ZKBS
Zentrale Kommission für die Biologische Sicherheit

September 2025

Monitoring Synthetic Biology 2024

Highlights

In 2024, the ZKBS working group on Synthetic Biology selected **225 publications** from the monitoring process for detailed analysis. Of these, **28 were chosen** to be presented as short summaries on the ZKBS-homepage, offering an overview on and insight into the global developments in the field of Synthetic Biology.

The conclusion from the monitoring is that all research approaches as of December 31, 2024 considered here from the research fields of synthetic biology defined by the ZKBS are still regulated by existing legal regulations, in particular the GenTG.

Mirror Life

The ZKBS took notice of the comment on the development of "**mirror bacteria**" by Adamala *et al.* [1] published as a policy forum in Science in December 2024 and authored by 38 scientists from different countries and disciplines, as well as of the accompanying technical report on mirror bacteria [2].

The comment outlines potential risks posed by artificially created mirror bacteria composed of mirror-image amino acids, sugars, etc. According to the authors, the technical realization of mirror life is still at least a decade away. However, they wish to engage early with the scientific community, policymakers, research funders, industry, civil society, and the public to discuss potential negative aspects of mirror bacteria, e. g. immune evasion or harmful effects to ecosystems. The authors recommend that mirror organisms should not be created unless there is evidence that mirror life would not pose extraordinary dangers. For related technologies, which might be used to create potential therapeutic substances, the authors do not recommend any restrictions.

The ZKBS Working Group on Synthetic Biology has intensively discussed the comment and has published a first assessment on its homepage. As "mirror life" is going to be a matter of scientific as well as societal debate, ZKBS will consecutively and considerately monitor mirror life's worldwide development and report on this issue at ZKBS' website.

Two Highlight Publications

The ZKBS would like to consider two original articles as Synthetic Biology highlights in 2024.

At first, **Chen *et al.*** [3] reported on the first multi-cellular organism with a partially artificial chromosome. The terrestrial moss *Physcomitrium patens´* chromosome 18 was chemically synthesized and significantly reduced in size. Following this reduction, the moss grew without discernible phenotyping effects. The work is planned to be continued in the synthetic moss genome project that aims at ultimately replacing the full genome with a synthetic one.

The second paper the ZKBS would like to highlight is the work of **Gao *et al.*** [4], in which photosynthetic cyanobacteria modified to secrete glucose and ATP were introduced as endosymbionts into yeast cells. The yeast cells being mitochondria-deficient could only survive without external carbon sources when the cyanobacteria supplied glucose and ATP. These chimeras were stably formed, grew using solely CO_2 and light, and were able to produce natural biosynthetic products under photosynthetic conditions.

Detailed report on the monitoring of individual papers in the respective category of Synthetic Biology

Synthesis of genes and genomes

A designer synthetic chromosome fragment functions in moss (Chen *et. al.* 2024) [3]: The authors have constructed the first living multi-cellular organism, the terrestrial moss *Physcomitrium patens*, that carries a partial artificial chromosome. A region of 155 kilobases (kb), which spans about one third of an arm of chromosome 18 was replaced with a redesigned, simplified, chemically synthesized fragment of roughly 68 kb. The semi-syn18L moss shows normal wildtype growth, produces spores and maintains an epigenetic landscape similar to the wildtype. Chen *et al*. substantially simplified the genome without discernible phenotypic effects, implying that many transposable elements may minimally impact growth. They also introduced other sequence modifications, such as PCR tags, gene locus swapping and stop codon substitutions. The results lay the foundation for the synthetic moss genome project (SynMoss). The next phase of the project will focus on the complete replacement of chromosome 18 with chemically synthesized sequences, followed by full synthetic genome replacement.

