Recent Developments in Synthetic Biology

Nikolay Tzvetkov
Bulgarian Ministry of Environment and Water

What is Synthetic Biology?

“Synthetic biology is a further development and new dimension of modern biotechnology that combines science, technology and engineering to facilitate and accelerate the understanding, design, redesign, manufacture and/or modification of genetic materials, living organisms and biological systems” (CBD, 2016)

“Synthetic biology is the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms.” (EU scientific committees (SCHER, SCENIHR, SCCS, 2014)

What is Synthetic Biology?

“Synthetic biology is an emerging area of research that can broadly be described as the design and construction of novel artificial biological pathways, organisms or devices, or the redesign of existing natural biological systems. ” (Royal Society, 2007)

“The defining feature of Synthetic Biology is the introduction into living systems (ecosystems, organism, cells) of functionally important components (organisms, proteins, nucleic acids, lipids, metabolites, etc.) or processes (metabolic or signal transduction pathways, gene circuits, etc.) that do not exist in nature or are significantly different from those that exist in nature. (Bulgarian reply to CBD Notification 2018-103, 2019)

Scope of Synthetic Biology
Enabling Technologies

Synthetic Biology uses many of the technologies that are used in classical Genetic Engineering.

Respectively, many of the technologies used in Synthetic Biology are also used in other fields.

Those technologies are the Enabling Technologies.

The Enabling Technologies are not Synthetic Biology *per se* and do not define the field.

Scope of Synthetic Biology
Enabling Technologies

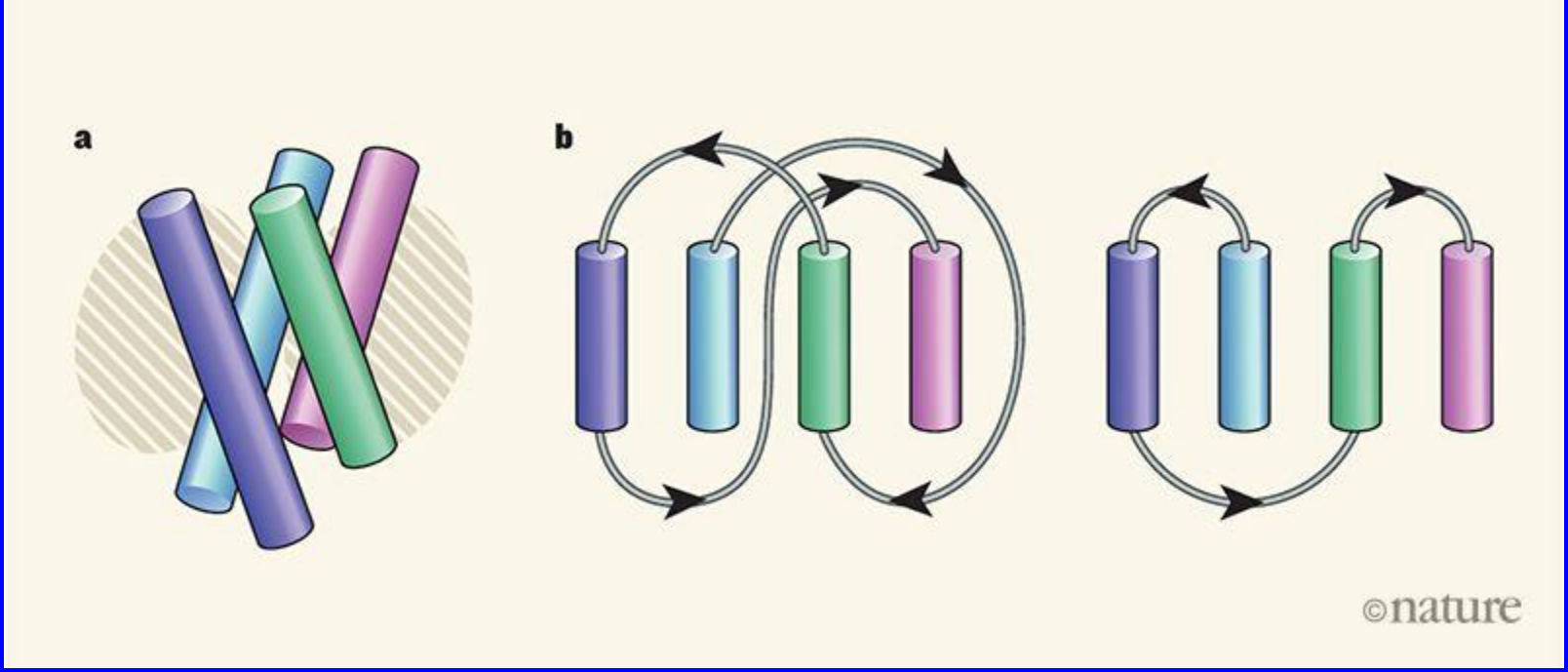
- **DNA sequencing, assembly and annotation**
- **DNA synthesis**
- ***In vivo* manipulation of genomic DNA that allow inter alia simultaneous introduction of small changes at multiple well-defined sites (Gene and Genome Editing)**
- **Computational techniques for *de novo* design of biological macromolecules, metabolic pathways or other biological process with predetermined properties**

Scope of Synthetic Biology Applications

- Design of biological macromolecules (proteins, DNA and RNA) with new properties
- Creation of synthetic biological circuits
- Creation of new metabolic pathways
- Biological macromolecules containing non-natural amino acids and nucleotides (Xenobiology)
- Synthetic genomics
- Minimal and synthetic cells and viruses
- Ecosystem engineering

