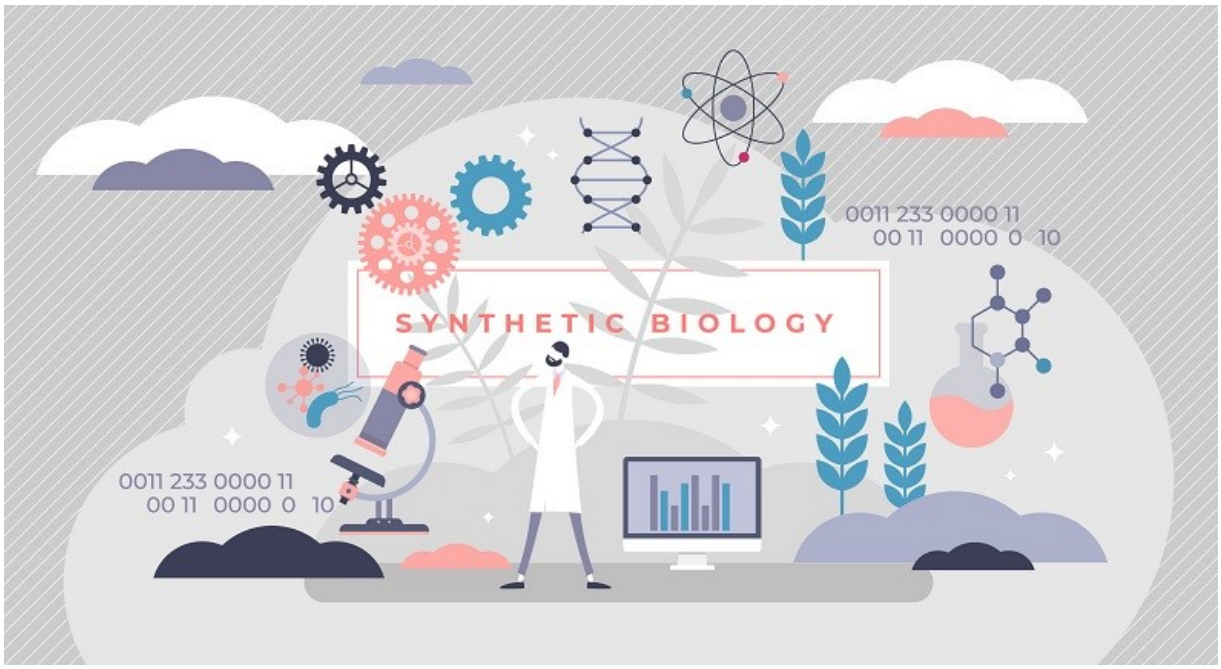
Synthetic Biology



3rd Interim report of the Central Committee on Biological Safety

Period: June 2018 - December 2021

ZKBS
Zentrale Kommission für die Biologische Sicherheit

Ref. 46012, July 2022

Contents

1 Introduction

The Central Committee for Biological Safety (ZKBS) has been monitoring synthetic biology for more than ten years in order to competently and critically accompany current scientific developments and to evaluate them with regard to biosafety. In 2012 and 2018, the first and second interim reports on this monitoring were published. While the first report focused on research activities in Germany, the second interim report looked at scientific developments worldwide.

The ZKBS has conducted a continuous monitoring of publications on synthetic biology since the second interim report. Selected publications which, in the opinion of the ZKBS, are particularly typical and relevant for the individual research subfields of synthetic biology are regularly presented on the ZKBS´s homepage (https://www.zkbs-online.de/ZKBS/EN/SyntheticBiology/Current_developments/Current_developments_node.html) .

This report briefly presents the developments in the various research subfields on the basis of the publications selected by the ZKBS for the homepage, evaluates them with regard to a potential threat to biological safety and assesses whether they are covered by the scope of the Genetic Engineering Act (GenTG) and the European Directives, respectively.

For an introduction to the field of synthetic biology, please refer to the interim reports already published.

