit 23S rRNA A2503 > m2A methylase: unidentified	**Unknown**	–
Large subunit 23S rRNA U2504 > pseudoU synthetase	**Unknown**	–
All 33 large subunit ribosomal proteins (1–7, 9–11, 13–25, 27–36)	+ except 25, 30	+

Minimum self-replicating system

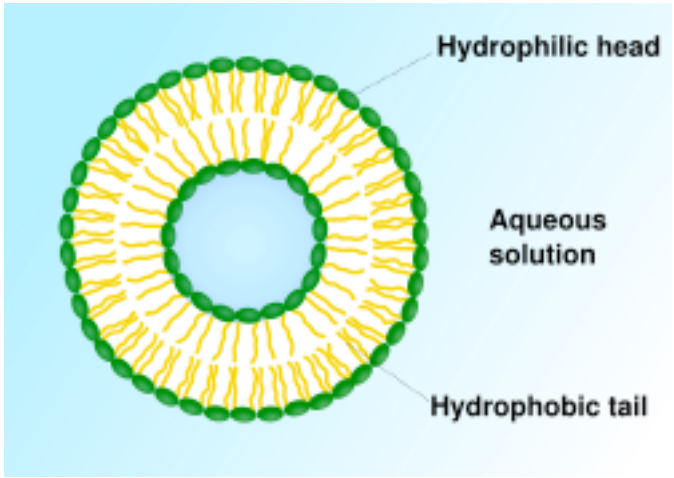
Translational initiation factor 1	+	+
Translational initiation factor 2	+	+
Translational initiation factor 3	+	+
Translational elongation factor Tu	+	+
Translational elongation factor Ts	+	+
Translational elongation factor G	+	+
Translational release factor 1	+	+
Translational release factor 2	–	+
Translational release factor Gln methylase	+	+
Translational release factor 3	–	+
Ribosome recycling factor	+	+
33/45 tRNAs (see Figure 3)	Set of 29	+
tRNA C34 > lysidine synthetase	**Unidentified**	+
tRNA A34 > I deaminase	**Unidentified**	+
tRNA U34 > cmo5U (=V) synthetases: unidentified	–	–
tRNA U34 > 2sU Cys desulfurase	–	+
tRNA U34 > 2sU synthetase	**Unidentified**	+
tRNA U34 > cmnm5U GTPase	**Unidentified**	+
tRNA U34 > cmnm5U synthetase	**Unidentified**	+
tRNA cmnm5U34 > nm5U > mnm5U synthetase	**Unidentified**	–
tRNA G37 N1-methylase	+	+
tRNA A37 > t6A N6-threonylcarbamoyl-A synthetase: unidentified	**Unidentified**	–
tRNA A37 > i6A synthetase	–	+
tRNA i6A37 > s2i6A > ms2i6A synthetase	–	+
All 22 aminoacyl-tRNA synthetase subunits (20 enzymes)	+ except Gly sub., Gln	+ except Gly sub., Ala
Met-tRNA formyltransferase	+	+
Chaperonin GroEL	+	+
Chaperonin GroES	+	+

151 genes=38 RNAs + 113 proteins

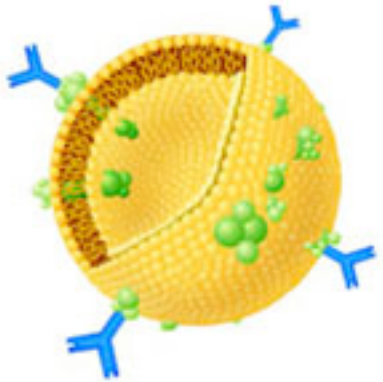
Synthetic biology highlights

- Minimal cell
- Synthetic cell
- Artificial cell (biomemetic chemistry)