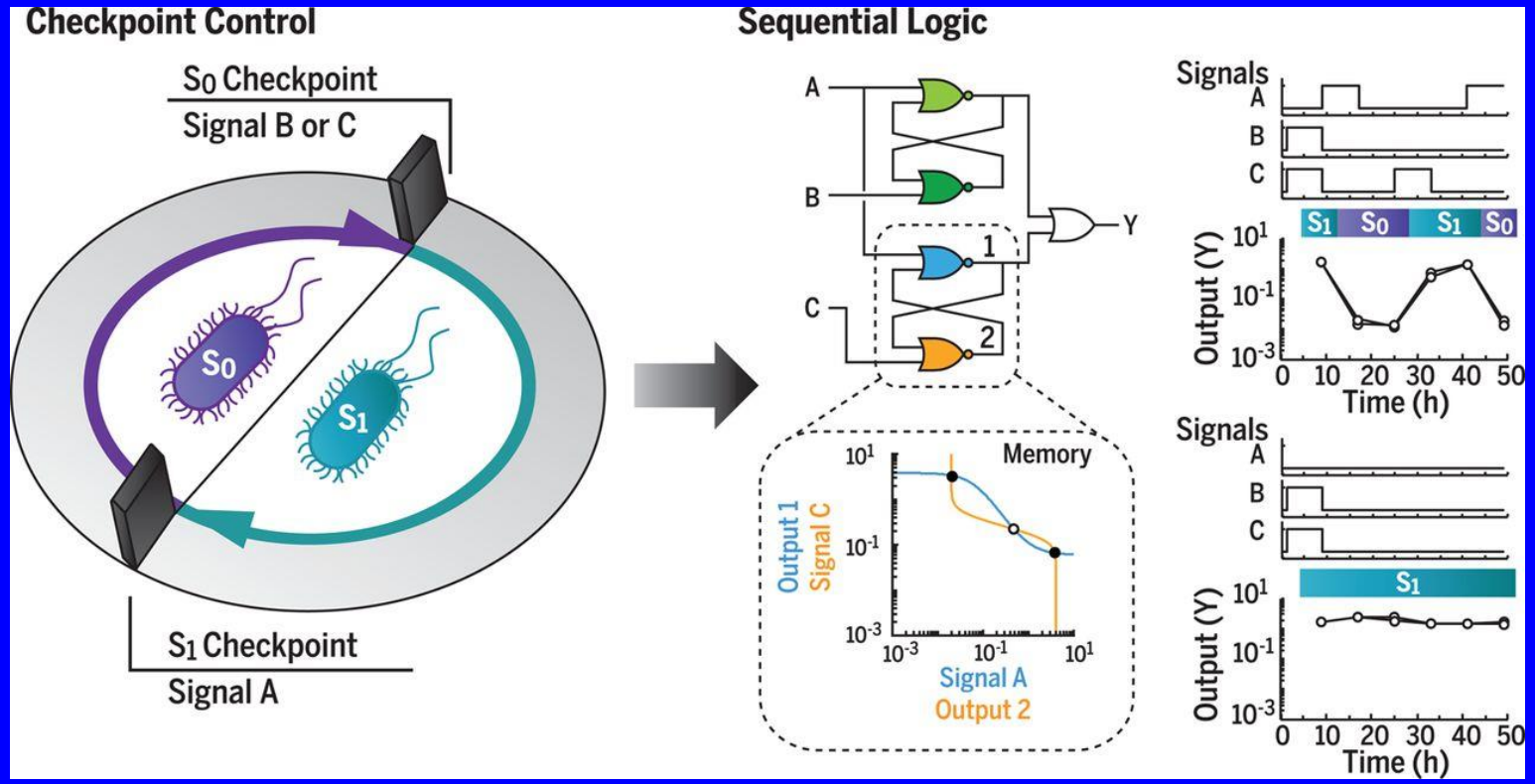
Novel Biological Macromolecules

De novo design of potent and selective mimics of IL-2 and IL-15, Silva *et al.*, Nature 565 (7738), 186-191



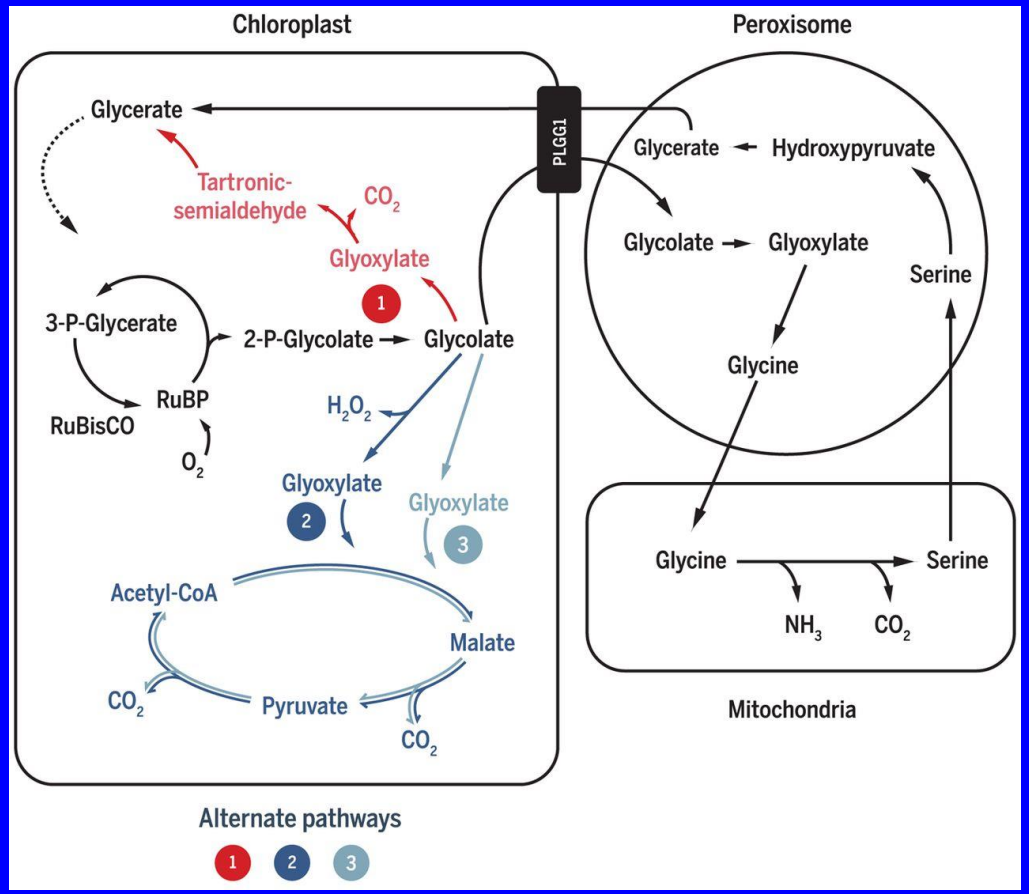
Novel Biological Circuits

Cellular checkpoint control using programmable sequential logic, Andrews, Nielsen and Voigt, Science 361 (6408), eaap8987



