ROADMAP
2024-2027

CENTRE FOR LIVING TECHNOLOGIES

Bio-based engineering for human and environmental health

April 2023

A. MISSION AND OBJECTIVES

The four institutions with the strategic alliance present complementary strengths in Molecular Life Sciences. The Centre for Living Technologies (CLT) was formed with the vision to capitalize and expand strengths across the Alliance institutions in order to establish a leading role for the Alliance in the ongoing synthetic biology revolution.

Mission of the Center for Living Technologies

The core mission of the Centre for Living Technologies is to develop and exploit next-generation approaches to control (multi)cellular behavior to improve human and environmental health. These experimental living technologies act as enabling technologies for innovations and advancements in the areas of Preventive Health and Circular Society.

To achieve this mission, the Centre for Living Technologies focuses its activities on two major pillars. The first is on human capital (education, cross-institutional scientific communities and interactions with relevant stakeholders), and aims to build cross-institutional communities centered around living technologies and synthetic biology domains. The second pillar centers on technological capital (biobanks, automation platforms, new molecular tools) that support excellence of research activities.

In seeding round 1, these two pillars were organized around three distinct activities:

1. Organize **platform activities** to connect researchers and exchange knowledge and expertise;
2. Establish and reinforce **mission-driven research groups** in the field of Living Technologies;
3. Establish **accessible facilities** for integrated molecular, cellular and tissue engineering.

This first seeding round of the CLT enabled us to explore and establish new networks, appoint new research groups (as part of activity 2) and high-end technology infrastructures (as part of activity 3).

For the coming strategic period 2024-2027, we aim to strengthen and expand the buildup of human capital and cross-institutional networks, and to further implement technological capital established over the previous years in the research activities across the 4 institutions. Importantly, we have successfully obtained third-party funding that will help to compensate for the reduced seeding funds in period 2.

The main CLT objectives for 2024-2027 are:

- Intensifying the initiated research connections between the four institutes and the newly formed research-groups
- Integrating cellular engineering and ecosystems engineering into educational activities
- Better embedding CLT technology infrastructures within the 4 partner institutions

In the following, we will outline our ambitions in the area of research, education and impact, which will be built upon our successes in the first strategic period.

Research

Over the past decade, synthetic biology has been revolutionizing the biological sciences by integrating historically disparate fields such as biotechnology, information technology, manufacturing and automation. Viewing biology as technology that can be reprogrammed by targeted genetic modifications not only enables studying outstanding biological problems in completely novel ways, it also enables to engineer biological systems with novel functions that could address critical societal problems (Figure 1).

Figure 1: *Synthetic biology can provide solutions for a wide variety of societal problems*

In addition to engineering new functions into cells, new biological functions can also result from a rational design effort that affect how cells function in synthetic multicellular environments. For example, *in vitro* cultured stem cells can form 3D organ-like structures termed organoids, which recapitulate essential features of biology, disease and treatment thereof. Such patient-derived cell models are anticipated to revolutionize drug development, will be critical to prioritize innovative synthetic biology approaches for therapeutic development and will integrate into healthcare systems as living diagnostics or therapies. Likewise, in the microbiology domain efforts for the design of synthetic microbial consortia/microbiomes have been initiated for applications in industrial biotechnology for the production of chemical building blocks, to address environmental issues such as bioremediation and CO_2 fixation, or to promote *e.g.* gut health.

When founding the Centre for Living Technologies in 2020, we recognized synthetic biology and the systematic design of "Living Technologies" as an exciting new research direction that could mobilize and integrate existing expertise and strengths of the four partner institutes, and add a completely new dimension and perspective to the molecular life science research in all four institutes.

1. Platform activities

In order to bring together EWUU researchers working towards biobased technologies and firmly establish synthetic biology as a new theme, the CLT organized/co-sponsored variety of programs. List of some highlights of 2021 -2023 activities:

- *Triannual CLT meetings* for the members and invited researchers from the EWUU institutes to discuss their research and explore future collaborations for joint proposals and funding.
- *Dutch iGEM Meet* to provide a common platform for all the Dutch iGEM teams to discuss their project, brainstorm and explore partnerships.
- *Sand-pit event* on 'Synthetic Microbial Communities - Novel avenues for biobased processes?' aimed towards building a multidisciplinary research consortium for advancing the fundamental understanding of synthetic microbial communities.
- *Seminars and symposia* on mission-driven research topics of CLT.
- *Representation at conferences*: CLT was represented in over 15 poster and networking sessions at numerous conferences, including NWO Biophysics, Utrecht Science for Life, EWUU AI for Health, Gordon Conferences, etc.

In addition to the above activities, during the coming funding period we plan to organize regular interactions of CLT researchers with other working groups particularly circular society, preventive health and AI. This will help in creating synergy between the activities/research direction of the working groups. We also plan to organize hands-on workshops for the members of the Alliance

institutes (Master students to young faculty members) on topics such as deep learning/AI, expansion microscopy, microfluidics, biosensors/inducible tools, image analysis, organoid cultures, biobanking, light microscopy.

2. Mission-driven research

In addition to initiating multiple research lines ourselves, we hired junior group leaders with specific expertise. Dr. Francesca Grisoni has established the Molecular Machine Learning group at the TU/e which uses deep learning models to screen and design new small molecule drugs to combat diseases like cancer. Her position is secured by the department beyond the Alliance-funded period. At UU, the Verweij group focuses on an innovative approach to control (sub)cellular dynamics within the context of entire organisms (i.e. zebrafish). The focus will be on controlling the secretion and targeting of extracellular vesicles using optogenetic and chemogenetic approaches. Also at UU, the Biology Department has guaranteed to continue the Tenure-Track position beyond the Alliance-funded period. Dr. Grisoni and Dr. Verweij were both awarded ERC Starting grants. At WUR, Dr. Martijn Diender, initially supported by CLT, was able to secure his own funding via a Veni grant. Dr. Diender will setup novel cultivation techniques for anaerobic microbes, including microfluidics. The Microbiology chair at WUR has guaranteed long-term funding for incorporation of Martijn Diender as a Tenure Tracker (permanent staff) in the coming years.

