Science Forum

Building a community to engineer synthetic cells and organelles from the bottom-up

Oskar Staufer[1,2,3*], Jacqueline A. De Lora[1], Eleonora Bailoni[4], Alisina Bazrafshan[5], Amelie S. Benk[1], Kevin Jahnke[1], Zachary A. Manzer[6], Lado Otrin[7], Telmo Díez Pérez[8], Judee Sharon[9], Jan Steinkühler[10], Katarzyna P. Adamala[9,11], Bruna Jacobson[8], Marileen Dogterom[12,13], Kerstin Göpfrich[1,2], Darko Stefanovic[8], Susan R. Atlas[8], Michael Grunze[1,2], Matthew R. Lakin[8], Andrew P. Shreve[8], Joachim P. Spatz[1,2,3,14*], Gabriel P. López[8*]

1 Max Planck Institute for Medical Research, Heidelberg, Germany
2 Max Planck School Matter to Life, Heidelberg, Germany
3 Max Planck Bristol Center for Minimal Biology, University of Bristol, Bristol, United Kingdom
4 University of Groningen, Groningen, The Netherlands
5 Emory University, Atlanta, GA, United States
6 Cornell University, Ithaca, NY, United States
7 Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany
8 University of New Mexico, Albuquerque, NM, United States
9 University of Minnesota, Minneapolis, MN, United States
10 Northwestern University, Evanston, IL, United States
11 Build-a-Cell Research Coordination Network
12 Delft University of Technology, Delft, The Netherlands
13 BaSyC Consortium, Delft, The Netherlands
14 Heidelberg University, Heidelberg, Germany

* Correspondence should be addressed to:
oskar.staufer@mr.mpg.de
joachim.spatz@mr.mpg.de
gplopez@unm.edu

One-sentence summary: The creation of synthetic cells and organelles will open doors to new collaborative and funding opportunities.

Abstract

Employing concepts from physics, chemistry and bioengineering, 'learning-by-building' approaches are becoming increasingly popular in the life sciences, especially with researchers who are attempting to engineer cellular life from scratch. The SynCell2020/21 conference brought together researchers from different disciplines to highlight progress in this field, including areas where synthetic cells are having socioeconomic and technological impact. Conference participants also identified the challenges involved in designing, manipulating and creating synthetic cells with hierarchical organization and function. A key conclusion is the need to build an international and interdisciplinary research community through enhanced communication, resource-sharing, and educational initiatives.

Philosophy of the field and past achievements

Physicists and engineers traditionally focus on the non-living world and apply model systems with reduced complexity to capture the essentials of a living entity, and to gain mechanistic insights into higher-order processes on a micro-, meso- and macroscale. This approach is also becoming more popular in the life sciences, where a ‘learning-by-building’ strategy is used to design and construct synthetic cells and organelles of reduced but defined complexity (Jia et al., 2019; Ausländer et al., 2017). Early national and transnational initiatives, such as the Max Planck School ‘Matter to Life’ (https://mattertolife.maxplanckschools.org), the Build-A-Cell research coordination network (https://www.buildacell.org/), the Building a Synthetic Cell (BaSyC) research program (https://www.basyc.nl/about-basyc/), and the International Genetically Engineered Machine (iGEM) competition (https://igem.org/Competition), have been established to advance training, research and collaboration in this exciting new field.

Cells are the basic units of life. But their intricate structure and the tightly orchestrated interplay of individual molecular components within cells are far from basic. Most cellular phenomena are not understandable through intuition but require complex analytical systems to provide a mechanistic description of the processes forming living matter. Yet, within the complexity of living cells hide the answers to some of the most fundamental questions in the life sciences, from the emergence of life and the transition from inanimate matter to life, to the development and cure of diseases.

These compelling and profoundly difficult questions reflect a vision for the future of the field as expressed by the SynCell2020/21 early-career panelists. The philosophical and ethical considerations underlying these questions, e.g., the misuse of synthetic cells for biological warfare, the impact of synthetic cells on natural environments, or the unpredictable nature of completely new life forms, are notable for their contrast with technological and engineering-focused objectives (Gallup et al., 2021).

Using principles from biology and engineering, interdisciplinary research teams have applied synthetic cells to construct materials and hierarchical structures with life-like properties that recreate essential features of living cells and reach beyond the capabilities of natural cells (Figure 1) (Elani et al., 2018; Krinsky et al., 2018; Rampioni et al., 2018; Staufer et al., 2021; Liangfei Tian et al., 2019). Cellular functions entail a diverse spectrum of modular components that regulate cell behavior. These include the ability to process chemical and physical signals from extracellular stimuli (information processing and decision making), cellular motions and adaptation to the environment (molecular adaptivity), cellular replication through division (proliferation) and nutrient uptake and garbage disposal (energy homeostasis). These abilities have helped to guide the functional design of synthetic cells and their equipment with modules that allow them to interact with the extracellular environment and to discriminate extracellular signals. For instance, extracellular morphogen sensing systems have been implemented in protocells that are able to change their structure and to discriminate between uni- and counter-directional morphogen gradients (L. Tian et al., 2019). Tactile behavior in the form of chemo- and phototaxis has also been engineered into synthetic cell surrogates (Bartelt et al., 2018; Pan et al., 2019).

Broadly speaking, there are two primary approaches to construct low-complexity synthetic cells: top-down and bottom-up (Ausländer et al., 2017). Top-down approaches make use of existing living cells and sequentially remove individual components such as single genes (Lachance et al., 2019). This process can be iterated until reaching the absolute lowest point of complexity required for a cell to live. Analogous to synthetic lethality experiments in model organisms, top-down approaches provide descriptive insights into which parts of a cell are most crucial. However, it can be harder to obtain a systems-level understanding of how the parts work together.

In contrast, the bottom-up approach rationally combines non-living molecules in an understand-by-design approach to activate and exhibit the behaviors of living cells within artificial structures (Buddingh et al., 2017). A common defining element of cellular life forms is the ability to replicate a compartmentalized information-storing and self-sustaining, out-of-equilibrium system that manifests itself in specific characteristics, which can be selected in an evolutionary process (Benner, 2010; Damiano et al., 2020; Porcar et al., 2011). This could be achieved by engineering a compartmentalized entity that exhibits a metabolism for reproduction purposes and environmental adaptation. For example, a lipid membrane vesicle that can grow and divide by catabolizing external substrates and harboring DNA-encoded genes that specify the enzymes required for catabolism and reproduction. The advantage of the bottom-up approach is that every component of the created system can be located and defined in a quantitative manner, together with specified interactions between molecules.

But regardless of the approach – top-down or bottom-up – the knowledge gained from building synthetic cells has the potential to provide fundamental insights into life and to shape technologies of global impact, such as new vaccination strategies (Dormitzer et al., 2013), routes to overcome antibiotic resistance (Wu et al., 2017), new manufacturing pipelines for synthetic materials (Le Feuvre et al., 2018), and alternatives to petrochemicals (Shih, 2018).

Within the last decade, the field of building synthetic cells and organelles has achieved several major technological breakthroughs. A minimal synthetic cell consisting of only 473 genes, capable of metabolizing and reproducing, has been constructed using a top-down approach (Hutchison et al., 2016). With the objective of engineering a minimal synthetic cell, synthetic chromosomes have been designed to generate artificial genetic blueprints for operating synthetic cell systems (Greene et al., 2019). In addition, droplet-based synthetic cells with an artificial photosynthetic metabolism that can bind CO_2 have been created, and synthetic cell systems for the scalable bio-production of natural plant products have also been built (Miller et al., 2020). Bioengineering concepts emerging from such studies have guided the implementation of new, application-focused technologies, e.g. cell-free expression systems. And most recently, the advanced synthetic genetic codes and technologies to rewire translational processes of protein production have provided the foundation for RNA-based SARS-CoV-2 vaccines (Karikó et al., 2008; Mulligan et al., 2020). Together with liposomal and lipid nanoparticle technologies, which program cellular uptake and processing of the RNA content, these vaccines have been a vital tool in the fight against COVID-19 (Park et al., 2021).

