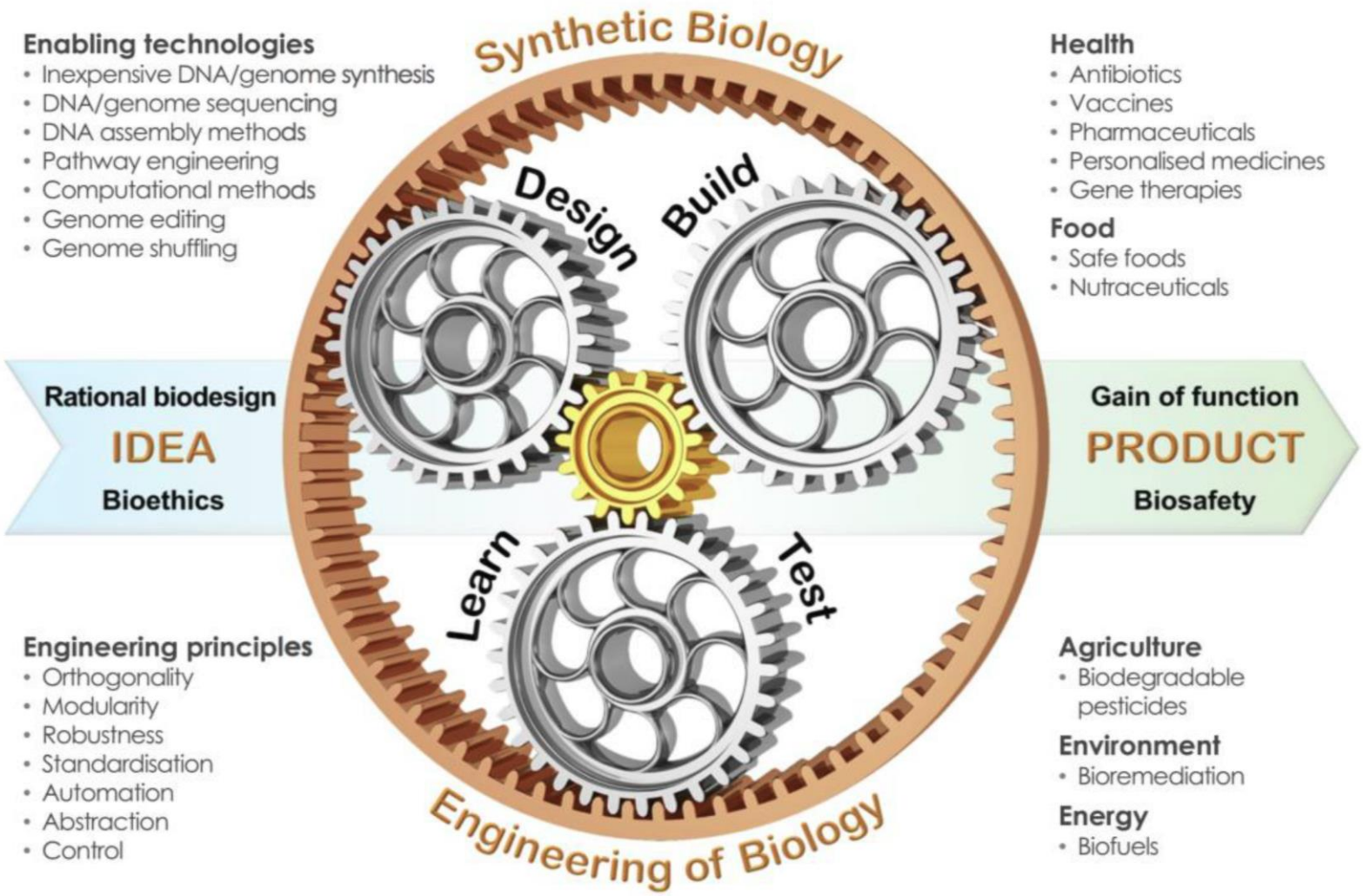
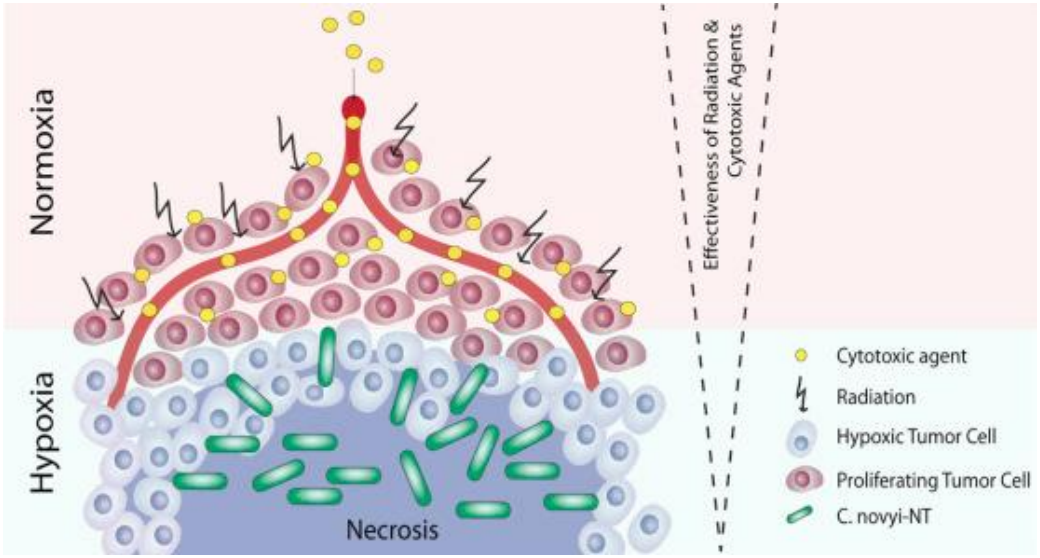
Applications in synthetic biology



Tumor targeting bacteria

Tumor targeting bacteria: an emerging strategy that represents vial alternative to molecular targeted therapy and immunotherapy

- Cancer cells within hypoxic areas are dormant but viable and these areas are often responsibel for clinical relapse
- Hypoxic areas are poorly accessible to systematically delivered therapeutic and oxygen (important fro radiotherapy)
- Hypoxic/necrotic areas are critical niche for bacteria colonization – they are rapidly cleared in the circulation and normal tissues
- Many anaerobic bacteria were shown to target solid tumors – *Salmonella, Clostridium, Listeria...*



Engineering towards improving therapeutical potential:

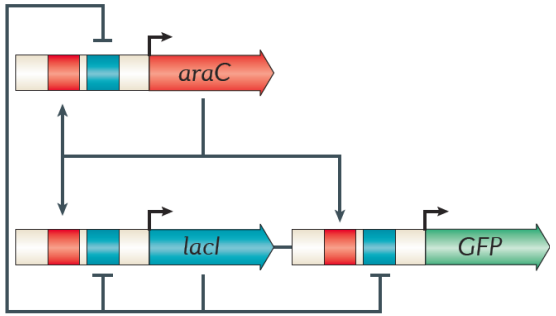
- ***Improving safety*** – reducing pathogenicity, exotoxin deletion, ...
- ***Increasing tumor targeting*** – surface display of antitumor antibodies, systems inducible by tumor-associated facotrs, ...
- ***Effector systems*** – cytotoxins, prodrug-converting enzymes, delivery of expression cassettes to targeted cells, expression of tumor antigens or immunomodulators to stimulate immune system (e.g. tetanus oxoid)
- ***Targeting tumor stroma*** – disruption of tumor vasculature, improving bacterial colonization (enzymes degrading extracellular matrix)

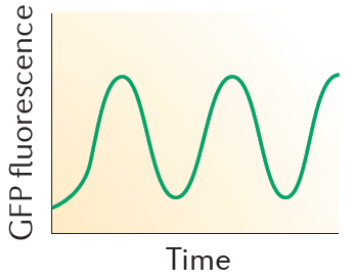
Zhou et al., Nat Rev Cancer 2018

Bacteria programmed to limit bacterial growth while continuously producing and releasing cytotoxic agent

Quorum sensing based oscillator

Positive-negative feedback loop oscillator

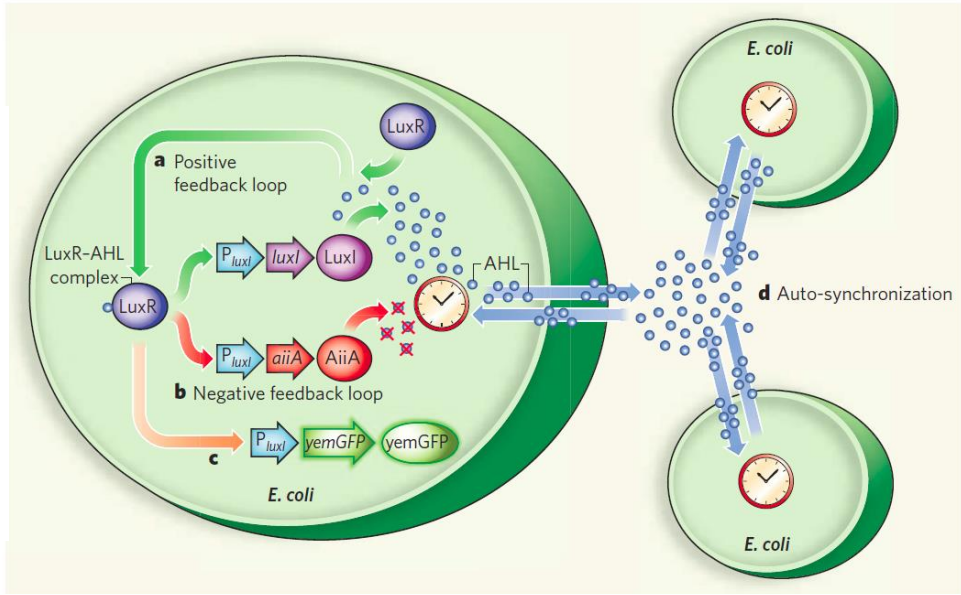




- Oscillations are robust, persistant and hereditary
- Oscillations can be adjusted by temperature, growth media, or concentration of transcription inducing molecules
- However, these are still intracellular oscillators – **how to coordinte entire cell populations?**

Quorum sensing: communication system between bacteria based on constantly secreted signalling molecules. The concentration of the signal depends on density of the bacteria; when it exceeds a certain threshold, it triggers transcriptional activation that controls some form of collective action (eg biofilm formation).