Liposomes

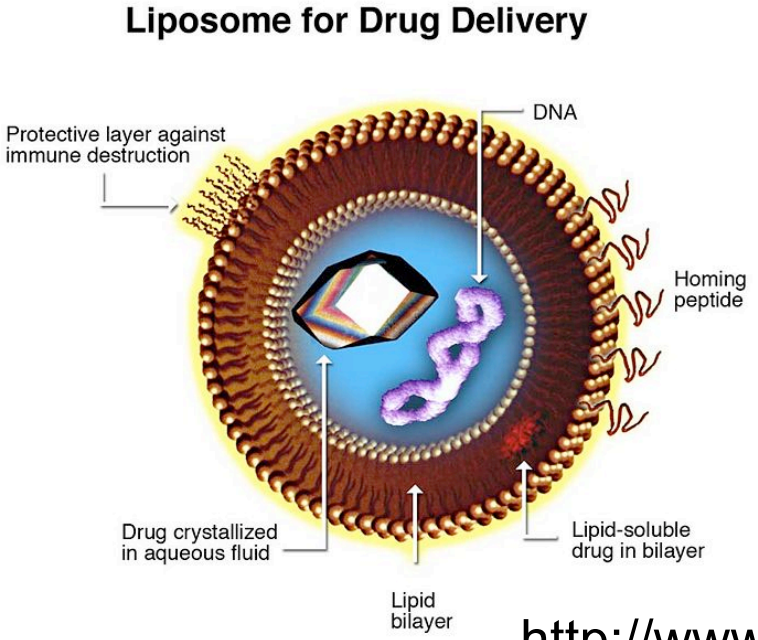


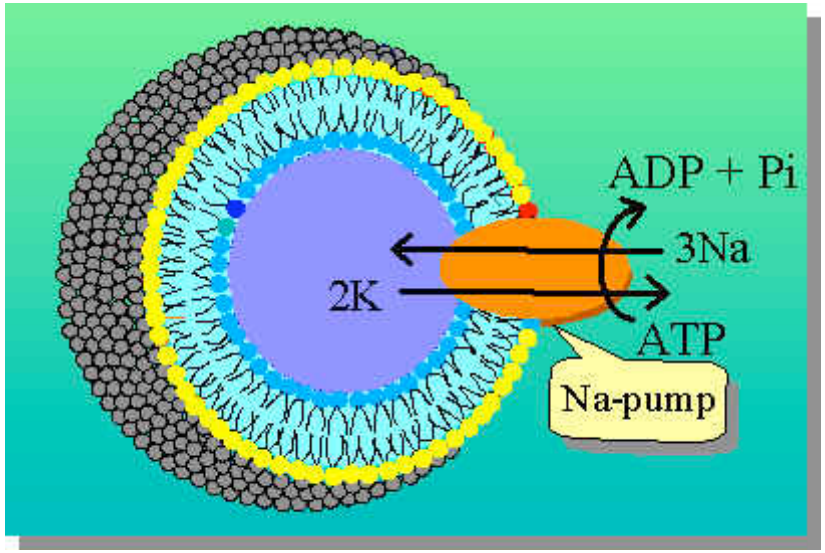
proteoliposome



immunoliposome

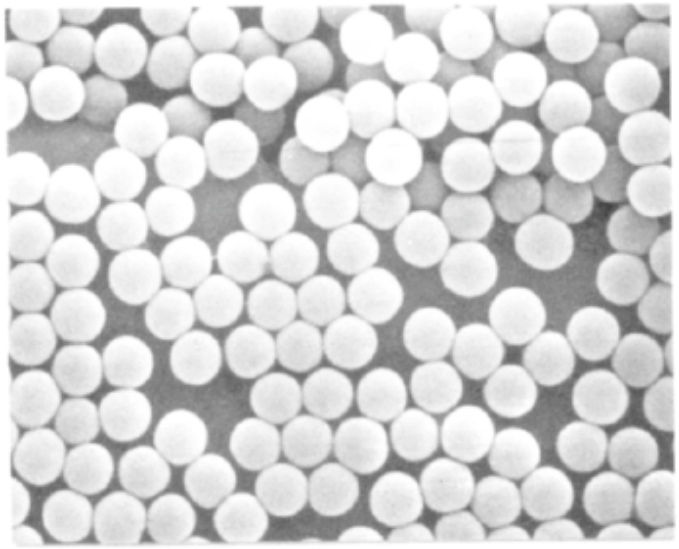
Encapsula NanoSciences

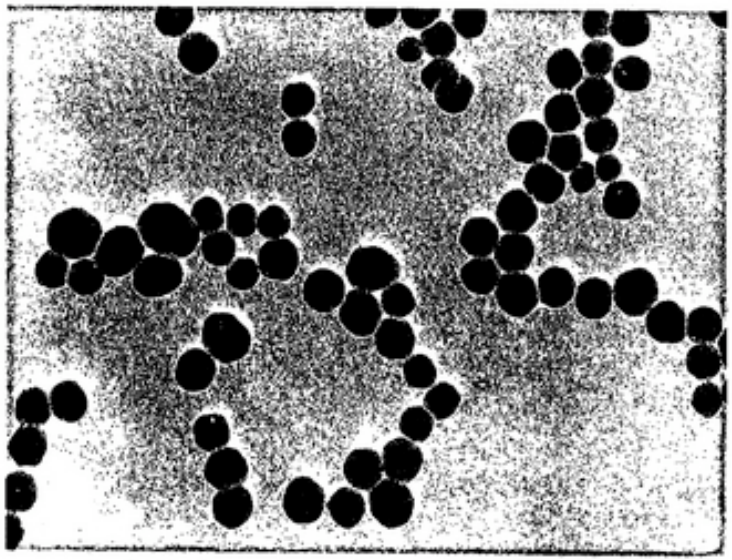


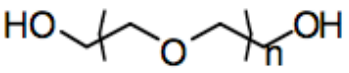