Exploring the fundamentals of life as illustrated in these brief examples requires diverse skill sets for designing and engineering experimental systems. It further needs an unbiased and creative mind with a strong interdisciplinary background to successfully integrate aspects of physics, chemistry, biology, and the information sciences. Similar to fields that explore artificial intelligence and neuromorphic computing (Valeri et al., 2020), students attracted to synthetic cell research often share enthusiasm and interests that go beyond their primary disciplines, incorporating aspects of philosophy and cognition within their research (e.g., the 'Synthetic Biology, Politics and Philosophy' workshop held at BrisSynBio (Meacham et al., 2020). This cross-fertilization provides unique opportunities for attracting young researchers into the field of engineering synthetic cells and organelles (see Box S1 in Supplementary file 1 for selected quotations from young researchers who participated in the SynCell2021 Workshop).

Recent research directions and bottlenecks

The creation of compartmentalized, cell-mimicking structures and the integration of coupled transcription-translation (TXTL) systems has seen substantial progress (Garamella et al., 2019). However, considerable challenges, some seemingly paradoxical, remain. Many of these were highlighted at the recent SynCell2020/21 conference (see Figure 1–figure supplement 1 and Tables S1–S5 in Supplementary file 1 for listings of the conference program and links to recordings) and have been extensively reviewed recently (Gallup et al.; Meng et al., 2020).

One of the most demanding challenges remains the coupling of information-encoding systems with self-replicating cell-like entities (Walker et al., 2017), which can be framed in terms of von Neumann´s abstract generality about the logic of cell-like, self-replicating automata (Neumann et al., 1966). Such entities require both a mechanism to copy the cellular architecture and functionalities that allow copying of genetic information specifying cellular structure and function. Such units need molecular systems that link the functional parts of a synthetic cell to a decoding mechanism that reads the genetic instructions required to autocatalytically build a new cell. They also require a molecular module that copies and reinserts a transcript of the (genetic) instruction into the synthetic daughter cell (Olivi et al., 2021). This is the logical basis of self-reproduction.

The first steps towards the synthetic construction of such systems were presented at the conference. DNA-encoded genetic systems represent just one implementation of self-reproduction, leaving ample room for designing alternatives to fulfil the basic conditions for a 'living', synthetic cell (Dreher et al., 2021; Otrin et al., 2021). In addition, synthetic cells will also require control programs to orchestrate the interconnected processes of sensing, response and metabolism necessary for replication and other processes relevant for life-like behavior (Lakin et al., 2016; Li et al., 2021; Steinkühler et al., 2020).

Important progress reported at the meeting also included the engineering of synthetic structures with hierarchical organization inspired by eukaryotic life forms. Several implementations of such systems, such as hierarchical intrinsically disordered protein and nucleic acid droplets generated within synthetic cell-like compartments, were presented (Simon et al., 2017). These efforts are aimed at deconvolving the organizational principles of life, including the highly dynamic cross-scale architecture of eukaryotic and multicellular organisms, most apparent during embryogenic development and tissue regeneration.

How the structural organization of subcellular, cellular and tissue components is hard-wired and how degrees of plasticity in respective structures are regulated, are problems of such immense complexity for which approaches including multi-centered global screening efforts have not been able to resolve the underlying principles. New methods based on *in vitro* synthetic model systems of lower complexity may provide new insights into these processes.

For example, a pivotal driving force behind tissue organization consists of genetic feedback loops based on reaction-diffusion processes and hysteresis, as first proposed by Alan Turing in his work on the chemical basis of morphogenesis in the mid-20th century (Turing, 1952). This is a prime example of how reductionist approaches in the form of precisely defined models can be applied to the study of complex behaviors in biological systems. Researchers in synthetic biology have recently recreated Turing patterns from protein-based systems and used these to study decision-making during cellular organization and symmetry breaking (Glock et al., 2019). This underscores both the fundamental impact of the questions asked in the field and their longstanding relevance that argues for the need to pursue novel theoretical, computational and experimental approaches by unbiased young scientists working in integrative research communities.

Other approaches have contributed insights into the spatio-temporal dynamics and organization principles of membrane-less organelles (Simon et al., 2017). Until recently, studying such dynamic structures in living cells has mostly been limited by a lack of perturbation capabilities and the undefined chemical environment within the cytosol. However, through *in vitro* reconstitution of intrinsically disordered protein / nucleic acid systems in isolated low-complexity environments, quantitative insights into the molecular and thermodynamic principles needed for assembly and homeostasis of phase-separated organelles has been achieved (Linsenmeier et al., 2019). Understanding the hierarchical organization principles of life will ultimately enable the formulation of the principal laws of decision-making within living matter, and the basis of information processing and signal integration within cell collectives (Staufer et al., 2018; Staufer et al., 2019).

Engineering synthetic cells and organelles is not solely directed towards investigating biological principles, but also holds promise for practical applications. This offers the opportunity to explore an extensive technical repertoire. For instance, microfluidic approaches have been developed to assemble synthetic cells with adjustable and tunable composition. Specifically, cell-sized compartments in the form of water-in-oil droplets that contain proteins, lipids or nucleic acids, provide means of engineering systems capable of genetic information processing and artificial genotype-to-phenotype coupling, where selection is exerted at the level of the synthetic cells´ phenotype but propagation of a selected trait depends on the relevant genetic information being carried forward (Miller et al., 2020; Staufer et al., 2021; Staufer et al., 2020; van Vliet et al., 2015; Weiss et al., 2017). Such droplet-based approaches have also been adopted for lipid membrane engineering (Lussier et al., 2020; Steinkühler et al., 2020). Similarly, DNA nanotechnology has allowed to combine programmable molecular architectures with extrinsically controlled functions (Bazrafshan et al., 2020; Jahnke et al., 2020). In a combinatorial approach, integration of DNA nano-architectures with synthetic cells has synergized top-down and bottom-up strategies (Jahnke et al., 2021). These examples demonstrate the potential for technology innovation originating from the field.

Although the reductionist approach pursued in the field of engineering synthetic cells and organelles has proven to be powerful for several lines of research, it is also confronted with systematic limitations. Foremost, as an engineering approach that iteratively reduces the complexity of living systems, the construction of synthetic cells will always be subjected to the problem of 'hidden variables'' (Garcia et al., 2016). Unobservable or unidentified molecular components of importance to natural systems might be underrepresented or absent in the recreated *in vitro* system. Such unknown variables could contribute to the stochastic behavior of a system, and biological phenomena could potentially be masked behind noise effects in synthetic cells and organelles.

Moreover, living cells cannot only be considered as the simple sum of their parts. Emergence in living systems is poorly defined and understood, and the possibility to capture this aspect by a modular approach remains to be evaluated. A second systematic limitation of the reductionist approach lies in the fact that reduced complexity does not directly imply increased understanding of a biological phenomenon. This is well illustrated by the creation of minimal bacterial genomes with only 473 genes (Hutchison et al., 2016). Despite the remarkably reduced complexity and size of this genome, 149 of the genes are still of unknown function. Addressing these limitations provides opportunities for ongoing research and collaborative efforts, even as progress continues in advancing understanding and functional applications of synthetic cells and organelles.

Future perspectives and community

Engineering approaches set the stage for implementing synthetic functional modules capable of performing specific functions in synthetic cells. The successful combination of all individual elements within a single entity will be key to assemble synthetic living cells. This, in turn, requires integrated inter-laboratory solutions that allow for off-the-shelf unification of individual modules. Exchanging expertise between laboratories and universal module interfaces will be essential and will enable broad participation in the field.