Open-ended molecular recording of sequential cellular events into DNA (Loveless *et al.*, 2025) [5]: The authors present the DNA recorder peCHYRON (**p**rime **e**diting **C**ell **H**istor**Y** **R**ecording by **O**rdered **N**ucleotide insertion), that inserts durable mutations in a cell´s genome upon transient biological events, that can later be reconstructed by DNA sequencing. The system uses a prime editor, a nickase Cas9-reverse transcriptase fusion that can insert precise mutations specified by a prime editing guide RNA (pegRNA). peCHYRON functions as follows: a pegRNA targets a 20 bp locus in a cell´s genome and inserts a variable 3-nt sequence encoding up to 6 bits of information together with a 17-nt propagator sequence that serves as the target for the next insertion step. After each insertion, the previous propagator sequence is no longer adjacent to the PAM-sequence and thus inactive, while the new propagator sequence is adjacent to the PAM and active. Each editing step inserts a new 3-nt sequence with the new propagator sequence as a record of an event. Insertions are sequential and can be propagated without end. This allows the temporally resolved recording of multiple cellular signals in mammalian cells. The authors showed that the constitutive expression of pegRNA collections generates insertion patterns for the straightforward reconstruction of cell lineage relationships whereas the inducible expression of specific pegRNAs results in the accurate recording of exposures to specific biological stimuli.

Convenient synthesis and delivery of a megabase-scale designer accessory chromosome empower biosynthetic capacity (Ma *et al.* 2025) [6]: Reconstructing complex traits in new host organisms using large-scale genetic information remains a significant challenge. In their work Ma *et al.* developed a CRISPR/Cas9-mediated haploidization method that bypasses the natural

process of meiosis. Based on the programmed haploidization in yeast, they further developed a method designated HAnDy (Haploidization-based DNA Assembly and Delivery in yeast) that enables efficient assembly and delivery of large synthetic DNA, with no need for elaborate *in vitro* manipulations. Using HAnDy, a *de novo* designed 1,024 Mb synthetic accessory chromosome (synAC) encoding 542 exogenous genes was parallelly assembled and then directly transferred to six phylogenetically diverse yeasts. The synAC enhances host adaptation and expands the metabolic network, enabling the production of valuable compounds. This approach should facilitate the assembly and delivery of largescale DNA for expanding and deciphering complex biological functions.

Synthesis of molecular switches and genetic circuits

Engineered receptors for soluble cellular communication and disease sensing (Piraner *et al.* 2025) [7]: The authors modified the so-called synthetic intramembrane proteolysis receptor (SNIPR) (Zhu *et al.* 2022) [8], a receptor that can be activated not only by membrane-bound ligands, but also by soluble ones. SNIPR uses domains from the regulated intramembrane proteolysis proteins Notch and Robo1 coupled to a ligand binding domain such as single-chain variable fragment or nanobody and a transcription factor that is activated when a ligand is bound. It binds natural and synthetic soluble factors. SNIPR was shown to localize chimeric antigen receptor T cells to solid tumours expressing soluble disease-associated factors. Furthermore, the authors developed orthogonal synthetic signalling networks for cellular communication and environmental interactions. SNIPRs could be used in cells for therapeutic purposes or to study biological interactions.

Synthetic protein circuits for programmable control of mammalian cell death (Xia *et al.* 2024) [9]: The authors built a genetic circuit that induces cell death in mammalian cells. Cells can regulate cell death amongst others via either apoptosis or pyroptosis. Unlike apoptosis, which does not alert the immune system, pyroptosis releases damage-associated molecular patterns that stimulate the immune system and cause inflammation. The circuit termed “synpoptosis” circuit was built using modified apoptosis- (caspase-3) and pyroptosis (gasdermin)-inducing proteins. Both proteins were modified for protease-controlled expression/repression and showed the desired action in different cell types. The synpoptosis circuit was also modified to selectively recognize and eliminate specific target cells or as a tool for the establishment of synthetic killer cells. For this purpose, sender cells were engineered to express virus-like particles containing the synpoptosis circuit to enter and eliminate receiver cells. The synpoptosis circuit is thus a step forwards to programmable control of mammalian cell death, for example in a future context of treating cancer, autoimmune diseases and infection.