From 2023, we have initiated new research on plant synthetic biology within CLT connecting UU, WUR and TU/e. In addition, researchers from different institutions within CLT are actively collaborating on a broad mix of different projects. For many of these projects that were initiated within the CLT we have successfully secured additional funding. This will enable us to continue with these existing research lines despite the decrease in direct funding from the Alliance.

3. Facilities

To ensure that the methods established in our research lines can be rapidly disseminated to the wider research community, we established different facilities across the four institutions of the Alliance (Figure 2, 3, 4 and 5).

Figure 2: *Facilities established within the CLT*

Molecular engineering

The Utrecht Nanobody Facility offers technology for the selection and production of nanobodies for academic and non-academic researchers. In 2021, we initiated the integration of this facility into the CLT. From 2022, the facility has been managed and operated by CLT staff and current efforts are aimed at establishing animal-free selections using synthetic libraries. At TU/e, we have also setup a molecular engineering facility that specializes in chemical biology and DNA nanostructure fabrication.

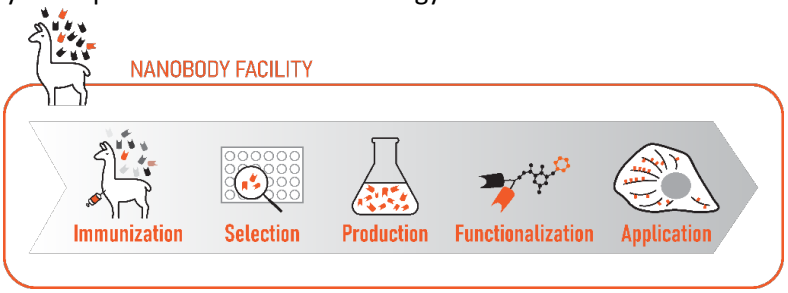


Figure 3: *Overview of the nanobody facility*

Cellular engineering

The Cellular Engineering Facility (UU) provides technologies for real-time monitoring and on-demand modulation of cellular functions. It serves as a Centre of expertise for advanced synthetic cell biology and genetic modification. It provides existing and develops novel synthetic systems, advice in designing and troubleshooting experiments, and develop on-demand reporter cell lines and specialized tools. In collaboration with the group of Jeffrey Beekman (UMCU), the facility started to apply engineering strategies to more complex models, such as patient-derived airway cells. WUR has long-standing experience and facilities for synthetic engineering tool development (*e.g.* CRISP-Cas).

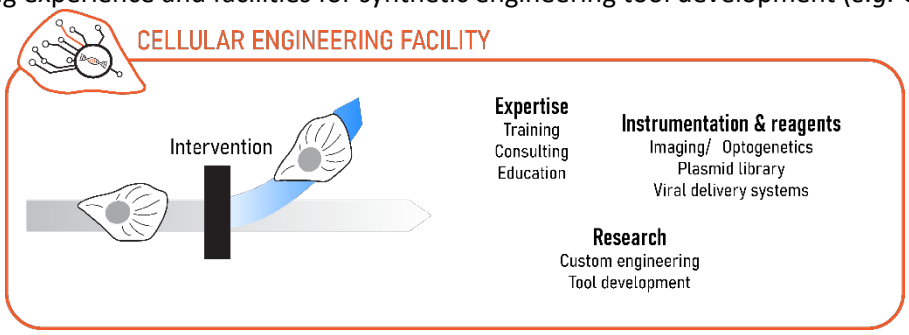


Figure 4: *Activities of the cellular engineering facility*

Disease model screening and analysis

The facility serves as a center for expertise for the development and use of primary human cell systems (e.g. organoids) in culturing, screening, imaging and quantification. The CLT has aligned with UPORT to ensure standardization of cell biobanking. The facility is currently being upgraded with an automated robotic platform for sample handling and imaging. Apart from the screening infrastructure, a biobank coordinator has been trained to facilitate activities on sharing of biomaterials, and build associated infrastructures (new biobanks and legal/ethical procedures).

Synthetic microbial communities

WUR has broad experience in working with anaerobic microbial systems. Within CLT we increased capacity for anaerobic cultivation of microbes and microbial communities at different scales. Microfluidics, for cultivation and live-cell imaging, is currently under development as a line in CLT, merging the experience between WUR-UU-UMCU. We also streamlined connections with external infrastructures, such as Unlock (at WUR) for increased capacity and expertise in what concerns microbial cultivation.

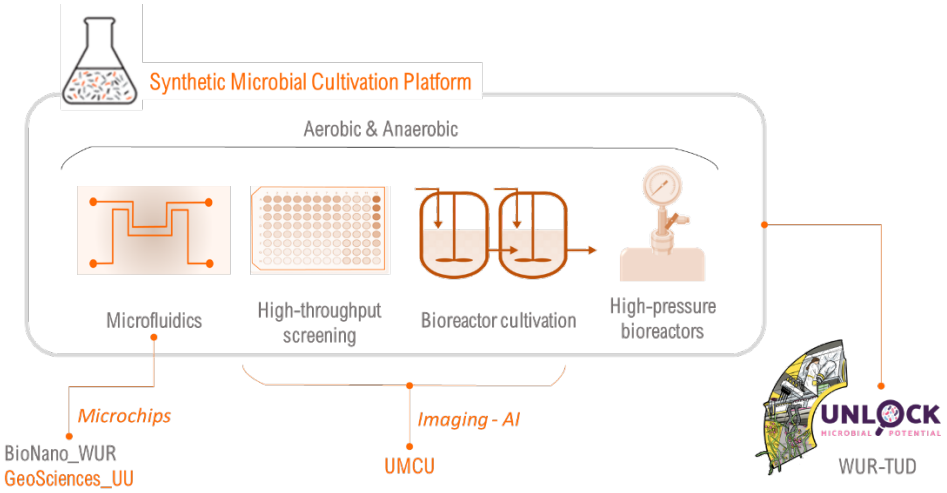


Figure 5: *Overview of the platform for synthetic co-cultivation and links with the EWUU institutions*