2 Developments in the research subfields of synthetic biology and evaluation by the ZKBS

So far, there is no generally accepted definition of synthetic biology. In its monitoring, the ZKBS focuses on five areas that are generally seen by researchers and other stakeholders as the research fields of synthetic biology. These are:

- Design and synthesis of genes and genomes
- Design of genetic signalling circuits
- Design of customised metabolic pathways
- Minimal cells: Genome reduction and generation of protocells
- Xenobiology.

In addition, since 2018 the ZKBS has also listed publications on methods impacting on synthetic biology on its homepage. The results of the continuous monitoring are briefly summarised below. For a complete list of the short summaries, please refer to the ZKBS´s homepage (https://www.zkbs-online.de/ZKBS/EN/SyntheticBiology/Current_developments/Current_developments_node.html) .

2.1 Design and synthesis of genes and genomes

Some of the developments already described in the first two reports were continued and advanced. For example, a synthetic *Escherichia coli* genome with *a* size of 4 Mbp was synthesised, in which two codons were recoded [1]. DNA as a storage medium was used to dynamically store large amounts of data or to record cell lineage [2, 3]. Synthetic promoters, developed among other things for plants [4] and for *Saccharomyces cerevisiae* [5], contribute to standardisation in the synthesis of genes and genomes.

Assessment of the ZKBS: As already described in the last two interim reports, the possibilities for synthesising complete genomes are constantly progressing. However, a *de novo* design of genomes has still not been reported, meaning that *in vitro* synthesised genomes continue to be built on the basis of natural genomes. This means that a comparative risk assessment is still possible.

The *in vitro* synthesis of genes and genomes *per se* does not fall under the GenTG as long as the nucleic acid segments produced are not introduced into the genome of living organisms. If newly synthesised and modified genomes are introduced into living organisms, this constitutes a genetic engineering operation according to the GenTG, unless these modifications can occur naturally by mating or natural recombination.

2.2 Design of genetic signalling circuits

Foreseen applications of genetic circuits are in medicine and environmental diagnostics. On the one hand, genetic circuits seem to be becoming more complex; on the other hand, efforts towards a more stable expression of the circuits and their outputs as well as a regulation by novel inputs are described. In addition, more applied examples can be observed. Examples of complex circuits are the processing of multiple inputs by a metabolic perceptron [6] or flexible logic gates constructed from proteins and used for post-translational control [7]. Stable gene expression independent of copy number or the localisation of a circuit in the genome has been described, for example, by Segall-Shapiro *et al*. [8] or Frei *et al*. [9]. Novel inputs for circuits can be, for example, electrical signals [10], photothermal signals via LED light [11], or light [12, 13]. Genetic circuits are being explored for use in cancer therapy. They can be used to measure the concentration of surface proteins (e.g. antigen density) or microRNAs in a cell and thus make tumour cells specifically recognisable, which can mediate their targeted killing [14-18]. Another example of an application are genetic switches that are incorporated into tissue to act as wearable sensors for small molecules such as toxins [19].

Assessment of the ZKBS: In the design of genetic circuits, precisely defined DNA segments, usually well characterised in terms of function, are combined with each other. The circuits are often introduced into model organisms that have long been known in research, using a biological safety measure. As already stated in the last two interim reports, genetically modified organisms are produced in the process that fall within the scope of the GenTG.

2.3 Design of customised metabolic pathways

Tailored metabolic pathways are used, for example, to increase biomass production in plants or to establish new pathways for CO_2 fixation [20-22]. A new carboxylation pathway for improved CO_2 fixation [23] can be combined with the CETCH cycle described in the last interim report [24]. The production of economically interesting molecules, such as synthetic cannabinoids in *S. cerevisiae* and the aromatic substance vanillin from a PET degradation product, was also described [25, 26]. Metabolic pathways were placed into vesicles to prevent a toxic effect of the resulting products [27].

