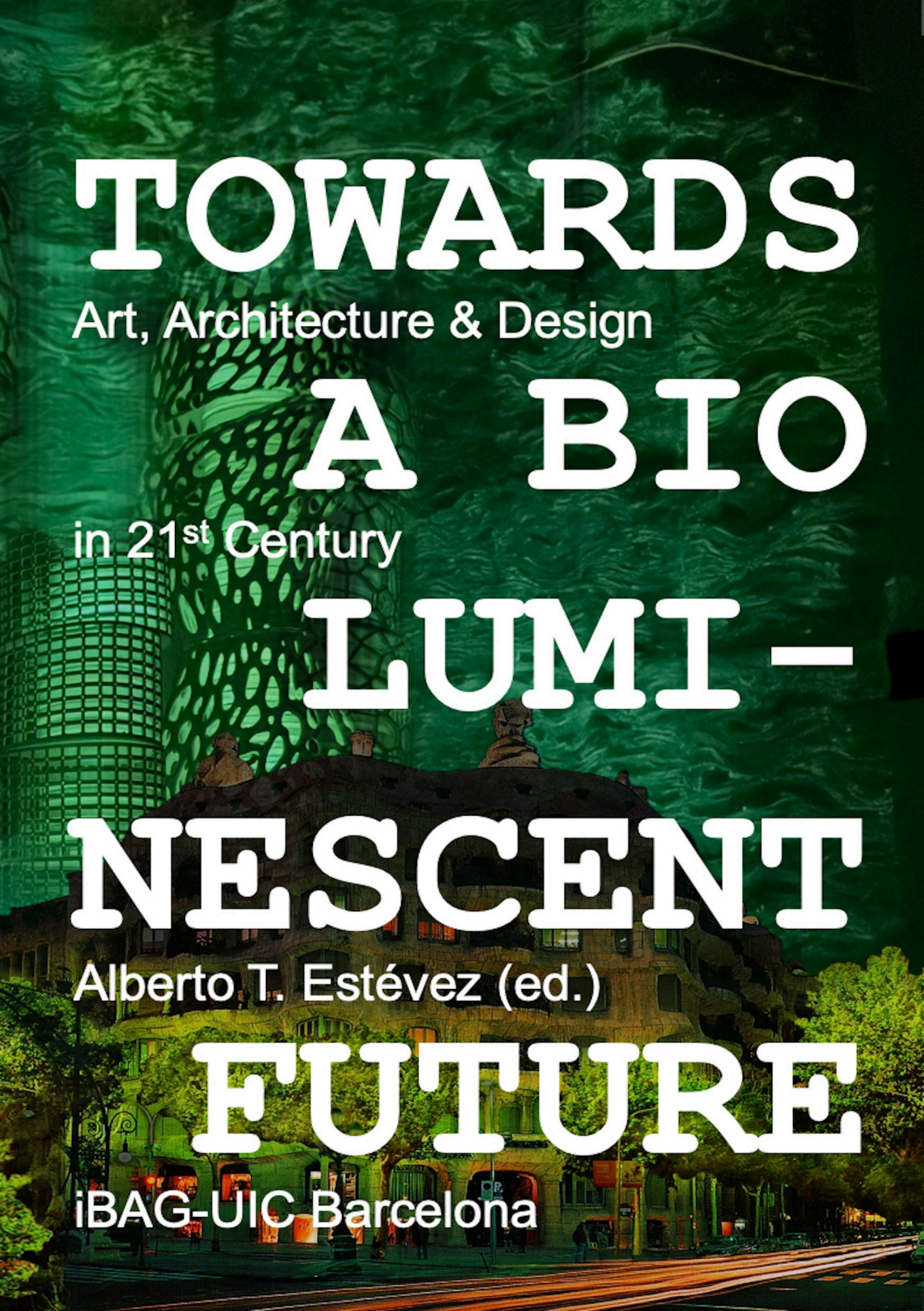
TOWARDS
Art, Architecture & Design
A BIO
in 21st Century
LUMI-
NESCENT
Alberto T. Estévez (ed.)
FUTURE
iBAG-UIC Barcelona

Alberto T. Estévez (ed.)

TOWARDS A BIOLUMINESCENT FUTURE:

Art, Architecture & Design in 21st Century

iBAG-UIC Barcelona, 2026
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that constitute the iBAG-UIC Barcelona.

This book owes its
gratitude to all who have collaborated
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BioLumCity Project

Alberto T. Estévez

Summary
Barcelona, 2023-2026

The BioLumCity Project, directed by Alberto T. Estévez, is the 6th phase (2023-2026) of the Genetic Barcelona Project, that since 2003 proposes an advanced and feasible integration of real bioluminescence into architecture and urban design, bridging biotechnology, digital fabrication, and aesthetic research. This 6th phase first focuses on naturally occurring bioluminescent organisms, particularly *Vibrio fischeri*, selected for its relative intensity, durability, and ease of implementation in non-illuminated interiors. To overcome the intrinsic limits of bacterial luminosity and the perceptual challenges posed by contemporary urban lighting, the research also develops a genetically enhanced bioluminescent system in the microalga *Chlamydomonas reinhardtii*, aiming at greater light efficiency and environmental performance.

Bioluminescent organisms—both non-modified bacteria and genetically engineered microalgae—are embedded within digitally fabricated, transparent bioreceptive panels ranging from object scale (lamps, furniture) to architectural and urban elements (storefronts, façades). Designed through bioinformed algorithms, these panels embody principles of harmony, continuity, organicity, complexity, and fractality, ensuring functional performance and perceptible beauty even in daylight, when luminescence is not visible (with Yomna K. Abdallah: computational design and bioengineering).

Over two years, iterative experimentation led to optimized culture media and the positive selection of highly luminous clones derived from the original *Vibrio fischeri* ES114 strain. The resulting system achieves sustained luminous effectiveness for nearly 20 days, with sufficient intensity to define spaces for human movement without artificial lighting (with Aranzazu Balfagón and Marta Serra: microbiology and genetics). Parallel genetic research introduced a fungal bioluminescent pathway (HispS, H3H, Luz, CPH) into microalgae through codon-optimized synthetic genes, enabling the prospect of autonomous, photosynthetically powered biolight

production combined with high CO_2 fixation capacity and valuable biomass generation (with Jae-Seong Yang's CRAG group: genetics). The project culminated in an exhibition, seminar, and publication titled "Towards a Bioluminescent Future: Art, Architecture & Design in 21st Century" (21st-23rd January 2026), presenting prototypes and sustainability insights. Funded by the Fritz und Trude Fortmann-Stiftung, BioLumCity advances innovative construction concepts and proposes a paradigm shift toward regenerative, living light infrastructures for the 21st century**: a true manifesto to open people's eyes to the new realities that we must build in our times.**

The two teams: iBAG-UIC Barcelona and CRAG.

Alberto T. Estévez, poster for the seminar / exhibition “Towards a Bioluminescent Future: Art, Architecture & Design in 21st Century” (21st-23rd January 2026), 2025 (with image of the 1st and photos of the 6th phase of the Genetic Barcelona Project).

BioLumCity Project

Alberto T. Estévez
Yomna K. Abdallah
Aránzazu Balfagón
Marta Serra

Introduction (abstract)

The BioLumCity Project wants to offer an advanced way of real bioluminescence application in architecture and urban design. First, attending to the already existing natural sources of bioluminescence, choosing among the most suitable living organism for the case, bioluminescent bacteria, enough effective in not illuminated interiors. But, given the limitations of its relatively weak luminescence, and also since the current urban artificial light does not allow bioluminescence to be perceived, it is proposed also to create a genetic modification in microalgae searching for a greater light efficiency. While on the other hand, genetically modified organism and those that are not, both for a greater lighting effect, will be supported on panels of different scales, from objects scale, lamps and furniture, to showcases, storefronts, window displays and facades scale. Digitally manufactured panels in a very special way, with transparent material and biolearned algorithms, as we are used to design, that assure the functional efficiency and the perception of beauty. So, using digital design development, the panels - in every scale - emerge with the values of harmony, unity, continuity, organicity, diversity, dynamic, complexity, fractality: the values of an “objective beauty” that every living being has in nature: values to be seen also by day, when bioluminescence can't be perceived.

So, we have prepared some 3D prototypes with not modified already existent bioluminescent bacteria, as they are today available, integrated into architecture, in panels, furniture and lamps. Looking also for the most efficient panels in terms of bioluminescence, and with deep research around how to improve durability and bioluminescent efficiency in bacteria: we can comment here now the success in luminosity that we have achieved, after multiple experiments in media and in bacteria selection (setup of *Vibrio fischeri* with variations to check the best efficiency version conditions). And, at the same time, we did deeper research, a genetical research of bioluminescence in microalgae, and also an approach of bioluminescence in ornamental plants.

That said, succinctly and quickly, as planned, we did the application of *Vibrio fischeri* on panels of different scales (city and home) and an efficiency verification of the panels. We did as well the digital design of bioreceptive

panels and prototypes with different scales (city and home), and the 3D digital fabrication of bioreceptive panels and prototypes with different scales (city and home). All of this is here documented, also with a multitude of photographs.

Thus, it can be said that, as of today, the final versions of the bioreceptive panels and prototypes with different scales (city and home: see photos of the panels and lamps), are considered to be achieved, as well as the best version achieved in terms of the maximum possible effectiveness in the bioluminescence of *Vibrio fischeri*, both in terms of its intensity and duration. When it has been achieved that they maintain their luminous effectiveness for almost 20 days, with sufficient intensity to mark spaces and rooms without artificial light for human walking use.

Specifically said, we reach a clone of *Vibrio fischeri* that comes from multiple selections of the most bioluminescent colonies during these two years, along with the marine broth under optimal conditions (see photos). The strain we were working with is a positive selection for bioluminescence that we have performed in our laboratory, starting from the original *Vibrio fischeri* strain ES114.

Choosing first bioluminescent bacteria because is the most effective natural already existing being in terms of light intensity, durability, ease of installation and maintenance. And definitively choosing microalgae because are photosynthetic organisms that exhibit significantly higher CO2 fixation efficiency than terrestrial trees, absorbing several hundred times more CO2 gas within the same volume of space. By leveraging these advantages, our objective is to engineer microalgae and ecosystem capable of producing bioluminescence for both CO2 removal and biolight functionality. Furthermore, the biomass of *Chlamydomonas reinhardtii* is rich in proteins and carbohydrates, and it also contains beneficial pigments such as chlorophyll and carotenoids. This significantly enhances the economic value of this microalgae's biomass.