Maintaining and extending these four facilities requires highly skilled staff, equipment, reagents and other consumables. In the previous funding period, we have worked hard to make our facilities future-

proof by secured additional funding. In several cases, we have been able to convince our departments that the temporary positions funded by the Alliance should be converted into permanent positions after the first funding period. This will allow us to keep these facilities running during the coming funding period.

Education

Over the past years, the CLT has contributed to the development of several educational modules between the different partner institutions.

- *iGEM* CLT is the main sponsor of both the TU/e and WUR iGEM teams. iGEM is the premier student competition in the field of synthetic biology. The CLT has organized the Benelux iGEM Jamboree where student teams from Belgium, the Netherlands and Luxembourg present their project before going to the main iGEM event in Paris. In 2022, the TU/e iGEM team has won the undergraduate iGEM competition with their project on engineered cells to treat autoimmune disease.
- *Courses* Together with the working group on Education, we selected a number of courses related to synthetic biology that are now accessible for students from the four institutions.

Impact

In the past four years, CLT has generated impact both inside and outside academia and we expect this to continue over the next strategic period.

Academic impact has been generated by publishing in peer-reviewed journals, presentation of research talks at scientific conferences and obtaining prestigious grants and prizes by researchers from CLT. From an infrastructure perspective, CLT contributed to sharing of patient-derived cells from UMCU to academic labs of veterinary sciences and the beta faculty in UU and to multiple institutions throughout Europe. We helped to establish a new organoid kidney biobank (UMCU) and are working on standardized methods to collect, catalog and distribute materials efficiently. This way, we hope to make our biobank materials more accessible for researchers throughout Europe. We are also pursuing a legal framework to make it easier to share materials and knowledge within the alliance institutions.

Outside academia, CLT is generating impact by connecting to therapeutic development pipelines of companies. Joint research opportunities with biotech companies within the alliance environment [Genmab (Utrecht); Cytosmart (Eindhoven)], and beyond [Recode Therapeutics (US, mRNA therapy, close to first contract); Spirovant (US, gene therapy, close to first contract); AstraZeneca (UK, granted)] are being explored or have started. CLT will also connect to a national roadmap 'grootschalige wetenschappelijke infrastructure' initiative (in collaboration with Leiden and Rotterdam) to support a nationwide infrastructure for complex stem cell models and AI-based image analysis from such in vitro models. This infrastructure will help to connect and disseminate knowledge and assets from the EWUU alliance to both academic and commercial research within EWUU and beyond. At WUR, infrastructure for microbial cultivation was incremented at different scales, from microfluids cultivation chambers for live visualization of individual cells to lab-scale bioreactors (including high-pressure bioreactors, a unique environment for microbial studies). In addition, a collaboration with the national infrastructure Unlock (https://m-unlock.nl/) was established. Regarding microbial systems, discussions on the creation of a Microbiome Centre at WUR are ongoing where CLT could also play a role. List of key research awards and outputs are as follows:

Grants and Awards:

- Veni (M. Diender)
- Vidi (F. Verweij)
- Vici (L. Kapitein)
- ITN SYNSENSO (T. de Greef)
- 2x ERC StG (F. Grisoni, F. Verweij)

- National roadmap GWI, joint collaboration between UMCU, LUMC, EMC and hDMT (J. Beekman - S. van Beuningen)
- Health Holland – PPP (TKI) (J. Beekman - S. van Beuningen)
- Emily's Entourage (J. Beekman)
- SARS-COV2 (Health Holland and ZonMW, J. Beekman and L. Kapitein)
- Lush Young Researcher prize (F. Grisoni)
- KNAW Early Career prize (F. Grisoni)
- TU/e Groundbreaking Researcher Award (T. de Greef)

Patents:

- Provisional patent on DNA data storage (T. de Greef)
- Provisional patent on a novel synthetic microbial co-culture for the production of bioplastic precursors (D. Sousa)
- Filed patent on reference grid assembly for use in expansion microscopy (L. Kapitein)

Startup:

- Beekman co-founded FAIR therapeutics, a clinical stage biotech company with the aim to develop accessible CF medications through personalized medicine; FAIRTX has currently acquired a seed investments of 4,8M euro for the start of a Phase 2b study end of 2023.

B. OVERVIEW OF CONTRIBUTING (RESEARCH) GROUPS

This LT workgroup started with the following persons:

- Lukas Kapitein, UU
- Jeffrey Beekman, UMCU
- Diana Machado de Sousa, WUR
- Tom de Greef, TU/e

UU and UMCU

Key areas of research at **UU** and **UMCU** are regenerative medicine and stem cells, one health, personalized medicine and fundamental life sciences (Science for Life). To capitalize on existing strengths and enable collaborations between different groups, Utrecht Science Park houses numerous facilities that offer specific resources and instrumentation for research on the molecular, cellular, tissue and organismal scale (https://www.utrechtlifesciences.nl/facilities). This includes facilities for organoid research, advanced high-resolution microscopy to follow molecular dynamics inside living cells and tissue, as well as facilities for structural biology to understand the structure and dynamics of biomolecules at the atomic level.