Synthesis of customized metabolic pathways

Advancing the scale of synthetic biology via cross-species transfer of cellular functions enabled by iModulon engraftment (Choe *et al.* 2024) [10]: The authors analysed transcriptome databases and identified so-called iModulons. These are sets of co-expressed but potentially independently regulated genes that are responsible for a specific cellular metabolic function. Genes that have not yet been annotated are also recorded. The identification of iModulons enables the precise identification of genes that are required for the transfer of complex cellular

functions between different species. iModulons can be further improved with adaptive laboratory evolution, so that the new host organism makes optimal use of the transferred metabolic pathway under selection pressure and that host factors which need to be adapted can be identified. In the present approach, three metabolic pathways and one antibiotic resistance trait were transferred from *Pseudomonas* to *E. coli*. This cross-species transfer of metabolic pathways is fast and precise and could be a valuable tool to generate specific production strains.

De novo design of proteins housing excitonically coupled chlorophyll special pairs (Ennist *et al.* 2024) [11]: Ennist *et al.* have functionally incorporated the central reaction centre of the native photosynthetic LH1−RC complex from purple nonsulfur bacteria (*Blastochloris viridis*), consisting of a synthetic 'special pair' of chlorophyll, into designer proteins. The special pair is excited by light energy, and the resulting charge separation initiates the electron transfer cascade that leads to the production of energy-rich compounds in the cell. The authors synthesised a c2-symmetrical protein that binds two chlorophyll molecules close together and in the same orientation as in the native special pair. Spectroscopy showed that the chlorophylls are excitonically coupled, and fluorescence lifetime imaging demonstrated energy transfer. Due to its symmetry, the synthetic special pair could also be successfully incorporated into larger subcomplexes. An octahedral nanocage generated with the synthetic protein, 24 synthetic chlorophyll molecules, and a special pair at each end is a first step towards a *de novo* design of photosynthetic compartments corresponding to thylakoids or chromatophore vesicles. The study lays the foundation for investigating the photophysics of special pairs, regardless of the complexity of native photosynthetic proteins, and represents the first step towards the development of synthetic photosystems for new energy conversion technologies.

New-to-nature CO_2-dependent acetyl-CoA assimilation enabled by an engineered B12-dependent acyl-CoA mutase (Schulz-Mirbach *et al.* 2024) [12]: Acetyl-CoA is a key intermediate in C1 carbon assimilation, in which C1 is incorporated into higher compounds and biomass after its formation. In aerobic metabolic pathways, a part of the previously fixed CO_2 is released again during further processing. In the present study, the authors have developed the synthetic lactyl-CoA mutase (Lcm) module to prevent this loss. The Lcm module establishes a direct connection between acetyl-CoA and pyruvate without releasing CO_2. To achieve this, a new synthetic coenzyme B12-dependent mutase was used, which converts 3-hydroxypropionyl-CoA into lactyl-CoA. The 2-hydroxyisobutyryl-CoA mutase from *Bacillus massiliosenegalensis* was used as a basis. The catalytic efficiency of Lcm in *E. coli* was improved via adaptive evolution and hypermutation with the eMutaT7 system. The Lcm module still needs improvement of its efficiency, stability, and oxygen tolerance, but is thought to be used as an alternative route for various microbial production applications in the future.

Proto-, minimal-, and synthetic cells

Genetically programmed synthetic cells for thermo-responsive protein synthesis and cargo release (Monck *et al.* 2024) [13]: The paper describes the development of synthetic cells that control protein expression through a temperature-sensitive mRNA element. The authors used an RNA thermometer, consisting of a ribosome binding site (RBS) and a complementary anti-RBS sequence located in the 5´ untranslated region between promoter and gene of interest (Jia *et al.* 2019) [14]. The complementary sequences form a hairpin that is only opened when a certain temperature is reached. This system was used in combination with a cell-free protein expression

system in giant unilamellar vesicles. As a proof-of-concept, the pore protein α-hemolysin from *Staphylococcus aureus* was expressed under control of the thermometer. Upon temperature elevation, the vesicles synthesized the pore protein and a small fluorescent cargo protein was released from the vesicles. This technique could be used for example in biomedicine to create customizable synthetic cells that release their cargo upon a specific temperature.