System to improve the lighting efficiency. To generate bioluminescence in microalgae, we need to introduce external genes related to luminescence since wild-type microalgae lack the inherent ability for this phenomenon. We used the well-characterized bioluminescent pathway from fungi, involving

four molecules and four enzymes (HispS, H3H, Luz, and CPH). Specifically, hispidin synthase (HispS) that synthesize hispidin from caffeic acid, the hydroxylase enzyme (H3H) converts hispidin to 3-hydroxyhispidin, known as luciferin, which is then oxidized by the enzyme Luz, resulting in a light-producing reaction called caffeoyl pyruvate. The corresponding decarboxylase enzyme, CPH, regenerates caffeic acid from caffeoyl pyruvate. However, while these characterized genes have been successfully tested in higher plants, they have not yet been tested in microalgae. Therefore, to enable their expression in *Chlamydomonas*, we created synthetic genes with optimized codons for microalgae. The Jae-Seong Yang Lab of CRAG has already characterized strong promoters that work better than conventional promoters, so we anticipate high-level expression of the system. (It will not be necessary to detail here now in this introductory abstract the rest of the technical specifications about the process).

Sustainability. The project addresses at least 10 of the Sustainable Development Goals of United Nations, f. ex. the goals number 2, 4, 7, 9, 11, 12, 13, 14, 15, 17:
GOAL 2: Zero Hunger (achieving food from a sustainable agriculture of algae). **GOAL 4:** Quality Education (giving quality education about sustainability, teaching all this to undergraduate and postgraduate students at ESARQ-UIC Barcelona School of Architecture, opening their eyes, broadening their horizons). **GOAL 7:** Affordable and Clean Energy (creating a lighting system, natural, renewable and without electricity). **GOAL 9:** Industry, Innovation and Infrastructure (building sustainable resilient infrastructure, and foster innovation). **GOAL 11:** Sustainable Cities and Communities (making cities and human settlements inclusive, safe, resilient and sustainable). **GOAL 12:** Responsible Consumption and Production (ensuring sustainable consumption of natural not electrical and non-polluting energy). **GOAL 13:** Climate Action (being an action and products that combat climate change and its Impacts). **GOAL 14:** Life Below Water (promoting a water living system for sustainable development). **GOAL 15:** Life on Land (promoting sustainable use of terrestrial ecosystems, combat desertification, and halt and reverse land degradation, and halt biodiversity loss). **GOAL 17:** Partnerships to Achieve the Goal (strengthening the means of implementation and revitalize the global partnership for sustainable development, In this case, by bringing together a private foundation, a private university, and a public research center).

It all ended with the exhibition (21st-23rd January 2026), seminar (21st January 2026), and this book, “Towards a Bioluminescent Future: Art, Architecture & Design in 21st Century” (also online), with the results of this Project, with prototypes, images and explanations of the sustainability importance of bioluminescence. The importance of transferring and disseminating the results of every research or action is widely recognized by official institutions. Therefore, we have prepared this exhibition, seminar and book, with explanations of different experts from the fields of biology, art, architecture and design. Organized in our University (Universitat Internacional de Catalunya), in Barcelona. As well that can be offered to different educational institutions, as cultural centres, schools and universities. And the book can be sent worldwide to the main libraries and universities, and deposit in a book website for be bought online, as we already do with our book series. And if desired, the genetic research and prototypes could conclude as patents and commercialization.

This research project, prepared since 2023, was kindly funded by the foundation Fritz und Trude Fortmann-Stiftung for a period of two years (beginning February 2024 - end January 2026), after winning the corresponding publicly announced competition. The research project substantiates the foundation's purpose of promoting research and product development in the field of construction through efficient building material and construction concepts.

So, we would like to express our sincere gratitude to the Fritz and Trude Fortmann Foundation for its generous support. Thanks to this funding, we successfully published our research in some indexed journals, international congresses, and exhibitions, mentioning always the Foundation. We believe these contributions will significantly advance the promotion of science and research in the field of construction for the promotion of building culture and materials, as is the purpose of the Foundation. Definitively, it is very relevant research, which is **a true manifesto to open people's eyes to the new realities that we must build in our 21st century.**

The following pages explain now more in detail the work done for the BioLumCity Project from the Alberto T. Estévez group at iBAG-UIC Barcelona (Barcelona, Spain), explained in two parts: a first general part and the second part titled PANDORA Project, as a branch inside the BioLumCity Project. After these two parts comes in a separate chapter a third part, as detailing the BioLumCity Project from the Jae-Seong Yang group at CRAG (Barcelona, Spain).

BioLumCity Project: Integrating Bioluminescence into Architecture & Urban Design for Sustainability

Bioluminescence Integrated in Art, Architecture and Design for Passive Lighting in Future Cities

Director: Alberto T. Estévez

Problem: The reliance on non-renewable and non-sustainable sources of energy for architectural and urban lighting.

Objective: focus on bioenergy research applications in passive lighting through the integration of bioluminescent bioactive agents (naturally or genetically modified bacteria, yeast, algae and plants) for architectural and urban lighting.

Secondary objective 1: boost the efficiency of bioluminescence activity of naturally bioluminescent bacteria *Aliivibrio fischeri* in terms of light intensity, duration and ease of architectural and urban scale applications (iBAG-UIC Barcelona).

Secondary objective 2: designing bioreceptive architectural elements to host the naturally bioluminescent bacteria *Aliivibrio fischeri* and guarantee affordability, availability, ease of implantation and maintenance of these elements, with solid and slurry phase medium (iBAG-UIC Barcelona).

Secondary objective 3: synthesizing bioluminescent ornamental plants through genetic modification by the integration of the Lux-operon to facilitate the autonomous growth, bioluminescence activity and reproduction of these genetically modified plants: Pandora Project (iBAG-UIC Barcelona / XTART).

Secondary objective 4: synthesizing bioluminescent bacteria, yeast and algae through genetic modification for accelerated and autonomous reproduction and enhanced bioluminescence intensity (CRAG).

Secondary objective 5: designing biocontainers as architectural elements to host the genetically modified bacteria, yeast and algae to guarantee affordability, availability, ease of implantation and maintenance of these elements: liquid phase medium (iBAG-UIC Barcelona).

Literature review
(justification, analyses, learned lessons and defined trajectories)

Current research trajectories are focused on achieving sustainability through the intelligent usage of non-renewable energy resources and the development of novel alternative renewable energy systems. This has been one of the primary research focuses over the last 25 years and remains so to this day. Most sources of alternative renewable energy typically rely on centralized energy management, storage, and distribution systems, as seen in solar, wind, and hydroelectric power stations, each of which requires complex and non-eco-friendly manufactured parts and electronics that necessitate an expert to implement, operate, and harness their generated power. Although these systems may work for large-scale commercial architectural and urban applications, such as factories, the complexity of these systems hinders their domestic use on a small-scale, average user level for urban and architectural purposes. For example, one study (Atasu, et al., 2021) [1] reported a high cost of managing solar panel waste, imposing environmental and economic concerns in terms of managing these non-organic or eco-digestible wastes. The recycling cost of one panel was usually USD 20-USD 30, in comparison to the USD 1-USD 2 cost of sending that same panel to landfill. Similarly, Kuby Renewable Energy Ltd. (2019) [2] detailed the components of a standard solar panel, exhibiting their high energy demand for manufacturing their silicon semiconductors as well as their incorporation of hazardous chemicals that require strict control in the manufacturing process. Similarly, wind power is costly due to the need to build remotely located stations due to their high noise and threat to wildlife [3], and their efficiency in terms of power generation is also questionable [4]. This deficiency in the performance, sustainability, and economic competence of solar and wind power has mainly led to a shift toward searching for bioenergy solutions, for instance, modern solid and liquid bioenergy [5].

The trajectory of bioenergy research and applications incorporates various and expanding options by exploiting biomass in microbial fuel cells for generating light and/or electricity from non-pathogenic microbial strains. For example, in 2007-2008, the bacterial “Barcelona Biolamps” of Alberto T. Estévez [6], employed the *Aliivibrio fischeri* bacterial strain in bioreactors to fully illuminate an apartment with living light for first time in the history. While Abdallah, et al., 2019 [7] employed a cathodic immobilized laccase-secreting, non-pathogenic fungal strain *Aspergillus sydowii* NYKA 510 in a

single-chamber microbial fuel cell to generate 0.76 V, 380 mAm−2, 160 mWm−2, and 0.4 W at 2000 Ω, which was later employed in a self-sufficient lighting unit employing a system of two sets of four MFCs each, connected in series, for electricity generation. Despite the successful implantation of these bacteria- and fungi-powered devices, their non-photosynthetic characteristics have hindered the autonomous durability of these biobatteries or biolamps. This has led to the focus on algae-powered bioreactors for energy generation as an integral sustainable solution, where the benefits include not only the generation of sustainable, self-maintained, and self-sufficiently generated bioelectricity but also carbon dioxide mitigation, oxygen, and industrially valuable enzyme production and even the production of nutritious elements such as proteins [8]. Still, these liquid batteries did not solve the complexity of the above operation and maintenance problems. It might have complicated the issue by introducing these wet liquid battery systems that operate using living organisms that are sensitive to their environmental conditions. Added to this is their low energy production and the high cost of electrolyte membranes [9], adding further constraints to their architectural and urban applications, as reported in [10], with the criteria for the implementation of these bioactive liquid systems in architecture and urban applications taking into consideration their life cycle and maintenance rate and boosting their energy output in reasonable applications.

Therefore, a third research trajectory sought to implement the intrinsic nature of bioemitted light, known as “bioluminescent” activity, at the material-scale level. For example, Mitiouchkina, et al., 2020 [11] engineered tobacco lines expressing a fungal bioluminescent system [12], which converts caffeic acid present in all plants into luciferin and reports self-sustained luminescence that is easily visible to the naked eye, in the search for integrating fluorescent and bioluminescent genes in plants. However, such endeavours did not consider architectural or urban applications by proposing the genetic modification of plants or trees that are growing or can be adopted on the urban scale. Therefore, an easier and more sustainable procedure was proposed in the “Genetic Barcelona Project” of Alberto T. Estévez, by applying genetic modification to lemon trees to make them autonomously fluorescent, in a first phase (2003-2006) in 7 lemon trees, which was achieved by introducing the GFP gene into their DNA, searching a renewable, sustainable, autonomous, and passive source of urban lighting [13]. These genetically modified lemon trees were kept alive for more than 13 years and would still be alive today if it were not for the need to relocate the research work and its infrastructure. They remained actively fluorescent at night despite their trivial level of emitted light intensity, which could only be sensed in total darkness. These steps fuelled research interest in biomanufacturing novel, advanced bioluminescent or fluorescent materials.