Assessment of the ZKBS: For the development of novel customised metabolic pathways, existing genes are modified or genes from another organism are introduced into an already existing organism. This could enable the specific formation of hazardous substances. However, the production and handling of these organisms are fully covered by the GenTG.

2.4 Minimal cells: Genome reduction and generation of protocells

Top down: Production of minimal cells by genome reduction

Reduced genomes have been developed for yeast *S. cerevisiae,* among others, by bundling essential genes into a superchromosome [28] or by reducing the size of one of the 16 chromosomes by 58 % [29]. In the field of bacteria, for example, the genome of *Caulobacter ethensis* was reduced and an additional 56 % of all codons were replaced by synonymous codons [30].

Bottom up: Production of protocells

The production of minimal cells from biological building blocks focuses on the development of various functions that "viable" synthetic cells require. This may include the formation of compartments such as an artificial cell nucleus [31], structures for cell division [32] or cell movement [33, 34] or for energy supply [35, 36]. Many working groups are also studying communication between protocells, e.g. with the help of DNA [37, 38] or small molecules [39].

Assessment of the ZKBS: Minimal organisms produced by the targeted reduction of the genome are generally less adaptable to their environment, which is accompanied by reduced fitness and possibly also reduced pathogenicity. Most of these organisms can only survive under defined conditions, which is why an increased threat to biological safety cannot be identified. As provided for in the GenTG, the hazard potential can be well assessed by comparing the organisms with the original organisms.

"Bottom up" organisms designed from scratch are not covered by the GenTG, which is applicable to organisms whose "genetic material has been modified in a way that does not occur under natural conditions by mating or natural recombination" (§ 3 GenTG). According to the GenTG, the risk assessment is based on the known hazard potential of the donor and recipient organism. Protocells that are not based on a natural organism are therefore not covered by the GenTG.

To date, however, no protocell has been described that can replicate autonomously and can be considered as an organism. No risk to biosafety is currently expected from this field.

2.5 Xenobiology

In the field of xenobiology, some approaches on genetic code modification have been described. These included, for example, the development of a fail-safe code, in which each amino acid is encoded by only one four-base codon, which cannot be converted into another codon by mutation and is thus intended to protect against spontaneous mutation [40]. Also, an eight-letter alphabet in which the naturally occurring bases A, T, C and G are supplemented by synthetic nucleotides [41], or new base pairs on the basis of metals [42] have been described. Work on tRNAs that recode sense codons, for example, [43] or decode four-base codons [44] complement this work. In addition, some research on the insertion of non-canonical amino acids into proteins has been described. These were aimed, for example, at auxotrophy [45]. Bacteria that can synthesise a 21st amino acid and insert it into a protein have also been described [46].

Assessment of the ZKBS: The approaches pursued in the field of xenobiology are used to produce organisms whose genetic material has been altered in a way that does not occur under natural conditions through mating or natural recombination. In this context, non-natural bases are also regarded as genetic material. They are therefore subject to the GenTG.
Many applications from the field of xenobiology also aim for orthogonality and thus reduced interaction with natural organisms. This can lead to an increase in biosafety.

2.6 Methods impacting on synthetic biology

Since the second interim report in 2018, the ZKBS has also included publications on methods impacting on synthetic biology in its continuous monitoring. These are divided into applied methods and *in silico*, i.e. computer-assisted, methods.

Applied methods

In this area, research on the integration of nucleic acid segments using CRISPR/Cas9 [47], a sequencing method for a non-natural base [47] or approaches on data storage in DNA [48, 49] are listed. Techniques on the formation, division and colony formation of protocell precursors were also described [50–52].

In silico methods

Genetic data from synthetic biology applications can be converted into the *Synthetic Biology Open Language* (SBOL) for standardised data exchange. In order to make the often disordered datasets in repositories such as SynBioHub more accessible, Zhang *et al*. [53] developed a programme for sorting these data sets.