http://www.biophys.au.dk/Person/fc/fceng.htm

Sol-gel process

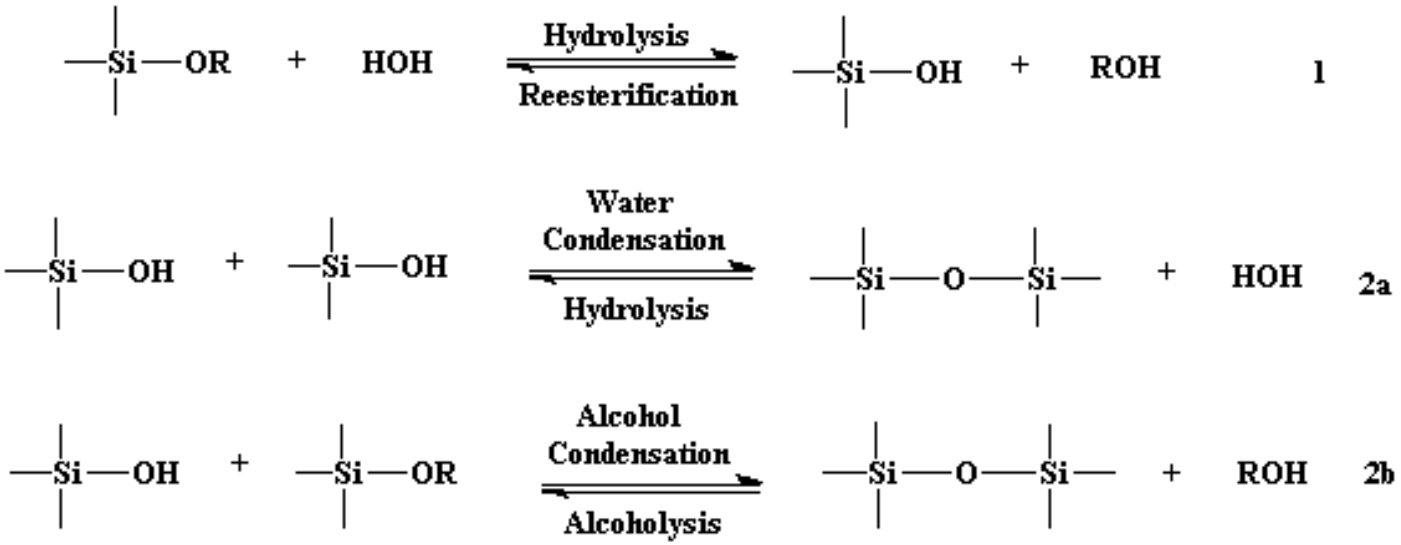




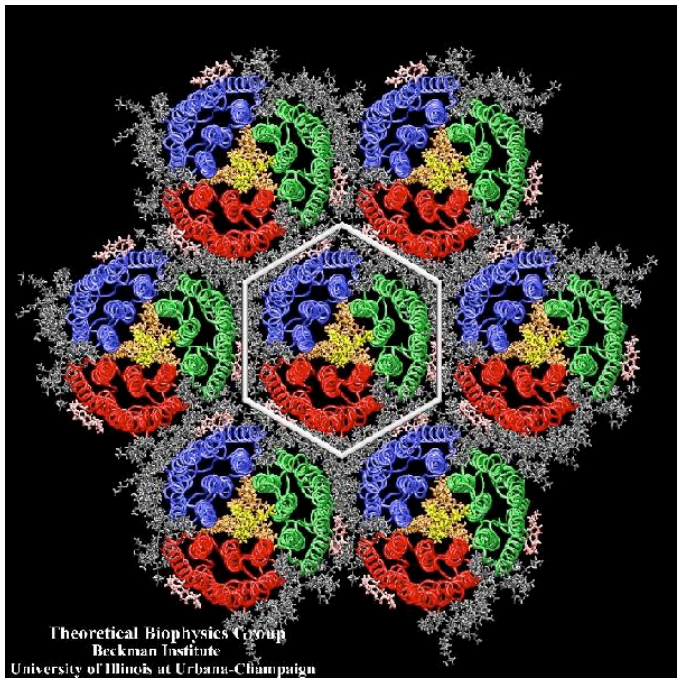


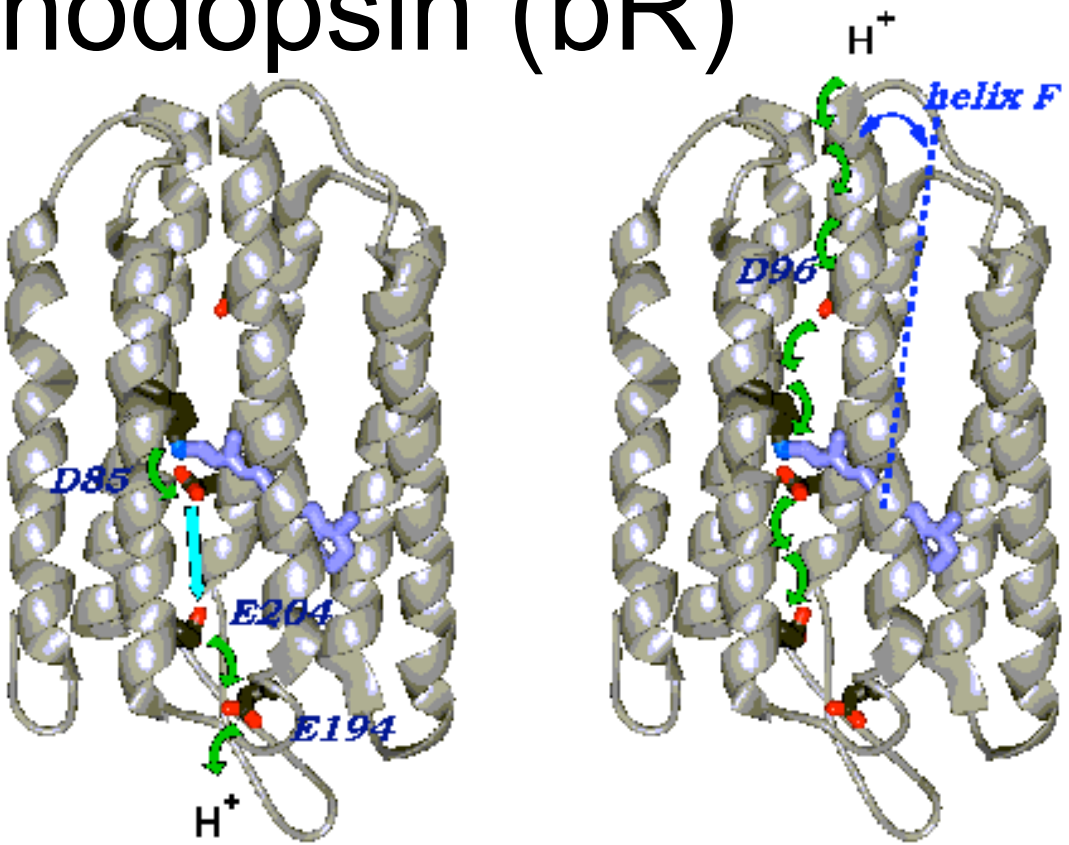
PEG

Silica particles



Bacteriorhodopsin (bR)