Figure 1 | All together now. Hasty and colleagues[1] have constructed a network of genes and proteins in *Escherichia coli* that acts as a molecular clock and can be synchronized across a population of the bacteria. **a**, When bound to the diffusible molecule acyl-homoserine lactone (AHL), the constitutively expressed LuxR receptor initiates a positive feedback loop by triggering expression of LuxI, the enzyme that synthesizes AHL. The expression of LuxI is driven by P_{luxI}, a DNA promoter sequence. Large arrows indicate DNA promoter sequences or genes. **b**, The LuxR–AHL complex also kicks off a time-delayed negative feedback loop by activating the P_{luxI}-driven production of AiiA, a protein that degrades AHL. **c**, In the final section of the network, the LuxR–AHL complex triggers P_{luxI}-driven expression of a green fluorescent protein (yemGFP). **d**, The dynamic interactions of the positive and negative feedback loops produce regular pulses of AHL, which act as time-keepers in the molecular clock. The AHL molecules diffuse out of each cell and across the membranes of neighbouring bacteria, where they stimulate the expression of yemGFP. All the cells of the *E. coli* population simultaneously send and receive AHL, providing a signal that enables each individual clock to self-adjust and auto-synchronize with the others, as demonstrated by the coordinated pulses of fluorescence produced by the bacteria.



Danino et al., Nature 2010; Fussenegger, Nature 2010

Bacteria synchronized for drug delivery

Bacteria programmed to limit bacterial growth while continuously producing and releasing cytotoxic agent

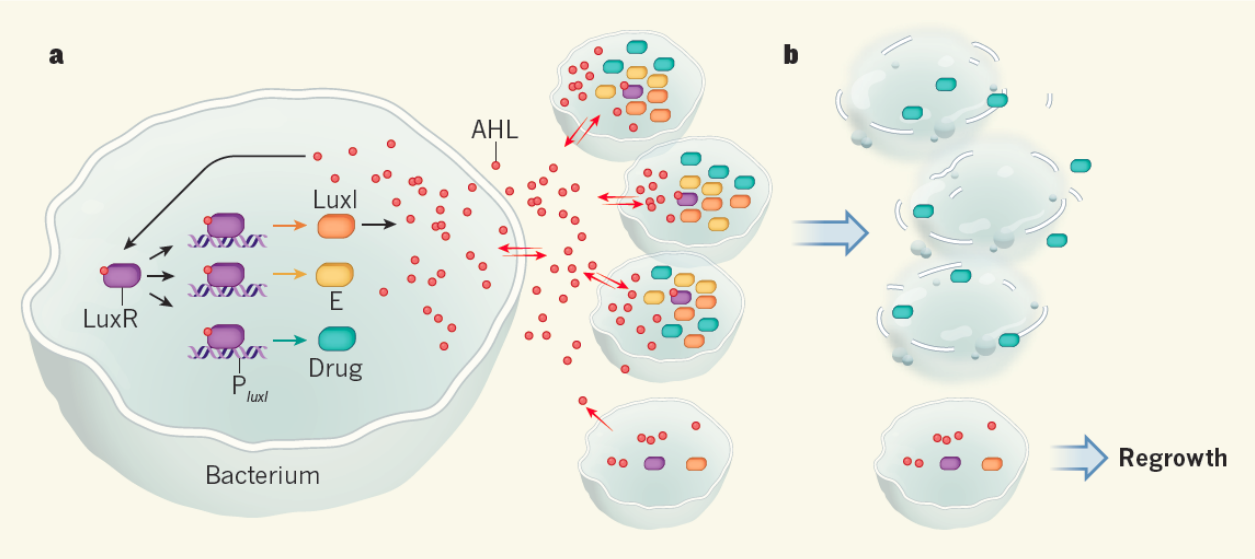


Figure 1 | Synchronized cyclical lysis and drug release. Din *et al.*[1] constructed a genetic circuit in tumour-targeting bacteria that mediates drug production and release through cell lysis in synchronized, repeated cycles. **a**, Binding of the signalling molecule AHL to its receptor protein LuxR leads to their subsequent interaction with, and activation of, the promoter DNA sequence P_{luxI}. This promoter drives expression of the gene that encodes the enzyme LuxI, which catalyses AHL synthesis, thus generating a feed-forward loop. The promoter also drives expression of genes that encode a bacterial toxin (drug) to kill cancer cells, and protein E, which releases the drug through bacterial-cell lysis. AHL can diffuse freely in and out of cells (red arrows). When the density of the bacterial population is low, AHL primarily diffuses out of the cell and the circuit is not active. When population density increases, intracellular AHL accumulates, reaching a threshold concentration that activates the circuit in most cells. **b**, The cells lyse in synchrony, releasing a burst of the drug. The few bacteria that survive kick off another cycle.

Components:

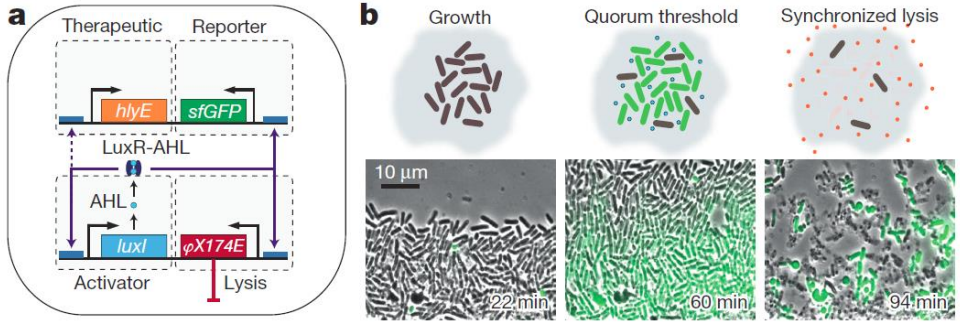
LuxI – production of acetyl homoserine lactone (AHL)

LuxR – AHL receptor, transcriptional activator

Φ184 E – bacteria lysis protein

sfGFP – superfolder GFP (rapidly folding GFP variant)

hlyE – pore forming anti-tumor toxin Haemolysin E



Din et al., Nature 2016; Zhou, Nature 2016

Remote detection of buried landmines using a bacterial sensor

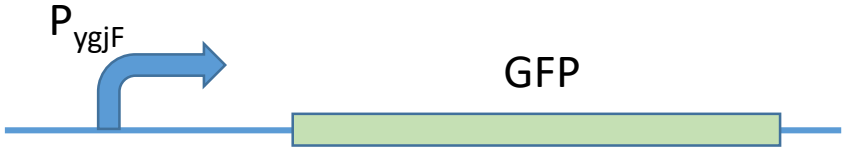
Detection of 2,4-dinitrotoluene and 2,4,6-trinitrotoluene residues

Screening of *E.coli* promoter library for response to DNT and TNT

yqjF and *ybiJ* promoters
(detect a volitile products of DNT degradation)

Directed evolution of the *yqjF* promoter

Improved robustness and sensitivity of the *yqjF* promoter



DNT/TNT bacterial sensor

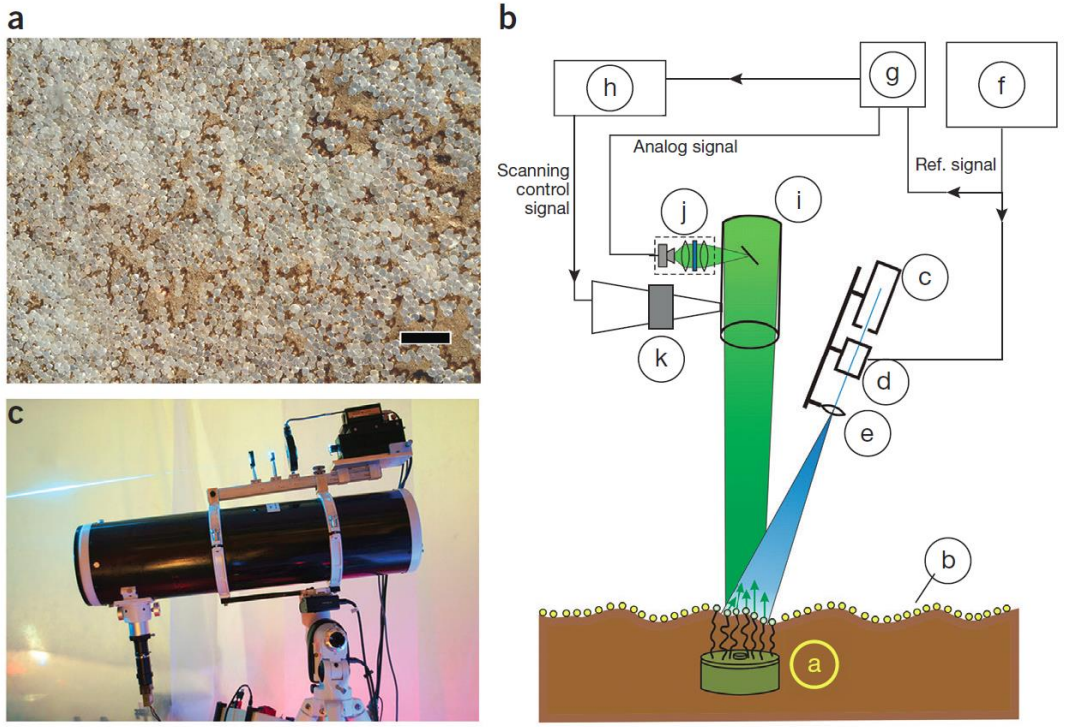
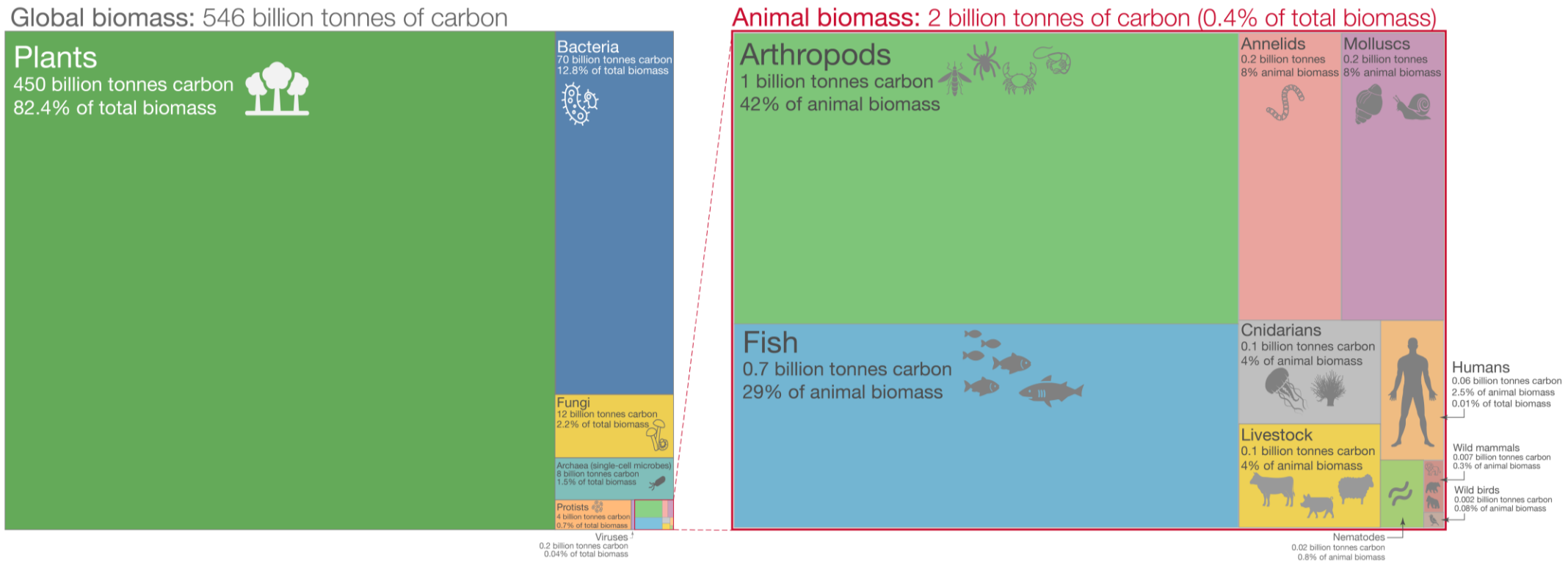