Lukas Kapitein, UU

Kapitein's research group has a strong track record in developing novel technological approach to answer key questions on the organization and dynamics of complex cells, such as neurons. In particular, the group specializes in super-resolution microscopy and high-resolution live-cell imaging. When combined with cellular engineering and innovative optogenetic approaches this enables dissecting cause-consequence relationships through well-controlled perturbations with high spatiotemporal precision.

Jeffrey Beekman, UMCU

Beekman's research group focuses on developing cell-based disease models, with a focus on rare genetic disease. His research is set by patient priorities and bridges academia, private and clinical domains (biotech, clinicians, regulatory authorities). Their primary cell models include intestinal stem cell organoids and primary airway stem cell culture models that are used for the study of pathophysiological mechanism, drug development and personalized medicine. He is currently expanding his disease modeling activities from simple genetic diseases to more complex genetic diseases and infectious diseases.

WUR

WUR is known for its focus on life sciences, which is patent in its mission motto 'to explore the potential of nature to improve the quality of life'. Research core domains are: natural resources and living environment; food, feed and biobased production; and society and well-being. In this frame, WUR aims to generate and apply knowledge and assist with education to address current challenges in biodiversity, climate, sustainability and circular economy, bio-circular agri-food systems and healthy and secure food.

Diana Machado de Sousa, WUR

D. Sousa leads the Microbial Physiology group that focusses on studying the physiology of the anaerobic microorganisms and anaerobic microbial communities (natural or synthetic) that play an important role or have potential for application in sustainable circular economy approaches. The main research interests are the physiology and functional genomics of syntrophic fatty-acid degraders and anaerobic microorganisms converting C1 compounds (carbon monoxide, syngas) and biotechnological applications of synthetic anaerobic microbial networks for the production of chemical building blocks.

TU/e

Research at the **TU/e** is less cell-oriented and more focused on biomaterials and the design of modified molecules and new functional materials, molecular diagnostics and chemical biology. Much of this expertise and the corresponding principal investigators are part of the Institute for Complex Systems (ICMS) and are integrated in the TU/e Strategic Area Health. Several of these areas are key drivers in two national Gravitation programs, i.e. the research center for Functional Molecular Systems (FMS) and the Materials-Driven Regeneration (MDR) program which focus on supramolecular chemistry, regenerative medicine, biomaterials discovery & characterization, nanomedicine and chemical biology. The TU/e is aiming to be the leading university in the Netherlands on artificial intelligence and machine learning and has recently bundled its expertise on these topics in the Eindhoven Artificial Intelligence Systems Institute (EAISI).

Tom de Greef, TU/e

T. de Greef is Full Professor Synthetic Biology at the department of Biomedical Engineering. Work in the Synthetic Biology group is directed at the bottom-up construction of basic cellular functions from well-characterized biological components, and the development of novel biological computing devices that can enhance signal-processing capabilities of natural and synthetic cells. This involves the design and construction of integrated genetic, protein or DNA-based circuits, either in a cell-free environment or in living cells, capable of autonomously performing useful functions. Ultimately, advances in synthetic biology will allow the development of functional living and hybrid technologies such as biological robots, synthetic cells or augmented natural cells, that can be interfaced with the human body to detect disease biomarkers and allow autonomous, closed-loop therapeutic functions.

Junior CLT and other associated members

Francesca Grisoni, TU/e

F. Grisoni is Assistant Professor at TU/e where she leads the Molecular Machine Learning team. Her team focuses on developing and applying data-driven methods to design novel molecular entities and unveil structure-activity relationships of small molecules and peptides. With research located at the interface between chemical biology and AI, they aim to augment human intelligence in drug and molecule discovery.

Frederik Verweij, UU

F. Verweij is Assistant Professor leading the group Extracellular Vesicle Biology. His group focusses on small nanometer-sized Extracellular Vesicles (EVs) to understand the intercellular communication in

multicellular life forms. They cover aspects from single-cell up to organismal level to decode molecular mechanisms in source and receiver cells that underly the biological role of this mode of communication in health and disease.

Lapin Dmitry, UU
D. Lapin is Assistant Professor in the group Translational Plant Biology at UU. His research focuses on understanding the plant immune system and how plants recover from the infections, which is an important target of crop improvement. Current research is based not only on the model organism *Arabidopsis thaliana* but also on lettuce, which is an emerging model organism for the largest family of plants – *Asteraceae.*

Renze Heidstra, WUR
R. Heidstra is Associate Professor in the Plant Developmental Biology cluster at WUR. He investigates the molecular mechanisms controlling cell fate specification, a process that is taking place during embryogenesis but is reiterated during the life of the plant to generate the adult architecture. Fate decisions are also instrumental during regeneration of a plant from somatic cells, a process important for modern plant propagation and breeding techniques.

Nico J. Claassens, WUR
N. J. Claassens is Assistant Professor in the Laboratory of Microbiology. A core activity of his group is to perform systems-wide metabolic engineering using novel high-throughput genome editing techniques (e.g. recombineering and CRISPR-Cas based) in bacteria. Specifically he focuses on engineering synthetic pathways to support the efficient use of next-generation, sustainable feedstocks, including CO_2 and one-carbon-substrates, such as formate and methanol. Furthermore, his work includes the optimization of protein expression (including enzymes and membrane proteins) via both rational design and randomization strategies targeting gene regulatory elements and codon usage. Nico has also supported the initiation of the Dutch synthetic biology association SynBioNL in 2022, to which he is an advisory board member. In addition he is one of the coordinators of the Wageningen iGEM teams (international synthetic biology student competition).