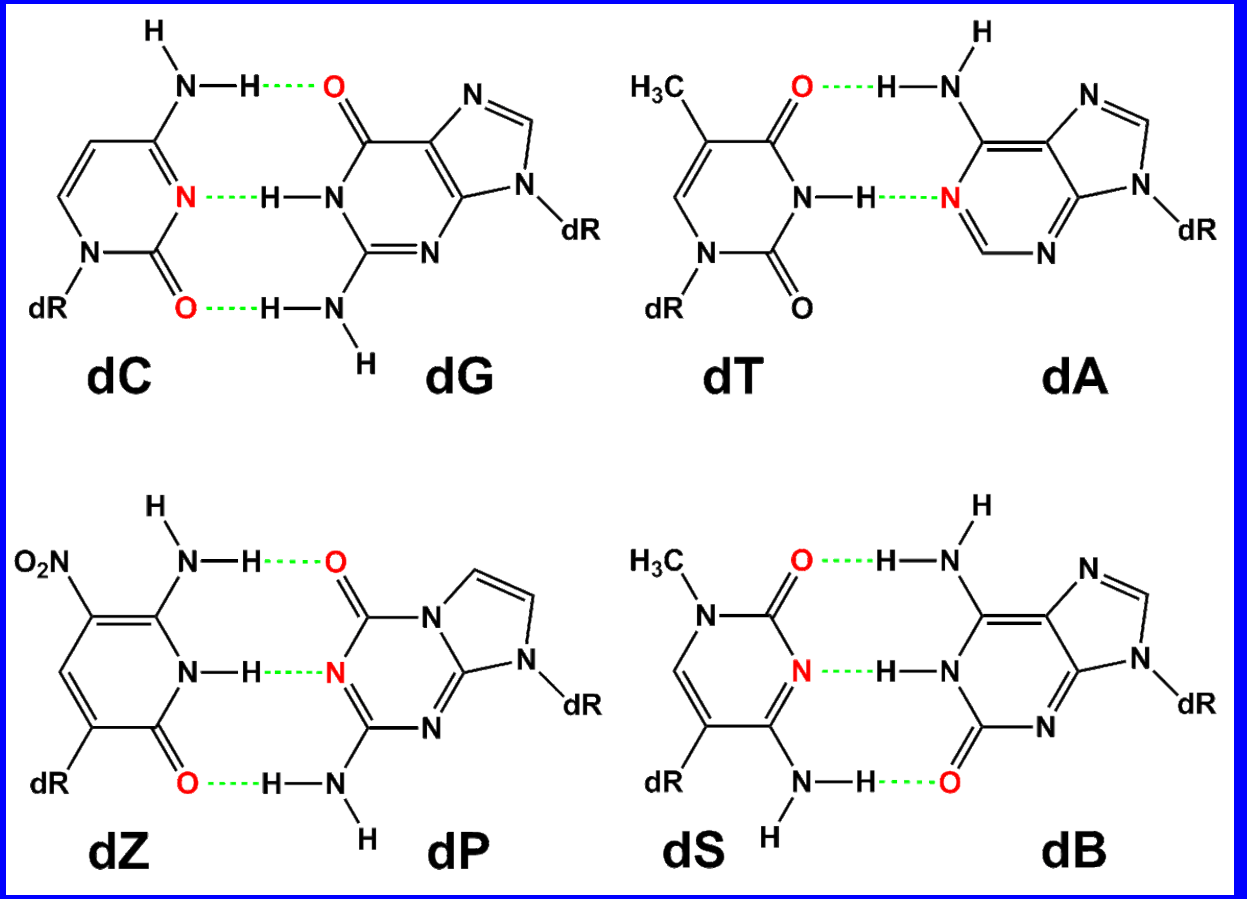
Novel Metabolic Pathways

Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field, South et al., Science 363 (6422), eaat9077