For example, a group of researchers [14] explored the idea of making of a bioluminescent micro-architecture, exploring the use of light-emitting bacteria as an architectural material by investigating the 3D printing of bioluminescent bacteria. The project employed cutting-edge 3D bioprinting technologies to create the bacterial culture and carrier media using a collaborative robot with a bespoke micro-dispenser to liberate the form of the medium at an architectural scale of fabrication. However, the feasibility of scaling-up the project to architectural-scale applications remains questionable due to the complex biomanufacturing processes, which can only be performed inside a laboratory under strict sterile conditions and with expert management of the sensitive bioprinting process, which itself imposes stress on the viability of the bacterial cells in the culture. This is due to the well-known rheological deferential effect pre-, during, and post-printing caused by the pressure at the 3D bioprinter extruder tip [15]. Additionally, the scale of the developed bacterial bioink is inapplicable, as the hydrogel base cannot sustain itself alone, especially when the number of 3D-printed layers becomes higher at the architectural scale, due to its poor rheological properties which were not measured in the reported project. In addition, there is a potential need for crosslinking this reported bioink to sustain its form post-printing, which might again affect the viability of the cells. And the possible contamination of this biocompatible hydrogel, which might lead to the coexistence of undesired bacterial and fungal pathogenic and harmful strains. Still, that is not everything; the feasibility of bioluminescent activity operation varies definitely across varied scales because of the spaces between the cells, oxygen, nutrient exposure, cell migration, and signalling. Similarly, Thomsen et al., 2020 and Tyse et al., 2022 [16,17] projects like “Bioluminescent micro-architectures: Imprimer la lumière”, which examined bioluminescent bacterial cultures as an architectural material, suffered from the same issues of the non-feasible scalability of the proposed materials and procedures on an architectural scale. Despite the research merits in terms of developing computational models for simulating the behaviour, growth rates, and lifespan of the proposed bacterial materials and taking into consideration the ecosystem in which the light-emitting metabolisms take place, as well as their limited lifespan. However, there is a deviation between the computational simulations and the living cultures, where the most significant deviation between the simulation and real observations is seen in the first hours due to the lag phase, which the simulation does not consider. In addition, there are imperfections in the 3D-printed media compared to its twin 3D model, where the thickness of the extruded media is not consistent. Therefore, such speculations of unrealistic upscaling from the lab scale to the architectural scale must be reevaluated. Therefore, the current research aims to minimize the wide gap between research projects with their pilot-scale micro-models and the architectural scale by understanding the customization and application potential of this bioactive material.

The limitations of this 3D-printed bioluminescent biomaterial trajectory imply the need for customizing and testing these bioink materials in terms of their rheology, biocompatibility, cell viability, activity post-printing, cell migration, and behaviour on a scale adequate for architectural applications before attempting to propose them for architectural applications [18].

Engineered bioluminescent biomaterials, either incorporating a naturally bioluminescent or a GMO organism, are considered the best trajectory for implementing bioluminescent activity on the material-scale level. However, there are still several considerations to be addressed before introducing these bioactive materials into the built environment with direct contact with people.

For example, the specific chemical, physical, and physiological characteristics of the employed bioluminescent microbial strains must be understood, which vary between bacteria, fungi, and algae in terms of their bioluminescent activity stimuli, intensity, and duration. In addition, the maintenance and co-existence of these living materials must be carried out in a safe way for the bioactive agent and for users. Moreover, the limits of the physical translation and morphogenesis of these living materials in the built environment are crucial, as their physical properties will determine their physical existence and functionality within the environment. These aspects and more must still remain under extensive study before these bioluminescent materials can be implemented in the architectural and urban built environment. Therefore, scaffold structures can offer a possible durable and refillable physical transient between bacterial bioink and its environment of implementation, offering customized, sustainable encapsulation of bioactive bioluminescent organisms.

These scaffolds for bioluminescent bioink are borrowed from the field of tissue engineering, where living cells or bioinks are seeded on scaffolds that are both compatible with the cells' behaviour and provide a physical form for these cells to proliferate and differentiate, thereby creating the required tissue [20]. In addition, they offer easier, more reliable procedures and easier maintenance of these living cultures than bioreactor devices.

This customized scaffold design was proposed in the research article 3D-Printed Bioreceptive Tiles of Reaction-Diffusion (Gierer-Meinhardt Model) [21] by adopting a mixed-algae culture with varied algal strains, ranging from filamentous to unicellular, to form a dense mesh of a photosynthetic bioactive scaffold that sustains the existence of the bioluminescent unicellular algae resting on a 3D-printed PLA tile. Although the results of such methods were successful in preserving the life of the bioluminescent algae, the bioluminescent activity was trivial and could not be sensed with the naked

eye. Thus, in the current work, the objective is to incorporate the bioluminescent bacterial strain *Aliivibrio fischeri*, which is more efficient in terms of its emitted bioluminescent light intensity and duration [22], in the form of a bioactive solid media in petri dishes containers (biolamps) or in the form of slurry bioink layered on a PLA scaffold that will host the system. This approach facilitates the implementation of this system on both the architectural and urban scales, as it requires minimal one-step maintenance for recharging these bioluminescent solid-slurry media containers and tiles with new media. Moreover, in the case of the bioreceptive panels, they can be incorporated on the urban scale with varied options of enclosure by using ion-exchange membranes or dense polycarbonate glass.

Figure 1 exhibits a collection of research trajectories for obtaining biolight developed since 2003 by Estévez, 2005, 2006, 2007 [13], and later by Abdallah, et al., 2019; Jaafari, et al., 2021; Estevez, and Abdallah, 2024, and Abdallah, and Estévez, 2023 [7,8,19, 21], from 2019 to 2024. These involve using bioluminescent bioreactors, genetically modified plants with the GFP gene or with the *Aliivibrio fischeri* genes responsible for bioluminescence, bioelectricity from fungi and algae in MFCs, and bioluminescence panels obtained by immobilization of naturally bioluminescent unicellular marine algae entrapped in a mixed-filamentous-green-algae culture**.**

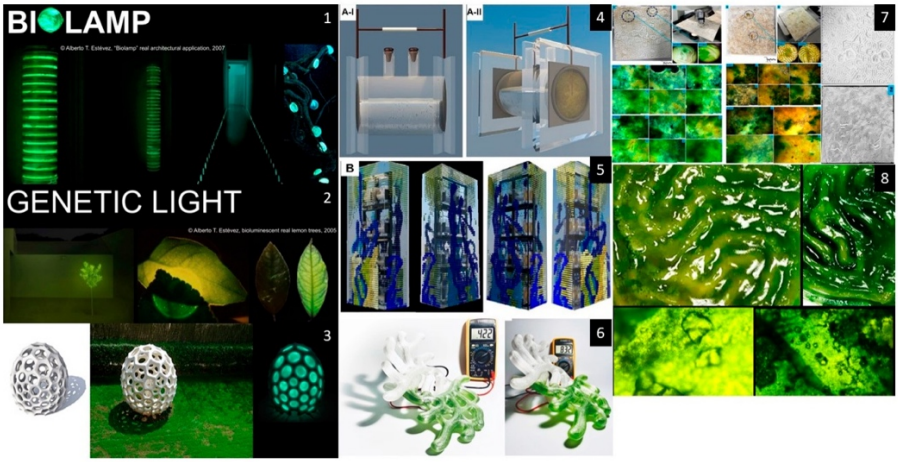


Figure 1. Images from the authors of varied research trajectories on developing biolight through natural and genetically modified bioluminescence and fluorescence activity, bioelectricity, and bioluminescence symbiosis on architectural and urban scaffolds: **1.** © Alberto T. Estévez, Biolamps (Genetic Barcelona Project, 2nd phase, 2007-2010): Bioluminescent batteries applied in the Biolamp 2 (left), in a Skirting Board Biolamp (center), and in the Roots Biolamp (right); photo was made with a conventional reflex camera (human eye vision). **2.** © Alberto T. Estévez, Genetic

Barcelona Project, 1st phase, 2003-2006. Left, manifesto image, light from trees produced with GFP (green fluorescent protein). Genetic creation of bioluminescent plants for urban and domestic use. Comparison between a lemon tree leaf with GFP and another without GFP from the same lemon tree type. Center, photo by Alberto T. Estévez with a conventional reflex camera, and right, photo by Alberto T. Estévez and Josep Clotet with a special UV photo camera. **3.** © Alberto T. Estévez, Barcelona Biodigital Pavilion, Barcelona, 2008-09. After a "biolearning" process (in this case, from microscopic structure research on radiolarians and pollen), CAD-CAM technologies were applied for producing directly real 1:1 scale architecture. Left, digital drawing; center, real photo, directly digitally fabricated with CNC and actually installed on a large architectural scale; right, image of a table lamp with application of Biolamps for digital fabrication with a 3D printer on a small-object scale. **4.** Lighting unit design utilizing ACMSC-MFC with *A. sydowii* NYKA 510. Two different views of an ACMSC-MFC modelled by [7] (A-I and A-II). **5.** Mixed approach of patterned customized mass method for a lighting unit design utilizing an ACMSC-MFC system. (B) The final design of a self-sufficient lighting unit powered by ACMSC-MFC from different viewpoints, designed by [7], using Rhinoceros 3D+ Grasshopper. **6.** A photosynthetic-algae-based MFC produced by [8], producing sufficient electrical current for domestic applications. **7.**, **8.** Three-dimensionally printed bioreceptive tiles as scaffolds hosting a mixed algal culture to create symbiosis between photosynthetic filamentous green algae and unicellular bioluminescent algae, exhibiting two reaction-diffusion-based customized patterns employed in bioreceptive tiles to immobilize the exact multi-scale lengths of the typically hosted algal strains, accompanied by microscopy images of the cultures immobilized on the tiles, showing the bioluminescent activity of the hosted bioluminescent algae strain *Pyrocystis fusiformis* [21]. By the authors.

Learned lessons and defined trajectories in the BioLumCity Project

1. Reliance on *Aliivibrio fischeri* as the natural bioluminescent bioagent for integration in architectural elements for passive lighting. Thanks to its durable bioluminescence activity in terms of intensity and duration. In addition to its safety, ease of culturing and maintenance.

2. Reliance on solid-to-slurry culture media as a durable and manageable method of implementing the bioluminescent bacterial culture in architectural elements for passive lighting. Thanks to its durability, easy maintenance, ease of application and free multi-axial orientation of their designated host.

3. The reliance on slurry culture media opens research trajectory of biomaterials based on biomanufactured bioinks and customized scaffolds, and the methods of biofabrication.

4. Integrating genetically modified bioluminescent plants and microorganisms in the built environment requires further research on their safety, environmental interactions and their life-cycle analysis.

5. Designing biocontainers for liquid culture media weather for naturally or genetically modified bacteria, yeast or algae have more constraints in their application in the architectural built environment due to the orientation limitations, the need of enclosure and the complex process of refilling.