Figure 1 Optical scanning system and alginate-encapsulated bacterial sensors. (**a**) Biosensor beads spread over target areas. Scale bar, 2 cm. (**b**) Schematic of the optical scanning system. Key. (a) Buried landmine; (b) beads containing encapsulated bacteria; (c) laser system producing a Gaussian beam with 0.5 W at 473 nm; (d) laser modulator; (e) optical aiming system; (f) oscillator; (g) digital data acquisition card; (h) computer; (i) collecting telescope; (j) detection module; (k) scanning apparatus. (**c**) Photograph of the optical scanning system.

Belkin et al., Nat Biotech 2017

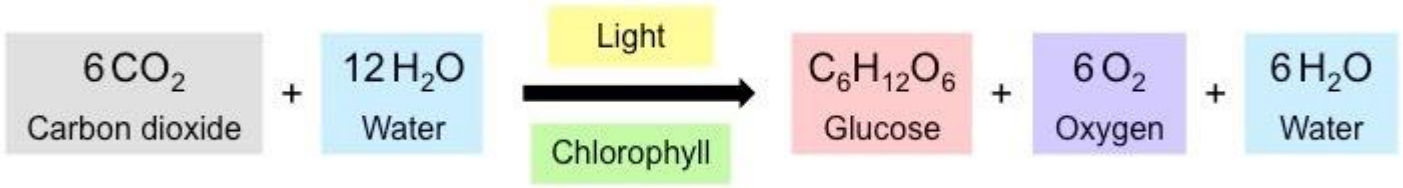
Applications of synthetic biology in plants

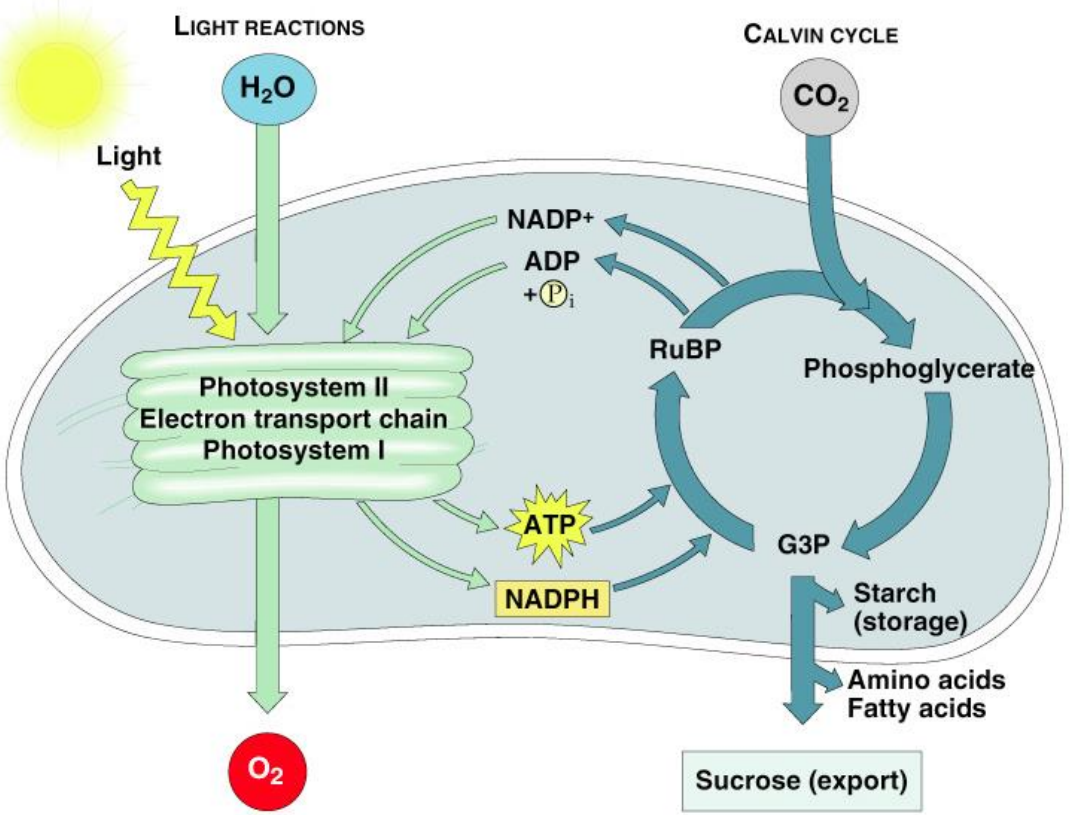
The distribution of Earth´s biomass



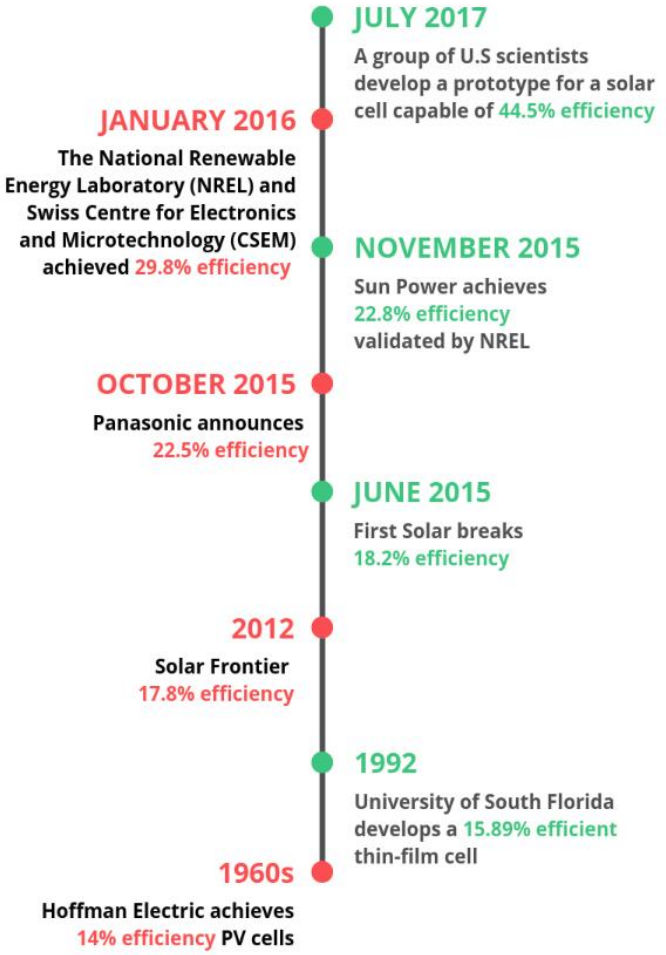
Source: Based on data from Bar-On, Y. M., Phillips, R., & Milo, R. (2018). The biomass distribution on Earth. *Proceedings of the National Academy of Sciences.* Icon graphics sourced from Noun Project (2018).
This is a visualization from OurWorldinData.org, where you find data and research on how the world is changing.