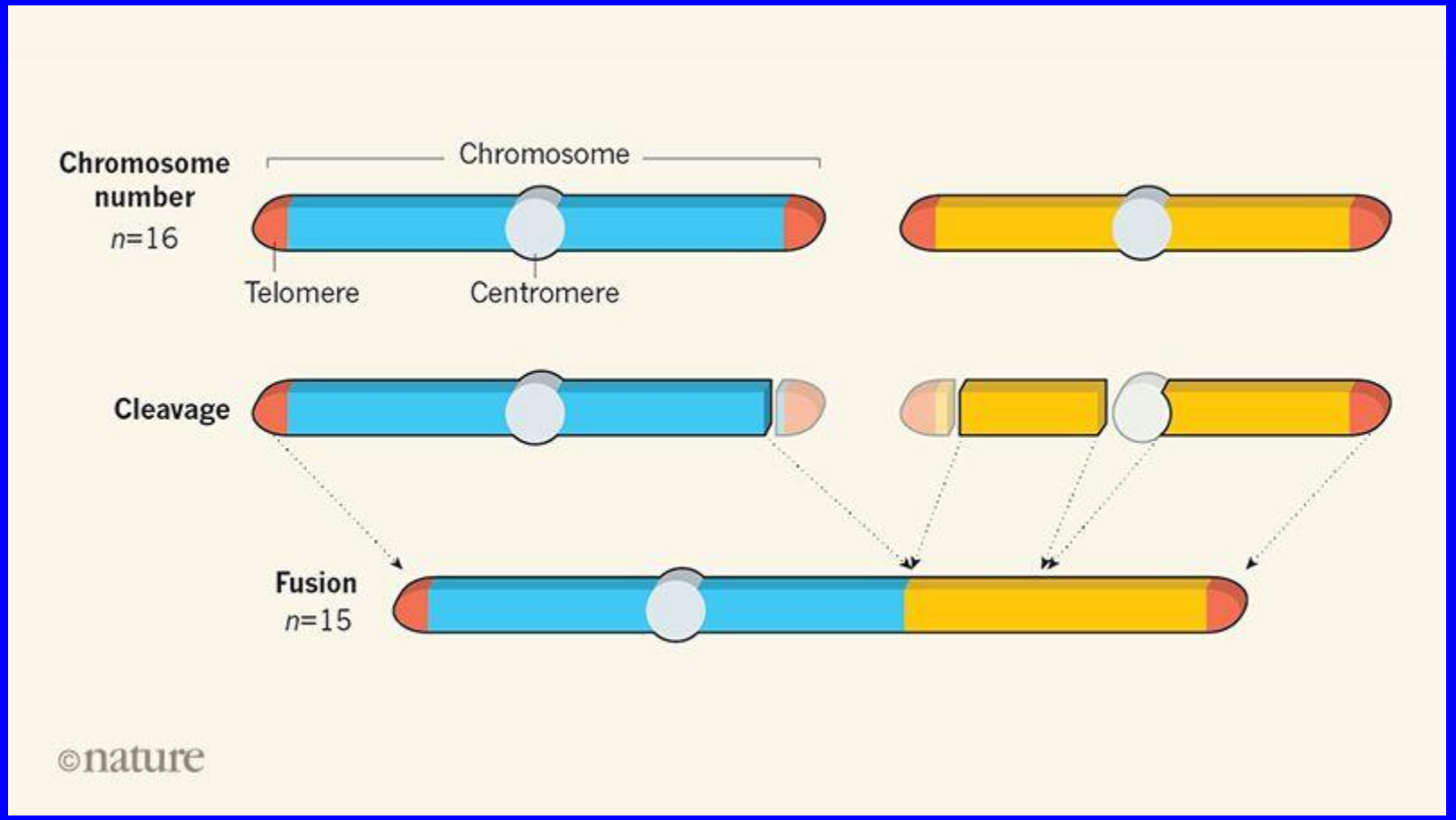
Xenobiology

Hachimoji DNA and RNA: A genetic system with eight building blocks, Hoshika et al., Science 363 (6429), 884-887



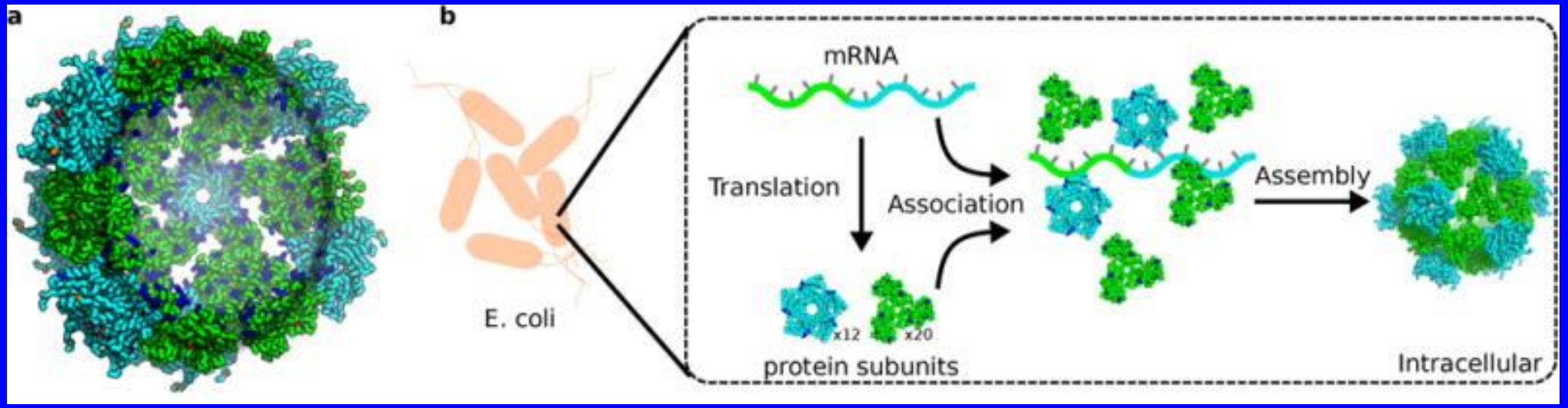
Synthetic genomics

Creating a functional single-chromosome yeast, Shao et al., Nature 560 (7718), 331-335



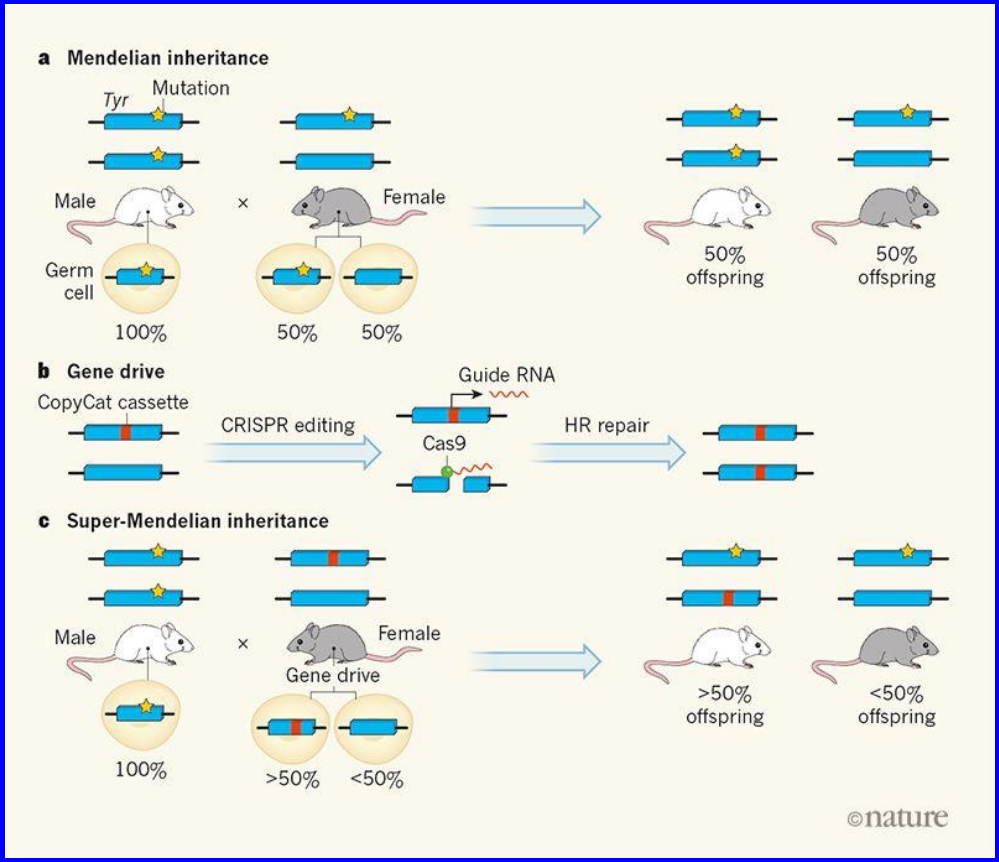
Minimal and synthetic cells and viruses

Evolution of a designed protein assembly encapsulating its own RNA genome, Butterfield et al., Nature 552 (7685), 415-420