Following these main trajectories the iBAG-UIC Barcelona team achieved the 4 mentioned objectives as follows**.**

Methodology (materials and methods)

The iBAG-UIC Barcelona team managed and achieved the secondary objectives number (1, 2, 3 and 5), while the secondary objective 4 is managed by the CRAG team.

The first and second secondary objectives were tackled in parallel following these steps:

Step 1: Optimizing the culture media for the naturally bioluminescent bacterial strain *Aliivibrio fischeri* CECT 524 in liquid, solid media (petri dish of 10 cm and 15 cm diameters) and in slurry media (hydrogel).

A total of 15 mL of *Aliivibrio fischeri* strain ES144/NCIMB1281 (*Aliivibrio fischeri* CECT 524) was obtained in freeze-dried form from CECT (Colección Española de Cultivos Tipo). Three different culture media were tested to enhance the bioluminescence activity of the *Aliivibrio fischeri*: Luria Bertani Broth (LB), LB Broth, and Lennox (Condalab) + Sodium Chloride AGR (Labkem). The sea salt concentrations in the LB media were varied by 15%, 20%, and 25%, tested both in a liquid culture and in petri culture of 10 cm plates mixed with agar (in triplicate). Tryptone Soy Broth (TSB) used dextrose as the carbon source, sodium chloride as the osmotic balance agent, and dipotassium phosphate as a buffering agent. The standard Marine Broth (MB) contained minerals and salts simulating seawater and peptone and yeast extract as nitrogen sources. The salt and mineral concentrations were adjusted to simulate the composition of seawater. Each of the three-culture media was tested in liquid (150 mL/flask) and solid forms, mixed with agar-agar BAC, providing firmness and prolonging the bioluminescence of the organism. The petri dishes containing the medium and *Aliivibrio fischeri* CECT 524 were sealed in sterile conditions and incubated at room temperature (25°C) to promote colony growth after 24

hours post-inclusion. The bioluminescence activity was activated and lasted for seven days. Observations were made in a dark chamber for a week to monitor the bioluminescence. To determine the optimal medium for the *Vibrio*, the optical density (OD) was measured to compare the conditions and duration of the bioluminescence of the bacterial cultures under the three different media.

Optimizing the culture media of the bioluminescent urban tiles hosting *Aliivibrio fischeri* CECT 524 in bioink

The optimized MB (Marine Broth) media were mixed with four different concentrations of MB with 100%, 50%, 40%, and 35% of agar-agar BAC to optimize the media-to-agar concentration ratio and to create a gel bioink for application on the urban screen panels (tiles), balancing the medium density based on the feedback of the bioluminescence activity test of the *Aliivibrio fischeri* CECT 524 culture on the tiles. The pH of the media was adjusted with 1M NaOH or KOH to pH 6, 7, and 8, and the media were autoclaved for 20 minutes at 121°C and placed in a 47°C water bath in triplicate to maintain the media at an optimal temperature, thereby preventing gelling. *Aliivibrio fischeri* CECT 524 was inoculated individually in each plate. Using a micropipette, 200 µL of glycerol stock (prepared with 50% *Aliivibrio fischeri* CECT 524 and 50% glycerol) and 20 mL of Marine Broth with agar-agar BAC at various percentages were added at 47°C to avoid killing the organisms.

Step 2: Biocompatible design and 3d printing of the scaffold: the bioreceptive urban screen panel.

The design concept of the urban screen panels is developed from the physiochemical field as a form of diffusion to facilitate the integration of a stain of augmented bioluminescent bacteria, *Aliivibrio fischeri*, in the built environment. The field attractors were chosen for the current design case. To explore different topologies in a gradient methodology, this study moved from localized cellular entrapment to smoother gradient topologies achieved by the field, as learned from nature. Two form-finding steps were employed: the first step employed diffusion-based generative design models to generate several design iterations for the bioreceptive tiles, while the second step optimized the form-finding equation parameters to develop a more continuous topology. The results of the design form-finding informed the third design phase of the algorithmic customization, where four additional design iterations were reconstructed from 2D to 3D. The software packages used were Rhinoceros 3D, Grasshopper, and Scripting in Python. Following this phase, the design iteration C2 was customized to be 3D-printed by adding transitional parallel topological steps (curves) to maximize the entrapment of the bacterial culture, which is crucial for sustaining the bacterial culture's

viability and augmenting its bioluminescent intensity and duration. The modified version of the C2 design model was 3D-printed as a bioreceptive tile measuring 18 × 18 × 0.5 cm and featuring a rectangular micro-texture grid of 0.1 × 0.1 cm. This was 3D-printed with a Felix Tec 4 singular-head 3D printer with translucent PLA at a 210°C printing head temperature and a 70°C printing bed temperature.

Inoculation of the urban tiles (3d culture of bioluminescent *Aliivibrio fischeri* CECT 524 in sol-gel bioink on the 3d printed scaffolds)

Three 3D-printed urban screen panels (tiles) were cut into smaller pieces, each measuring 5 × 5 cm, into four distinct parts (P1, P2, P3, and P4), following a rectangular 90° grid to be contained within a 15 cm petri dish size and for the biocompatibility of the deferential topology of the tile design to be evaluated in terms of boosting the bioluminescence activity of the hosted bioluminescent bioink of the *Aliivibrio fischeri* CECT 524. The 3D panels were pre-cooled on a cold plate (−32°C) and manually inoculated using a serological pipette with the *Aliivibrio fischeri* bioink, which had been previously cultured in Marine Broth (MB) with 40% agar at 37°C to boost the bacterial cells' anchorage first on the micro-textured 3D-printed panels, and then the agar medium was poured over the plates, ensuring uniform coverage of the structures. The medium was allowed to solidify before incubating the plates at 26°C.

Three-dimensionally printed urban screen panels' bioluminescence activity

The cultured 3D-printed tiles (in parts) inoculated with the *Aliivibrio fischeri* + MB optimized media bioink were monitored in a dark chamber at room temperature and recorded using a LENS WIDE setup with the following settings: EV 0, ISO 4000, exposure time 20 s, F AUTO, and WB 2200. The inoculated 3D-printed panel parts (P1, P2, P3, and P4) were kept enclosed within the petri dish space without sealing. Light emission observations were conducted for up to 14 days post-inoculation to evaluate the intensity and durability of the bioluminescence.

Bioluminescent bioreceptive panels design variations

The bioreceptive panels (tiles) were processed to offer variations in their design by customizing field-based pattern with differential growth patterns resulting in 3 different variations that were 3D printed using the same translucent PLA filament, in addition to testing other colours of the PLA filaments such as white, black, transparent, and blue green. Each of these different colours of the PLA filament were measured in terms of their

luminosity and reflectivity using a spatial luminometer in a completely dark room, where all the different colours recorded 0.002 Lux after 3 seconds and at room temperature except for the black PLA filament which recorded 0.

Similarly, the different designs of the bioreceptive panel were measured in dark room before the addition of the bioluminescent bacterial slurry culture, to test the effect of the design on the reflectivity and luminosity of the panels alone as a control group. Using the spatial luminometer. Again, all the 3D printed tiles in the different PLA filaments including translucent, transparent, white and blue-green recorded 0.002 Lux while the black 3D printed tiles recorded 0.

Step 3: Designing biolamps as biocontainers for *Aliivibrio fischeri* solid culture (petri containers).

The Voronoi pattern was decided to be applied as 3D Voronoi structure of the biocontainers (biocups) to hold the optimized solid cultures of the bioluminescent bacteria *A. fischeri* (5 petri dishes per cup). Thus, the dimensions of the biocontainers were decided based on the standard dimensions of the petri dishes to facilitate and standardize the adaptation and commercialization of the biolamp design developed in the current work. These dimensions were adjusted to host two different sizes of petri dishes: 10 cm x 1 cm and 14 cm x 1 cm respectively. Each biocup (biolamp) is designated to host 5 petri dishes to facilitate the possibility of maintenance and recharging nutrients and managing possible waste, while providing sufficient light intensity per each unite facilitating the possibility of separate maintenance and manipulation of the bioluminescent cultures as well. These biocups or biocontainers were designed to be assembled around a slandered stainless-steel bar of 40 cm hight and 2 cm diameter to hold around it 5 biocups (biocontainers unites) in a fractal relevance with the five contained petri dishes in each biocup, given in one fully assembled biolamp 25 petri dishes to offer boosted bioluminescent light intensity that can be actually applied in the architecture interior spaces or urban scale.

The biocup formal generative design went through several iterations-3D printing-testing feedback loop to test the efficiency of customized Voronoi design to achieve more and wider porosity to maximize the bioluminescent light from the biolamp; lesser material and production time as markers of controlling the cost of mass production and feasibility of commercializing the design; structural efficiency and durability. The main iterations in this design family based on Voronoi pattern were 4 iterations: V1, V2, V3 and V4. The variation of the iterations depended on varying the reinforcement between double wall or single wall Voronoi, straight or curvy Voronoi lines, and full or hollow Voronoi pipes or struts. Moreover, several filaments were tested in

the 3D printing process mainly focusing on transparent, and translucent filaments to further maximize the obtained light from the bioluminescent bacterial cultures hosted in the biolamp.

The design concept was developed inspired from the Bochum Building of the Fritz and Trude Fortmann Foundation, and as second family of variations was employing a rectangular grid as the main pattern. This design concept included several iterations as well, varying the porosity and profile shape of the grid struts as well as varying the enclosure of the bottom of the biocups. The biocups followed the exact same dimensions of the Voronoi biocups to fit the function of hosting the solid cultures of the bioluminescent bacteria.

The current design phase develops and tests various designs of biocups, caps, and possible bases. The caps design is to ensure further protection to each biocup while rotating them around the steel bar, to avoid undesired moving of the contained petri dishes of the solid bioluminescent bacterial cultures, since the petri dishes will not be sealed. While the base design will enable the hybrid integration of the genetically modified plants with the bioluminescent genes to be integrated in the same biolamp design where it will not only produce light, but as well consume carbon dioxide and produce oxygen for integral sustainable bioluminescent solution from architectural to urban scale.

Step 4: Designing biocontainers for liquid culture of naturally and genetically modified bioluminescent bacteria, yeast and algae.

In this step, the biocontainer design criteria required a hermetic design to enclose the liquid culture of the genetically modified bioluminescent algae and bacteria.

The biocontainer for liquid culture design biomimicked the human heart anatomical model to function as biocontainers for bioluminescence and Microbial Fuell Cells for bioelectricity generation on an urban scale installation. As a manifestation of the significant multidisciplinary value of the heart, not only as an emotional and moral symbol but also as the most efficient bioreactor, and to increase awareness about sustainable, renewable alternative energy sources.