Raymond Staals, WUR
R. Staals is Assistant Professor at the Laboratory of Microbiology at WUR. His research revolves around protein engineering and fundamental studies on bacterial defense systems, with a long-lasting expertise on type III CRISPR-Cas systems in particular. During the last few years, he developed several genetic engineering, diagnostic and biosensing applications that evolved from the obtained fundamental knowledge on these and other CRISPR-Cas systems.

Clara Belzer, WUR
C. Belzer is Associate Professor at the Laboratory of Microbiology. Belzer specializes in microbial diversity, molecular genetics and the microbiome of the digestive tract, among other fields. Her research focuses on the influence of nutrition on the microbiome of babies and the influence of the microbiome on disease development. She previously worked as a postdoctoral researcher at Harvard Medical School, and at Erasmus MC Rotterdam, where she researched gut health.

Maria Suarez Diez*, WUR*
M. S. Diez is Professor and Chair Systems and Synthetic Biology at WUR. Her research focuses on building and using mathematical and statistical models to gain systems level understanding of the dynamics of cellular networks. She has extensively worked in generating and deploying models of metabolism, in studying microbial communities and on large scale integration of heterogeneous datasets. Her work exploits semantic technologies to develop powerful and scalable bioinformatics systems to facilitate prediction of complex phenotypes from multiple heterogeneous sources.

Martijn Diender (WUR - prospective TT)
M. Diender is a Veni grant awardee at the Laboratory of Microbiology, WUR. He specializes on microbes that use carbon monoxide (CO) as their food source and how these can be applied for biotechnological applications (e.g. production of chemical building block). His Veni research aims to study CO-utilizing microbes that can link their metabolism to metal reduction and how this type of metabolism exactly functions on a molecular level. In addition, Martijn works on developing new anaerobic cultivation and screening methods, such as anaerobic microfluidics and anaerobic high-throughput cultivation.

Sam van Beuningen, UMCU
Sam van Beuningen is a senior data scientist affiliated with the Department of Pediatric Pulmonology (UMC Utrecht) and the Regenerative Medicine Center Utrecht. He has expertise in molecular biology, advanced microscopy and data science and focuses on developing automated image analysis pipelines based on deep-learning. He provides extensive support on the development of primary cell and organoid based disease models and high-content-screening assays, quantitative imaging projects, automation, and FAIR data solutions.

Wilco Nijenhuis, UU
W. Nijenhuis is a senior scientist and a facility manager in the nanobody and cellular engineering facilities at Utrecht University. His research focuses on organelle interdependence and transport-related diseases using optimized optogenetic heterodimerization systems. He aims to create new synthetic systems, visualize and control cellular pathways within advanced (epithelial) model systems, and develop a center of expertise on inducible synthetic tools as part of the Utrecht cellular engineering facility.

CLT members at the CLT Meet (Dec 2022, Social Impact Factory, Utrecht)

C. COLLABORATION STRATEGY

Research within the CLT has a strong fundamental character. However, Living Technologies have a range of emerging applications that could transform society by addressing critical societal issues in the area of health and green society. This is evident, for example, by two recent EU Pathfinder calls in this area, i.e. the EU Pathfinder *Engineered Living Materials* and *DNA-based Digital Data Storage* calls where CLT members were involved in defining the scope of these projects. In the past years, CLT has established various collaborations with industry including Microsoft Research, Novartis, IBM research, Danone, Torrgas, Paques Global, OnePlanet and Genmab. We will continue to disseminate our scientific results to these industrial partners and involve them in new calls that require industrial partners to further translate our fundamental discoveries to emerging applications.

The joint collaboration of UMCU/UU (Beekman/Kapitein) is highly interesting for biotech companies in the field of airway diseases as we can offer a wide variety of outstanding technologies from the basic research to applied domain. AstraZeneca has already started an investigation into RSV that for now focused on the applied domain. Especially in the context of rare genetic airway diseases such as primary ciliary dyskinesia, our joint activities are highly appreciated by clinicians and biotech industries, and we are building a joint research effort in this domain that bridges the entire research area from basic research to clinical observational and intervention trials. At the level of policy makers, we will collaborate with EMA to work towards a first regulatory approval of organoid-based disease models for personalized drug testing. We will also raise awareness on the importance of Living Technologies to policy makers within the European Research Agencies and NWO with the goal of establishing specialized calls on this topic. Finally, we will also raise awareness on Living Technologies to the broad public both via our iGEM teams and by establishing contacts with the Rathenau institute who are currently charting both the EU and Dutch landscape on applications of synthetic biology.

To further strengthen the connections between the four institutions of the Alliance, we will actively seek opportunities to collaborate with the other alliance initiatives, in particular the initiatives on Preventive Health, Circular Society and Artificial Intelligence. As can be seen in our agenda for the coming years (section D below), several topics for collaboration have already been identified during the preparation of the current Roadmap. To further develop these collaborations, we will schedule regular meetings with the representatives of the different initiatives.

D. AGENDA 2024-2027

Research

In the past four years, CLT has focused on three major research themes 1) Cellular engineering, 2) Engineered cellular communities and 3) Synthetic morphogenesis. We will continue the basic research that we initiated within these themes, mostly funded by external funds acquired during the course of the first funding period. In the period 2024-2027 we foresee an integration of these topics with efforts within the preventive health and circular society initiatives, as discussed below. We furthermore foresee a key role in various emerging artificial intelligence technologies in cellular imaging and protein design to accomplish these goals and will align our efforts with the EWUU AI initiative.

Preventive Health

Synthetic biology and living technologies offer new solutions for preventive health which are beyond reach with current approaches. The CLT focus on developing early innovations within these domains, up until proof-of-concept and validation within a laboratory environment (Technology Readiness Levels 1-4). This focus on fundamental research and early innovation aligns with current programs of our institutions such as ICAT, and aim to ensure that the pipeline for innovations within the preventive healthcare domain remains filled. CLT activities will focus on the development and application of living technologies and synthetic biology in the context of target identification and validation, drug development efforts and precision medicine applications based on advanced diagnostic and therapeutic (living) innovations.