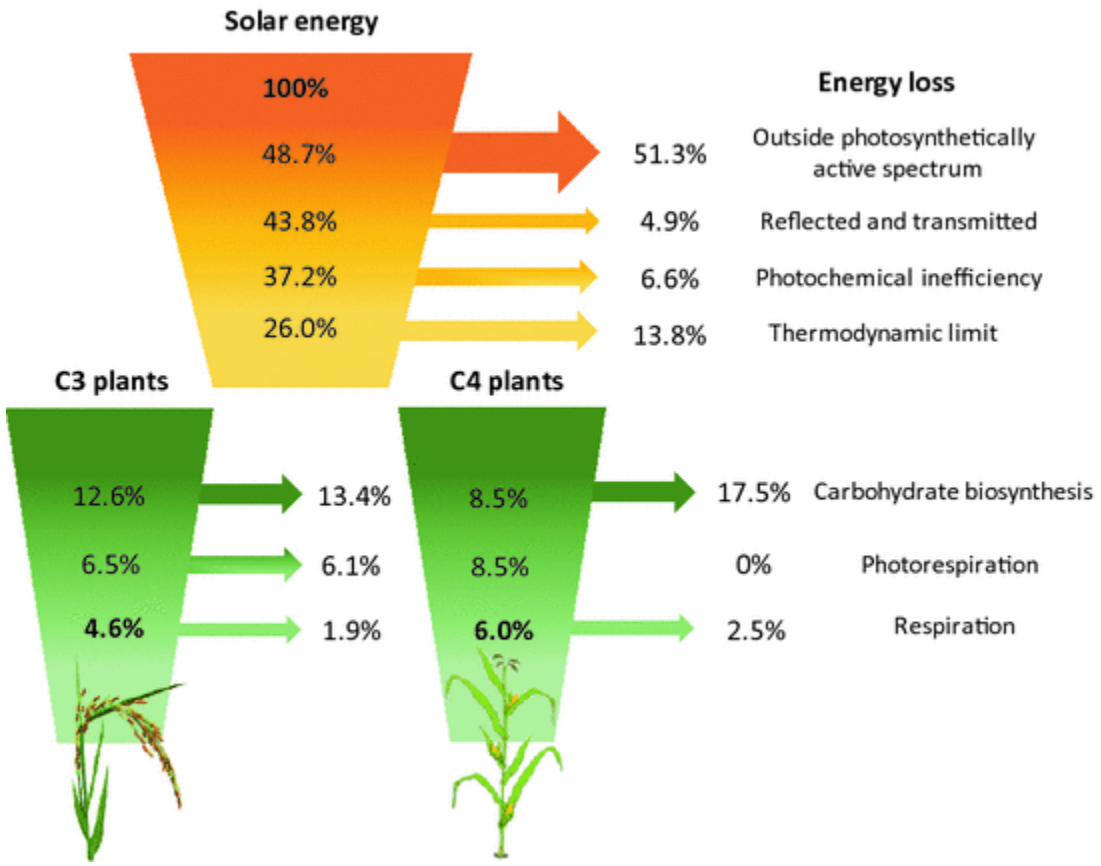
Photosynthesis





Efficiency of photosynthesis





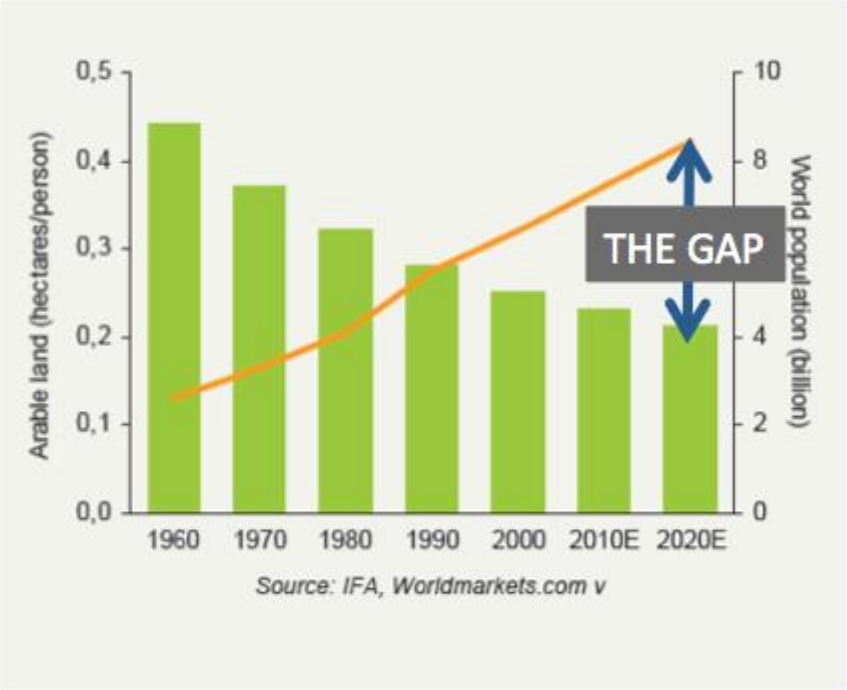
Do we need to increase efficiency of photosynthesis

CLOSE THE GAP!

Very limited potential to increase arable land

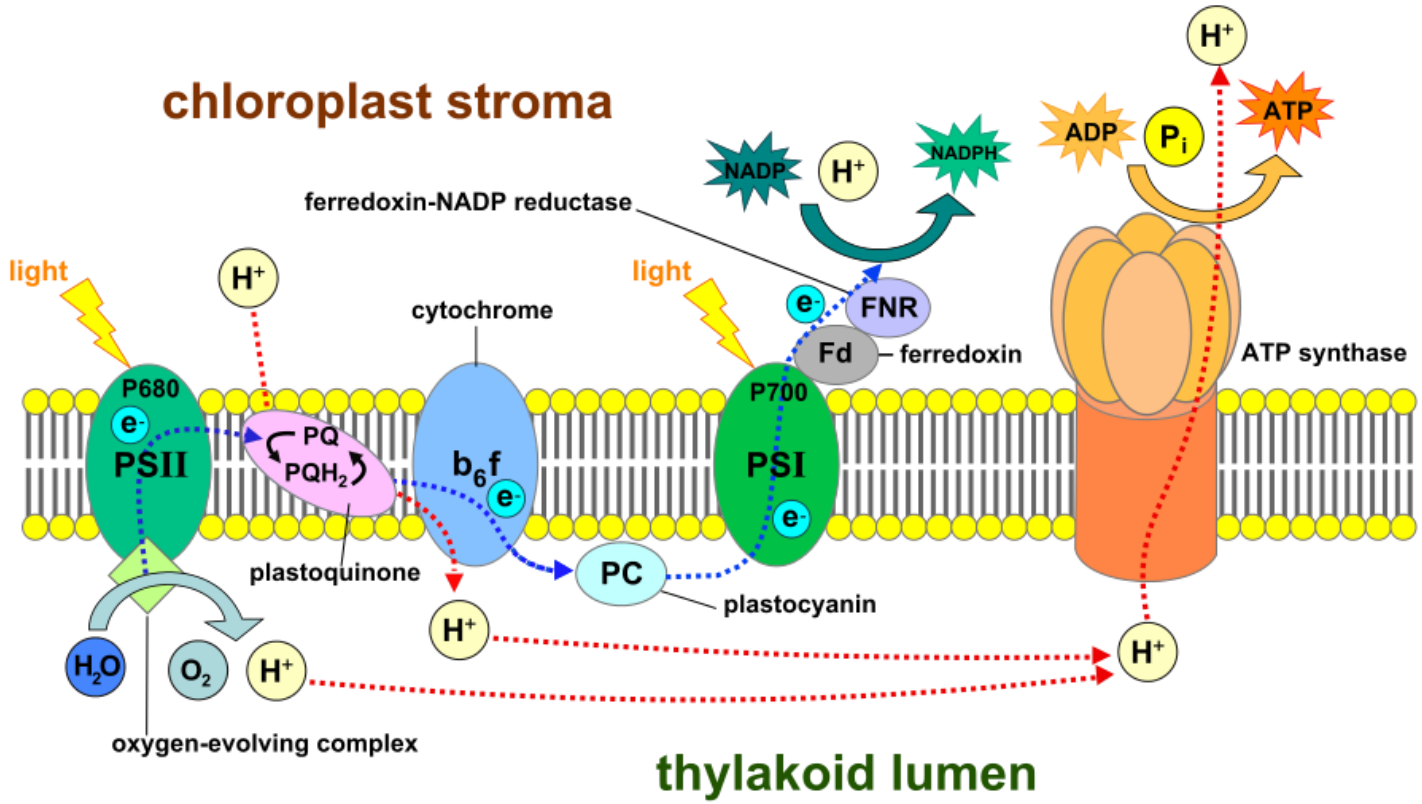
Improved living standards increase protein consumption per person requiring more grain for animal feed

The only solution is to increase agricultural productivity



Source: Norman Borlaug statement on the basis of 2005 food production level.

Photosynthesis: light reaction



Photosystem I (PSI) is naturally adapted for highly efficient light harvesting and charge separation. PSI operates with a quantum yield close to 1, and currently, no synthetic system has approached such remarkable efficiency.

Light reaction of photosynthesis as target for synthetic biology

1. Enhancement of the process *in vivo* to increase the efficiency of light use to increase biomass and crop yields.

Increasing spectral range at which photosynthesis operates: e.g. cyanobacteria that employ plant-derived LHCs as antennae to shift the light saturation of photosynthesis to higher intensities, or the integration of cyanobacterial chlorophyll d and chlorophyll f (which can absorb far-red and near infrared light) into algal or plant photosynthesis to expand the spectral region available to drive photosynthesis.

2. Coupling of the light reactions of photosynthesis to previously unconnected pathways will enable to utilize the reducing power directly to produce large amounts of high-value compounds *in vivo*.

3. The high efficiency and robustness of PSI should allow it to be used in hybrids with biotic or abiotic components to generate hydrogen, simple carbon-based solar fuels, or electricity in vitro.