The design follows the heart's four-chambered morphology, as a biomimetic, four-chamber microbial fuel cell (MFC) employing bioluminescent bacterial strain *Aliivibrio fischeri* CECT 524, where the morphology of the heart achieves best bioluminescence and bioelectricity generation. Thanks to the varied surface-to-volume ratio increased at between the top and reduced at the bottom of the heart shape, allowing for lower solution´s resistance to the

electrons transfer in the case of bioelectricity and enhanced diffusion of oxygen in the media for better bioluminescence activity.

The Heart Shelter system was the Heart Bricks, employing 3D printed reconstructs of CT-scanned trabecular bone in the form of a brick to hold inside it each heart-shaped biocontainer.

The Urban Heart Bioreactors: biomimetic bioreactor design for bioluminescence and bioelectricity

Despite the significance of biomimetic heart bioreactors in regenerative medicine, tissue engineering and artificial organs, however, a biomimetic heart scheme has never been employed in designing a Microbial Fuel Cell or a bioenergy bioreactor before up to our knowledge. Thus, we first introduced the Urban Hearts Bioreactors Project in the SiGraDi 2024 Conference exhibition held at Universitat Internacional de Catalunya, Barcelona in November 2024. Where one of the main first generated visualizations of the project was employed as the main SiGraDi 2024 conference poster using the heart to symbolise the perfect and forever existing intelligent bioreactor system that we as humans live by its function (Figure 13). The Project is also developed as part of the funded research project BioLumCity funded by Fritz und Trude Fortmann Stiftung to develop bioluminescent materials and systems for architecture and urban scale applications.

Looking to the highly complex and coordinated system of the human heart anatomy and function, it can be approximated as a two-chamber fuel cell, referring to the left and right hearts as anode and cathode chambers in a fuel cell. Where the deoxygenated blood collected as a waste from all the body organs (except for the lungs as described above) is purified through an auxiliary system (the lungs) and the oxygenated blood collected from the lungs is redistributed all over the body again. This is an example of an urban heart system collecting wastes from all over the urban context (for example a residential block or in a city plaza) and exploiting it as growth media for the certain used microbial strains in the biocontainer and/or MFC to produce the designated needed energy (biolight and/or bioelectricity), oxygen, food and industrially valuable elements and redistribute these throughout this specific urban applications in the city. Serving the core objective in the current work which is the sustainable decentralized generation of biolight and bioelectricity through a system that works passively and can be implemented in any location freely to overcome the limitations of centralized generation and distribution of energy. Since the later lacks the local moderation of the network, power distribution and maintenance; leading to generalized black

outs (e.g., the general electricity cut out that hit Spain, Portugal and south France last April 2025).

Nevertheless, in order to apply such a complex model (the human heart) on the urban scale as a fuel cell, simplification and modification of the model in addition to the redesign of the traditional fuel cell is mandatory. In a MFC the chamber form and configuration significantly affect its electrical current and overall performance by influencing factors like oxygen diffusion, internal resistance, electrode surface area, and the balance between anodic and cathodic reactions [23]. Therefore, the typical heart form is the most intelligent to biomimic in designing MFC chambers. Since the flipped conoid form of the heart varies where the surface-to-volume ratio which is decreased in the bottom to decrease the solution (anolyte and catholyte) resistance to the electrons and is increased on the top which is closer to the bridge that connects that anode to cathode and to the wire where the electrons flow, which leads to optimized performance of the MFC and higher and more stable electrical current output. Furthermore, the same form of the heart-shaped biocontainer is the most intelligent for the bioluminescent bacteria that requires oxygen as part of its bioluminescent enzymatic reaction of the luciferin-luciferase enzymes to produce the biolight, this means the larger surface-to-volume ratio on the upper part of the flipped conoid in the heart shape container contributes to better oxygen diffusion in the media and better bioluminescence activity of the bioluminescent bacteria on the top layers of the media [24; 25]. For example, in the case of a In a MFC microbial fuel cell the two chambers (anode and cathode) are separated; and they are either connected by a salt bridge or by an ion-exchange membrane [26]; which is similar in essence to the lungs function of gas exchange. The ion-exchange membrane although if its significance in optimizing the efficiency of MFC be replacing the salt bridge in polarizing the battery and transferring electrons through the minimized distance with less resistance. However due to its relatively high cost and lesser sustainability in performance; since it needs to be changed periodically due to chemical and physical changes [27], it was not considered in the current phase of the urban hearts biocontainers/ MFCs a crucial role in passing the produced electrons or protons from the anode to the cathode in order to accumulate the electrical current [22; 18]. Similarly, the typical anatomical human heart model was simplified by reducing complex parts as the arteries and the pumping system as well as the auxiliary purifying system which is the lungs. This means that a MFC can be reduced from the detailed anatomical composition of the heart. Thus, the authors employed the following steps to adjust the human heart model to the urban biocontainer/ MFC model:

1. The four chambers of an anatomical heart model were translated into 4 full hearts shaped biocontainers (20 x 20 x 20

cm) 3D printed in translucent and red translucent PLA (Figure 14) and connected (each two biocontainers are connected as an anode and cathode of the MFC). This configuration generated 2 MFC batteries with flexibly rechargeable biocontainers. Allowing for the duality of the bioluminescence and bioelectricity activity of the system hosting the bioluminescent bacterial culture *Aliivibrio fischeri* CECT 524 at the current research phase. The *Aliivibrio fischeri* CECT 524 is not pathogenic and exists already as marine bacteria in nature, and lives in symbiosis with marine animals, thus there is no hazards related to using it [28]. Still, the proposed urban hearts bioreactors accept flexible application of wide arrange of bioluminescent and/or bioelectricity generating microorganism such as bacteria, algae and fungi.

2. The arteries and veins from the human heart model were abstracted as the infill, outlet, and bridge for the MFC in silicon tubes (of 3 mm diameter). To passage the designated fluid to the specific chamber. These tubes will further facilitate the rechargeability of the urban hearts bioreactors (MFC).

3. A pumping system that is essential for the functioning of the human heart model was not applied in the Urban Hearts' bioreactors (MFCs), since they work passively based on the chemical reactions occurring at the anode and cathode to trigger the bioluminescence activity and/or generate the bioelectricity through the oxidation-reduction reaction or luciferin-luciferase enzymatic reaction.

4. A supporting system was designed to hold and enclose the biocontainers; mimicking the human heart model that is enclosed inside the ribcage. Therefore, the authors designed the urban hearts' bioreactors sheltering which will also identify their physical implementation in the urban context as follows:

The Heart Shelter Brick (Figure 15) of 22 x 20 x 22 cm: the bricks resemble the trabecular bone tissue that has a customized cave space to encompass the heart biocontainer. Each heart-shelter brick has an opening at the top to allow passing the tubes (to infill, discharge and connect the biocontainer to its twin to compose the MFC. The heart - shelter brick design was developed from the 3D reconstructs of the CT (computerized tomography) scanned bovine femur bone from the trabecular structural motif level. This is intended to guarantee enhanced mechanical strength of the brick while achieving porous and light weight structure. The 3D reconstructs were modified and customized to the scale and size of the heart biocontainer scale using Rhinoceros 3D+ Grasshopper+ Python.

The trabecular bone biocontainer

Another biocontainer design followed a biomimicry approach informed by the same results of the micro computed tomography imagery of bovine femur bone that was used to design the heart shelter bricks. The biocontainer resulting from combining the vertical extension of a bovine femur with the trabecular perforations on one side to provide points for assembly of many biocontainer units, as well as to facilitate the handling of the device for discharging and recharging the bioluminescent culture media.

The design process was based on customizing the Micro-CT 3D reconstructs of the bovine femur through AI-guided 3D mesh modification in Rhinoceros 3D+ grasshopper + weaverbird plugin, and the AI software Meshy. Ai. The design was 3D printed using translucent and luminous PLA filaments and the XL Felix 3D printer.

Figure 16 exhibits the 3D printed bone-shaped biocontainers that were exhibited in the Heart of Architecture Exhibition held at Universitat Internacional de Catalunya, Saló de Graus, Barcelona, February 2025.

Results of Steps 1 & 2

Three-dimensionally printed biocompatible scaffold: the Urban Screen Tile

The first design phase mixed the reaction diffusion polar-periodic pattern with the field gradient effect. However, the results of this phase were limited to localized cellular entrapment patterns rather than the required smooth gradient transition of a continuous topology. The resulting patterns of this first design phase suffered from discontinuities in their topologies and separated wells that hindered the bacterial culture's chemotaxis behaviour necessary for augmenting the bioluminescence intensity. The second design phase reached several design iterations of field patterns with a more continuous topology, as exhibited in Figure 2 (1, 2). These results of field-continuous topology-based patterns informed the algorithmic customization design phase (Figure 2 (3)), reaching design iteration C2 (Figure 2 (4)), which was 3D-printed as a bioreceptive tile measuring 18 × 18 × 0.5 cm with a rectangular micro-texture grid measuring 0.1 × 0.1 cm, including the addition of transitional parallel topological steps (curves) (Figure 2 (6)) to maximize the entrapment of the bacterial culture and to enable dense biofilm formation for sustaining the bacterial culture's viability and augmenting its bioluminescence intensity and duration.

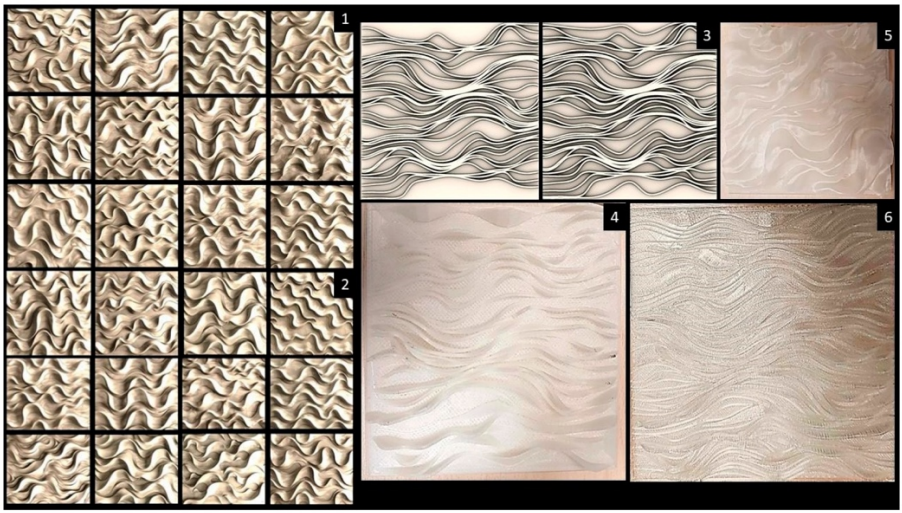


Figure 2. The generative design process of field-topology-based bioreceptive tiles for immobilizing bioluminescent bacteria *A. fischeri.* **1.**, **2.** Twenty-six different design iterations originating from the second design phase, where the diffusion parameters were modified to create a smooth and open topological transition in the fields pattern. **3.** The algorithmic customized design of the bioreceptive tile based on the field-continuous topology pattern developed in Rhinoceros 3D, Grasshopper, and Python, featuring a dense curvilinear pattern and a more continuous topology, which qualifies it as a bioreceptive surface for bioluminescent bacteria. **4.** The 3D-printed bioreceptive tile design C2 and its further enhanced version with added curvilinear offsets for enhanced entrapment of the bacterial culture, as exhibited in **6.**, the two tiles were 3D-printed with translucent PLA with a micro-texture of a rectangular grid: each tile had dimensions of 18 × 18 × 0.5 cm. **5.** Another iteration of C2, varying the diffusion parameters to fuse the curves into continuous topologies; this 3D-printed iteration was tested separately and will be exhibited in a future study. By the authors.