Discussions during the SynCell2020/21 revealed several fundamental strategic frameworks and infrastructure that are needed to achieve such a successful integration of the global community. Firstly, in the interest of effective paywall-free knowledge transfer among researchers, open-access data repositories are needed. This will facilitate transfer of experimental protocols and sharing of data and blueprints for synthetic cell modules, effectively boosting access of interested students to the field. Moreover, standardization efforts that strive to provide universal norms for the design and assembly of synthetic cell modules and interfaces need to be developed. Specific implementations of such platforms could be arranged, inspired by the collaborative software development and version control platform GitHub, which has experienced community-wide acceptance within computer science and engineering fields. The Build-A-Cell network has embraced this approach, and has begun to assemble such open-access repositories (https://www.buildacell.org/).

Secondly, engineering synthetic cells and organelles will be a model for new transcontinental educational modalities. SynCell2020/21 was organized by the National Science Foundation (USA) and the Max Planck Society (Germany). It also received support from national research programs, e.g., the Build-A-Cell network (USA-based) and the BaSyC program (Netherlands; Figure 1–figure supplement 1). Presentations by leading researchers in student-centered tutorials were a focus of the conference framework. Community-driven education programs for specialized training in relevant domains (biology, physics, chemistry, microbiology, molecular biology, biophysics, computer science or ethics) will be key for equipping new generations with the necessary skills to successfully engineer living synthetic cells and organelles. International workshops and research summer schools will be important to develop a coherent, long-lasting community that fosters cross-generational collaborations among scholars.

At present, only a limited number of training and graduate programs focused on the engineering of synthetic cells and organelles have been established, such as the Max Planck School 'Matter to Life', the Cold Spring Harbor Laboratory Summer School on Synthetic Biology, and research programs supported by the U.S. National Science Foundation 'Rules of Life' initiative. Their successful implementation will not only nurture the next generation of scientists but will also train a cohort of researchers to enable industrial applications. If possible, future events should be organized between all major research and teaching initiatives (see online-only Box 2) to bring together the global expertise and emerging talent, and to promote a broad distribution of thought leadership across institutions as the field continues to grow and develop.

Lastly, following the learning-by-building approach, the field awaits a steadily growing demand for an integrated research infrastructure that provides computational power and specialized courses in molecular and genetic design. This includes molecular modelling of large-scale, whole-cell models to predict the interactions of engineered components with host cells. Access to advanced computational facilities and enhanced algorithms for simulations based on machine learning and optimization techniques, will greatly expand the scope for designing and constructing synthetic cells and organelles. Dedicated research centers, such as the Max Planck–Bristol Center for Minimal Biology, could provide such facilities, as well as technical support for the increasingly important administrative aspects to the field, including technology transfer procedures, handling of intellectual property issues, and curation of specialized genetic parts and molecular module libraries specified for the field (inspired by biobanks such as Addgene and large-scale gene and genome synthesis 'bio foundries', such as those funded by the Department of Energy in the US).

For all the proposed measures, commitment and support from funding bodies, political and regulatory authorities, and universities with established teaching infrastructure, will be essential. Especially to successfully install a strategic, open-source platform for synthetic biology and student exchange programs, like the ones between the University of New Mexico and the Max Planck Society.

We also observe that connections to and inspiration drawn from other research communities will be important. For example, research advances addressing origin-of-life questions, the basic principles of life, and the exploration of eukaryogenesis, connect many scientific themes that arise in the study of synthetic cells and organelles. This was highlighted by the observation that many SynCell2020/21 participants are also active in these related communities. Furthermore, expanding the research community in synthetic cells to connect to these and other related scientific communities opens additional opportunities for research support, including that available from private and philanthropic foundations. Recent examples of such initiatives include the 'Life? – A Fresh Scientific Approach to the Basic Principles of Life' program, supported by the Volkswagen Foundation, and the 'Project on the Origin of the Eukaryotic Cell', sponsored jointly by the Gordon and Betty Moore Foundation and the Simons Foundation.

Specific measures will include a joint program between the research initiatives mentioned in point ii), aimed towards continued organization of the SynCell conference as a think-tank for community building and research exchange. Moreover, the Build-a-Cell initiative has initiated several focused working groups, e.g., working towards collection and annotation of synthetic cell subtype components or towards establishing *in silico* modeling frameworks of synthetic cells with predictable behavior. These groups provide an optimal platform to develop future cross-scale-organized infrastructure that will be able to manage between different stakeholders from academia, industry and political authorities, while also serving as an advisory council representing the field's interest. Furthermore, concentrated efforts will be made to raise awareness in academic faculties and scientific societies towards the importance of establishing relevant teaching schemes in graduate and undergraduate programs.

A compelling model for developing and sharing modular tools across the diverse synthetic biology community can be found in the design of the original Unix multi-user operating system and subsequent community-driven, evolutionary development of Linux (Raymond et al., 2001). Unix's 'graceful facilities' enabled users to create complex programs by using software 'pipes' to compose simple modules together: at the same time, the operating system was designed to facilitate communication among programmers as 'the essence of communal computing' (https://www.youtube.com/watch?v=qdEmbqGyeWY). Linux emerged from an unprecedented, worldwide open-source effort by volunteer programmers. These core values of streamlined, modular design and enthusiastic, open, collaborative development can similarly inform and shape progress in the synthetic cell community.

Conclusion

SynCell2020/21 demonstrated remarkable engagement of a large and geographically diverse community, and potential for global collaboration and transcontinental knowledge-sharing as the foundation for future success in the field. Importantly, a collaborative and well-trained community, including a new generation of young scholars, will be able to communicate the societal impacts of engineering synthetic cells and organelles responsibly and effectively to the public. Particularly with respect to questions of how to share intellectual property to benefit humanity while continuing to reward innovation, biosafety, biosecurity and other unique ethical and philosophical considerations, including the most fundamental question of all: 'what is life?'

Acknowledgments

The authors thank all the speakers and participants of the SynCell2020/21 and associated activities for their critical input, inspiring discussions and engaged participation. The National Science Foundation (CBET-1841170), the Max Planck Society, the New Mexico Consortium and the University of New Mexico are acknowledged for their financial support.

Authors contributions:

O.S., S.R.A., M.R.L., A.P.S. and G.P.L. conceptualized the manuscript and figures.

O.S. wrote, and S.R.A., M.R.L., A.P.S. and G.P.L edited the initial drafts of this manuscript.

K.P.A., M.D., J.D.L, E.B., A.B., A.S.B., K.J., Z.A.M., L.O., K.G., J.S., J.St. and J.P.S. provided written input or were panelists in the workshop leading to this article.

J.D.L., T.D.P., K.P.A., B.J., D.S., S.R.A., M.D., M.G., M.R.L., A.P.S., D.S., J.P.S. and G.P.L. conceptualized the International Conference on Engineering Synthetic Cells and Organelles and A.P.S, D.S., M.G., J.P.S. and G.P.L. acquired funding.

J.D.L. and T.D.P. organized the conference and workshop leading to this article.

All authors participated in the workshop leading to, and commented on the final draft of, this article.

Additional Files

Supplementary file 1

Table S1. Presentations at SynCell2020.

Table S2. Presentations at the SynCell2021 Spring Lecture Series with Build-A-Cell.

Table S3. Featured and Contributed Oral Presentations at SynCell2021.

Table S4. Poster Presentations at SynCell2021.

Table S5. Lightning Talks at SynCell2021. (Presenters chosen by jury from poster presenters.)

Box S1. Quotations from the early-career panelists of the SynCell20/21 workshop.