Direct coupling to previously unconnected pathways

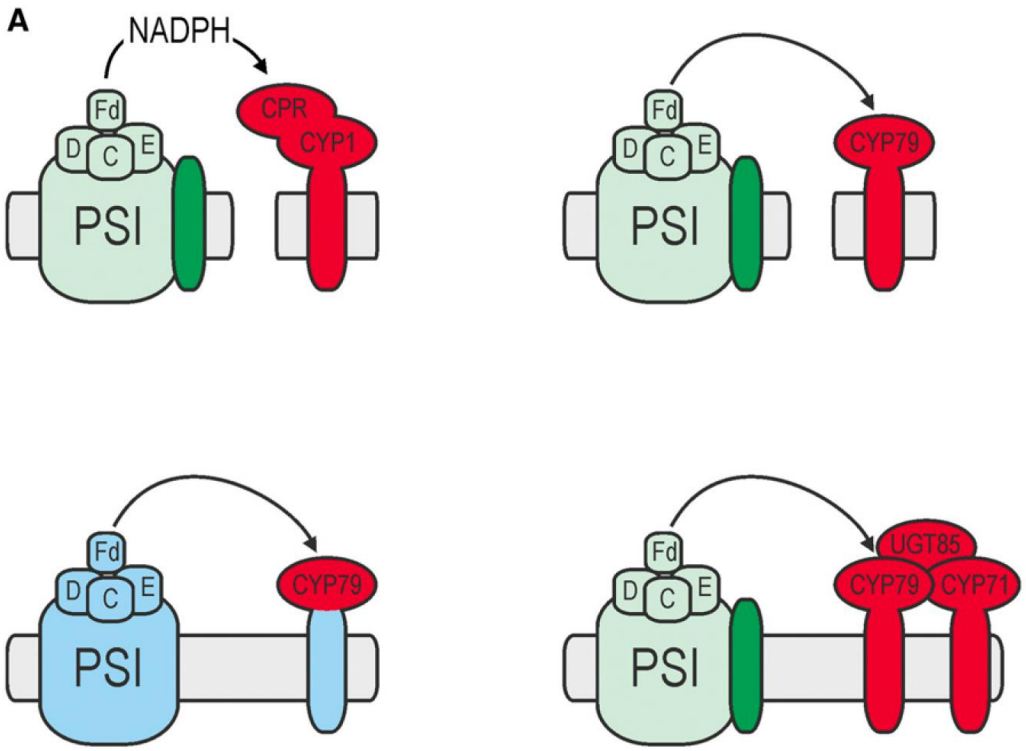


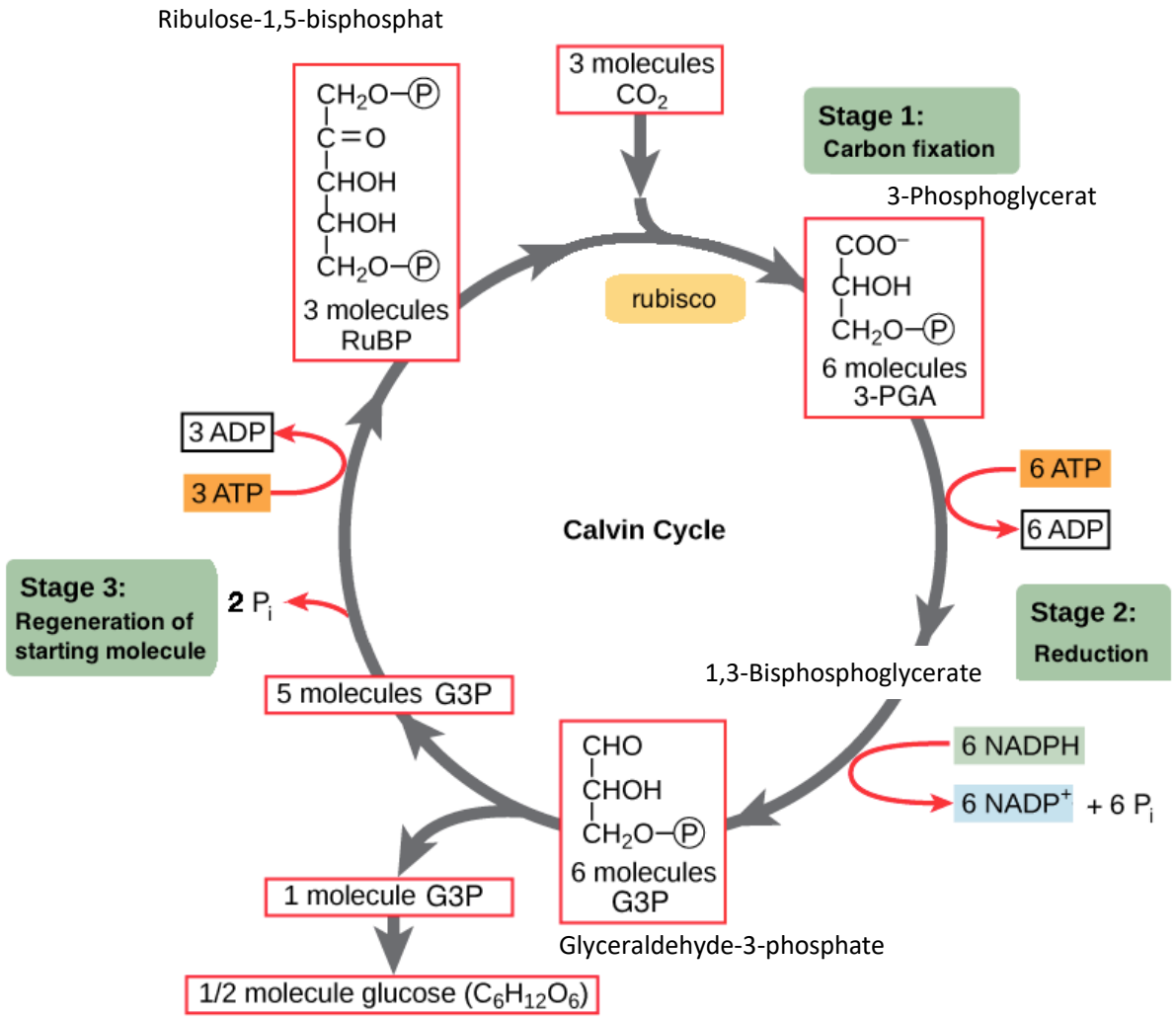
Figure 4. Design of bio-bio hybrids. A, Harnessing the reducing power of photosynthesis for cytochrome P450 enzymes (red) has been demonstrated in vitro and in vivo. In vitro approaches were based either on a CPR-CYP1A1 fusion protein that could use NADPH produced by photosynthesis or on CYP79A1 that can utilize photoreduced ferredoxin directly. The latter approach also was realized in vivo by fusing CYP79A1 to ferredoxin (not shown) or the PSI subunit PsaM. The most elaborate approach reported utilizes three enzymes (CYP79A1, CYP71E1, and UGT85B1) to couple dhurrin synthesis with photosynthesis. B, PSI-hydrogenase complexes are based either on genetic fusions of the hydrogenase (Hyd) to PsaE or ferredoxin or on covalent linking of the hydrogenase and PSI via a molecular wire. Currently, these bio-bio hybrids function only in vitro.

P450s are monooxygenases that insert an oxygen atom into hydrophobic molecules, which enhances their reactivity and hydrophilicity. They act on various endogenous and xenobiotic molecules and their extreme versatility and irreversibility of catalyzed reactions make these enzymes very attractive for use in biotechnology, medicine, and phytoremediation.

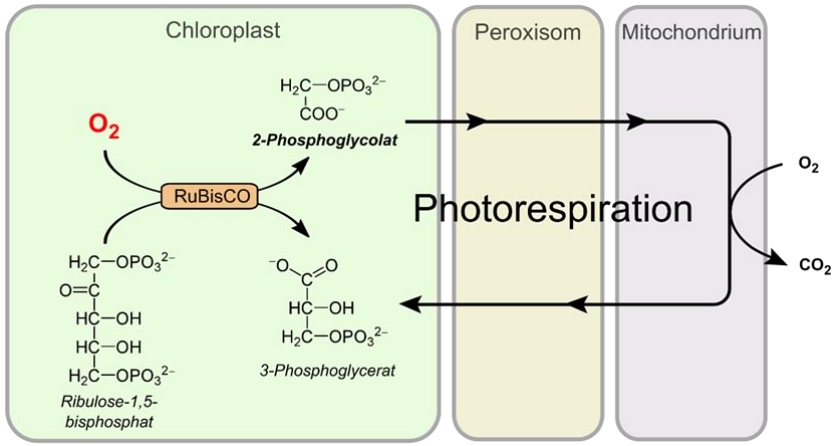
Attemts to couple PSI directly with hydrgenases to produce H_2 – fuell cells.

Leister, Plant Phys 2019

Photosynthesis: dark reaction



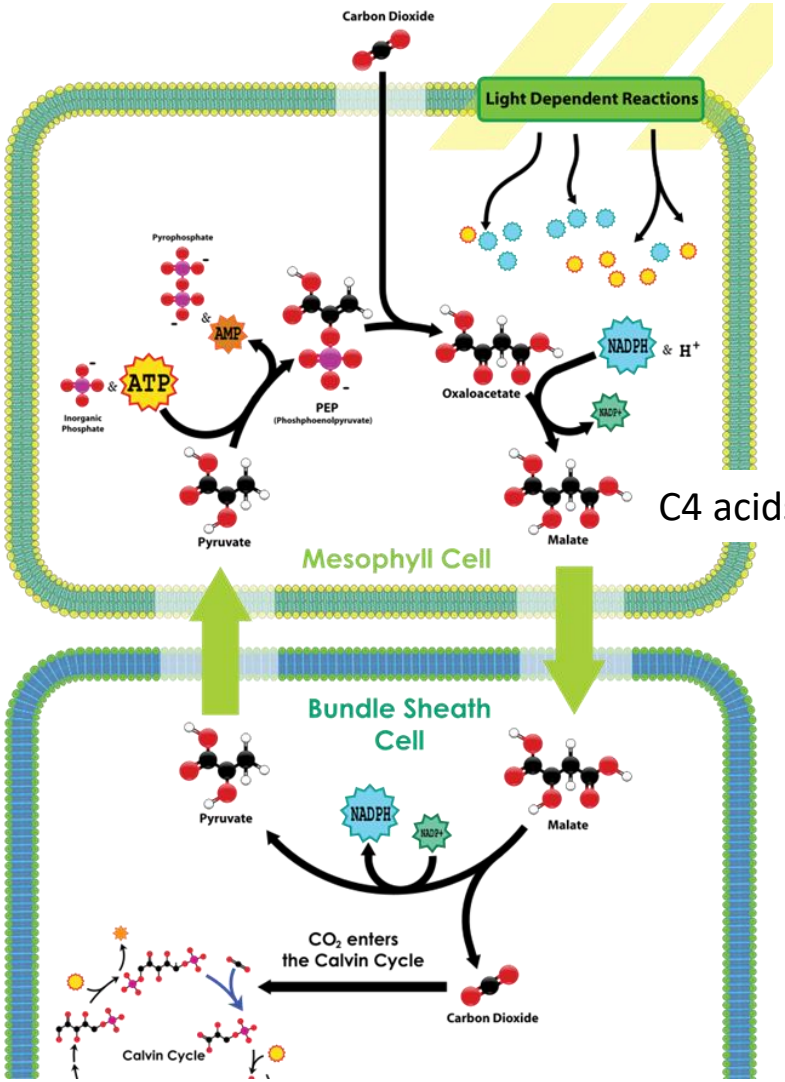
Oxygenation: up to 25% RuBisCo reactions



Around 25% of carbon fixed by photorespiration is re-released as CO_2 and nitrogen.