Ecosystem engineering (Gene Drives)

Super-Mendelian inheritance mediated by CRISPR–Cas9 in the female mouse germline, Grunwald et al., Nature 566 (7742), 105-109



The Future of Synthetic Biology

- ***In vivo*** **synthesis of non-natural nucleotides and amino acids using** ***de novo*** **designed metabolic pathways and enzymes**

- **Designed viruses that carry various cargo, e.g. novel enzymes repairing defects in the genome, circuits that detect oncogenic changes in the cells, gene drive systems that combine horizontal and vertical inheritance, etc.**

Regulatory Considerations (1)

- The term Synthetic Biology can be misleading
- Engineering Biology is probably a better term to capture the nature of recent developments is modern biotechnology
- Most organisms created through the use of Synthetic Biology are GMOs/LMOs in regulatory sense and the techniques for risk assessment that we use now will be still applicable

Regulatory Considerations (2)

- Living systems are complex
- The profound manipulations done through Synthetic Biology will result every now and then in unexpected effects with unexpected magnitudes
- That will be even more important issue when a few different techniques and approaches are combined

Regulatory Considerations (3)

- When we assess the effects of the organisms developed through Synthetic Biology we will rely more on modelling
- Modelling is always limited and the crucial limit is the scope and quality of empirical date we use
- Balance between the need to obtain data that is a broad as possible under most realistic conditions and the risk of unexpected effects with serious consequences

Regulatory Considerations (Coda)

Bold Precaution

Thank you

Slide 1

The presentation addresses briefly three topics related to Synthetic Biology:

1. What is Synthetic Biology and what is it not?
2. What are different technologies and applications that fall within the scope of Synthetic Biology? I will also try to illustrate each subfield with examples of recent developments, mainly things that have been published last two years 9as long as we can consider that recent).
3. What are the regulatory implications of those developments in Synthetic Biology?

Slide 2

The CBD operational definition of Synthetic Biology adopted in 2016 and definition used by three of the EU scientific committees are very similar. They emphasize on the use of technological and engineering approaches in modifying living organisms.

I find those two definitions to be too broad and too fuzzy, so almost everything in the field of genetic engineering can be considered Synthetic Biology. What is probably much more important is to show what is really new and unique in the recent developments in the field.

Slide 3

The definition of the UK Royal Society and the one used by the Biological Diversity Unit of the Bulgarian Ministry of Environment and Water are focused on the fact that we are at the stage where can start develop living systems and biological materials that go beyond Nature. This is probably the most important, and in my opinion most exciting, feature of Synthetic Biology. We can be creative not only by just combining and recombining what is already out available from the Nature, but we can create truly novel living organisms or novel components with functional importance.

Slide 4

The next important question is what is included in the scope of Synthetic Biology and respectively what is not.

Many of the technologies used in Synthetic Biology are also used in classical Genetic Engineering and other fields of modern biology. Often they are used in exactly the same way.

I will call such technologies Enabling Technologies, but I do not think that those technologies define or are Synthetic Biology as such. Not every application that uses them necessarily falls within the scope of Synthetic Biology.

Slide 5

Some examples of such Enabling Techniques are:

1. DNA sequencing, assembly and annotation that allow very large amounts of data to be generated and interpreted in short time and at low price;
2. DNA synthesis that allows large fragments of DNA to be produced *in vitro* accurately and cheaply;
3. Gene/Genome Editing allows *in vivo* manipulation of genomic DNA, incl. introduction simultaneously of a number of small changes at multiple well-defined sites;
4. *De novo* design of biological macromolecules, metabolic pathways or other biological process with predetermined properties using computational techniques.

All those techniques have other non-Synthetic Biology applications.

Slide 6

We can try to illustrate the scope of Synthetic Biology with some examples of applications within Synthetic Biology and illustrate each of them with some recent example.

Slide 7

Under "Novel Biological Macromolecules" I am referring development of macromolecules, e.g. proteins that can catalyze chemical and metabolic reactions, incl. novel ones, interact with cellular compounds and change their activity, etc. Such molecules can be designed *de novo* using the principles of physical and quantum chemistry and/or can be done by the use of *in vitro* and *in vivo* evolutionary approaches.

The example on the slide is a very elegant study that demonstrates that it is possible to design *de novo* potent and selective mimics of IL-2 and IL-15. Those mimics can be completely unrelated (sequentially and structurally) to their natural counterparts, but are able to interact and activate the same cellular receptors. At the same time they have superior therapeutic properties (IL-2 is used for example to treat some forms of cancer) and significantly reduced side effects.

Slide 8

Synthetic biological circuits can be created using various macromolecules (enzymes, gene expression regulators, etc.) that when combined in specific ways can form regulatory circuits inside the cell with specific properties, e.g. oscillators, logic switches, etc. That allows cells to react in new ways to various stimuli. The creation of such regulatory circuits is enabled by molecular design, information technology and the principles of electronic engineering.

The study shown on the slide demonstrates that it is possible to build programmable genetic sequential logic circuits inside the cell. The state of a circuit depends on the current inputs as well as the input history (memory). Such circuits can perform regulatory functions similar to the biological checkpoint circuits of living cells and allow them to proceed through succession of defined states when appropriate set of stimuli is present.

Slide 9

Novel Metabolic Pathways can be introduced in the cell and new substances can be synthesized or metabolized, e.g. pollutants with a long life into the environment. The creation of those pathways is enabled by the principles of organic chemistry coupled with design of macromolecules with new properties and new regulatory chains.

In the study on the slide, the authors engineered more efficient photorespiratory pathways into tobacco while inhibiting the native pathway. That allowed more efficient recapture of the unproductive by-products of photosynthesis with less energy lost. Even more importantly those transgenic tobacco plants were ~40% more productive than wild-type on the field.

Slide 10

It is possible to introduce in the cellular proteins and nucleic acids novel amino acids and nucleotides that do not occur in nature. This can extend the functional repertoire of those macromolecules.

Example shown presents DNA and RNA-like molecules that contain eight different nucleotides (four natural and four non-natural) that form four orthogonal pairs. Those nucleic acids could

be replicated and transcribed efficiently *in vitro*. That can significantly increase the information density of genome if successfully introduced *in vivo*.

Slide 11

It is already possible to synthesize *in vitro* complete viral and prokaryotic genomes, and eukaryotic chromosomes that in some cases can be successfully introduced into the cell to substitute or supplement its natural genetic material. That allows the genome as a whole to have precisely defined, pre-determined sequence, and hence specific properties.