Economical competence and enclosure and assembly in the built environment

The bioreceptive tile design is suitable for mass production using affordable 3D printing tools and materials, requiring a moderate fabrication time of 4 hours and a 300% increase in printing speed per tile. To reduce the printing time per tile by increasing the speed of the 3D printing process, thus proving the compatibility of the tile´s design with high definition, accurate printing settings and the high resolution and precision of the employed 3D printer were used in the current study. An average desktop FDM 3D printer, which costs EUR ~350 (Shenzhen Creality 3D Technology Co, Ltd. Shenzhen, China, https://www.creality.com/), can be used to print an unlimited number of these tiles. The PLA filament used to print one tile costs EUR 4. While

machine wear-out costs can be added to the overall cost of the tiles, which includes the electrical power and the effects of the 3D printing job on machine wear-out, this cost is defined by the manufacturer. It can be estimated as a percentage of the material cost or can be equal to the material cost. In the current work, the wear-out cost is defined as equal to the material cost due to the high printing speed wear-out effect. Therefore, the total production cost for one bioreceptive tile is EUR 8. In some cases, the designer´s cost can be added to the overall cost of the tile; however, in this work, only the actual production costs are presented, consistent with the purpose of the decentralized production of this bioreceptive tile for bioluminescence integration in the built environment.

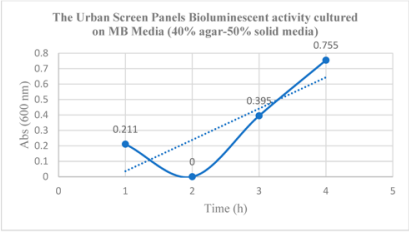
On the other hand, the costs of culturing the bioluminescent bacteria strain *Aliivibrio fischeri* used in the current study are also not estimated from the production cost of the tiles, since it can be obtained from friendly microbiology labs in universities and research centers, and even if purchased, starter kit only costs EUR ~340, including the media components (Fisher Scientific S.L. 28108-Alcobendas, Madrid, Spain / Thermo Fisher Scientific Inc., Waltham, MA, USA), which can be used to regenerate the bacterial culture unlimitedly. Such bioreceptive tiles do not need to be in bioreactor containers, since the bioluminescent bacteria can grow and glow on solid media, as shown in the current work. Furthermore, these bioreceptive tiles can be used as cladding for any facade and can be enclosed by a wide array of membranous materials, such as ion-exchange membranes [23], to ensure their safe integration in the built environment. There are a variety of ion-exchange membranes that range in price from EUR 1.5 per $1m^2$ to EUR 500 (Fuel Cell Store, © 2025., Bryan, TX, USA). This production cost of the bioluminescent tile holds economic competence in comparison to the production and operation costs of a bioreactor, since for 3D printing a hermetic bioreactor with the same field pattern, the production cost per bioreactor tile will be multiplied by 6 (6 × EUR 4 = EUR 24), having the six sides of a simple cuboid or a box bioreactor. This leads to an increase in the overall production cost, considering the machine wear-out factor, which is estimated equally to the material cost, giving an overall cost of EUR 48. There is also the cost of auxiliary system components, including circulation and effluent, which consist of pipes, valves, and filters, and the complexity of discharging the effluent and recharging the system with fresh media. All these factors qualify the bioreceptive tiles developed in the current study as a more durable and easily manageable system than bioreactors.

A fully assembled 1:1 architectural-scale urban screen is exhibited in Figure 3, side by side with the details of the developed tile, featuring its square micro-texture as the building-block scaffold of this bioluminescent urban screen system.

Figure 3. The urban screen system is designed for hosting the bioluminescent bacterial strain *A. fischeri* for integration in the built environment and in architectural and urban applications. **1.** One 3D-printed bioreceptive tile of the bioluminescent urban screen, showing the varied topology between peaks and wells and interstitial spaces, as well as the micro-textured square grid for better anchorage of the hosted bacterial strain. **2.** A fully assembled urban screen panel measuring 170 × 90 × 4 cm, incorporating the 3D-printed tiles with varied PLA filaments between translucent, transparent, and fluorescent, which will be tested for their effect on the bioluminescent activity of the hosted in *A. fischeri* and exhibited in a future study**.**

Optimized culture media of the bioluminescent bacteria *Aliivibrio fischeri* CECT 524

The Marine Broth (MB) yielded a higher growth rate of *Aliivibrio fischeri* CECT 524, with the bioink 3D culture showing visible bioluminescent emitted light but with fluctuations in absorbance, where a negative OD value at 18 h was recorded, probably due to nutrient consumption by the growing bacteria causing the solution to become clear (Figure 4, Table 1). From the different agar concentrations analyzed, the 40% to 50% agar concentration for the solid media applied to the urban screen panels achieved the best results in terms of enabling the bioluminescent chemotaxis behaviour due to the enhanced mobility of A. fischeri at lower agar concentrations, which ensured better nutrient availability. No differences in bioluminescence expression were observed between NaOH and KOH used as pH adjustment agents applied to the bioink media on the urban screen panels.



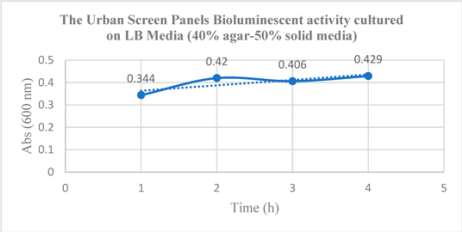


Figure 4. The bioluminescence activity of the urban screen panels under MB media vs. LB media. **1.** The urban screen panels' bioluminescence activity when cultured on MB media (40% agar-50% solid media), exhibiting the highest maximum Abs as an indication of the *A. fischeri* bacterial culture density and activity when cultivated on Marine Broth reaching 0.755 Abs, despite the fluctuations on the second hour that is attributed to the lag phase. **2.** The urban screen panels' bioluminescence activity when cultured on LB media (40% agar-50% solid media) showing the highest reached Abs of 0.429 which is lower than in the case of Marine Broth Culture media. The unit Abs is absorbance, a quantity that measures how much light a substance absorbs at a particular wavelength. It is a logarithmic measure, often called optical density (Molecular Devices, 2024: https://www.moleculardevices.com/technology/absorbance, accessed on 5 September 2025), with higher Abs values indicating more light absorption by the sample and less light passing through. The solid lines is the estimated Absorbance (Abs) values in time (h), while the dotted lines is the deducted function representing the overall behaviour of the bioluminescent bacterial culture on the tiles; which are a growth function in the first "The urban screen panels' bioluminescence activity when cultured on MB media (40% agar-50% solid media)", and almost a fixed function in the second "The urban screen panels' bioluminescence activity when cultured on LB media (40% agar-50% solid media)".

The Urban Screen Panels Bioluminescent Activity Cultured on MB vs. LB		
	MB	LB
Time (h)	Abs (Absorbance) 600 nm	Abs (Absorbance) 600 nm
13 h (08:00 am)	0.211	0.344
18 h (13:00 pm)	Negative	0.420
21 h (16:00 pm)	0.395	0.406
23 h (18:00 pm)	0.755	0.429

Table 1. The urban screen panels' bioluminescence activity when cultured on MB vs. LB, as measured by absorbance at 600 nm at 5, 3, and 2 h, respectively.

The LB Broth media ranked second in in terms of the achieved culture growth and bioluminescence activity, with the optimal sea salt concentration achieved by 15 g/L sodium chloride AGR. The TSB media recorded negative results.

Three-dimensionally printed urban screen panels with bioluminescence activity

The best results in terms of the bioluminescence activity of the *Aliivibrio fischeri* CECT 524 was blue-green light at ~490 nm, which was more visible with the naked eye on the P1 part of the urban screen panel (tiles) (Figure 5), with a higher emission of light, enough to illuminate the tile surface space after only 3-10 s when placed in a dark room and with a 10-s exposure time captured with a standard digital camera. A longer duration of luminescence activity, lasting up to 10 days without refilling the growth medium, was demonstrated by the MB media slurry spray, in comparison to the other three parts (P2, P3, and P4), which lasted between 3 and 7 days of visible bioluminescence activity. It was observed that the maximum light emission was visible at the thin curvilinear peaks of the 3D-printed field patterns, as they were more durable in terms of their bioluminescence activity in comparison to the 3D surfaces of the wells and the projected strips, as exhibited in Figure 5, showing the density of the projected peaks that were >0.5 cm in height, the projected strips of ≤0.3 cm, and the wells at each of the four parts of the 3D-printed bioreceptive panel with its field-based pattern.

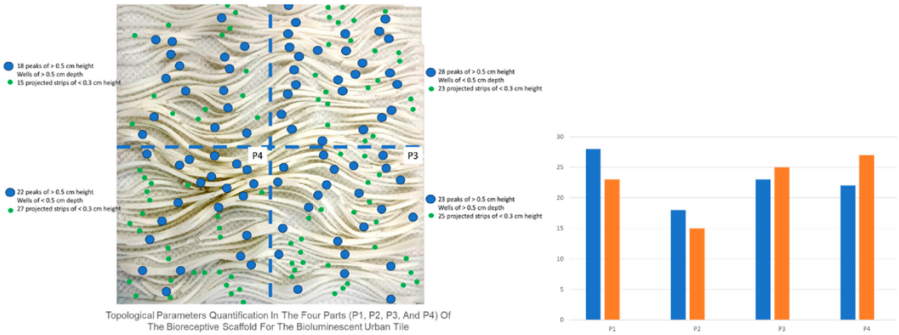


Figure 5. the field-based pattern density and topological level distribution in each of the four parts of the original bioreceptive 3D-printed panel, serving as a scaffold for the bioluminescent bioink. In each of the four parts, the number of peaks > 0.5 cm, the projected strips of ≤0.3 cm, and the varied-size wells are exhibited to justify the best bioluminescent activity results obtained on P1. The blue dots represent the number of peaks, and the green dots represent the number of projected strips, while the wells were not assigned to a specific color since any interstitial spaces between the peaks or strips were considered wells. P1 excelled in terms of the number of peaks with 28 peaks that were higher than 0.5 cm, thanks to their short extension in comparison to the continuous peaks of P2. P1 also had tighter wells with widths of less than 0.5 cm, which enhances cell attachment and culture anchorage. Finally, the moderate number of projected strips in P1, combined with the tight wells and the high peaks, yielded the best bioluminescence durability and intensity in comparison to P2, P3, and P4. By the authors.