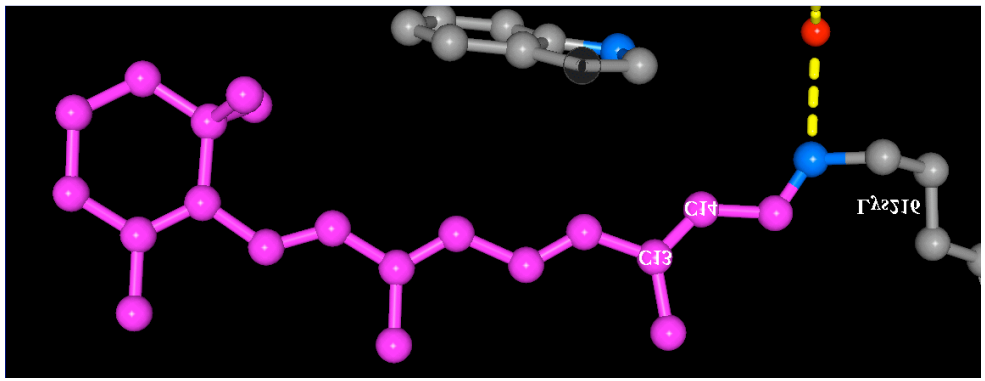


http://anx12.bio.uci.edu/~hudel/br/

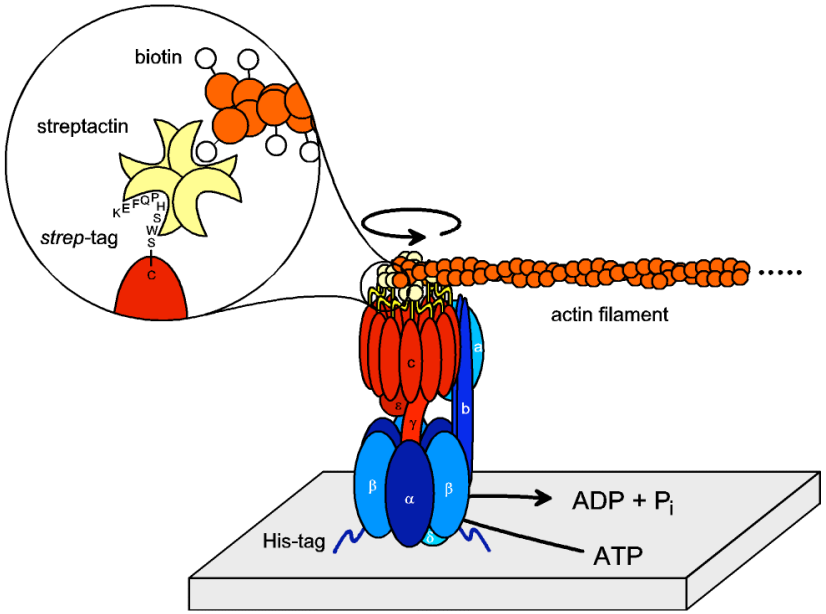
Proton pump in halobactaria (archaea)

Retinal cofactor

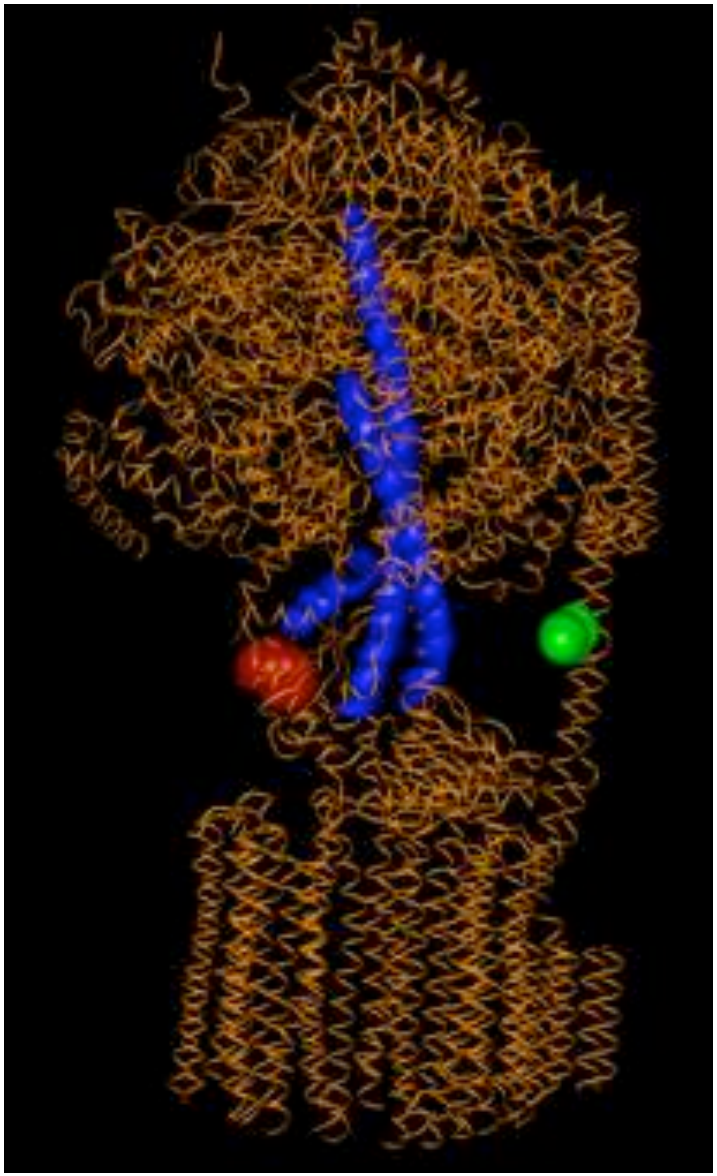
New on DVD?