In the example shown, yeast cells with a single chromosome were created. In that case gene editing techniques were used and not total synthesis of chromosomal DNA. Nevertheless it clearly demonstrates that it is possible to do very profound *in vivo* reorganizations of eukaryotic genomes.

Slide 12

Those studies aim to define the minimum requirements for the existence of cell-like or virus-like life forms using knowledge from the field of physical chemistry, biochemistry, cellular biology and evolutionary biology. Top-down approaches, where components are removed from the cell or the virus can be used to define the minimal set of genes that allow an organism to replicate in a certain environment. Alternatively, using bottom-up approaches, components can be assembled in ways that will result in replicating entities.

The study shown on the slide demonstrates that it is possible to develop synthetic nucleocapsids that can package their own RNA genome inside the cells. This is an example of *de novo* design of virus-like structures by bottom-up approach. While not a real synthetic virus, as it lacks the ability to leave and enter the cells, the entity developed is a very important step in that direction.

Slide 13

It is possible to introduce into the environment organisms that have been modified in such a way that their interactions within their population or with populations of other species are changed in specific ways. Gene drives are an example for such a manipulation, but there are other possibilities as well.

Most of the research on gene drives at present is done on insects (mainly on important disease vectors and pests). The study shown describes the first gene drive system in mammalian species. In principle, it can be possible to develop gene drive system for any sexually reproducing organism.

Slide 14

The future of Synthetic Biology is hard to predict. But some speculations may not be entirely out of place.

I expect that many of that different fields of research will come together and this will increase enormously the opportunities (and the risks) to manipulate living organisms. Two speculative examples might be helpful to illustrate that point:

1. So far in most cases non-natural nucleotides and amino acids that incorporate in the biological macromolecules, should be fed into to the cell from outside. This of course makes such a cell non-viable in natural environment. But a metabolic pathway can be designed, in principle, to produce such molecules *in vivo* . The reactions of such a pathway can be catalyzed by natural enzymes and/or by enzymes designed *de novo*.

2. Another possibility would be to design artificial viruses with synthetic capsids that include proteins that bind to specific cellular receptors. That way one can introduce into specific cells, e.g. cancer cells, various genetic cargo. That can be a novel circuit that will detect if actual oncogenic transformation has occurred and if necessary can kill the cell. Or it can be a system that can repair the DNA defects, if present. One can even imagine that artificial viruses can carry gene drive systems. This will allow certain traits to spread both horizontally (via the virus) and vertically (via sexual reproduction).
Of course, that is only speculation, but if I have to make a guess, we will see developments similar to the ones just described 5-10 years from now.

Slide 15
Finally I want to share some thoughts how those developments in Synthetic Biology can be relevant from regulatory perspective.
The term Synthetic Biology can be misleading, if we want to understand the recent developments in modern biotechnology. If we want to emphasize on the ability to created, to engineer living system with novel properties that do not exist in nature, the term Engineering Biology might be a better one.
Most or probably even all organisms created through the use of Synthetic Biology so far are GMOs/LMOs in regulatory sense and the techniques for risk assessment that we use now will be still applicable.

Slide 16
Living systems are complex by nature. Their manipulation, especially in profound ways as we can using Synthetic Biology, will result in unexpected effects with unexpected magnitudes. That will be even more important issue when a few different techniques and approaches are combined.

Slide 17
In the future, when we assess the effects and risks associated with such organisms developed through Synthetic Biology we will rely more on modelling. Modelling is limited and a critical limitation is the scope and quality of empirical date used to develop and run those models. It will be very important to collect as much data under as realistic conditions as possible. At the same time, we need to find a balance between that objective and the need to minimize the risks of unexpected effects with serious consequences.

Slide 18
This leads me to the end. What I think we need is what I call Bold Precaution.
We should not shy away from releasing organisms developed through Synthetic Biology into the environment, but we should design those releases in such a way that we get as much useful data as possible, with as little risk as possible.
For example, when we talk about gene drives in mosquitos, we may need to releases modified animals that carry the gene drive we plan to use for eradication, but with cargo being GFP or some other useful marker. This will allows us to follow how such gene drive spreads in the environment, in time and space.

Slide 19

With the presentation I tried to illustrate, what I think the most important feature of Synthetic Biology, namely the ability to manipulate living systems in ways that go beyond nature.
Synthetic Biology is not a single technology, but rather a number of application fields, which I tried to illustrate with some recent studies.
Finally, I tried to share some of my thoughts about the challenges that Synthetic Biology poses to the regulators and how we might address them

Thank you for your attention.

Recent publication in the field of Synthetic Biology (up to 30^{h} September 2019)

The list covers some articles published in the period in the period December 2017 –September 2019 and is by no means complete. It represents publications in the field of Synthetic biology that I find interesting.

Macromolecular Design

De novo design of tunable, pH-driven conformational changes, Boyken *et al.*, Science 364 (6441), 658-664
pH-driven conformational transitions with tunable cooperativity and pH set point are created using computational protein design.

Design and evolution of an enzyme with a non-canonical organocatalytic mechanism, Burke *et al.*, Nature 570 (7760), 219-223
An enzyme with a non-canonical organocatalytic mechanism was generated by introducing Nδ-methylhistidine into a designed active site.

Evolution of a designed protein assembly encapsulating its own RNA genome, Butterfield et al., Nature 552 (7685), 415-420
The paper presents the development of synthetic nucleocapsids that can package their own RNA genome. This is an example of de novo design of virus-like structures.

De novo design of a fluorescence-activating β-barrel, Dou *et al.*, Nature 561 (7724), 485-491
The first demonstration of accurate *de novo* design of β-barrel proteins that can bind small molecule ligands and activate them.

Programmable CRISPR-responsive smart materials, English *et al.*, Science 365 (6455), 780-785
CRISPR-associated nucleases are used to control multiscale properties of DNA-based materials.

High-Throughput Investigation of Diverse Junction Elements in RNA Tertiary Folding, Knight Denny *et al.*, Cell 174 (2), 377-390
This paper demonstrates the relationships between sequence, structure and energetic in RNA and can provide basis improved design of RNA molecules with specific functions, e.g. switches.

De novo protein design by citizen scientists, Koepnick *et al.*, Nature 570 (7761), 390-394
Proteins were designed *de novo* by players of the online protein-folding game Foldit, incl. a protein with entirely new fold.