Box S2. Quotations from the early-career panelists of the SynCell20/21 workshop on the future challenges in the field.

References

Ausländer, S., Ausländer, D., & Fussenegger, M. (2017). Synthetic Biology—The Synthesis of Biology. *Angewandte Chemie International Edition*, *56*(23), 6396-6419. https://doi.org/10.1002/anie.201609229

Bartelt, S. M., Steinkuhler, J., Dimova, R., & Wegner, S. V. (2018). Light-Guided Motility of a Minimal Synthetic Cell. *Nano Lett*, *18*(11), 7268-7274. https://doi.org/10.1021/acs.nanolett.8b03469

Bazrafshan, A., Meyer, T. A., Su, H., Brockman, J. M., Blanchard, A. T., Piranej, S., Duan, Y., Ke, Y., & Salaita, K. (2020). Tunable DNA Origami Motors Translocate Ballistically Over µm Distances at nm/s Speeds. *Angewandte Chemie International Edition*, *59*(24), 9514-9521. https://doi.org/https://doi.org/10.1002/anie.201916281

Benner, S. A. (2010). Defining life. *Astrobiology*, *10*(10), 1021-1030. https://doi.org/10.1089/ast.2010.0524
Buddingh, B. C., & van Hest, J. C. M. (2017). Artificial Cells: Synthetic Compartments with Life-like Functionality and Adaptivity. *Accounts of Chemical Research*, *50*(4), 769-777. https://doi.org/10.1021/acs.accounts.6b00512
Damiano, L., & Stano, P. (2020). On the "Life-Likeness" of Synthetic Cells [Opinion]. *Frontiers in Bioengineering and Biotechnology*, *8*(953). https://doi.org/10.3389/fbioe.2020.00953
Dormitzer, P. R., Suphaphiphat, P., Gibson, D. G., Wentworth, D. E., Stockwell, T. B., Algire, M. A., Alperovich, N., Barro, M., Brown, D. M., Craig, S., Dattilo, B. M., Denisova, E. A., De Souza, I., Eickmann, M., Dugan, V. G., Ferrari, A., Gomila, R. C., Han, L., Judge, C., Mane, S., Matrosovich, M., Merryman, C., Palladino, G., Palmer, G. A., Spencer, T., Strecker, T., Trusheim, H., Uhlendorff, J., Wen, Y., Yee, A. C., Zaveri, J., Zhou, B., Becker, S., Donabedian, A., Mason, P. W., Glass, J. I., Rappuoli, R., & Venter, J. C. (2013). Synthetic generation of influenza vaccine viruses for rapid response to pandemics. *Sci Transl Med*, *5*(185), 185ra168. https://doi.org/10.1126/scitranslmed.3006368
Dreher, Y., Jahnke, K., Bobkova, E., Spatz, J. P., & Göpfrich, K. (2021). Division and Regrowth of Phase-Separated Giant Unilamellar Vesicles. *Angewandte Chemie International Edition*, *60*(19), 10661-10669. https://doi.org/https://doi.org/10.1002/anie.202014174
Elani, Y., Trantidou, T., Wylie, D., Dekker, L., Polizzi, K., Law, R. V., & Ces, O. (2018). Constructing vesicle-based artificial cells with embedded living cells as organelle-like modules. *Sci Rep*, *8*(1), 4564. https://doi.org/10.1038/s41598-018-22263-3
S. I. Walker, P. C. W. Davies, & G. F. R. Ellis, Eds.(2017). From Matter to Life: Information and Causality. Cambridge University Press. https://doi.org/DOI: 10.1017/9781316584200
Gallup, O., Ming, H., & Ellis, T. Ten future challenges for synthetic biology. *Engineering Biology*, https://doi.org/https://doi.org/10.1049/enb2.12011
Garamella, J., Garenne, D., & Noireaux, V. (2019). TXTL-based approach to synthetic cells. *Methods Enzymol*, *617*, 217-239. https://doi.org/10.1016/bs.mie.2018.12.015
Garcia, H. G., Brewster, R. C., & Phillips, R. (2016). Using synthetic biology to make cells tomorrow's test tubes [10.1039/C6IB00006A]. *Integrative Biology*, *8*(4), 431-450. https://doi.org/10.1039/C6IB00006A
Glock, P., Ramm, B., Heermann, T., Kretschmer, S., Schweizer, J., Mücksch, J., Alagöz, G., & Schwille, P. (2019). Stationary Patterns in a Two-Protein Reaction-Diffusion System. *ACS Synth Biol*, *8*(1), 148-157. https://doi.org/10.1021/acssynbio.8b00415
Greene, A., Pascarelli, K., Broccoli, D., & Perkins, E. (2019). Engineering Synthetic Chromosomes by Sequential Loading of Multiple Genomic Payloads over 100 Kilobase Pairs in Size. *Molecular Therapy - Methods & Clinical Development*, *13*, 463-473. https://doi.org/10.1016/j.omtm.2019.04.006
https://igem.org/Competition.
https://mattertolife.maxplanckschools.org.
https://www.basyc.nl/about-basyc/.
https://www.buildacell.org/.
https://www.youtube.com/watch?v=qdEmbqGyeWY.
Hutchison, C. A., Chuang, R.-Y., Noskov, V. N., Assad-Garcia, N., Deerinck, T. J., Ellisman, M. H., Gill, J., Kannan, K., Karas, B. J., Ma, L., Pelletier, J. F., Qi, Z.-Q., Richter, R. A.,

Strychalski, E. A., Sun, L., Suzuki, Y., Tsvetanova, B., Wise, K. S., Smith, H. O., Glass, J. I., Merryman, C., Gibson, D. G., & Venter, J. C. (2016). Design and synthesis of a minimal bacterial genome. *Science*, *351*(6280), aad6253. https://doi.org/10.1126/science.aad6253

Jahnke, K., Ritzmann, N., Fichtler, J., Nitschke, A., Dreher, Y., Abele, T., Hofhaus, G., Platzman, I., Schröder, R. R., Müller, D. J., Spatz, J. P., & Göpfrich, K. (2021). Proton gradients from light-harvesting E. coli control DNA assemblies for synthetic cells. *Nat Commun*, *12*(1), 3967. https://doi.org/10.1038/s41467-021-24103-x

Jahnke, K., Weiss, M., Weber, C., Platzman, I., Göpfrich, K., & Spatz, J. P. (2020). Engineering Light-Responsive Contractile Actomyosin Networks with DNA Nanotechnology. *Advanced Biosystems*, *4*(9), 2000102. https://doi.org/10.1002/adbi.202000102

Jia, H., & Schwille, P. (2019). Bottom-up synthetic biology: reconstitution in space and time. *Curr Opin Biotechnol*, *60*, 179-187. https://doi.org/10.1016/j.copbio.2019.05.008

Karikó, K., Muramatsu, H., Welsh, F. A., Ludwig, J., Kato, H., Akira, S., & Weissman, D. (2008). Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. *Mol Ther*, *16*(11), 1833-1840. https://doi.org/10.1038/mt.2008.200

Krinsky, N., Kaduri, M., Zinger, A., Shainsky-Roitman, J., Goldfeder, M., Benhar, I., Hershkovitz, D., & Schroeder, A. (2018). Synthetic Cells Synthesize Therapeutic Proteins inside Tumors. *Adv Healthc Mater*, *7*(9), e1701163. https://doi.org/10.1002/adhm.201701163

Lachance, J.-C., Rodrigue, S., & Palsson, B. O. (2019). Minimal cells, maximal knowledge. *Elife*, *8*, e45379. https://doi.org/10.7554/eLife.45379