Assessment of the ZKBS: In the applied methods listed here, either the genetic material of an existing organism is modified in a way that does not occur under natural conditions through mating

or natural recombination, so that the GenTG applies, or methods are developed to generate protocells to which the GenTG does not apply (see section 2.4). The *in silico* method described does not involve handling an organism, so that the GenTG does not apply and no risk to biosafety occurs.

3 References

1. **Fredens J, Wang K, La Torre D de, Funke LFH, Robertson WE, Christova Y, Chia T, Schmied WH, Dunkelmann DL, Beránek V, Uttamapinant C, Llamazares AG, Elliott TS, Chin JW** (2019). Total synthesis of Escherichia coli with a recoded genome. *Nature* **569**(7757):514–8.
2. **Lin KN, Volkel K, Tuck JM, Keung AJ** (2020). Dynamic and scalable DNA-based information storage. *Nat Commun* **11**(1):2981.
3. **Chow K-HK, Budde MW, Granados AA, Cabrera M, Yoon S, Cho S, Huang T-H, Koulena N, Frieda KL, Cai L, Lois C, Elowitz MB** (2021). Imaging cell lineage with a synthetic digital recording system. *Science* **372**(6538).
4. **Wang K, La Torre D de, Robertson WE, Chin JW** (2019). Programmed chromosome fission and fusion enable precise large-scale genome rearrangement and assembly. *Science* **365**(6456):922–6.
5. **Kotopka BJ, Smolke CD** (2020). Model-driven generation of artificial yeast promoters. *Nat Commun* **11**(1):2113.
6. **Pandi A, Koch M, Voyvodic PL, Soudier P, Bonnet J, Kushwaha M, Faulon J-L** (2019). Metabolic perceptrons for neural computing in biological systems. *Nat Commun* **10**(1):3880.
7. **Chen Z, Kibler RD, Hunt A, Busch F, Pearl J, Jia M, VanAernum ZL, Wicky BIM, Dods G, Liao H, Wilken MS, Ciarlo C, Green S, El-Samad H, Stamatoyannopoulos J, Wysocki VH, Jewett MC, Boyken SE, Baker D** (2020). De novo design of protein logic gates. *Science* **368**(6486):78–84.
8. **Segall-Shapiro TH, Sontag ED, Voigt CA** (2018). Engineered promoters enable constant gene expression at any copy number in bacteria. *Nat Biotechnol* **36**(4):352–8.
9. **Frei T, Cella F, Tedeschi F, Gutiérrez J, Stan G-B, Khammash M, Siciliano V** (2020). Characterization and mitigation of gene expression burden in mammalian cells. *Nat Commun* **11**(1):4641.
10. **Krawczyk K, Xue S, Buchmann P, Charpin-El-Hamri G, Saxena P, Hussherr M-D, Shao J, Ye H, Xie M, Fussenegger M** (2020). Electrogenetic cellular insulin release for real-time glycemic control in type 1 diabetic mice. *Science* **368**(6494):993–1001.
11. **Aratboni HA, Rafiei N, Khorashad LK, Lerma-Escalera AI, Balderas-Cisneros FdJ, Liu Z, Alemzadeh A, Shaji S, Morones-Ramírez JR** (2021). LED control of gene expression in a nanobiosystem composed of metallic nanoparticles and a genetically modified E. coli strain. *J Nanobiotechnology* **19**(1):190.

12. **Hörner M, Jerez-Longres C, Hudek A, Hook S, Yousefi OS, Schamel WWA, Hörner C, Zurbriggen MD, Ye H, Wagner HJ, Weber W** (2021). Spatiotemporally confined red light-controlled gene delivery at single-cell resolution using adeno-associated viral vectors. *Sci Adv* **7**(25).

13. **Nakanishi H, Yoshii T, Kawasaki S, Hayashi K, Tsutsui K, Oki C, Tsukiji S, Saito H** (2021). Light-controllable RNA-protein devices for translational regulation of synthetic mRNAs in mammalian cells. *Cell Chem Biol* **28**(5):662-674.e5.