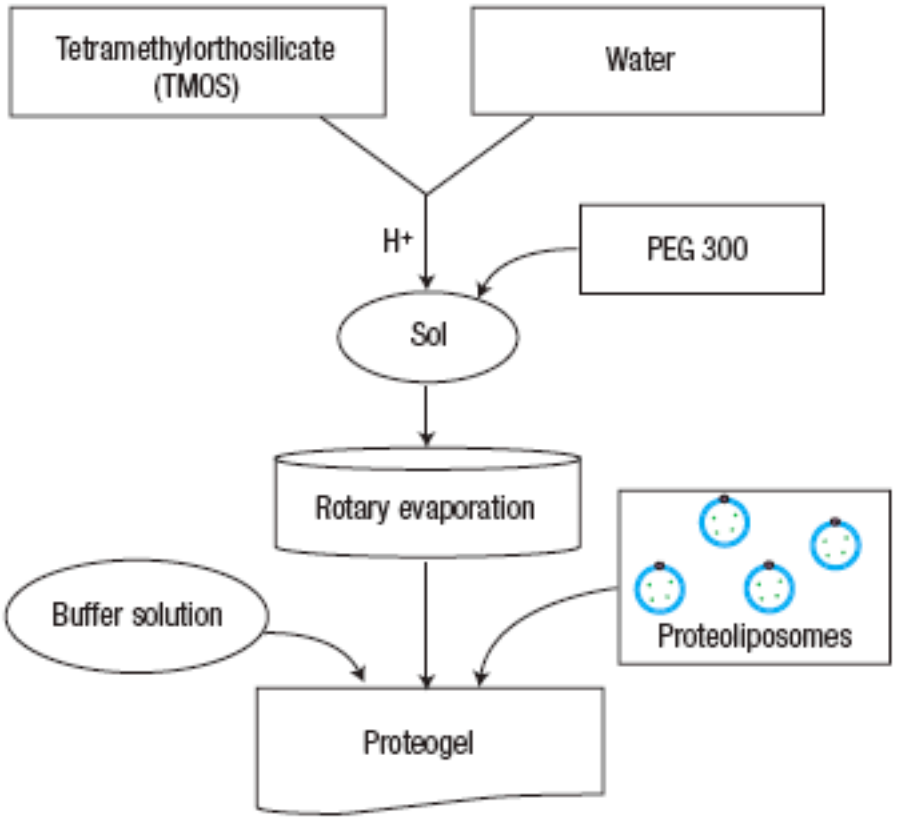
FoF1 ATP synthase

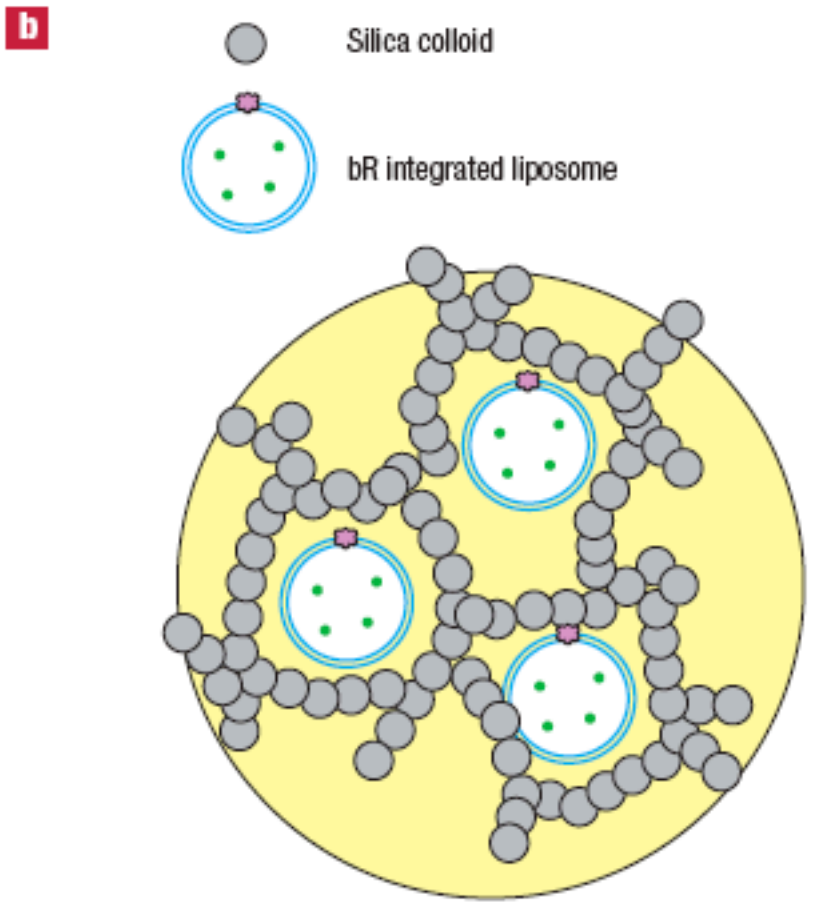


(Wolfgang Junge)