De novo design of bioactive protein switches, Langan *et al.*, Nature 572 (7768), 205-210
A technique for the de novo design of switchable protein systems controlled by induced conformational change is presented.

An ultra-stable gold-coordinated protein cage displaying reversible assembly, Malay *et al.*, Nature 569 (7756), 438-442
A stable artificial protein cage with novel non-natural geometry, whose assembly and disassembly can be controlled is presented.

Induction of Potent Neutralizing Antibody Responses by a Designed Protein Nanoparticle Vaccine for Respiratory Syncytial Virus, Marcandalli *et al.*, Cell 176 (6), 1420-1431
The article describes computationally designed self-assembling nanoparticle that displays multiple copies of a trimeric viral protein that induces potent neutralizing antibody responses.

Random access in large-scale DNA data storage, Organick et al., Nature Biotechnology 36 (3), 242-248
200 MB of digital data is stored in DNA, randomly accessed and recovered using an error-free approach.

Biotechnological mass production of DNA origami, Praetorius *et al.*, Nature 552 (7683) 84-87
This paper presents a method for scalable productions of large DNA molecules of arbitrary sequence that can be used for production of complex nanostructures.

De novo design of potent and selective mimics of IL-2 and IL-15, Silva *et al.*, Nature 565 (7738), 186-191
The paper demonstrates that *de novo* design can used to develop potent and selective mimics of natural signaling molecules. Such molecules can be completely unrelated to the natural counterpart and might have superior therapeutic properties.

Diverse and robust molecular algorithms using reprogrammable DNA self-assembly, Woods *et al.*, Nature 567 (7748), 366-372
A set of 355 self-assembling DNA 'tiles' is presented that can be reprogrammed to implement many different computer algorithms.

Synthetic Circuits and Signaling Pathways

Cellular checkpoint control using programmable sequential logic, Andrews, Nielsen and Voigt, Science 361 (6408), eaap8987
The paper presents genetic circuits that encode sequential logic to instruct cells to proceed through a linear or cyclical sequence of states.

A universal biomolecular integral feedback controller for robust perfect adaptation, Aoki *et al.*, Nature 570 (7762), 533-539
A synthetic gene circuit implementing an integral feedback topology is shown to achieve robust perfect adaptation in living cells.

Complex signal processing in synthetic gene circuits using cooperative regulatory assemblies, Bashor *et al.*, Science 364 (6440), 593-597
Tunable protein interactions are used to build gene circuits with nonlinear behaviors.

A compact synthetic pathway rewires cancer signaling to therapeutic effector release Chung et al., Science 364 (6439), eaat6982
A rationally designed synthetic pathway specifically detects an intracellular oncogenic state and rewires it for therapeutic outputs.

Programmable protein circuits in living cells, Gao *et al.*, Science 361 (6408), 1252-1258
The paper presents a scalable platform to facilitate protein circuit engineering for biotechnological applications based on orthogonal modular proteases.

Evolutionary Convergence of Pathway-Specific Enzyme Expression Stoichiometry, Lalane *et al.*, Cell 173 (3), 749-761
This paper identifies an important principle for building biological pathways that can significantly facilitate development of new synthetic pathways.

De novo design of bioactive protein switches, Langan *et al.*, Nature 572 (7768), 205-210

A technique for the de novo design of switchable protein systems controlled by induced conformational change is presented.

Rock-paper-scissors: Engineered population dynamics increase genetic stability, Liao et al., Science 365 (6457), 1045-1049
An ecological strategy to make stable synthetic gene circuits under selective pressure with high mutation rates is presented. Three strains of bacteria are used, each of which could kill or be killed by one of the other strains.

Engineering Epigenetic Regulation Using Synthetic Read-Write Modules, Park *et al.*, Cell 176 (1), 227-238
A synthetic epigenetic regulatory system in human cells using m6A DNA modification that allows construction of regulatory circuits is described.

Programmable RNA-Guided RNA Effector Proteins Built from Human Parts, Rauch *et al.*, Cell 178 (1), 122-134
A general strategy for engineering programmable RNA effectors is presented.

Engineered promoters enable constant gene expression at any copy number in bacteria, Segall-Shapiro, Sontag and Voigt, Nature Biotechnology 36 (4), 352-358
Designed promoters that utilize incoherent feedforward loop (iFFL) and maintain constant levels of expression at any copy number are presented.

Engineering a Model Cell for Rational Tuning of GPCR Signaling, Shaw *et al.*, Cell 177 (3), 782-796
Signaling pathway is engineered in yeast that can effectively detect various compounds.

Time-resolved protein activation by proximal decaging in living systems, Wang *et al.*, Nature 569 (7757), 509-513
A general strategy for in vivo protein activation using light-controlled proximal decaging is presented.

Circuit design features of a stable two-cell system, Zhou *et al.*, Cell 172 (4), 744-757
This paper demonstrates the principles on which stable cell-circuits can be formed. Similar principles can be used to design novel cell-circuits that do not exist naturally.

Synthetic Metabolic Pathways

Complete biosynthesis of cannabinoids and their unnatural analogues in yeast, Luo *et al.*, Nature 567 (7746), 123-126
The article presents engineering of a complex biosynthetic pathway in yeast.

Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field, South *et al.*, Science 363 (6422), eaat9077
The study demonstrates that engineered metabolic pathways can affect and improve the agricultural properties of plants.

Optogenetic regulation of engineered cellular metabolism for microbial chemical production, Zhao *et al.*, Nature 555 (7698), 683-687
The paper demonstrates the use of engineered metabolic pathways to control fermentation with only light.

Xenobiology (Non-natural Nucleotides and Amino Acids)

Design and evolution of an enzyme with a non-canonical organocatalytic mechanism, Burke *et al.*, Nature 570 (7760), 219-223
An enzyme with a non-canonical organocatalytic mechanism was generated by introducing Nδ-methylhistidine into a designed active site.

Designer membraneless organelles enable codon reassignment of selected mRNAs in eukaryotes, Reinkemeier, Estrada Girona and Lemke, Science 363 (6434), eaat2644
The article presents the design of an artificial, membraneless organelle into mammalian cells to perform orthogonal translation.

Hachimoji DNA and RNA: A genetic system with eight building blocks, Hoshika *et al.*, Science 363 (6429), 884-887
DNA and RNA with expanded genetic code from four to eight nucleotide letters that includes synthetic bases is presented.