3D DNA origami pincers that multitask on giant unilamellar vesicles (Zhan *et al.* 2024) [15]: The authors developed artificial, programmable DNA origami structures, so-called DNA origami pincers (DOPs), that enable the targeted control of the morphology of giant unilamellar vesicles (GUVs). The DOPs consist of three DNA bundles, with their inter-bundle angle precisely tuneable via the incorporation of specific DNA locking strands. Depending on the programmed pinching angle, varying degrees of membrane deformation were induced in the GUVs. Furthermore, multiple DOP units were interconnected on the vesicle surface, leading to the self-assembly of higher-order cage-like DNA architectures. During this oligomerization process, transient membrane pores were formed, allowing the influx of small molecules into the vesicle interior. The DNA cages were also capable of capturing lipid fragments and subsequently detaching from the membrane. This work demonstrates how DNA nanostructures could be employed in the future to enable precise membrane remodelling, controlled molecular transport, or the construction of synthetic cell-like systems.

Artificial membraneless organelles and compartments

Spatiotemporal regulation of cell fate in living systems using photoactivatable artificial DNA membraneless organelles (**Zhang *et al*. 2024) [16]:** The authors developed synthetic, DNA-based coacervates that function as membraneless organelles. These structures consist of long single-stranded DNA molecules generated via rolling circle amplification (RCA), which condense into stable coacervate droplets through liquid–liquid phase separation. By incorporating photoactive palladium nanoparticles, the coacervates enabled targeted drug release, such as doxorubicin, upon near-infrared irradiation. The particles were produced *in vitro* and introduced into murine tumor cells (4T1 cells) via endocytosis or injected intravenously into mice. There, they allowed for light-dependent, spatiotemporal release of the drug and selectively induced programmed cell death in tumor tissue.

Dipeptide coacervates as artificial membraneless organelles for bioorthogonal catalysis (Cao *et al*. 2024) [17]: The authors developed artificial coacervate droplets based on the dipeptide FF-OMe, a diphenylalanine (FF) derivative bearing a methoxy group (OMe) at its C-terminus. These droplets form via pH-dependent self-association and create a hydrophobic microenvironment that facilitates the encapsulation and catalytic activity of enzymes and hydrophobic catalysts in aqueous media. Used as microreactors, they significantly enhanced the efficiency of enzymatic and metal-catalyzed reactions in water. By combining them with polyelectrolyte-based coacervates, cell-like multicompartment systems were generated, in which the FF-OMe coacervates functioned as artificial organelles. Through chemical dimerization, the coacervates were stabilized and successfully introduced into HeLa cells, where they exhibited bio-orthogonal catalytic activity without affecting cell viability. The study demonstrates that minimalist peptide coacervates can serve as versatile, cell-compatible reaction compartments and artificial organelles in both cell-free systems and living cells.

Co-transcriptional production of programmable RNA condensates and synthetic organelles (Fabrini *et al*. 2024) [18]: The authors developed a modular system for the formation of programmable RNA condensates based on synthetic RNA nanostructures. These so-called RNA nanostars are composed of a single RNA strand that folds into a four-armed, star-shaped structure during transcription. Through specific base-pairing interactions, these nanostars self-assemble into larger complexes. Both *in vitro* and in synthetic cells (water-in-oil droplets), they formed membraneless RNA organelles with defined size, number, and composition. Embedded RNA aptamers enabled the targeted capture of fluorophores and proteins such as EYFP and streptavidin. The introduction of specialized linker RNA altered the interactions between condensates and allowed for the controlled formation of multiphase, structured assemblies. This study demonstrates how RNA nanotechnology can be used to create cell-like compartments suitable for the selective recruitment of functional molecules.

Towards chloroplastic nanofactories: formation of proteinaceous scaffolds for metabolic engineering (Dwyer *et al*. 2024) [19]: The authors established a synthetic bacterial microcompartment (BMC) within the chloroplasts of *Arabidopsis thaliana*. Due to its resemblance to a porous plastic ball, an icosahedral hollow structure with regular pores, it was termed a minimal wiffle ball. To construct it, two shell proteins (BMC-H and BMC-T1) from *Haliangium ochraceum* were genetically engineered to autonomously assemble into 40 nm icosahedral structures after import into chloroplasts. These structures resembled BMC shells and did not impair plant growth or chloroplast function. The study demonstrates that BMCs can be stably assembled as protein-based sub-compartments in plant cells and may be used to specifically modulate and optimize plant metabolic processes.