Lakin, M. R., & Stefanovic, D. (2016). Supervised Learning in Adaptive DNA Strand Displacement Networks. *ACS Synthetic Biology*, *5*(8), 885-897. https://doi.org/10.1021/acssynbio.6b00009

Le Feuvre, R. A., & Scrutton, N. S. (2018). A living foundry for Synthetic Biological Materials: A synthetic biology roadmap to new advanced materials. *Synthetic and Systems Biotechnology*, *3*(2), 105-112. https://doi.org/https://doi.org/10.1016/j.synbio.2018.04.002

Li, X., Rizik, L., Kravchik, V., Khoury, M., Korin, N., & Daniel, R. (2021). Synthetic neural-like computing in microbial consortia for pattern recognition. *Nat Commun*, *12*(1), 3139. https://doi.org/10.1038/s41467-021-23336-0

Linsenmeier, M., Kopp, M. R. G., Grigolato, F., Emmanoulidis, L., Liu, D., Zürcher, D., Hondele, M., Weis, K., Capasso Palmiero, U., & Arosio, P. (2019). Dynamics of Synthetic Membraneless Organelles in Microfluidic Droplets. *Angewandte Chemie International Edition*, *58*(41), 14489-14494. https://doi.org/https://doi.org/10.1002/anie.201907278

Lussier, F., Staufer, O., Platzman, I., & Spatz, J. P. (2020). Can Bottom-Up Synthetic Biology Generate Advanced Drug-Delivery Systems? *Trends Biotechnol*. https://doi.org/10.1016/j.tibtech.2020.08.002

Meacham, D., & Prado Casanova, M. (2020). Philosophy and Synthetic Biology: the BrisSynBio Experiment. *NanoEthics*, *14*(1), 21-25. https://doi.org/10.1007/s11569-020-00369-1

Meng, F., & Ellis, T. (2020). The second decade of synthetic biology: 2010–2020. *Nat Commun*, *11*(1), 5174. https://doi.org/10.1038/s41467-020-19092-2

Miller, T. E., Beneyton, T., Schwander, T., Diehl, C., Girault, M., McLean, R., Chotel, T., Claus, P., Cortina, N. S., Baret, J.-C., & Erb, T. J. (2020). Light-powered CO2 fixation in a chloroplast mimic with natural and synthetic parts. *Science*, *368*(6491), 649-654. https://doi.org/10.1126/science.aaz6802

Mulligan, M. J., Lyke, K. E., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Neuzil, K., Raabe, V., Bailey, R., Swanson, K. A., Li, P., Koury, K., Kalina, W., Cooper, D., Fontes-Garfias, C., Shi, P.-Y., Türeci, Ö., Tompkins, K. R., Walsh, E. E., Frenck, R., Falsey, A. R., Dormitzer, P. R., Gruber, W. C., Şahin, U., & Jansen, K. U. (2020). Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature*, *586*(7830), 589-593. https://doi.org/10.1038/s41586-020-2639-4

Neumann, J. V., & Burks, A. W. (1966). *Theory of Self-Reproducing Automata*. University of Illinois Press.

Olivi, L., Berger, M., Creyghton, R. N. P., De Franceschi, N., Dekker, C., Mulder, B. M., Claassens, N. J., ten Wolde, P. R., & van der Oost, J. (2021). Towards a synthetic cell cycle. *Nat Commun*, *12*(1), 4531. https://doi.org/10.1038/s41467-021-24772-8

Otrin, L., Witkowska, A., Marušič, N., Zhao, Z., Lira, R. B., Kyrilis, F. L., Hamdi, F., Ivanov, I., Lipowsky, R., Kastritis, P. L., Dimova, R., Sundmacher, K., Jahn, R., & Vidaković-Koch, T. (2021). En route to dynamic life processes by SNARE-mediated fusion of polymer and hybrid membranes. *Nat Commun*, *12*(1), 4972. https://doi.org/10.1038/s41467-021-25294-z

Pan, J., Du, Y., Qiu, H., Upton, L. R., Li, F., & Choi, J. H. (2019). Mimicking Chemotactic Cell Migration with DNA Programmable Synthetic Vesicles. *Nano Letters*, *19*(12), 9138-9144. https://doi.org/10.1021/acs.nanolett.9b04428

Park, K. S., Sun, X., Aikins, M. E., & Moon, J. J. (2021). Non-viral COVID-19 vaccine delivery systems. *Advanced drug delivery reviews*, *169*, 137-151. https://doi.org/10.1016/j.addr.2020.12.008

Porcar, M., Danchin, A., de Lorenzo, V., dos Santos, V. A., Krasnogor, N., Rasmussen, S., & Moya, A. (2011). The ten grand challenges of synthetic life. *Systems and Synthetic Biology*, *5*(1), 1. https://doi.org/10.1007/s11693-011-9084-5

Rampioni, G., D'Angelo, F., Messina, M., Zennaro, A., Kuruma, Y., Tofani, D., Leoni, L., & Stano, P. (2018). Synthetic cells produce a quorum sensing chemical signal perceived by Pseudomonas aeruginosa. *Chem Commun (Camb)*, *54*(17), 2090-2093. https://doi.org/10.1039/c7cc09678j

Raymond, E. S., & Young, B. (2001). *The Cathedral and the Bazaar: Musings on Linux and Open Source by an Accidental Revolutionary*. O'Reilly & Associates, Inc.

Shih, P. M. (2018). Towards a sustainable bio-based economy: Redirecting primary metabolism to new products with plant synthetic biology. *Plant Science*, *273*, 84-91. https://doi.org/https://doi.org/10.1016/j.plantsci.2018.03.012

Simon, J. R., Carroll, N. J., Rubinstein, M., Chilkoti, A., & López, G. P. (2017). Programming molecular self-assembly of intrinsically disordered proteins containing sequences of low complexity. *Nature Chemistry*, *9*(6), 509-515. https://doi.org/10.1038/nchem.2715

Staufer, O., Antona, S., Zhang, D., Csatári, J., Schröter, M., Janiesch, J.-W., Fabritz, S., Berger, I., Platzman, I., & Spatz, J. P. (2021). Microfluidic production and characterization of biofunctionalized giant unilamellar vesicles for targeted intracellular cargo delivery. *Biomaterials*, *264*, 120203. https://doi.org/https://doi.org/10.1016/j.biomaterials.2020.120203

Staufer, O., Dietrich, F., Rimal, R., Schröter, M., Fabritz, S., Boehm, H., Singh, S., Möller, M., Platzman, I., & Spatz, J. P. (2021). Bottom-up assembly of biomedical relevant fully synthetic extracellular vesicles. *Sci Adv*, *7*(36), eabg6666. https://doi.org/10.1126/sciadv.abg6666

Staufer, O., Hernandez, B. J., & Rustom, A. (2018). Protease-resistant cell meshworks: An indication of membrane nanotube-based syncytia formation. *Exp Cell Res*, *372*(2), 85-91. https://doi.org/10.1016/j.yexcr.2018.09.012

Staufer, O., Schroter, M., Platzman, I., & Spatz, J. P. (2020). Bottom-Up Assembly of Functional Intracellular Synthetic Organelles by Droplet-Based Microfluidics. *Small*, e1906424. https://doi.org/10.1002/smll.201906424

Staufer, O., Weber, S., Bengtson, C. P., Bading, H., Rustom, A., & Spatz, J. P. (2019). Adhesion Stabilized en Masse Intracellular Electrical Recordings from Multicellular Assemblies. *Nano Lett*, *19*(5), 3244-3255. https://doi.org/10.1021/acs.nanolett.9b00784

Steinkühler, J., Knorr, R. L., Zhao, Z., Bhatia, T., Bartelt, S. M., Wegner, S., Dimova, R., & Lipowsky, R. (2020). Controlled division of cell-sized vesicles by low densities of membrane-bound proteins. *Nat Commun*, *11*(1), 905. https://doi.org/10.1038/s41467-020-14696-0