As exhibited in Figure 5, the number of peaks was the primary parameter determining the optimal bioreceptivity for the bioluminescent bacterial bioink, which yielded the highest intensity and durability of blue-green emitted light, lasting for 10 days without the need for refilling in the case of P1, thanks to the thinner layer of the bioluminescent bacterial bioink at these peaks compared to the wells and the strips. This facilitated better oxygenation and the shortest path for the bacterial cells to glide through the 3D culture of the bioink and demonstrate their collaborative bioluminescence behaviour. Moreover, in P1, with the highest number of 28 peaks, the distribution of high peaks, with their short distances, increased the oxygen circulation, alongside the effect of the peaks in the topology, augmented by the interplay with the tighter wells and the moderate number of projected strips, i.e., 23 strips that are less than 0.3 cm in height. P4 ranked second in terms of bioluminescence efficiency thanks to its 22 peaks and tight wells. P3 came third, with 23 peaks that were higher than 0.5 cm. The wider wells of more than 0.5 cm in width hindered the bioluminescence chemotaxis behaviour and affected the anchorage of the 3D culture to the scaffold, despite the large number of the projected strips. P2 came last, with a lower number of peaks and wider wells that hindered the anchorage of the 3D cell culture within the bioluminescent bioink, proving the effect of the scaffold´s geometry on the bioluminescence efficiency of the bioink.

Figure 6 exhibits the culturing procedures for the cold-plate freezing of the 3D-printed tile pre-inoculation for sterilization and for sustaining the slurry culture added in step 2. Steps 3 and 4 exhibit P1 post-inoculation, and the blue-green light was emitted for 10 days without the need for refilling the media, proving the sustainability of this approach based on collaborating the customized geometrical design of the scaffold with the customized bioluminescent bioink. It also delivers lower maintenance costs and procedures than bioreactors and has self-sustaining structures, enabling architectural-and urban-scale applications. By the authors.

Figure 6. The bioluminescent urban screen culturing and bioluminescence activity: **1.** Cold-plate freezing of the 3D-printed tile, divided into four parts, for sterilization and preparation for adding the slurry culture of the bioluminescent bioink. The seeding process was followed by covering it with the media in 5 layers. **2.** The seeded urban panels' parts cultured in petri dishes. **3.** The P1 part of the urban screen panel seeded with the bioluminescent bacterial culture + media in agar. **4.** The same part under a conventional digital camera with an exposure time of 10 sec. in a dark room (as a naked human eye see it), exhibiting the visible bioluminescence activity, which lasted for 10 days without refilling. By the authors.

Figure 7. design variations for the bioreceptive panels, varying the design based on customizing fields-based patterns with differential growth patterns, also varying the colour, transparency and reflectivity of the used PLA filament in the 3D printing process of each of these design variations. By the authors.

Results of Step 3

Figure 8 exhibits a prototype of a biolamp containing 5 biocups with a total of 20 petri dishes and its immitted bioluminescence immediately responding to a dark room within 3-5 secs.

Figure 8. A prototype of biolamp design containing 4 biocups and a cylindrical hollow structural Voronoi base to act as scaffold for the genetically modified bioluminescent plants to grow and coil around them, as a hybridization between natural bioluminescence of bacterial strain *A. fischeri* and the GM bioluminescent plant. The base was replaced with a fifth biocup to add more light. **V.I2-BI** is the fully assembled biolamp with 4 biocups each containing 5 petri dishes of solid cultures of the bioluminescent bacteria *A. fischeri*. **V.I2** stands for Voronoi-Iteration 2, and BI stands for Bio-lamp. The **V.I2-BI d1** and **d2** are two details exhibiting the bioluminescence intensity of the biolamp achieved by the bioluminescent bacterial strain at hand. (Photos taked with conventional digital camera, f/1,6: center photo with an exposure time of 3 sec., as a naked human eye see it, and right photo with 10 sec. exposure). Below, different 3D printed variations of biocups to find the best. By the authors.

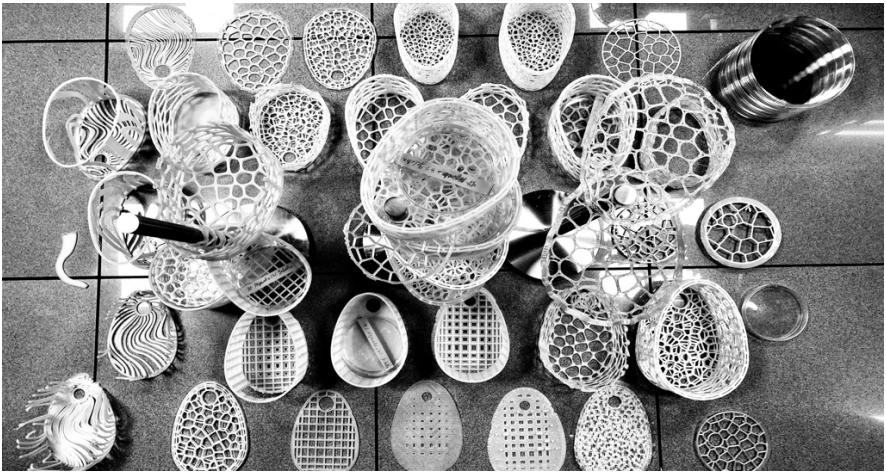


Figure 9 exhibits the main 4 design iterations of the Voronoi design family, while Figure 10 exhibits the same iterations in 3D printed models.

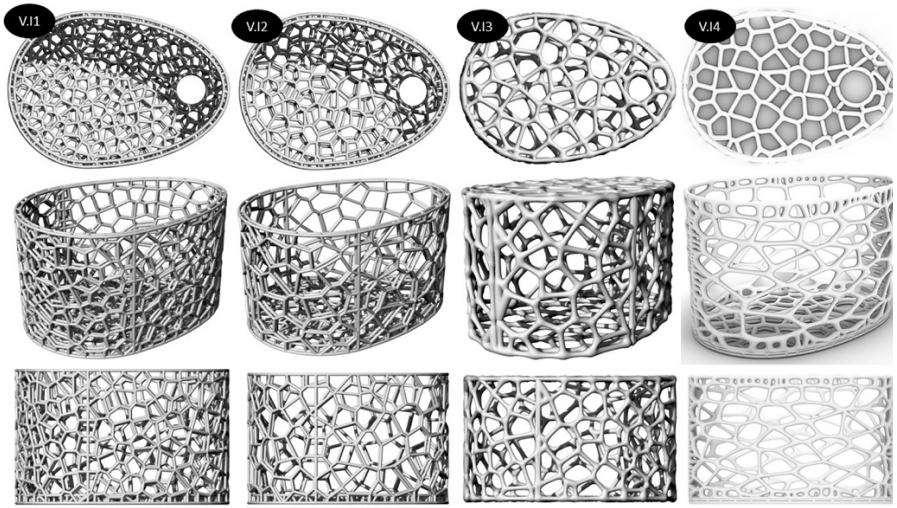


Figure 9. Four different iterations of the Voronoi design family of the biocups. **V.I1** exhibit the first design iteration using tight straight double walled Voronoi structure of 500 points population as base for the 3D Voronoi structure (exhibiting the top view plan, perspective and left side view), enhanced to **V.I2** with less density, wider double walled Voronoi structure of 300 points population base of the Voronoi structure. **V.I3** exhibits the shift to curvy (chamfered) single-walled hollow pipes Voronoi structure with 150 points population of the Voronoi structure evenly distributed, while **V.I4** is adding attractors of the Voronoi structure towards the borders of the biocup as an emphasize on the horizontal ends of each biocup. However, this iteration would result in uneven exposure of the immitted bioluminescent light, blocking light at the bottom and top of each biocup and resulting in lesser light amount. Thus, iteration **V.I3** was the one chosen for further customization and application in the biolamps.

Figure 10. The 3D printed Voronoi family iterations of biocup designs, by the authors.

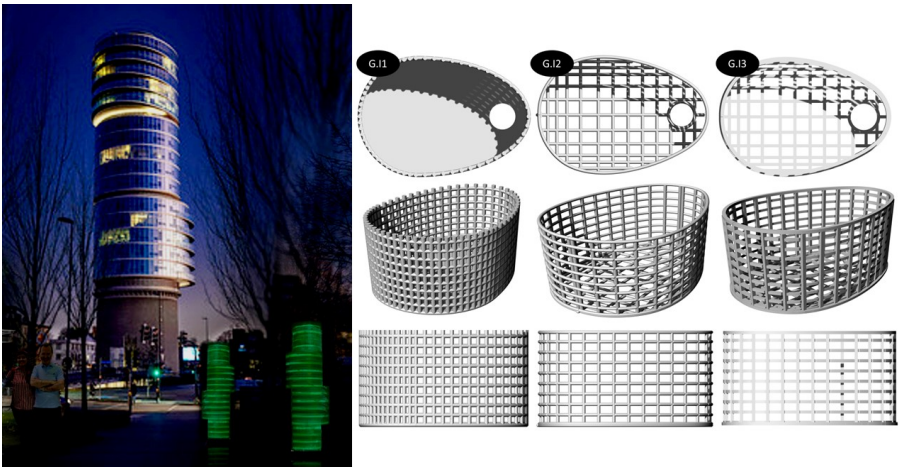


Figure 11. Design concept inspired from the Bochum building of Fritz and Trude Fortmann Foundation: left side, imagined urban biolamps with the Bochum building in the background. The right side are the iterations of the gride design family including **G.I1** with a dense vertical grid and closed bottom, **G.I2** is with wider openings and circular profile of the grid struts and with grided bottom, while **G.I3** is with moderate openings and square profile of the grid struts and a grided bottom which was the optimized design iteration in this design family. By the authors.

Figure 12. From left to right, the final family of Voronoi biolamps, the grid biolamp, the biocolumn, and below bacteria self-organized in Voronoi after two months growing, the radiolarian biolamp and the exhibition poster: all digitally designed and 3D printed for bioluminescence domestic and urban use. By the authors.