Living Diagnostics Synthetic biology devices including (patient-derived) living cells can act as biomarkers for disease or it's treatment, with improved sensitivity and specificity compared to current, non-living biomarkers. An advantage of using synthetic biological devices to diagnose disease in an early stage is the ability of these systems to identify different targets and integrate signals produced by each target in a more specific response. In the next four years, CLT will develop cellular communities based on mammalian and bacterial cells that can sense, amplify and process different types of biomarkers for early disease diagnostics. We will integrate our expertise in genetic

engineering, AI-driven protein engineering and imaging to develop cellular communities that can measure a diverse set of biomarkers with high sensitivity and specificity. As a readout, we will use both fluorescence-based outputs as well as genetic tape recorders whose output can be measured using next-generation sequencing. By integration of these cellular communities into materials, we will develop living biosensors that can measure biomarkers both within and outside the human body.

Organoid Technology Organoids are microscopic self-organizing, three-dimensional structures that are grown from stem cells in vitro. They recapitulate many structural and functional aspects of their in vivo counterpart organs. This versatile technology has led to the development of many novel human disease models that can be used to monitor disease progression as well as high-throughput drug screening. In the next four years, CLT will develop an integrated organoid technology platform that combines closed-loop automation, AI-assisted imaging and generative, AI-driven drug design for high-throughput screening of novel drugs for genetic diseases including cystic fibrosis, PCD and cancer. In parallel with preclinical activities in these models, we study how observations in these models can predict individual disease in the context of natural progression, or after treatment. This helps to better define individual disease progression and how to prevent or slow down disease through interventions such as life style changes, therapeutic selection, etc.

Circular Society

Engineering of synthetic microbial co-cultures (SMCs), i.e. artificially assembled mixtures of two or more microbial species, is an emerging field with promising applications. In biotechnology, SMCs can be used to develop novel bioconversion routes or to enhance catalytic performance – still, applications of SMCs is mainly restricted to food processing (starter cultures for fermentations). Application of SMCs for the synthesis of chemicals is still unexplored at industrial level, and an avenue we pursue in CLT.

Achieving the EU's climate and sustainability goals urges for new circular economy approaches. In the Netherlands, agreements on circular economy (Grondstoffenakkoord) and on mitigation of CO_2 emissions and climate change (Klimaatakkoord) were also signed. At CLT we address these urgent challenges for example by developing novel bioprocesses to convert C1-compounds (CO, CO_2) to useful chemicals. The scientific challenge lies in the use of SMCs, an approach that diverges from traditional bioprocesses where pure cultures of microorganisms are used. SMCs are powerful biocatalysts, considering their robustness and catalytic efficiency, and offer the potential to unlock C1 microbiological routes towards a variety of products. This paradigm shift, from mono- to mixed-culture cultivation, will lead to detailed understanding of microbial interactions, which are at the centre of the proposed research. This knowledge is essential to create optimal SMCs (for application in *e.g.* biotechnology (carbon capture, bioremediation), in health (probiotics), etc.), and also to comprehend microbial function in natural ecosystems (*e.g.* carbon and nutrient cycling in soils and oceans, functioning of gut microbiome).

Artificial Intelligence

The design of living technologies has undergone a rapid paradigm shift in recent years. While the design of new living technologies was historically done using an experimental approach based on trial-and-error, recently AI-driven approaches have shown to speed up the design-build-test cycle of living technologies resulting in a much faster development of new cell-based and molecular systems. In the next four years, we will use AI to design new proteins and cell-based systems de novo. At the TU/e, a new tenure track position will open on AI-driven protein engineering, and we will use this position to collaborate with the Alliance Artificial Intelligence initiative. Specifically, we will collaborate to develop new algorithms that will allow a closed-loop design-build-test-learn cycle for the development of new living technologies.

Research Facilities

In the period 2024-2027 we will continue to operate the established facilities using staff and post-docs. We will more broadly advertise our expertise within the Alliance to ensure that our unique combination of expertise will be optimally utilized within the alliance. In particular, we expect the following developments during the next phase.

1) *Molecular Engineering* At the TU/e, the molecular engineering will be staffed by a post-doctoral researcher during the next period. We will further expand this facility with expertise on biological lab automation and AI-assisted protein engineering via the recruitment of a new tenure-tracker and collaboration with the growth fund proposals Pharma NL and the Chemical Robotlab in which the TU/e participates. For the nanobody facility at UU, one technician hired from other funds will be responsible for selections using a newly established synthetic library and for supporting the educational activities within the Biotech hub.
2) *Cellular engineering* This facility will continue to function as an expertise centre for high-precision visualization and control of cellular systems. During the second funding period its manager will be funded directly by the Department of Biology of Utrecht University, while the technician will be hired from Alliance funds.
3) *Disease model screening and analysis* The facility serves as a center for expertise for the development and use of primary human cell systems (e.g. organoids) in culturing, screening, imaging and quantification. The CLT has aligned with UPORT to ensure standardization of cell biobanking. Activities will be centralized through UPORT or NPORT and a business model for biobanking will be developed so that cost price can be established for various materials from the biobank. The Living Technologies screening facility is capable of autonomous screening of cellular disease models using microscopy and various liquid handling devices all connected with a robotic set-up and control software. We will transform our AI in microscopy projects into a AI lab for Living Technologies to bring together AI knowledge and develop standardized disease model analysis pipelines using advance AI techniques. The Living Technologies screening platform together with an AI lab for Living Technologies brings high-throughput and standardized disease model research to the alliance and allows for improved healthcare by facilitating personalized medicine and drug discovery. To bring the screening platform into the next phase for advanced molecular and 3D cell screening we require an additional equipment investment of 0.5M. We will focus on a technician and postdoc position to run our facility in the next phase. In addition, to maintain and secure future access to our Living Technologies screening and AI facility we strongly believe in an senior scientist or PI staff position funded directly by the UMCU for future sustainability.
4) *Microbial cultivation and synthetic communities* The anaerobic facilities at WUR can provide on-demand personalized solutions for cultivation/screening/visualization of anaerobic microbes. The current infrastructure of this facility is state-of-the-art in the anaerobic microbiology field and we aim to further innovate and expand it in the coming period. We will continue developing microfluidics and imaging facilities in collaborations with partners at WUR, UU and UUMC. In addition, the collaboration with the Unlock program will be further established, expanding the options for anaerobic high-throughput analysis and microreactor cultivation studies. Together with Systems and Synthetic Biology group at WUR we develop models for the design of synthetic co-cultures. We want to further develop our facilities to study controlled cellular organization of microbes in co-cultures.