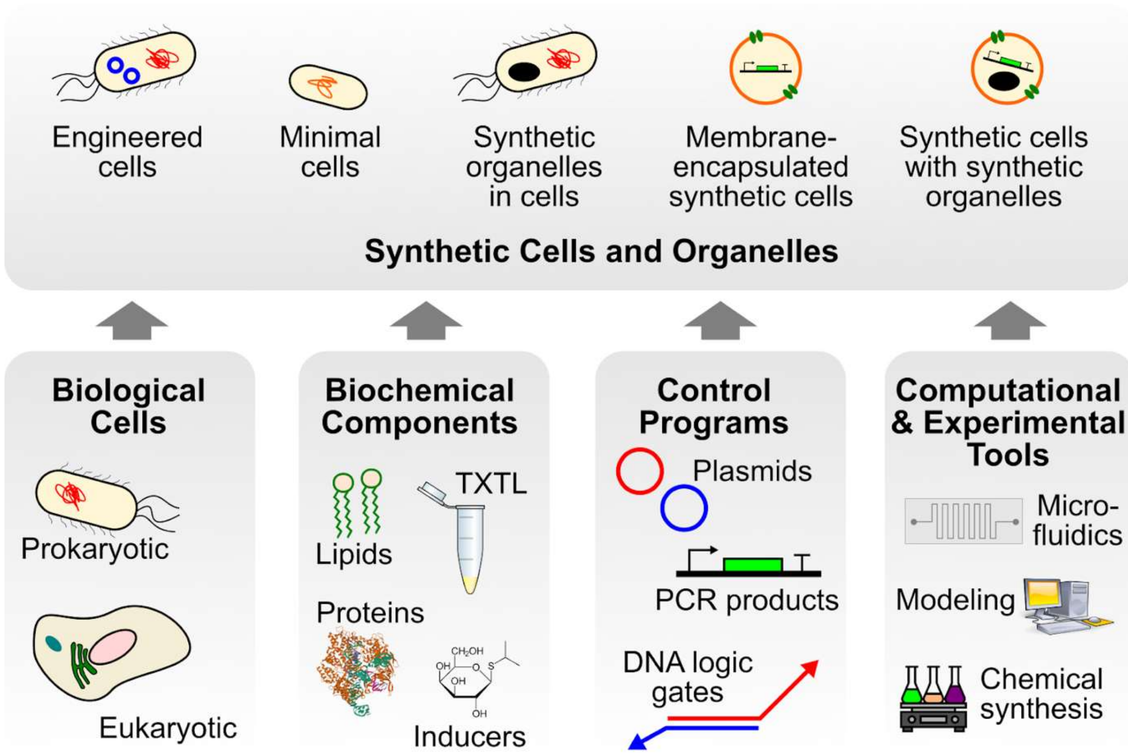
Tian, L., Li, M., Patil, A. J., Drinkwater, B. W., & Mann, S. (2019). Artificial morphogen-mediated differentiation in synthetic protocells. *Nat Commun*, *10*(1), 3321. https://doi.org/10.1038/s41467-019-11316-4
Tian, L., Li, M., Patil, A. J., Drinkwater, B. W., & Mann, S. (2019). Artificial morphogen-mediated differentiation in synthetic protocells. *Nat Commun*, *10*(1), 3321. https://doi.org/10.1038/s41467-019-11316-4
Turing, A. M. (1952). The chemical basis of morphogenesis. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *237*(641), 37-72. https://doi.org/doi:10.1098/rstb.1952.0012

Valeri, J. A., Collins, K. M., Ramesh, P., Alcantar, M. A., Lepe, B. A., Lu, T. K., & Camacho, D. M. (2020). Sequence-to-function deep learning frameworks for engineered riboregulators. *Nat Commun*, *11*(1), 5058. https://doi.org/10.1038/s41467-020-18676-2

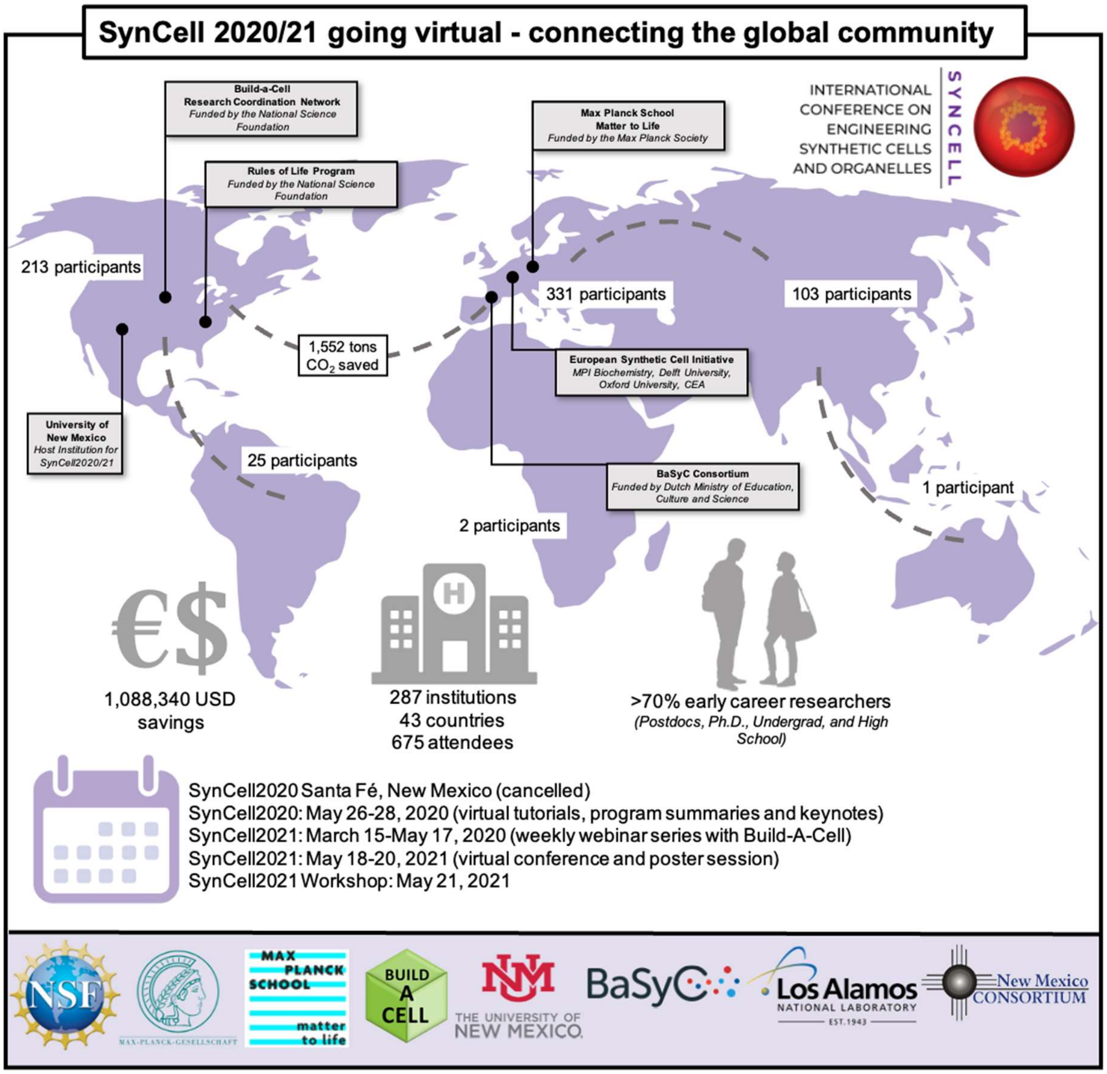
van Vliet, L. D., Colin, P. Y., & Hollfelder, F. (2015). Bioinspired genotype-phenotype linkages: mimicking cellular compartmentalization for the engineering of functional proteins. *Interface Focus*, *5*(4), 20150035. https://doi.org/10.1098/rsfs.2015.0035