Genetically programmed chiral organoborane synthesis, Kan *et al.*, Nature 552 (7683) 132-136
A genetically encoded platform for producing chiral organoboranes in bacteria that expands the chemical reactions that can be carried out in cell is presented.

Controlling orthogonal ribosome subunit interactions enables evolution of new function, Schmied *et al.*, Nature 564 (7736), 444-651
An ingenious *in vivo* system that can be used to evolve proteins with new functions, incl. containing non-natural amino acids is described.

Custom selenoprotein production enabled by laboratory evolution of recoded bacterial strains, Thyer et al., Nature Biotechnology 36 (7), 624-631
Selenocysteine is efficiently incorporated into recombinant proteins using evolved Escherichia coli strains.

Synthetic genomics

Synthetic sequence entanglement augments stability and containment of genetic information in cells, Blazejewski, Ho and Wang, Science 365 (6453), 595-598
Overlapping genes have been synthesized that enhance evolutionary stability and limit lateral dissemination in bacteria.

Total synthesis of *Escherichia coli* with a recoded genome, Fredens *et al.*, Nature 569 (7757), 514-518
Escherichia coli with a 61-codon synthetic genome that uses 59 codons to encode all of the canonical amino acids has been generated.

Human Artificial Chromosomes that Bypass Centromeric DNA, Logsdon *et al.*, Cell 178 (3), 624-639
An improved method to construct Human Artificial Chromosomes (HACs) is described.

Karyotype engineering by chromosome fusion leads to reproductive isolation in yeast, Luo *et al.*, Nature 560 (7718), 392-396

Creation of single chromosome yeast is presented. This demonstrates the feasibility of chromosomal engineering.

Creating a functional single-chromosome yeast, Shao *et al.*, Nature 560 (7718), 331-335
Work similar to Luo *et al.*; another demonstration of practicability of chromosomal engineering.

Programmed chromosome fission and fusion enable precise large-scale genome rearrangement and assembly, Wang *et al.*, Science 365 (6456), 922-929
Technologies to split, reorganize, and combine bacterial chromosomes to facilitate highly programmable genome engineering are explored.

Minimal and synthetic cells and viruses

Evolution of a designed protein assembly encapsulating its own RNA genome, Butterfield *et al.*, Nature 552 (7685), 415-420
The paper presents the development of synthetic nucleocapsids that can package their own RNA genome. This is an example of *de novo* design of virus-like structures.

Ecosystem engineering

Small-Molecule Agonists of *Ae. aegypti* Neuropeptide Y Receptor Block Mosquito Biting, Duvall *et al.*, Cell 176 (4), 687-701
Although not utilizing synthetic biology, the paper demonstrates how small-molecule compounds can be used to control disease vectors through altering their behavior.

Super-Mendelian inheritance mediated by CRISPR–Cas9 in the female mouse germline, Grunwald *et al.*, Nature 566 (7742), 105-109
A gene drive system in mammalians is described.

A CRISPR–Cas9 gene drive targeting doublesex causes complete population suppression in caged Anopheles gambiae mosquitoes, Kyrou et al., Nature Biotechnology 36 (11), 1062-1066
Complete population collapse of malaria vector *Anopheles gambiae* in cages is achieved using a gene drive that targets doublesex.

Rock-paper-scissors: Engineered population dynamics increase genetic stability, Liao et al., Science 365 (6457), 1045-1049
An ecological strategy to make stable synthetic gene circuits under selective pressure with high mutation rates is presented. Three strains of bacteria are used, each of which could kill or be killed by one of the other strains.

Transgenic *Metarhizium* rapidly kills mosquitoes in a malaria-endemic region of Burkina Faso, Lovett *et al.*, Science 364 (6443), 894-897
Transgenically expressing an insect-specific neurotoxin in an insect-pathogenic fungus increases its efficacy against malaria vectors.

Incompatible and sterile insect techniques combined eliminate mosquitoes, Zheng *et al.*, Nature 572 (7767), 56-61
This study demonstrates that mosquito population can be eliminated through inundative mass release of incompatible Wolbachia-infected males, which were also irradiated to sterilize any accidentally-released females.

Techniques

In vivo CRISPR editing with no detectable genome-wide off-target mutations, Akcakaya *et al.*, Nature 561 (7723), 416-419
A highly sensitive strategy that can robustly identify the genome-wide off-target effects of CRISPR–Cas nucleases *in vivo* is described. The paper shows show that appropriately designed guide RNAs can direct efficient *in vivo* editing with no detectable off-target mutations.

High aspect ratio nanomaterials enable delivery of functional genetic material without DNA integration in mature plant, Demirer et al., Nature Nanotechnology 14 (5), 456-464
High aspect ratio nanomaterials enable efficient delivery of DNA into mature plant cells in a species-independent and non-integrating manner for plant genetic engineering applications.

VEGAS as a Platform for Facile Directed Evolution in Mammalian Cells, English *et al.*, Cell 178 (3), 748-761
A system for directed evolution in mammalian cells is presented.

One-step genome editing of elite crop germplasm during haploid induction, Kelliher et al., Nature Biotechnology 37 (3), 287-289
Genome editing is induced in seeds during haploid induction, thereby enabling genome editing in monocot and dicot crops.

Transposon-encoded CRISPR–Cas systems direct RNA-guided DNA integration, Klompe *et al.*, Nature 571 (7764), 219-225
A programmable transposase integrates donor DNA at user-defined genomic target sites with high fidelity.

De novo DNA synthesis using polymerase-nucleotide conjugates, Palluk et al., Nature Biotechnology 36 (7), 645-650
An enzymatic approach enables synthesis of a defined DNA sequence using TdT with reversibly tethered dNTPs.

Scalable, Continuous Evolution of Genes at Mutation Rates above Genomic Error Thresholds, Ravikumar *et al.*, Cell 175 (7), 1946-1957
A system for scalable, continuous evolution of user-defined genes in vivo is described that allows routine, high-throughput evolution of biomolecular and cellular function to be carried out.

Controlling orthogonal ribosome subunit interactions enables evolution of new function, Schmied *et al.*, Nature 564 (7736), 444-651
An ingenious *in vivo* system that can be used to evolve proteins with new functions, incl. containing non-natural amino acids is described.

Predictable and precise template-free CRISPR editing of pathogenic variants, Shen *et al.*, Nature 563 (7733), 646-651
The paper presents an approach for precise, template-free genome editing.

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