Proteogel



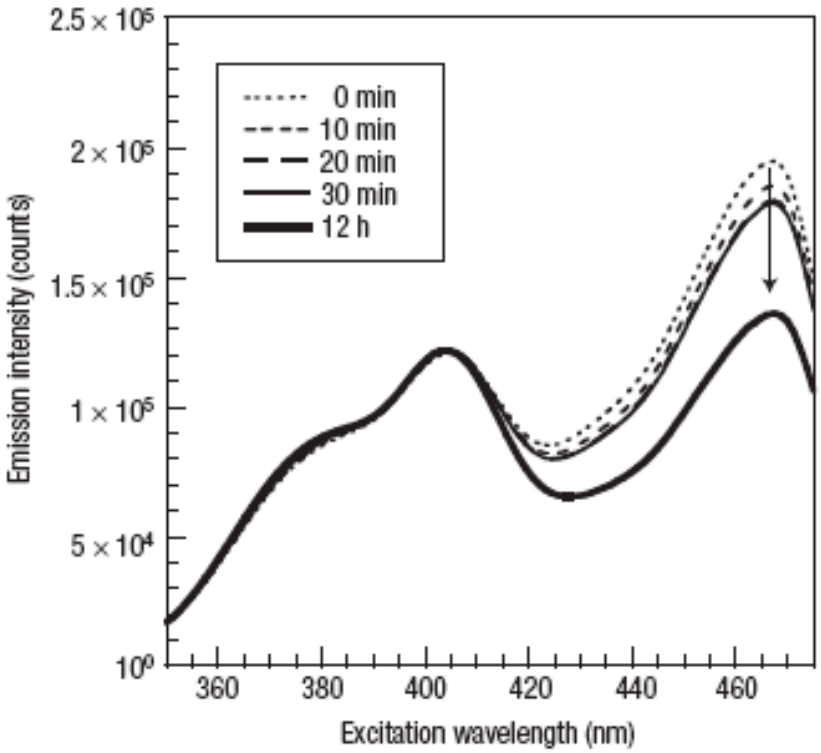


Nature Mat. 4:220

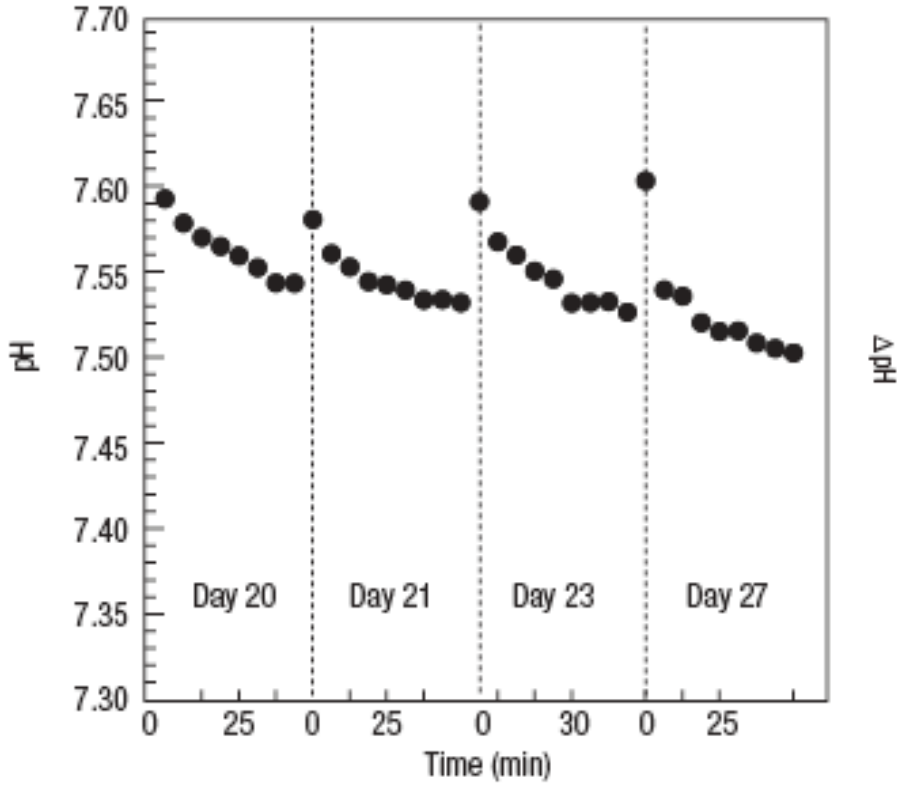
Gel “doped” with proteoliposomes

“cell” size: ~120nm

It can pump protons and make ATP !