Weiss, M., Frohnmayer, J. P., Benk, L. T., Haller, B., Janiesch, J. W., Heitkamp, T., Borsch, M., Lira, R. B., Dimova, R., Lipowsky, R., Bodenschatz, E., Baret, J. C., Vidakovic-Koch, T., Sundmacher, K., Platzman, I., & Spatz, J. P. (2017). Sequential bottom-up assembly of mechanically stabilized synthetic cells by microfluidics. *Nat Mater*. https://doi.org/10.1038/nmat5005

Wu, F., Bethke, J. H., Wang, M., & You, L. (2017). Quantitative and synthetic biology approaches to combat bacterial pathogens. *Current opinion in biomedical engineering*, *4*, 116-126. https://doi.org/10.1016/j.cobme.2017.10.007



Box 1 Research on engineering synthetic cells and organelles, as represented at SynCell2020/21, covers a wide range of experimental systems including engineered cells created using standard transformation techniques, minimal cells, membrane-encapsulated synthetic cells, and all of the above with the possible inclusion of engineered membraneless organelles that produce hierarchical structures. The common objective of the field is to engineer synthetic structures with defined complexity to mimic biological systems on multiple length scales. The creation and characterization of these experimental systems draws on a wide variety of interdisciplinary inputs, including biological cells, biochemical components (such as cell-free TXTL extracts), and control programs that encode desired behavior in a variety of formats. In addition, a broad range of computational and experimental tools are required.



Online-only box 2 The International Conference on Engineering Synthetic Cells and Organelles was originally scheduled to take place in Santa Fé, New Mexico (USA) in 2020 with 150 participants. The global pandemic necessitated the presentation of the program over the next year in a free, virtual format (SynCell2020/21), which greatly enhanced global participation. The world map depicts the origin and diversity of the participants as well as the major research and educational initiatives in the field of engineering synthetic cells and organelles. Logos indicate organizations that made the conferences possible.

Building a community to engineer synthetic cells and organelles from the bottom-up (Staufer *et al*. 2021 *eLife* 10:e73556)

Supplementary file 1

Table S1. Presentations at SynCell2020.
Table S2. Presentations at the SynCell2021 Spring Lecture Series with Build-A-Cell.
Table S3. Featured and Contributed Oral Presentations at SynCell2021.
Table S4. Poster Presentations at SynCell2021.
Table S5. Lightning Talks at SynCell2021. (Presenters chosen by jury from poster presenters.)
Box S1. Quotations from the early-career panelists of the SynCell20/21 workshop.
Box S2. Quotations from the early-career panelists of the SynCell20/21 workshop on the future challenges in the field.

Table S1. Presentations at SynCell2020.

Presenter	Type	Title	Date	Youtube Link
Reinhard Lipowsky	Tutorial	Understanding and controlling the morphological complexity of biomembranes	5/26/2020	https://www.youtube.com/watch?v=V5kHvKBevrM
Karin Jacobs	Tutorial	Unspecific interactions in or of cell-like compartments: theory & experiments	5/26/2020	
Michael Grunze	Program Highlight	Max Planck Matter-to-Life School	5/26/2020	
Joachim Spatz	Keynote	Bottom-up assembly of a cell	5/26/2020	
Darko Stefanovic	Tutorial	Development and application of DNA in nanoscale robotics	5/27/2020	https://www.youtube.com/watch?v=FM-70enAeFo
Jim Werner	Tutorial	Methods to visualize 3D dynamics	5/27/2020	
Marileen Dogterom	Program Highlight	BaSyC	5/27/2020	
Marileen Dogterom	Keynote	Building a functional cytoskeleton in synthetic cells	5/27/2020	
Drew Endy	Keynote	Upwelling — Challenges arising from the bottom of Earth's life well	5/28/2020	https://www.youtube.com/watch?v=SyqalYP-bFg
Laura De Laporte	Tutorial	Synthetic building blocks to grow functional tissues	5/28/2020	
Nick Carroll	Tutorial	Liquid/liquid phase separation of intrinsically disordered proteins	5/28/2020	
Kate Adamala	Program Highlight	Build-a-Cell	5/28/2020	
Andrew Ellington Kate Adamala Eberhard Bodenschatz Michael Grunze	Panel Discussion	Future synthetic cell technologies for mitigation of viral pandemics	5/28/2020	

Table S2. Presentations at the SynCell2021 Spring Lecture Series with Build-A-Cell.

Presenter	Title	Date	Youtube Link
Michael Jewett	Cell-free systems for synthetic cells, on-demand biomanufacturing, molecular sensing, and education	3/15/2021	Not posted.
Judee Sharon	DNA-mediated inducible liposome fusion	3/15/2021	https://www.youtube.com/watch?v=VmGcd-Dn4gs&t=1s
Ramin Golestanian	How living matter self-organizes while breaking action-reaction symmetry	3/22/2021	https://www.youtube.com/watch?v=djrwu4tL8go&t=1207s
Jan Steinkühler	Controlled division of cell-sized vesicles by low densities of membrane-bound proteins	3/22/2021	https://www.youtube.com/watch?v=eq_aojgf-Vo&t=1097s
Clyde Hutchison	Essential universal tasks for a minimal living cell	3/29/2021	https://www.youtube.com/watch?v=8cCj-SuMM5o&t=2156s
Oskar Stauffer	Bottom-up assembly of functional fully-synthetic extracellular vesicles	3/29/2021	https://www.youtube.com/watch?v=wSHczi0RKwM
Eberhard Bodenschatz	Synthonems: building synthetic cilia from the bottom up	4/5/2021	https://www.youtube.com/watch?v=DknSmix52Ck&t=133s
Lado Otrin	Artificial mitochondrion, bottom-up!	4/5/2021	https://www.youtube.com/watch?v=-RZdhF_X2W0
Felipe Garcia Quiroz	Membraneless and stimuli-responsive organelles	4/12/2021	https://www.youtube.com/watch?v=TPF9V20YW7c&t=1305s
Cesar Rodriguez-Emmenegger	Superselectivity in synthetic protocells	4/12/2021	https://www.youtube.com/watch?v=TPX0-c1Dxbc
Phillipe Bastiaens	A synthetic morphogenic perceptory system	4/19/2021	https://www.youtube.com/watch?v=qu6Akbg7FDM&t=455s
Zachary Manzer	Creating Biomimetic Interfaces Using the Cell-Free Synthesis of Transmembrane Proteins	4/19/2021	https://www.youtube.com/watch?v=GV5Ko-giT98
Andrew Ellington	Cell free operating systems for diagnostics	4/26/2021	https://www.youtube.com/watch?v=itZFfBC9Ihc&t=1575s
Amelie Benk	Controlled linkage of different proteins in Synthetic Cells	4/26/2021	Not posted.
Hendrik Dietz	Designing biomolecular devices and machines	5/4/2021	https://www.youtube.com/watch?v=g3FDadvwbAE
Kevin Jahnke	Proton gradients from light-harvesting E. coli control DNA assemblies for synthetic cells	5/4/2021	https://www.youtube.com/watch?v=s5ITxtjx9Fc
Neal Devaraj	Lipid sponge droplets as programmable synthetic organelles	5/10/2021	https://www.youtube.com/watch?v=9eBRysSAWXQ
Emiliano Altamura	Light-driven ATP production promotes mRNA biosynthesis inside hybrid multi-compartment artificial protocells	5/10/2021	https://www.youtube.com/watch?v=P__aO639YJY
Joachim Spatz	Mechanism in collective organizations of living and synthetic cells	5/17/2021	Not posted.
Ilia Platzman	Microfluidic approaches towards reconstitution of synthetic cells motility	5/17/2021	Not posted.