Nucleated synthetic cells with genetically driven intercompartment communication (Ioannou *et al*. 2024) [20]: The authors developed an easy-to-implement method for constructing nucleated synthetic cells (nSynCells) in the form of vesicle-in-vesicle structures. The nuclear and cytoplasmic compartments could be selectively loaded with different biochemical components. Within the inner compartment, the pore-forming protein α-hemolysin was constitutively expressed using the commercial PURExpress® TX-TL system and an α-hemolysin DNA plasmid. This protein formed functional pores exclusively in the inner membrane, enabling the directed diffusion of small molecules from the outer to the inner compartment. There, the signalling molecules could react with encapsulated enzymes and trigger an enzymatic reaction. The method requires no microfluidics and is based on a straightforward emulsion-centrifugation protocol. According to the authors, this represents the first demonstration of genetically programmed communication between compartments in synthetic cells, in which communication is enabled only after *in situ* expression of a membrane pore protein.

<u>Artificial endosymbiosis</u>

In 2024, three studies were published that describe the artificial reconstruction of endosymbiotic processes in eukaryotic cells. In these studies, both yeast and mammalian cells were made photosynthetically active by incorporating cyanobacteria or chloroplasts of algae. In Gao *et al*. [4], genetically engineered cyanobacteria (*Synechococcus elongatus* PCC7942) capable of secreting ATP and glucose were introduced into mitochondria-deficient yeast cells, resulting in stable yeast–cyanobacteria chimeras that could grow solely with light and CO_2. Hu *et al*. [21] first introduced *Synechocystis* PCC 6803 into murine macrophages and later into non-phagocytic mammalian cells, with human HEK293 cells proving particularly suitable for establishing long-

term endosymbiotic relationships. In Aoki *et al*. [22], chloroplasts from the red alga *Cyanidioschyzon merolae* were incorporated into hamster cells (CHO-K1) through co-cultivation, where they retained their internal thylakoid structure and photosynthetic activity for up to two days.

Xenobiology

Efficient genetic code expansion without host genome modification (Costello *et al.* 2024) [23]: Quadruplet codon usage is a strategy for genetic code expansion with non-native codons that is often less efficient than triplet decoding. The authors developed a strategy to improve incorporation of non-canonical amino acids (ncAAs) into proteins without altering the *Escherichia coli* host cell. They used a superfolder GFP reporter, in which the permissive Y151 was replaced by a quadruplet codon. This design will only allow GFP expression when the quadruplet codon is read by a tRNA. Furthermore, the codon usage of the five adjacent residues upstream and downstream was modified. With this approach, it could be shown that quadruplet decoding has a codon usage bias with a strong preference for a high-usage codon 3´ to the quadruplet codon and that off-target frameshifts could be avoided when high-usage codons were used. Furthermore, 12 mutually orthogonal transfer RNA (tRNA)-synthetase pairs were identified to incorporate ncAAs into proteins and five were evolved so that they can incorporate a repertoire of ncAAs at orthogonal quadruplet codons. Using these strategies, the authors built an *in vivo* biosynthesis platform to create > 100 new-to-nature macrocyclic peptides with up to three unique ncAAs. This could be a step towards building chemically diverse polymers in a programmable manner.

Adding α,α-disubstituted and β-linked monomers to the genetic code of an organism (Dunkelmann *et al.* 2024) [24]: To incorporate non-canonical monomers (ncMs, non-canonical amino acids or other molecules that are normally not incorporated into proteins) into proteins an orthogonal tRNA synthetase has to acylate the ncM to an orthogonal tRNA, which will then be bound by the ribosome for translation. ncM are often poor ribosomal substrates and cannot be acylated to the orthogonal tRNA efficiently. The authors developed a strategy to identify tRNA variants capable of ncM incorporation. They established a method that detects the acylation status of a tRNA, thus detecting whether the ncM is loaded onto the tRNA. Next, a fusion of a split tRNA and the mRNA of a pyrrolysyl-tRNA synthetase (PylRS) was created that allowed correlating the mRNA synthetase sequence with its activity. To identify new PylRS variants capable of incorporating ncMs, large libraries of these hybrid RNAs with mutated residues in the PylRS active site were generated and cultured with ncMs that are either non-translatable or poor ribosomal substrates. The acylated hybrid RNAs were then isolated and reverse transcribed to obtain the PylRS gene responsible for acylation. Using this approach, PylRS for the ncM classes β-amino acids, α,α-disubstituted amino acids and β-hydroxy acids were identified and some of them incorporated into proteins. This incorporation of new ncMs into proteins may be useful in creating new drug-like molecules in living cells.