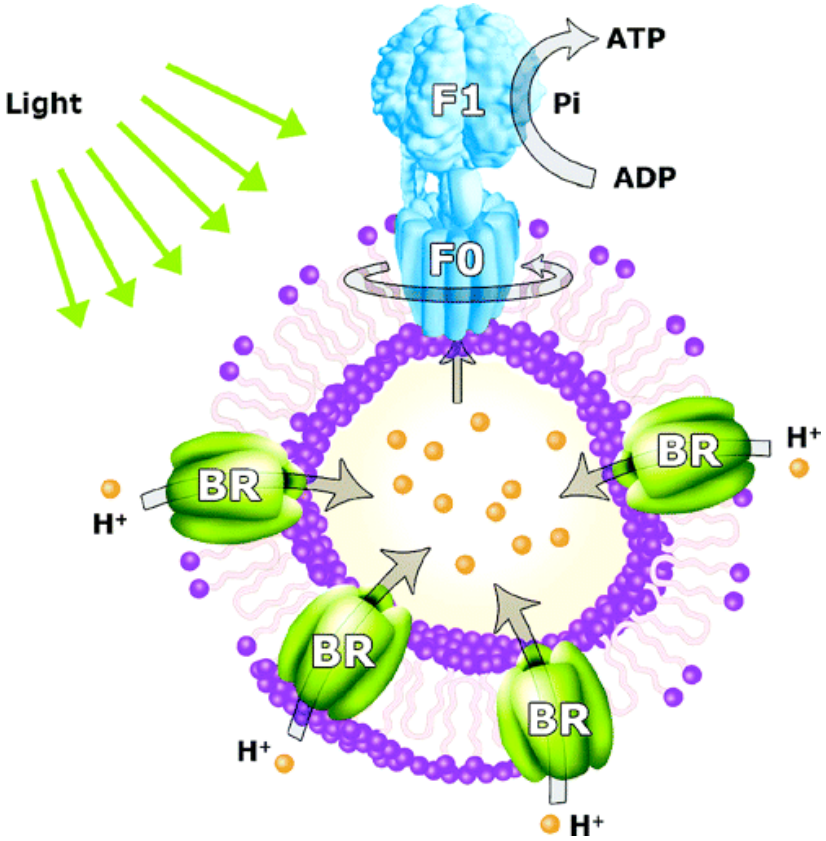


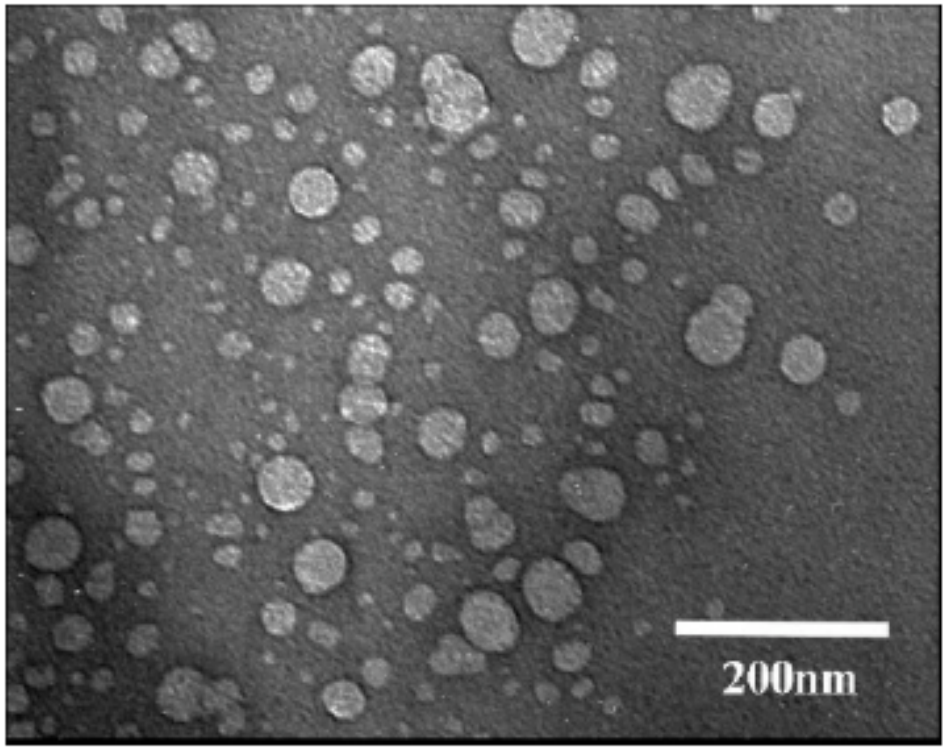
Fluorescence of pyranine



Charge-discharge cycles

Polymersome





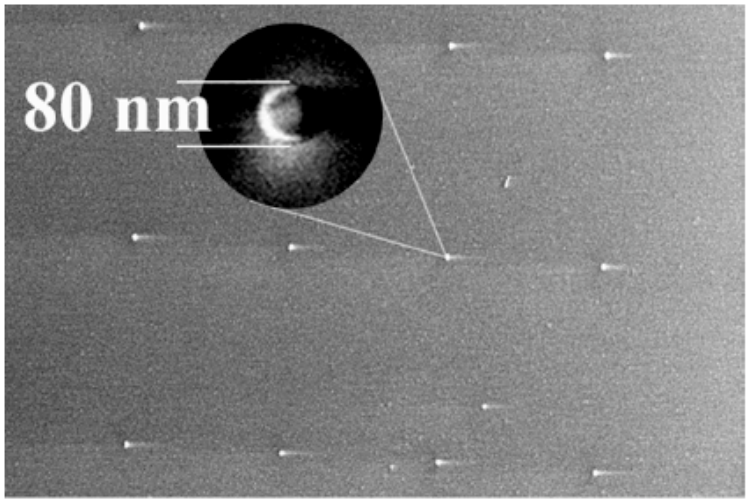
Membrane is a triblock copolymer!
Average size: 37nm

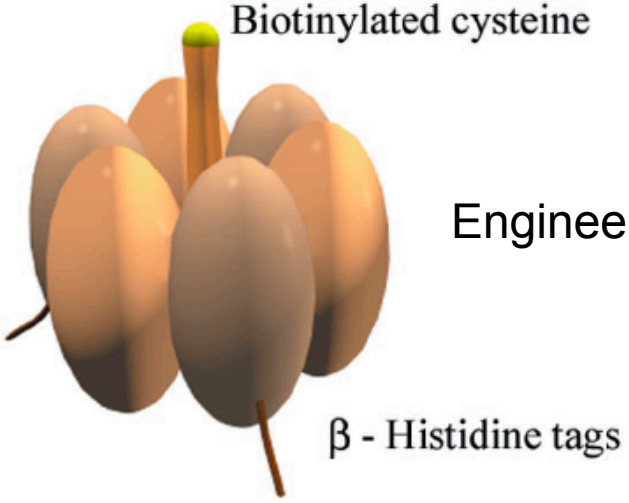
Nano Lett. 5:2528

Luciferase + luciferen was used to prove ATP production

F1-ATPase propeller

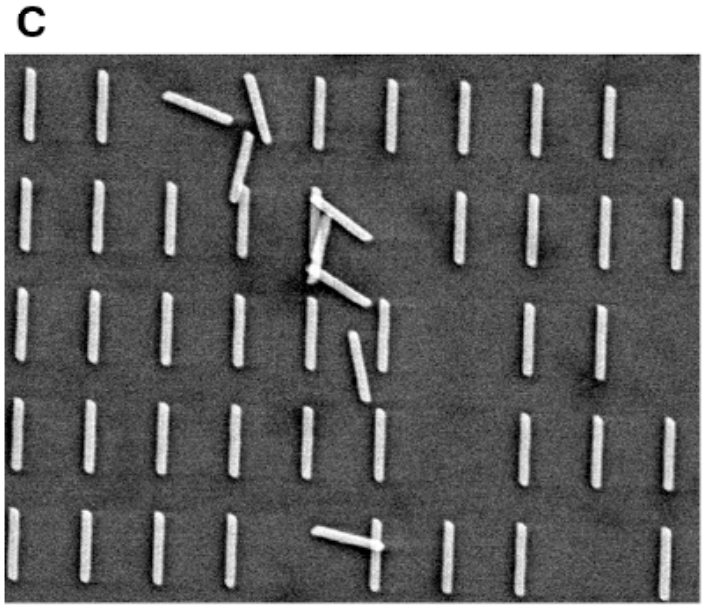
Ni post (height 200 nm, diameter 80 nm)