Strategies to improve the dark reaction

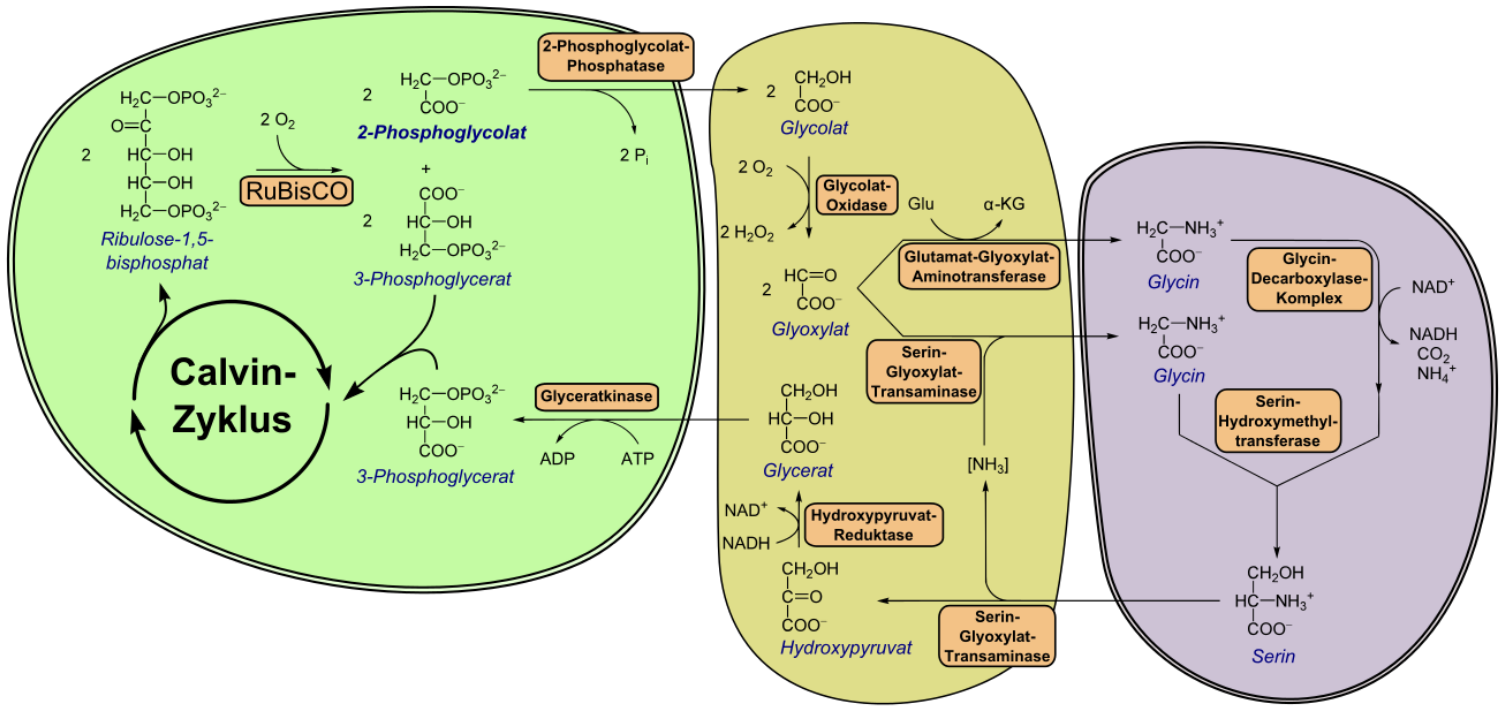
- **Engineering RuBisCo** for higher CO_2 specificity (ambient CO_2 concentrations are changing at a rate faster than plants can adapt to them, so replacing plant Rubisco with another variant could boost carbon fixation by up to 25%)
- **Optimizing expression of Calvin cycle enzymes:** at higher CO_2 is regeneration of RuBP rate limiting – Calvin cycle is not optimal and may limit photosynthesis. Specificity. Overexpression of sedoheptulose- 1,7-bisphosphatase and fructose-1,6-bisphosphatase increased photosynthetic rates and biomass in tobacco.
- **Establishing carbon concetrating mechanims** at teh sites of RuBisCo activity to inhibit oxygenation (carboxysomes, pyrenoids)
- **Carbon conserving photorespiration** engineering photorepisration bypass that does not lead to the release of CO_2
- **Engineering C4 photosynthesis** – independently evolved at least 66 times. It is a complex task that includes alteration of plant tissue anatomy, establishment of bundle sheath morphology, metabilic shuttling of intermediates between cells, as well as ensuring a cell type-specific enzyme expression. Alternative approach is **temporal decoupling** of light and dark reactions whereby malat synthesized during night is stored in vacuoles.



C4 photosynthesis is present in 3% of plants. It utilizes phosphoenolpyruvate carboxylase, the most efficient carbon fixation enzyme.

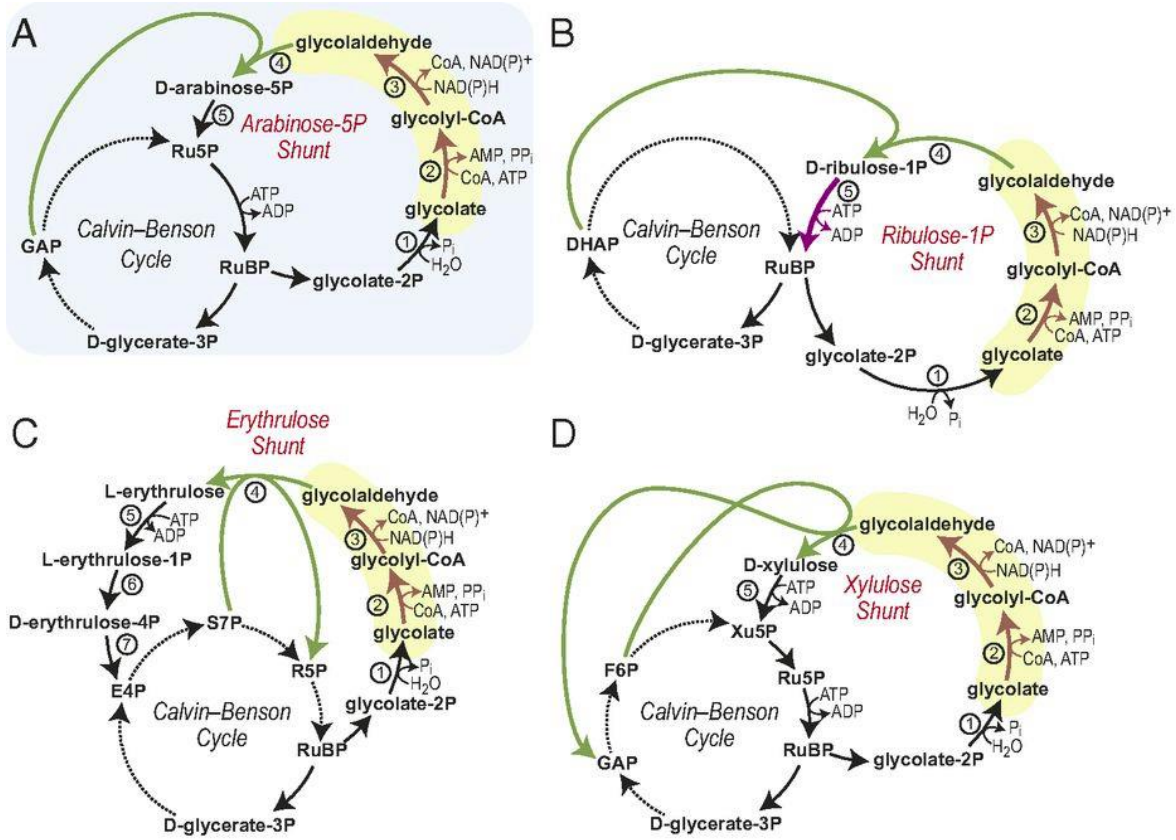
Kubis and Bar-Even, J Exp Bot 2019

Photorespiration



- Photorespiration assimilates 2-phosphoglycolate — the product of Rubisco's oxygenation reaction.
- It is vital to avoid the inhibitory effect of 2-phosphoglycolate and to recycle carbon back into the Calvin Cycle.
- Photorespiration is inefficient, as it dissipates energy and reducing power and releases carbon - one CO_2 per two glycolate molecules recycled.
- It is estimated that photorespiration leads to loss of up to 30% of the carbon fixed via photosynthesis

Design and *in vitro* realization of carbon-conserving photorespiration



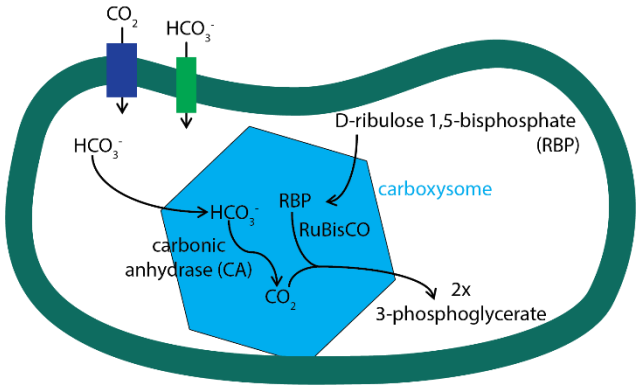
	①	②	③	④	⑤	⑥	⑦
(A)	2-phospho-glycolate phosphatase	glycolyl-CoA synthetase	glycolaldehyde dehydrogenase (CoA-acylating)	fructose-6P aldolase	arabinose-5P isomerase		
(B)				fuculose-1P aldolase	ribulose-1P kinase		
(C)				transketolase	L-eythrulose kinase	L-eythrulose-1P epimerase	D-eythrulose-4P isomerase
(D)				transaldolase	xylulose kinase		
(E)			glycolyl-CoA carboxylase	tartronyl-CoA reductase	tartronate SA reductase	glycerate 3-kinase	

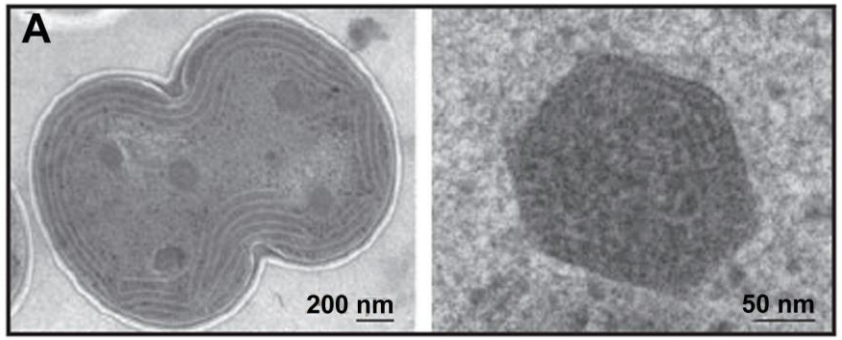
- Metabolic modelling to design possible photorespiration shortcuts that do not release CO2 (short, energetically feasible, limited overlap to endogenous metabolism)
- Pathway involving reduction of glycolate to glycoaldehyde most promising
- Required engineering novel enzymatic reaction: glycolate reduction to glycolaldehyde
- acetyl-CoA synthetase was engineered toward higher stability and increased catalytic efficiency with glycolate
- NADH-dependent propionyl-CoA reductase was engineered to use NADPH and for >10-fold in-creased selectivity for glycolyl-CoA over acetyl-CoA
- The glycolate reduction module was validated *in vitro*
- Theoretically, the synthetic pathways described here are expected to support a 10–60% increase in carbon fixation rate, which could provide a dramatic boost in plant growth and productivity.