Designing artificial fluorescent proteins and biosensors by genetically encoding molecular rotor-based amino acids (Hu *et al.* 2024) [25]: Fluorophores in fluorescent proteins like GFP are encoded by the primary amino acid sequence, that folds into a fluorescent rotor structure upon protein folding followed by spontaneous cyclization. The authors designed fluorogenic non-canonical amino acids with this rotor structure, the so-called fluorescent molecular rotor amino

acids (FMR-AAs). When integrated into a protein via expanded genetic code, the fluorophores transform proteins into fluorescent proteins. The technique was used to establish biosensors in mammalian cells, that detect, for example, changes in intracellular calcium concentration or protein interactions. That way, customized fluorescent proteins for molecular visualization and investigation can be created.

Methods with impact on Synthetic Biology

Establishing a synthetic orthogonal replication system enables accelerated evolution in *E. coli* (Tian *et al.* 2024) [26]: The authors established a synthetic DNA replication system in living *Escherichia coli* bacteria, that works independently of the host cell`s natural replication process. It is based on four genes essential for *in vivo*-replication of the bacteriophage PRD1 genome combined into a single replication operon controlled by an IPTG-inducible promoter and not copied by host polymerases. The operon-bearing *E. coli* were able to replicate a linear replicon, in which a *gfp* and a kanamycin resistance gene were expressed under control of constitutive promoters flanked by bacteriophage ITR sequences. The efficiency of linear replicon expression was improved by adding expression of a nuclease inhibitor, therefore protecting linear double-stranded DNA from degradation. A replicon of 16.5 kb was successfully replicated. Under selection pressure replication was stable for over 300 generations. When three orthogonal replicons were maintained simultaneously stability lasted over 100 generations. By adding an inducible dCas9 to repress the IPTG-responsive promoter the system allows regulation of the copy number and therefore evolutionary dynamics. Specific mutations in orthogonal DNA polymerases increase the mutation rates of the replicon 100 to 10,000-fold without affecting the mutation rate of the genome. The system therefore provides a platform for accelerated continuous mutational evolution that is simple, stable and scalable.

Oriented triplex DNA as a synthetic receptor for transmembrane signal transduction (Chen *et al.* 2024) [27]: The authors generated a synthetic receptor based on DNA. The transmembrane receptor is cholesterol-anchored across a lipid bilayer membrane and spans the membrane twice, with a third DNA strand facing outwards. A complementary helper sequence ensures the outward orientation. Induced by lowering the pH, conformational changes lead to a transition of the third strand from the outer to the inner membrane leaflet. This results in the receptor now spanning the membrane three times, starting transmembrane signal transduction. This work presents a versatile design for synthetic receptors characterized by modularity, programmability, and controllability.

Sequence modeling and design from molecular to genome scale with Evo (Nguyen *et al.* 2024) [28]: The authors trained an artificial intelligence (AI) model, named Evo, on millions of bacterial and phage genomes to enable predictions about DNA, RNA and protein functionalities as well as generation of complex systems based on CRISPR-Cas and transposable elements. Evo generated DNA sequences with plausible genomic architecture more than 1 megabase in length. The output was experimentally validated giving the first examples of protein-RNA and protein-DNA codesign via AI. Evo learns how small changes in DNA sequence affect the fitness of the whole organism. In conjunction with new techniques for large-scale genome modification, Evo expands the application possibilities in biotechnology and biological design to the scale of whole genomes.