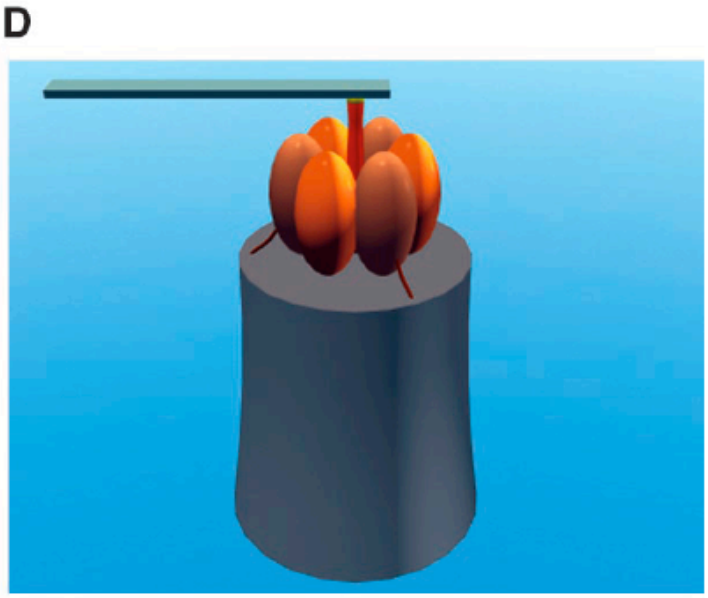




Engineered F1-ATPase

Carlo Montemagno

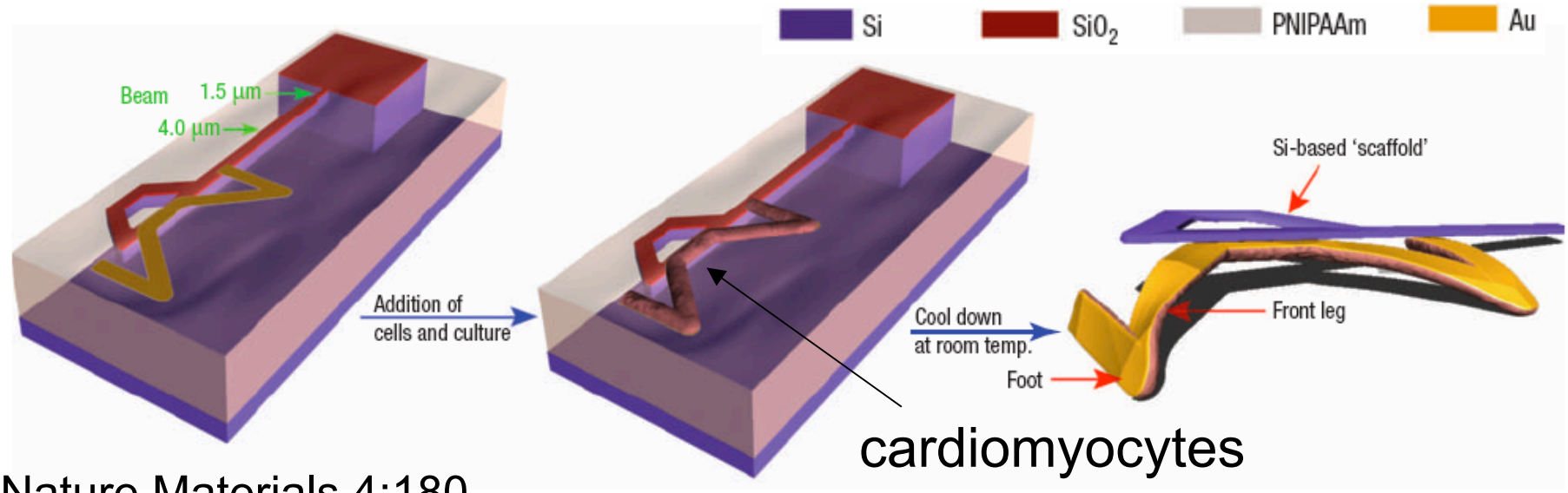




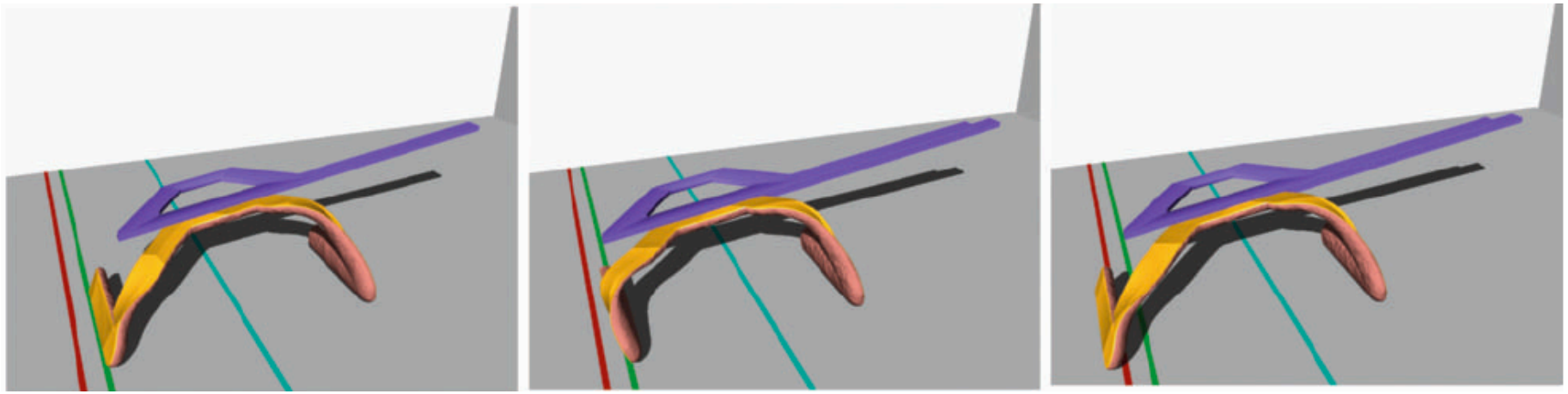
Nano-propeller

nanopropeller (length 750 to 1400 nm, diameter 150 nm).

Muscle powered walker



Nature Materials 4:180



138 μm long, 40 μm wide, and 20 nm/300 nm (Cr/Au) thick.