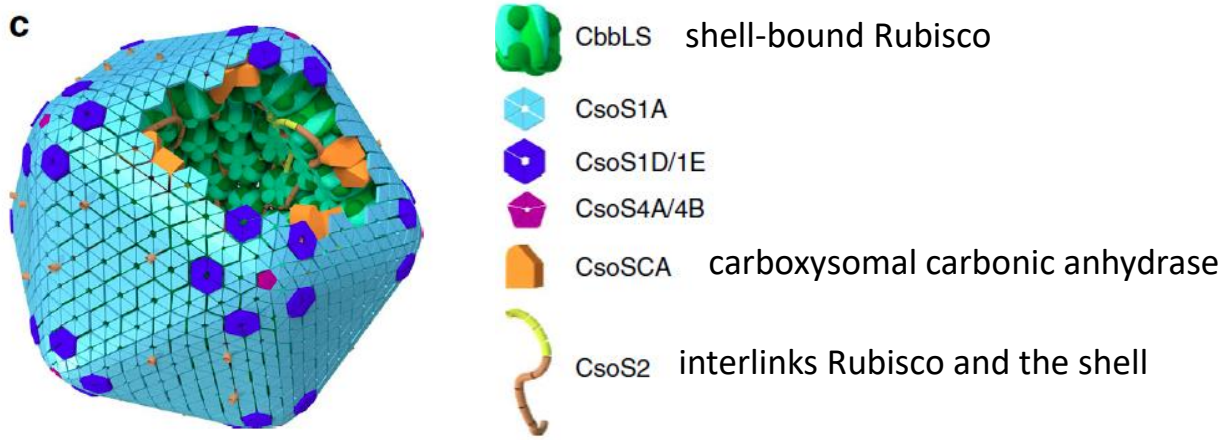
Trudeau et al., PNAS 2018

Carboxysomes in cyanobacteria

The cyanobacterial carbon concentrating mechanims is a single-cell, bipartite system that first generates a high intracellular bicarbonate (HCO3−) pool through action of membrane-bound inorganic carbon (Ci) transporters and CO2-converting complexes. This HCO3- pool is then utilized by subcellular micro-compartments called **carboxysomes**, which encapsulate the cell's complement of Rubisco. The carboxysome's outer protein shell enables diffusional influx of HCO3− and RuBP, where the former is converted to CO2 by a localized carbonic anhydrase (CA).







Carboxysome of *Cyanobium*

Engineering carboxysomes in tobacco chloroplasts

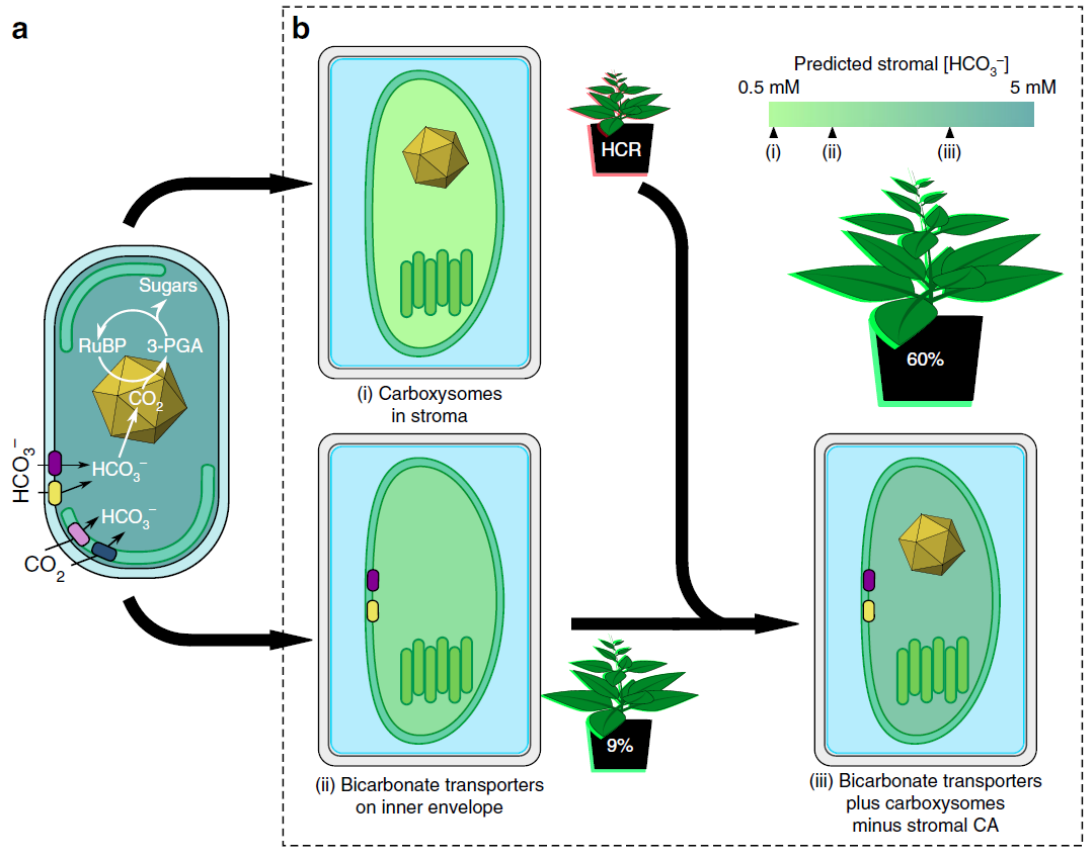
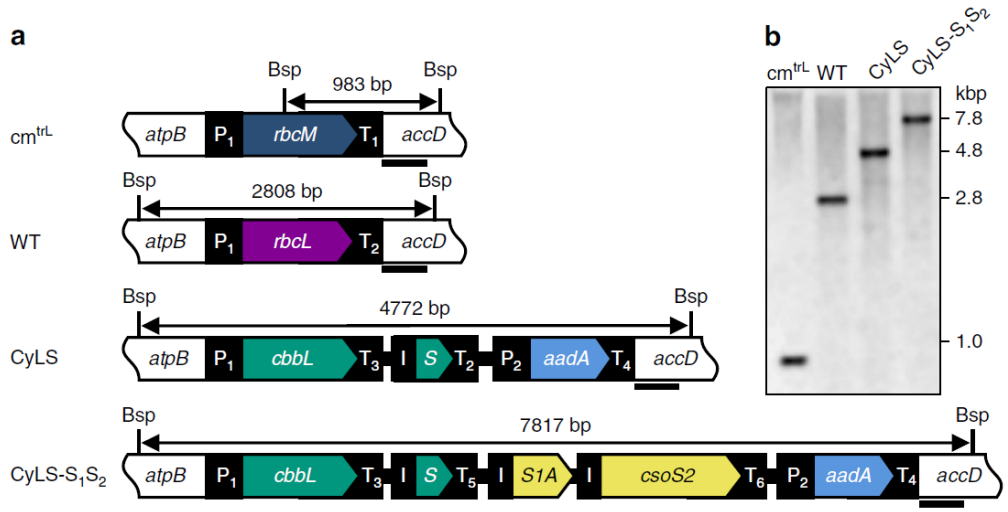
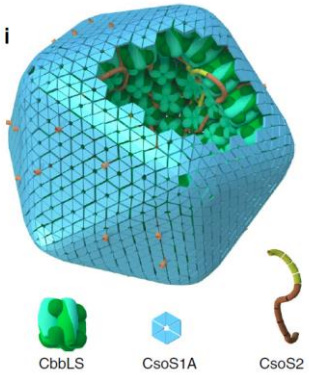


Fig. 1 Cyanobacterial CCM components for improved photosynthesis. Cyanobacterial CCMs (**a**) use bicarbonate (HCO_3^-) and CO_2 pumps on the plasma and thylakoid membranes, respectively, to elevate cytosolic HCO_3^-. This HCO_3^- pool is utilized by icosahedral-shaped Rubisco microcompartments called carboxysomes (yellow icosahedron), where HCO_3^- is converted to CO_2 by localized carbonic anhydrase (CA) and accumulates due to resistive CO_2 efflux. The carboxysome encapsulates the cell's complement of a high-catalytic-rate Rubisco, which operates at close to V_{max}, converting ribulose-1,5-bisphosphate (RuBP) to 3-phosphoglycerate (3-PGA) within the Calvin cycle. This mechanism leads to efficient photosynthesis and reduced nitrogen allocation compared to C_3 plants. A strategy to improve C_3 photosynthesis in plant cells (**b**, represented here as rectangular structures containing dual-membrane chloroplasts) using the cyanobacterial CCM recommends independent transfer of carboxysomes containing their cognate Rubisco to the chloroplast stroma (i) and HCO_3^- pumps (ii) to the chloroplast inner-envelope membrane. Successful transfer of HCO_3^- transporters alone (ii) should generate a moderately elevated stromal HCO_3^- pool (indicated by the change in colour shading of the chloroplast) and has a predicted photosynthetic improvement of 9%[15] due to the resulting net elevation of CO_2 near Rubisco. Expression of functional carboxysomes (i), or just their cognate Rubisco, in the chloroplast stroma should lead to a high CO_2-requiring (HCR) phenotype due to the characteristically high K_M and low specificity for CO_2 of carboxysomal Rubiscos and absence of an elevated HCO_3^- or CO_2 pool. However, in combination (iii), generation of high stromal HCO_3^- pool in the presence of functional carboxysomes, with stromal CA eliminated, is predicted to generate a stromal HCO_3^- concentration approaching 5 mM[16] and to increases in CO_2 fixation and yield of up to 60%[15]. **c** Carboxysomes of *Cyanobium* PCC7001, used in this study, consist of many thousands of polypeptides,