14. **Chung HK, Zou X, Bajar BT, Brand VR, Huo Y, Alcudia JF, Ferrell JE, Lin MZ** (2019). A compact synthetic pathway rewires cancer signaling to therapeutic effector release. *Science* **364**(6439).

15. **Huang H, Liu Y, Liao W, Cao Y, Liu Q, Guo Y, Lu Y, Xie Z** (2019). Oncolytic adenovirus programmed by synthetic gene circuit for cancer immunotherapy. *Nat Commun* **10**(1):4801.

16. **Lajoie MJ, Boyken SE, Salter AI, Bruffey J, Rajan A, Langan RA, Olshefsky A, Muhunthan V, Bick MJ, Gewe M, Quijano-Rubio A, Johnson J, Lenz G, Nguyen A, Pun S, Correnti CE, Riddell SR, Baker D** (2020). Designed protein logic to target cells with precise combinations of surface antigens. *Science* **369**(6511):1637–43.

17. **Williams JZ, Allen GM, Shah D, Sterin IS, Kim KH, Garcia VP, Shavey GE, Yu W, Puig-Saus C, Tsoi J, Ribas A, Roybal KT, Lim WA** (2020). Precise T cell recognition programs designed by transcriptionally linking multiple receptors. *Science* **370**(6520):1099–104.

18. **Hernandez-Lopez RA, Yu W, Cabral KA, Creasey OA, Lopez Pazmino MDP, Tonai Y, Guzman A de, Mäkelä A, Saksela K, Gartner ZJ, Lim WA** (2021). T cell circuits that sense antigen density with an ultrasensitive threshold. *Science* **371**(6534):1166–71.

19. **Nguyen PQ, Soenksen LR, Donghia NM, Angenent-Mari NM, Puig H de, Huang A, Lee R, Slomovic S, Galbersanini T, Lansberry G, Sallum HM, Zhao EM, Niemi JB, Collins JJ** (2021). Wearable materials with embedded synthetic biology sensors for biomolecule detection. *Nat Biotechnol* **39**(11):1366–74.

20. **Gleizer S, Ben-Nissan R, Bar-On YM, Antonovsky N, Noor E, Zohar Y, Jona G, Krieger E, Shamshoum M, Bar-Even A, Milo R** (2019). Conversion of Escherichia coli to Generate All Biomass Carbon from CO2. *Cell* **179**(6):1255-1263.e12.

21. **South PF, Cavanagh AP, Liu HW, Ort DR** (2019). Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. *Science* **363**(6422).

22. **Miller TE, Beneyton T, Schwander T, Diehl C, Girault M, McLean R, Chotel T, Claus P, Cortina NS, Baret J-C, Erb TJ** (2020). Light-powered CO2 fixation in a chloroplast mimic with natural and synthetic parts. *Science* **368**(6491):649–54.

23. **Scheffen M, Marchal DG, Beneyton T, Schuller SK, Klose M, Diehl C, Lehmann J, Pfister P, Carrillo M, He H, Aslan S, Cortina NS, Claus P, Bollschweiler D, Baret J-C, Schuller JM, Zarzycki J, Bar-Even A, Erb TJ** (2021). A new-to-nature carboxylation module to improve natural and synthetic CO2 fixation. *Nat Catal* **4**(2):105–15.

24. **Schwander T, Schada von Borzyskowski L, Burgener S, Cortina NS, Erb TJ** (2016). A synthetic pathway for the fixation of carbon dioxide in vitro. *Science* **354**(6314):900–4.

25. **Luo X, Reiter MA, d'Espaux L, Wong J, Denby CM, Lechner A, Zhang Y, Grzybowski AT, Harth S, Lin W, Lee H, Yu C, Shin J, Deng K, Benites VT, Wang G, Baidoo EEK, Chen Y, Dev I, Petzold CJ, Keasling JD** (2019). Complete biosynthesis of cannabinoids and their unnatural analogues in yeast. *Nature* **567**(7746):123–6.