Cell-type-directed design of synthetic enhancers (Taskiran *et al.* 2024) [29]: The authors designed enhancers (short DNA sequences that facilitate cell-type specific gene expression) with the help of already validated deep learning models. Three sequence design strategies have been used. The first strategy was a nucleotide-by-nucleotide sequence *in silico* evolution. It started from 500 bp random sequences and introduced mutations iteratively to target specific brain cells of the fruit fly or human cancer cells. To predict scores for the mutations, a deep learning model was used. The enhancers were then tested in transgenic lines. Additionally, it was tested if an enhancer that is active in a single cell type could be altered to become active in a second cell type as well as if an enhancer active in several cell types can be altered to become active in a single cell type. In a second strategy a combination of known activator motifs was used to design a cell-type-specific enhancer. In the third strategy generative adversarial networks have been used to generate functional and specific enhancers. In conclusion, this proof-of-concept study shows that enhancer design strategies are adaptable to different biological systems and even other species, including human.

Synthetic Biology with application perspectives

Incompatibility in cell adhesion constitutes a barrier to interspecies chimerism (Ballard *et al.* 2024) [30]: Blastocyst complementation is a possibility to address the shortage of donor organs for transplant-seeking persons. For this purpose, pluripotent stem cells (PSCs) are used to complement an organogenesis-disabled animal host-embryo with the objective of producing donor organs within the host. The technique has its limitations when applied across distantly related species such as mice and humans due to xenogeneic barriers. One of these barriers seems to be cell adhesion incompatibility. During development, epiblast cells are held together by strong anchoring junctions and the authors hypothesized that mismatched cell adhesion molecules might hinder the integration of human PSCs. To improve cell adhesion, they used nanobodies, which are single-domain fragments derived from camelid antibodies to achieve interspecies adhesion *in vitro*. Human PSCs were engineered to express a nanobody against GFP on their cell surface and were shown to bind mouse embryonic stem cells. *In vivo*, the nanobody-expressing human induced PSCs were injected into GFP-expressing mouse blastocytes and transferred into surrogate mothers. The resulting embryos were shown to contain more chimeric cells than control embryos. This technique could be used to advance interspecies organogenesis.

An electrogenetic interface to program mammalian gene expression by direct current (Huang *et al.* 2023) [31]: The authors developed an interface to link wearable electronic devices to medical interventions. The interface, called *direct current-actuated regulation technology* (DART), enables programming of transgene expression in human cells. Via direct current non-toxic levels of reactive oxygen species are generated that interact with a biosensor, a protein called KEAP1. KEAP1 releases the transcription factor NRF2 that translocates into the cell nucleus and binds to a synthetic promoter, resulting in NRF2-mediated reversible expression of a gene of interest. The system was tested *in vivo*. A battery pack providing direct current, wired via a manual ON/OFF power switch to two customized acupuncture needles located at an implantation site on the back of type 1 diabetic mice was used. The implantation site contained subcutaneously microencapsulated engineered human cells that express native KEAP1 and NRF2 and NRF2-mediated expression of the insulin gene. In type 1 diabetic mice, DART decreased high blood sugar levels by stimulating insulin expression. The link of analogue

biological systems with digital electronic devices facilitated by DART promises versatile applications for gene- and cell-based therapies, real-time dosing, and remote monitoring of patients through medical personnel.

Leveraging DNA-Encoded Cell-Mimics for Environment-Adaptive Transmembrane Channel Release-Induced Cell Death (Li *et al.* 2024) [32]: The authors established an artificial DNA-encoded T cell mimic model (ARTC). It imitates the function of cytotoxic T lymphocytes, important players in the immune systems response to tumors, by releasing the protein perforin, which causes membrane pores disrupting the homeostasis of target cells. ARTC is comprised of DNA-based spherical particles containing a double-strand DNA complex (IG) with three functional regions; the m* region for bridging, the i-motif DNA segment for response to changes in the pH and LG4, a guanine-rich DNA strand. Structural changes in IG as a response to a mildly acidic environment leads to the release of LG4 that exits the DNA sphere and anchors into the target cell membrane, forming a potassium ion channel. The following efflux of K+ disrupts the cell homeostasis, eventually triggering apoptosis of the target cell. Cell mimetics such as this are to be further developed to enable precise and controlled treatments to restore cell function.

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