Education

In the period 2024-2027 we will continue to integrate and expand the education linked to the CLT. Specifically, we would like to focus on the following aspects

Biotechnology Teaching Hub CLT members within the Faculty of Science at UU are directly involved in establishing a Biotechnology Teaching Hub to accommodate small scale research projects for groups of up to 20 students. The hub is embedded within the CLT Utrecht Nanobody and Cellular Engineering facilities. This creates synergy between project-driven

teaching courses and CLT cellular and protein engineering activities. To house the hub, dedicated lab spaces will be constructed at UU. Planning of the reconstruction effort is in an advanced stage and investments in dedicated equipment have already been made by UU Faculty of Science.

iGEM Currently, only WUR and TU/e have an active iGEM team. Next to providing active support of these teams in the next period, CLT will also establish a joint iGEM team between UU and UMCU. In this way, all four Alliance universities will have active iGEM teams which will contribute to a vibrant synthetic biology community within the Alliance.

MOOC Microbial Ecosystems Engineering Microbiology and Systems and Synthetic Biology at WUR plan to take initiative in the creation of a MOOC to offer within EWUU and outside with a focus on engineering of synthetic microbial communities. This will cover physiology, modeling, process engineering aspects, etc.

Erasmus summer school With its knowledge on anaerobic microbial production processes, WUR will contribute to the PhD summer school on Biological Carbon Capture Technologies (Erasmus Blended intensive program, coordinated by the University of Valladolid (Spain)); two CLT members will lecture C1-metabolism, gas fermentation and microbial production topics.

Coherent course packages for Master students In the next years we will develop a coherent master package for students interested in synthetic biology and living technologies. This coherent package consists of participating in the iGEM competion as well as following courses at WUR, TU/e and UU. Master students will follow a synthetic biology course at WUR with a strong emphasis on bacterial synthetic biology, a synthetic biology course at UU with a strong emphasis on mammalian cell-based systems and a synthetic biology course at the TU/e with a strong emphasis on biomolecular interactions. By combining these courses with the iGEM competition, students will both be trained at a theoretical and experimental level.

External collaboration

In the period 2024-2027 we will establish various collaborations with large consortia within the Netherlands to make the CLT more visible. Below, a summary is provided

1) *Growth fund consortia*. We will establish contacts and collaborations with the growth fund consortia PharmaNL and RobotLab. The PharmaNL consortia is building a large microfluidic screening facility at the TU/e to screen formulations for drug delivery. At the same time, the TU/e is developing an autonomous robotlab to synthesise and screen formulations via an AI-assisted closed loop strategy. The facilities and knowledge generated within these consortia could also be important for applications in synthetic biology, with applications in preventive health and circular society. CLT is strongly connected to a round 3 growth fund application that focuses on animal free innovations and to establish animal-free in vitro model technologies to which CLT and EWUU will connect (e.g. development of animal-component free culture media, in silico based drug development).
   WUR is involved in the growth fund *(GroenVermogenNL)* on hydrogen underground storage. In addition there have been contacts for participation in *FutureCarbonNL*, related with carbon capture and carbon fixation. These consortia and projects will allow a faster transfer of knowledge developed at CLT and are closely connected to circular society approaches.
2) *Gravitational consortium IMAGINE!* The mission of this consortium is to transform cell biology by shifting from studying cells on glass to studying cells in their natural environment. Next to innovative microscopy approaches, this requires the ability to establish well-controlled multicellular systems that are engineered to respond to specific stimuli, in order to dissect cause-consequence relationships. This will require the expertise of the CLT Cellular Engineering Facility.

Community building

In order to bring together EWUU researchers working towards biobased technologies and firmly establish synthetic biology, the CLT plans to organize events such as:

CLT meetings for the members and invited researchers from the EWUU institutes to discuss their research and explore future collaborations for joint proposals and funding.

Dutch iGEM Meet to provide a common platform for all the Dutch iGEM teams to discuss their project, brainstorm and explore partnerships.

Collaborating with SynBioNL Co-organizing synthetic biology conference and research talks with the Synthetic Biology Association of the Netherlands.

Engage with relevant scientific communities Organizing joint events to increase visibility and build living technology community.

Hands-on workshops for the members of the Alliance institutes (Master students to young faculty members) on topics such as deep learning/AI, expansion microscopy, microfluidics, biosensors/inducible tools, image analysis, organoid cultures, biobanking, light microscopy.

To further strengthen the connections between the four institutions of the Alliance, we will actively seek opportunities to collaborate with the other alliance initiatives, in particular the initiatives on Preventive Health, Circular Society and Artificial Intelligence. As can be seen in our agenda for the coming years, several topics for collaboration have already been identified during the preparation of the current Roadmap. To further develop these collaborations, we will schedule regular meetings with the representatives of the different initiatives.