26. **Sadler JC, Wallace S** (2021). Microbial synthesis of vanillin from waste poly(ethylene terephthalate). *Green Chem* **23**(13):4665–72.

27. **Reifenrath M, Oreb M, Boles E, Tripp J** (2020). Artificial ER-Derived Vesicles as Synthetic Organelles for in Vivo Compartmentalization of Biochemical Pathways. *ACS Synth Biol* **9**(11):2909–16.

28. **Shao Y, Lu N, Wu Z, Cai C, Wang S, Zhang L-L, Zhou F, Xiao S, Liu L, Zeng X, Zheng H, Yang C, Zhao Z, Zhao G, Zhou J-Q, Xue X, Qin Z** (2018). Creating a functional single-chromosome yeast. *Nature* **560**(7718):331–5.

29. **Luo Z, Yu K, Xie S, Monti M, Schindler D, Fang Y, Zhao S, Liang Z, Jiang S, Luan M, Xiao C, Cai Y, Dai J** (2021). Compacting a synthetic yeast chromosome arm. *Genome Biol* **22**(1):5.

30. **Venetz JE, Del Medico L, Wölfle A, Schächle P, Bucher Y, Appert D, Tschan F, Flores-Tinoco CE, van Kooten M, Guennoun R, Deutsch S, Christen M, Christen B** (2019). Chemical synthesis rewriting of a bacterial genome to achieve design flexibility and biological functionality. *Proc Natl Acad Sci U S A* **116**(16):8070–9.

31. **Niederholtmeyer H, Chaggan C, Devaraj NK** (2018). Communication and quorum sensing in non-living mimics of eukaryotic cells. *Nat Commun* **9**(1):5027.

32. **Litschel T, Kelley CF, Holz D, Adeli Koudehi M, Vogel SK, Burbaum L, Mizuno N, Vavylonis D, Schwille P** (2021). Reconstitution of contractile actomyosin rings in vesicles. *Nat Commun* **12**(1):2254.

33. **Ghosh S, Mohajerani F, Son S, Velegol D, Butler PJ, Sen A** (2019). Motility of Enzyme-Powered Vesicles. *Nano Lett* **19**(9):6019–26.

34. **Ahmad R, Kleineberg C, Nasirimarekani V, Su Y-J, Goli Pozveh S, Bae A, Sundmacher K, Bodenschatz E, Guido I, Vidaković-Koch T, Gholami A** (2021). Light-Powered Reactivation of Flagella and Contraction of Microtubule Networks: Toward Building an Artificial Cell. *ACS Synth Biol* **10**(6):1490–504.

35. **Berhanu S, Ueda T, Kuruma Y** (2019). Artificial photosynthetic cell producing energy for protein synthesis. *Nat Commun* **10**(1):1325.

36. **Biner O, Fedor JG, Yin Z, Hirst J** (2020). Bottom-Up Construction of a Minimal System for Cellular Respiration and Energy Regeneration. *ACS Synth Biol* **9**(6):1450–9.

37. **Joesaar A, Yang S, Bögels B, van der Linden A, Pieters P, Kumar BVVSP, Dalchau N, Phillips A, Mann S, Greef TFA de** (2019). DNA-based communication in populations of synthetic protocells. *Nat Nanotechnol* **14**(4):369–78.

38. **Yang S, Pieters PA, Joesaar A, Bögels BWA, Brouwers R, Myrgorodska I, Mann S, Greef TFA de** (2020). Light-Activated Signaling in DNA-Encoded Sender-Receiver Architectures. *ACS Nano* **14**(11):15992–6002.

39. **Buddingh' BC, Elzinga J, van Hest JCM** (2020). Intercellular communication between artificial cells by allosteric amplification of a molecular signal. *Nat Commun* **11**(1):1652.

40. **Calles J, Justice I, Brinkley D, Garcia A, Endy D** (2019). Fail-safe genetic codes designed to intrinsically contain engineered organisms. *Nucleic Acids Res* **47**(19):10439–51.