Long et al., Nat Comm 2018

Construcution of a minimal carboxysome in tobacco chloroplasts





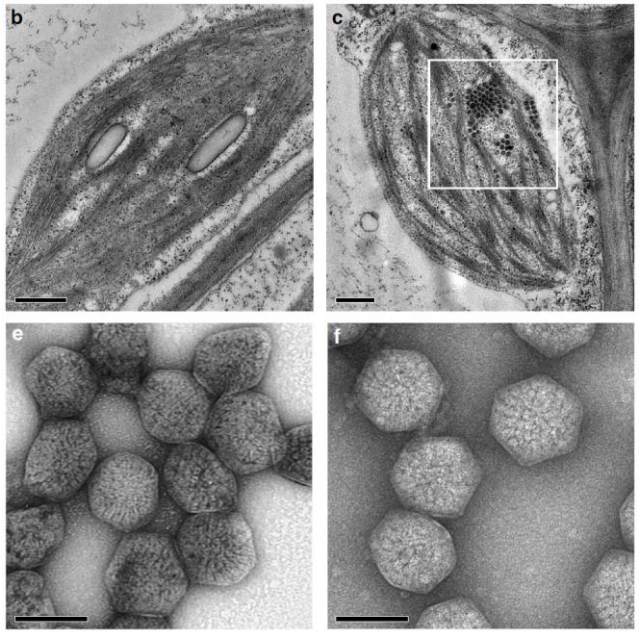
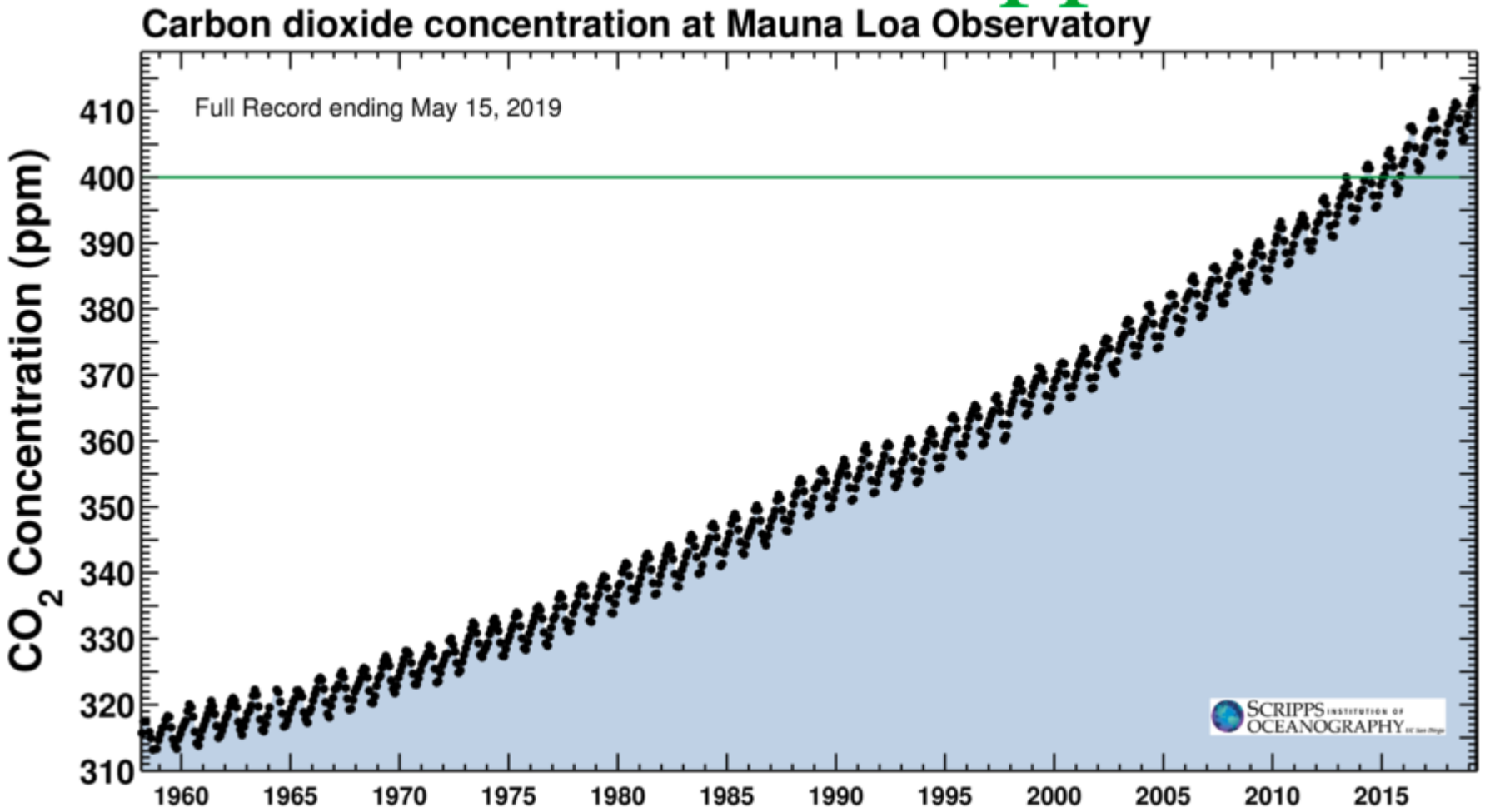


Fig. 2 Cyanobacterial Form-1A Rubisco and carboxysome gene constructs. **a** Plastome content at the Rubisco large subunit locus in the cm^{trL} (*Rhodospirillum rubrum* Rubisco—*rbcM*) master line[37], wild-type tobacco (WT) and transformant plants (CyLS and CyLS-S_1S_2). The recipient line, cm^{trL}, was transformed via homologous recombination with constructs CyLS and CyLS-S_1S_2 (GenBank accession numbers MH051814 and MH051815) containing *Cyanobium* PCC7001 Rubisco *cbbL* and *cbbS*, together with carboxysome shell gene *csoS1A* (*S1A*) and *csoS2* sequences, codon optimized for expression in *N. tabacum* chloroplasts. The *Cyanobium cbbL* sequence was also codon matched to the tobacco *rbcL* gene where there was amino acid identity. Transformation vectors also contained the *aadA* selection marker under control of the *NtPpsbA* promoter to allow growth of transformants on spectinomycin. Each transformation construct was flanked by partial plastome *atpB* and *accD* sequence, and transformants were generated using biolistic bombardment. The locations of promoters (P), terminators (T) and intercistronic expression elements (I) are indicated. P_1, *NtPrbcL*; P_2, *NtPpsbA*; T_1, *NtTpsbA* originally from pcmtrLA[37]; T_2, *NtTrbcL*; T_3, *AtTpetD*; T_4, *NtTrps16*; T_5, *AtTpsbA*; T_6, *CrTrbcL*. A DNA probe was constructed by PCR to anneal to the *accD* flanking region in each plastome (black bar), and the corresponding size of Bsp119I (Bsp) digestion fragments are shown for each genotype (in bp). **b** DNA blots of total leaf DNA digested with restriction enzyme Bsp119I and probed with the *accD* probe, indicating successful insertion of transgenes and loss of the cm^{trL} genotype. DNA fragment sizes in kbp are shown

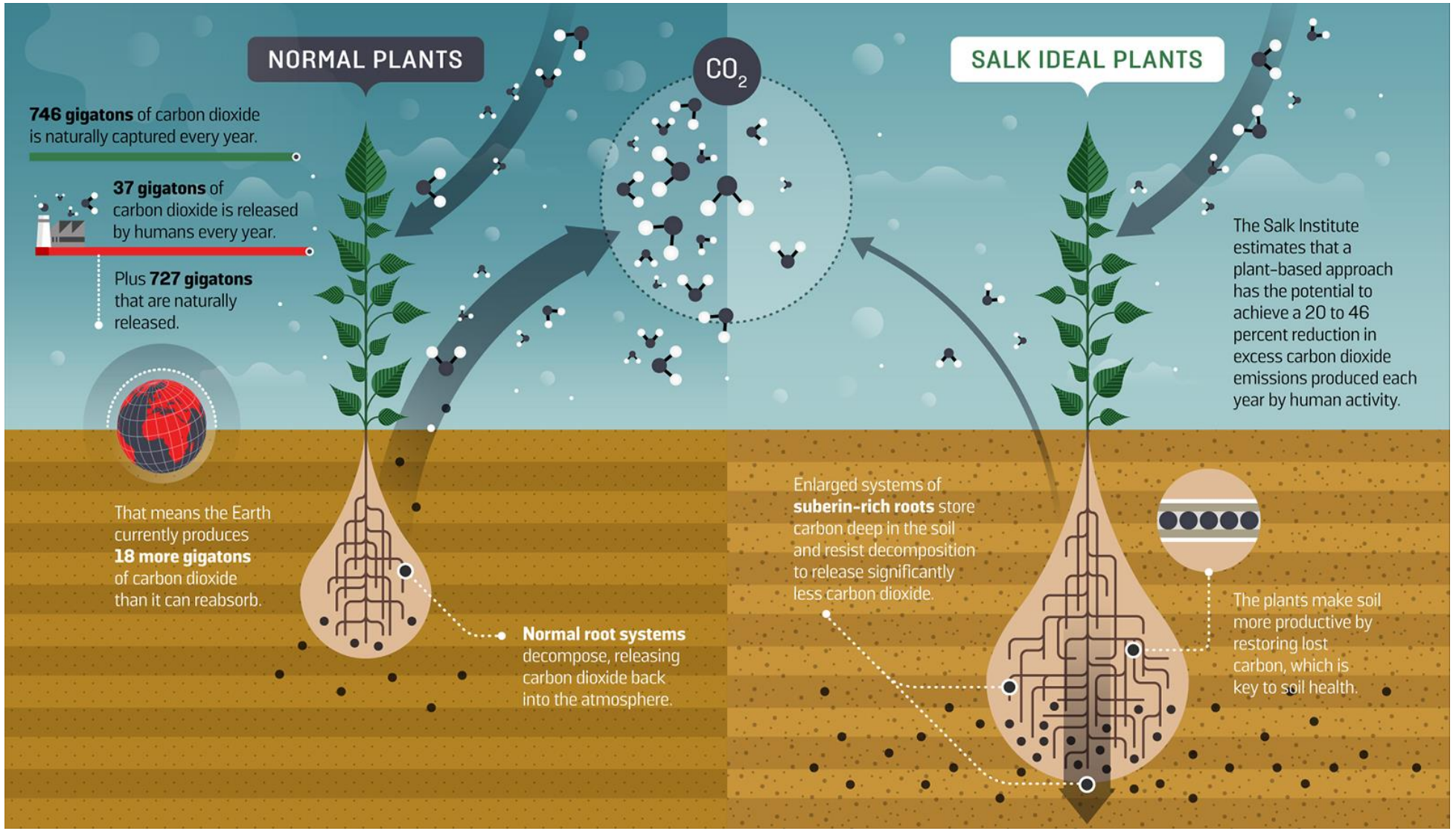
Long et al., Nat Comm 2018

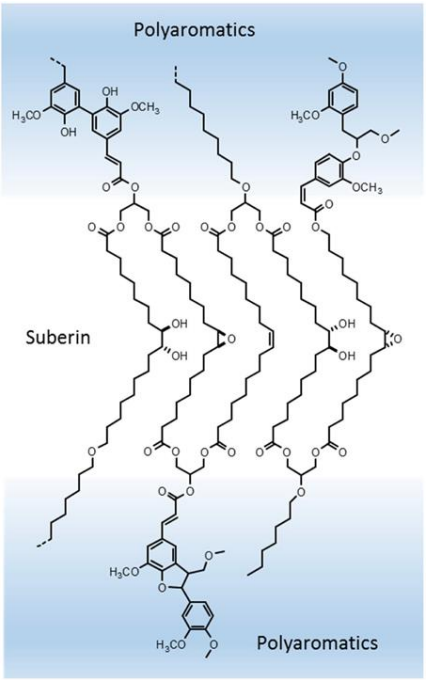
Latest CO_2 reading
May 15, 2019

415.70 ppm



SALK superplant to save the planet





Suberin resists decay.

Recommended reading:

Din M. et al. (2016) Synchronized cycles of bacterial lysis for in vivo delivery. Nature 536:81

Kubis A. and Bar-Even A. (2019) Synthetic biology approaches for improving photosynthesis. J Exp Bot 70:1425-33