E. MILESTONES AND DELIVERABLES

For the year 2024-2027, the following deliverables are expected.

Research

- We will foster and expand the Living Technologies community across the Alliance
- We will promote the facilities that we established in order to attract more users from across the four institutes. We anticipate that this will lead to joint publications (>5 per year) and joint applications for additional funding (>3 per year).
- We aim to regularly publish collaborative papers from the different CLT groups (>5 per year).

Education

- We aim to establish a coherent package of Master courses for students interested in synthetic biology and living technologies.
- We will also open the Biotech hub at UU to students from other institutions.
- To sort out the logistic challenges, we will also connect with the workgroup on education.
- Finally, we will explore the possibilities to establish a summerschool on organoids.
- We will establish a UU-UMCU iGEM team
- We expect three PhD candidates to defend their thesis in this period

Funding and connection with other working groups

- We will have regular meetings (>4 per year) with the representatives of the other initiatives within the alliance to identify possibilities for collaboration and cross-fertilization.
- We will establish a AI lab for Living Technologies and coordinate our efforts with the working group on AI.
- To secure external funding, we will participate in various grant applications, such as
    - SUMMIT proposal on microbial evolution (WUR, UU, RU, NIOZ)
    - SUMMIT proposal on regenerative medicine and living technologies (UMCU)

F. GOVERNANCE

The Supervisory Board consists of General Board members. The Supervisory Board meets at least twice a year. The role of the Supervisory Board is:

- to approve the roadmap and the annual agenda's
- to monitor progress on the roadmap
- to ensure commitment from the relevant faculties/science groups/divisions at TU/e, WUR, UU and UMCU.

Members of CLT Supervisory Board:

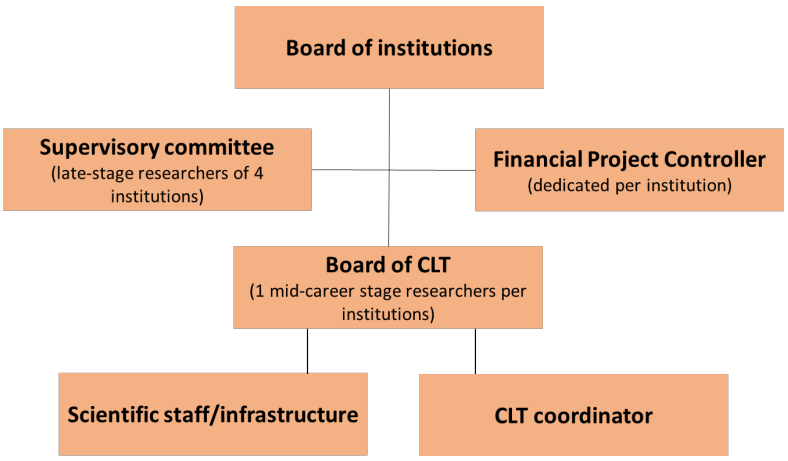
- Maarten Merkx (TU/e)
- Birgit Loos (WUR)
- Alain de Bruin (UU)
- Marianne Verhaar (UMCU)

Members of CLT steering committee:

- Tom de Greef (TU/e)
- Lukas Kapitein (UU)
- Jeffrey Beekman (UMCU)
- Diana Machado de Sousa (WUR)

G. MANAGEMENT

The overall organization of the CLT, in relation to the institution and scientific advisory committee follows the following scheme.

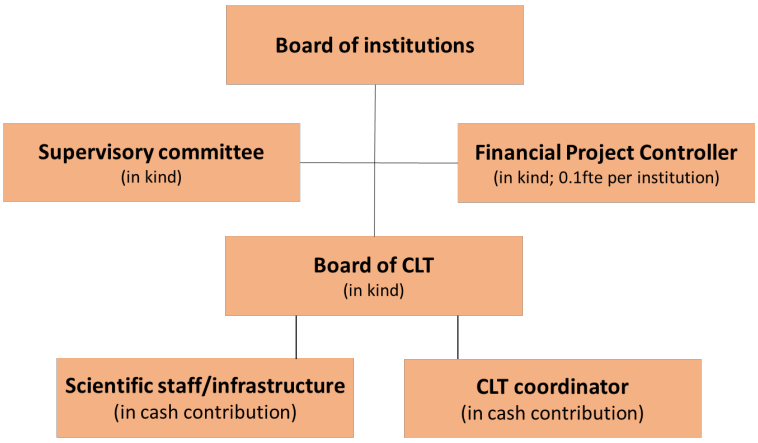


To organize the activities and management of CLT, we propose to appoint a project manager/ coordinator to coordinate the organizational aspects of our program. This person will have the following responsibilities:

- Advocate the CLT within our four institutes
- Proactively identify new opportunities for collaboration and funding
- Organizing platform activities such as meetings, workshops, training sessions, network events.
- Identifying funding opportunities and contribute to grant writing.
- Write reports
- Organization of dissemination and outreach activities, including social media.
- Coordinating with the Alliance office and other working groups.

- Providing and managing administrative support.

H. SUPPORT TEAM



I. FINANCE

Provided in the excel file.

Following consultation by Esther Stiekema, we request a higher budget for the first two years than initially foreseen by the executive board. The excess amount is the remainder of the budget from the first funding period, during which the Covid-19 pandemic caused a delay in recruitment and the start of contracts.

For the new road map, we request funding for the following:
- Programme coordination (0.8 fte), see above for tasks
- Research staff that will further develop the facilities and/or provide user support
- Other costs (travel, events, consultants, collaboration, etc.)
- Overhead on personnel costs (25%, following the Alliance regulations)