41. **Hoshika S, Leal NA, Kim M-J, Kim M-S, Karalkar NB, Kim H-J, Bates AM, Watkins NE, SantaLucia HA, Meyer AJ, DasGupta S, Piccirilli JA, Ellington AD, SantaLucia J, Georgiadis MM, Benner SA** (2019). Hachimoji DNA and RNA: A genetic system with eight building blocks. *Science* **363**(6429):884–7.

42. **Flamme M, Röthlisberger P, Levi-Acobas F, Chawla M, Oliva R, Cavallo L, Gasser G, Marlière P, Herdewijn P, Hollenstein M** (2020). Enzymatic Formation of an Artificial Base Pair Using a Modified Purine Nucleoside Triphosphate. *ACS Chem Biol* **15**(11):2872–84.

43. **Robertson WE, Funke LFH, La Torre D de, Fredens J, Elliott TS, Spinck M, Christova Y, Cervettini D, Böge FL, Liu KC, Buse S, Maslen S, Salmond GPC, Chin JW** (2021). Sense codon reassignment enables viral resistance and encoded polymer synthesis. *Science* **372**(6546):1057–62.

44. **DeBenedictis EA, Carver GD, Chung CZ, Söll D, Badran AH** (2021). Multiplex suppression of four quadruplet codons via tRNA directed evolution. *Nat Commun* **12**(1):5706.

45. **Koh M, Yao A, Gleason PR, Mills JH, Schultz PG** (2019). A General Strategy for Engineering Noncanonical Amino Acid Dependent Bacterial Growth. *J Am Chem Soc* **141**(41):16213–6.

46. **Chen Y, Tang J, Wang L, Tian Z, Cardenas A, Fang X, Chatterjee A, Xiao H** (2020). Creation of Bacterial cells with 5-Hydroxytryptophan as a 21st Amino Acid Building Block. *Chem* **6**(10):2717–27.

47. **Bourgeois L, Pyne ME, Martin VJJ** (2018). A Highly Characterized Synthetic Landing Pad System for Precise Multicopy Gene Integration in Yeast. *ACS Synth Biol* **7**(11):2675–85.

48. **Hamashima K, Soong YT, Matsunaga K-I, Kimoto M, Hirao I** (2019). DNA Sequencing Method Including Unnatural Bases for DNA Aptamer Generation by Genetic Alphabet Expansion. *ACS Synth Biol* **8**(6):1401–10.

49. **Koch J, Gantenbein S, Masania K, Stark WJ, Erlich Y, Grass RN** (2020). A DNA-of-things storage architecture to create materials with embedded memory. *Nat Biotechnol* **38**(1):39–43.

50. **Zhang Y, Wang F, Chao J, Xie M, Liu H, Pan M, Kopperger E, Liu X, Li Q, Shi J, Wang L, Hu J, Wang L, Simmel FC, Fan C** (2019). DNA origami cryptography for secure communication. *Nat Commun* **10**(1):5469.

51. **Li Q, Li S, Zhang X, Xu W, Han X** (2020). Programmed magnetic manipulation of vesicles into spatially coded prototissue architectures arrays. *Nat Commun* **11**(1):232.

52. **Dreher Y, Jahnke K, Bobkova E, Spatz JP, Göpfrich K** (2021). Division and Regrowth of Phase-Separated Giant Unilamellar Vesicles*. *Angew Chem Int Ed Engl* **60**(19):10661–9.

53. **Ip T, Li Q, Brooks N, Elani Y** (2021). Manufacture of Multilayered Artificial Cell Membranes through Sequential Bilayer Deposition on Emulsion Templates. *Chembiochem* **22**(13):2275–81.

54. **Zhang M, Zundel Z, Myers CJ** (2019). SBOLExplorer: Data Infrastructure and Data Mining for Genetic Design Repositories. *ACS Synth Biol* **8**(10):2287–94.