Table S3. Featured and Contributed Oral Presentations at SynCell2021.

Presenter	Type	Title	Date	Youtube Link
Neha Kamat	Featured	Membranes matter: designing bilayer membranes to control functions of artificial cells	5/19/2021	https://www.youtube.com/watch?v=h5abE8fBDRA&t=3467s
Jacqueline De Lora	Contributed	Rational design for assembly of biologically inspired compartments	5/19/2021	
Kerstin Gopfrich	Featured	A shortcut towards synthetic cell division	5/19/2021	
Matthew Good	Contributed	Engineered synthetic membraneless organelles built from self-assembling disordered proteins to regulate cellular function	5/19/2021	
Matt Lakin	Featured	Information processing in synthetic cells	5/19/2021	
Alisina Bazrafshan	Contributed	Programmable DNA origami motors	5/19/2021	
Kate Adamala	Featured	Lineage agnostic biology	5/20/2021	https://www.youtube.com/watch?v=tsez_iAzJ5E
Michael Levy	Contributed	Cell-free autonomous biogenesis of a ribosomal subunit	5/20/2021	
James Carothers	Featured	Synthetic cell systems for scalable bio-production of plant natural products	5/20/2021	
Eleonora Bailoni	Contributed	A multi-chamber flow dialysis setup for energy homeostasis in synthetic cells	5/20/2021	
Tobias Erb	Featured	Fixing CO2 fixation: Building an artificial chloroplast drop by drop	5/20/2021	
Roseanna Zia	Contributed	Cellular Stokesian dynamics": a computational model for biological cells	5/20/2021	

Table S4. Poster Presentations at SynCell2021.

Presenter	Type	Title	Date	Youtube Link
Alessandra Griffo	Poster	Insights on the effect of cholesterol and sphingomyelin on tension and elasticity of plasma-like freestanding model membranes from natural lipids	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=VnCqOzcBCSA
Jimin Guo	Poster	Biomimetic rebuilding of multifunctional red blood cells	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=_-rhtpkVwHE
Paola Albanese	Poster	Single compartment approach for photo-autotrophic protocell preparation	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=U_TaK9Z2WfI
Roberto Javier Brea Fernandez	Poster	Chemoselective generation of dynamic synthetic cells	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=-zMyvdkDSaM
Ahanjit Bhattacharya	Poster	Lipid Sponge Droplets as Programmable Synthetic Organelles	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=XeXkc-C6wNY
Telmo Diez Perez	Poster	Recombinant Intrinsically Disordered Proteins for Triggered Sequestration of Nucleic Acids via Liquid-Liquid Phase Separation	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=pa-iR9_5KpE
Nika Marušič	Poster	Charge vs. SNARE-mediated fusion of biomimetic polymer/lipid hybrid compartments: Which one is more efficient?	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=s0cSmIih6SA
Sadaf Pashapour	Poster	Generation of Extracellular Matrix Protein-based Microcapsules for Investigating Single Cells	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=XJoB_rkwJtM
Tobias Neckernuss	Poster	Monitor, categorize and manipulate label-free water-in-oil droplets in microfluidic systems	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=ggcjzq2yZyg
James Hindley	Poster	Building mechanosensitive signalling pathways in synthetic cells using membrane engineering	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=9SxuHz9WfCk
Jefferson Smith	Poster	Light-activated gene expression in synthetic cells	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=Lzx-Zl-8tC8
Michele Partipilo	Poster	A minimal pathway for the regeneration of redox cofactors	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=sBBj06mzJFo
Qi Wang	Poster	Influence of Breast Cancer Lipid Changes on Membrane Oxygen Permeability	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=7GMO2YN_hcc
Marco de Oliveira	Poster	Genetic circuits based on serine-integrases as regulatory networks in the minimal cell Mycoplasma mycoides JCVI-Syn3A	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=-stjWC7v-iQ
Satyam Khanal	Poster	Chemoenzymatic Generation of	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=a6BKQYCiHXo&feature=youtu.be

		Phospholipid Membranes Mediated by Type I Fatty Acid Synthase		
Hendrik Hähl	Poster	Pure protein bilayers and vesicles made from fungal hydrophobins: an alternative platform for synthetic cells	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=2meKpdRZ6SE
Sebastian Restrepo-Cruz	Poster	Tetraspanin Scaffold Regulation of Epidermal Growth Factor Receptor Biology on the Plasma Membrane	05/18/21 - 05/19/21	Not posted.
Franky Djutanta	Poster	Hydrodynamically-active oily ocean surface as a cradle for the emergence of life	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=TIC4bfBLBsY
Mark Walker	Poster	Engineering High Throughput Biosynthesis of Natural Product-Like Cyclic Peptides	05/18/21 - 05/19/21	https://www.youtube.com/watch?v=p-X8LUZjYC8

Table S5. Lightning Talks at SynCell2021. (Presenters chosen by jury from poster presenters.)

Presenter	Type	Title	Date	Youtube Link
Jefferson M. Smith	Lightning Talks	Light-activated gene expression in synthetic cells	5/20/2021	https://www.youtube.com/watch?v=vuwiYb5bUEE
Telmo Díez Pérez	Lightning Talks	Recombinant Intrinsically Disordered Proteins for Triggered Sequestration of Nucleic Acids via Liquid-Liquid Phase Separation	5/20/2021	
Sadaf Pashapour	Lightning Talks	Generation of Extracellular Matrix Protein-based Microcapsules for Investigating Single Cells	5/20/2021	
Hendrik Hähl	Lightning Talks	Pure protein bilayers and vesicles made from fungal hydrophobins: an alternative platform for synthetic cells	5/20/2021	

Box S1. Quotations from the early-career panelists of the SynCell20/21 workshop.

"In synthetic cell research, we are unlimited in creativity. We are challenged by the availability of resources."

"It is a field, in which you can be extremely creative and have a fling in many directions."

"All natural sciences will provide useful background since the field is so broad. The problem now is integration. We need to share protocols. The current approach of 1-1 sharing doesn't scale very well. We need to program more, design more."

"Things have developed very rapidly and people now build advanced things… we can think about 'click biology' not just 'click chemistry'. We need a streamlined platform to find building blocks and modules that can be combined together. The whole community could be engaged in this."

"I think the next generation of researchers will face the important challenge of bridging and merging different disciplines."

Box S2. Quotations from the early-career panelists of the SynCell20/21 workshop on the future challenges in the field.

"We still lack a lot of basic understanding of the components we are using in our synthetic cells. For DNA we see for example that we have already a quite good understanding, control, and cheap synthesis pipeline[s]. We are therefore able to build quite fast many new things using DNA. However for proteins, in which on a long timeframe [there may be]… more potential, we understand only fragments."

"A big challenge will be developing low-cost fabrication methods. Cell-free systems can be dramatically more inexpensive than other modes of production but further optimization in the context of coupling these with the required synthetic components will be necessary to reach a large scale."

"A major hurdle that needs to be overcome is a reliable and easy method for the encapsulation of any sort of cargo into vesicles without artefacts."

"At one point we need to combine all or at least some parts. Therefore we need to find ways to communicate and exchange, but also transfer the individual parts in easy to use modules."

"It might be helpful to form a greater network by uniting the already existing single communities while incorporating also smaller less well known groups, which might also have less financial possibilities."

"Especially the communication in between synthetic cell researchers and society needs to become clearer about our motivations and what we can and cannot do."