As can be seen in Figure 12, bottom left, the bacteria self-organize into a Voronoi pattern after two months of growth: digitized Voronoi pattern taken as a structure for the biolamps. Next to it is the radiolarian biolamp and above it to the right is the biolamp column.

Results of Step 4

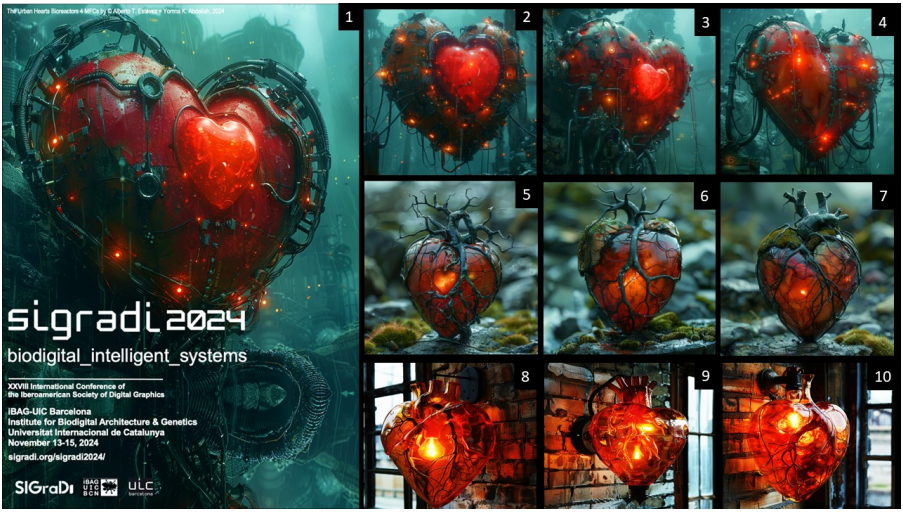


Figure 13. The Urban Hearts Bioreactors visualization presented at the SiGraDi 2024 exhibition held at Universitat Internacional de Catalunya, Barcelona in November 2024. **1.** The main poster of the SiGraDi 2024 conference on the theme of "Biodigital Intelligent Systems" exhibiting an abstract heart form with a bioluminescent red heart core in a vibrant red colour, while the entire heart bioreactor form is visualized in an urban scale with electrical connections to represent harnessing the generated electrical current from this 4-chamber Microbial Fuel Cell (MFC) (A microbial fuel cell is a liquid battery that employs bioagents such as microorganisms, like bacteria, algae, and fungi, to produce electricity through the oxidation-reduction reaction that occurs when the microorganisms consume the substrates in the growth media inside the microbial fuel cell. Typically, a microbial fuel cell is composed of two chambers: the anode and the cathode, which are usually connected by a bridge to facilitate the flow of electrons from the anode to the cathode which resulting in the electrical current. There are various configurations of the microbial fuel cells, including double chamber, single chamber, multi-cathode, and others) and redistribute it on an urban scale. **2.**, **3.**, and **4.** are different renders of the same concept of the Urban Hearts Bioreactors with variation of the central bioluminescent heart core and variation in the electrical fixtures. **5.**, **6.**, and **7.** are renders of an architectural element scale of the urban hearts' bioreactors with a biocontainer understanding of bioluminescent bacterial and/or algal cultures exhibiting a more anatomical detailed representation of the heart. **8.**, **9.**, and **10.** are renders of an interior architecture element scale that is employed as a passive lighting unit employing the generated bioluminescence and/or bioelectricity in the domestic use, where these hearts can be used as

biocontainers for bioluminescent cultures, a double or four chamber microbial fuel cell to light a bulb, or even a decorative glass container that can be used as an artistic lighting unit. The renders were generated using AI-Diffusion models fed by textual and visual prompts and the results were modified with Adobe Photoshop, by the authors.

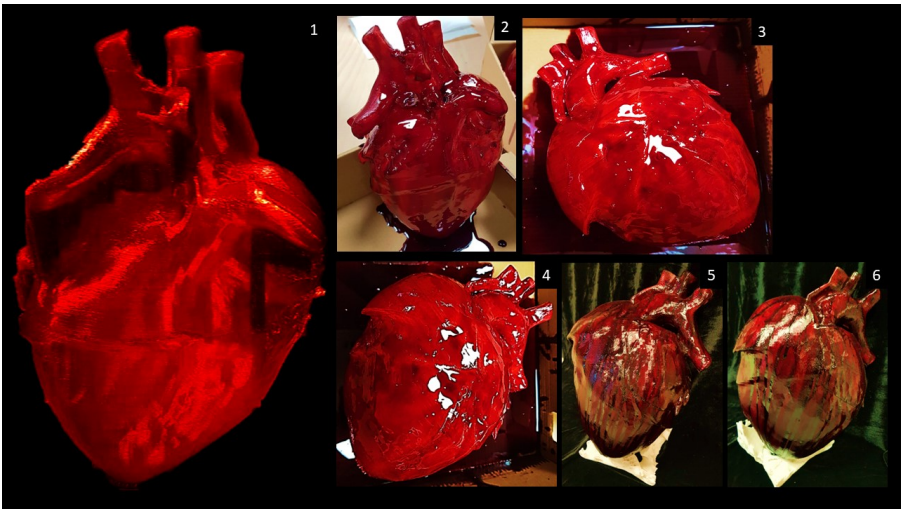


Figure 14. The Urban Hearts Biocontainers and MFC chambers 3D printing and colouring: varied scales of the 3D printed heart biocontainers. **1.** Is the urban scale 3D printed heart biocontainer of (40 x 40 x 50 cm) 3D printed in translucent red PLA using the Felix XL pro dual head printer. **2.**, **3.**, **4.** exhibits the architectural element scale heart biocontainer of (20 x 20 x 20 cm) 3D printed in translucent red PLA using Felix Pro Dual Head printer and finished with varnish (translucent carmen red and translucent) to conceal any pours and give the realistic effect of heart capillaries and blood mimicking the human heart. **5.** and **6.** are two views of the dried urban heart biocontainer on a 3D printed stand which is part of the Heart Shelter Brick. Below the Urban Hearts and Bone Biocontainers in the BioLumCity exhibition. By the authors.

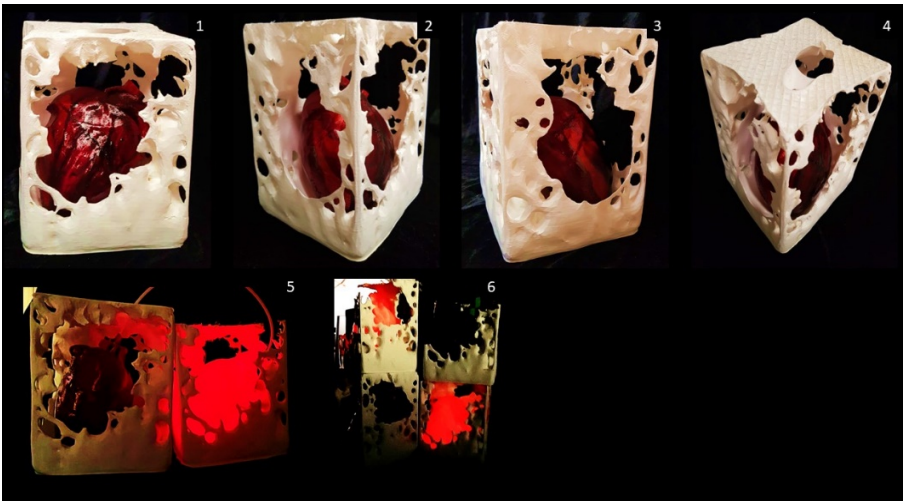


Figure 15. The Heart-Shelter Brick system. **1.**, **2.**, **3.** and **4.** are different perspectives of the 3D-printed heart shelter brick exhibiting its bone-mimicked porous structure developed from algorithmic modification of the CT scanned bovine trabecular bone structural motif, and exhibiting the fixed assembly of the heart biocontainer inside the customized cave within the brick shelter, where the heart biocontainer can be managed easily to be refilled with fresh bioluminescent media, however it can't be removed out of the shelter preventing its loss. **5.** and **6.** are the MFC arrangement of the heart bioreactors inside their shelter, showing the glowing bioluminescence of the hearts in a dark space in 3 seconds reaction activation time. By the authors.

Figure 16. The bone-shaped biocontainer for bioluminescent liquid culture. **1.** and **2.** are two views for the 1:10 scaled model of the bone shaped biocontainer. **3.** is the 3D printed models of the 1:2 scaled biocontainers in different printing stages and with varied PLA filaments including translucent, transparent and luminous filaments. The photo is taken from the Heart of Architecture Exhibition, held at Universitat Internacional de Catalunya, Barcelona, February 2025. By the authors.

Reporting outcomes (diffusion and scientific communication): publications and exhibitions

Along the two years duration of the funded research call, events and publications were conducted as a method of scientific communication of this research project. To promote the interest and awareness about bioluminescence and bioenergy as renewable, clean and cheap sources of energy. These diffusion activities included the following:

1. The official exhibition of the international conference of the Sociedad Iberoamericana de Gráfica Digital, SIGraDi 2024 Biodigital Intelligent Systems, held at Universitat Internacional de Catalunya, Barcelona, November 2024:
   http://www.sigradi.org/sigradi2024
   https://www.uic.es/es/agenda/sigradi-2024-biodigital-intelligent-systems

The exhibition included the first phase prototypes of the biolamps and the urban hearts biocontainers, as well as the first prototypes of the bioreceptive panels and one screen of the bioreceptive panels for application on urban scale. In addition to the official poster of the SIGraDi 2024 Biodigital Intelligent Systems conference as exhibited in Figure 17.

Figure 17. The communication of the BioLumCity Project at the SIGraDi 2024 conference. **1.** The official poster of the conference SIGraDi 2024 Biodigital Intelligent Systems, and **2.** from the exhibition showcasing the first prototypes of biolamps of petri dishes and the heart-shaped biocontainers for liquid bioluminescent culture.

2. The Heart of Architecture Exhibition held at Universitat Internacional de Catalunya, Barcelona, Spain, February 2025: https://www.uic.es/en/noticia/heart-architecture-heart-combines-biotechnology-sustainability-and-art-uic-barcelona.

The exhibition focuses on the concept of the heart as a bioreactor capable of pumping life through our body. Inspired by its morphology and functions, the heart-shaped biocontainer to incubate and cultivate strains of the liquid culture of bioluminescent bacteria and algae. The Heart of Architecture represents a metaphor. Just as the human heart pumps clean, oxygenated blood, this bioinspired system purifies polluted air and distributes it through the city's veins, creating a healthier, more sustainable environment. The exhibition included the heart-shaped biocontainers and the bone-shaped biocontainers.

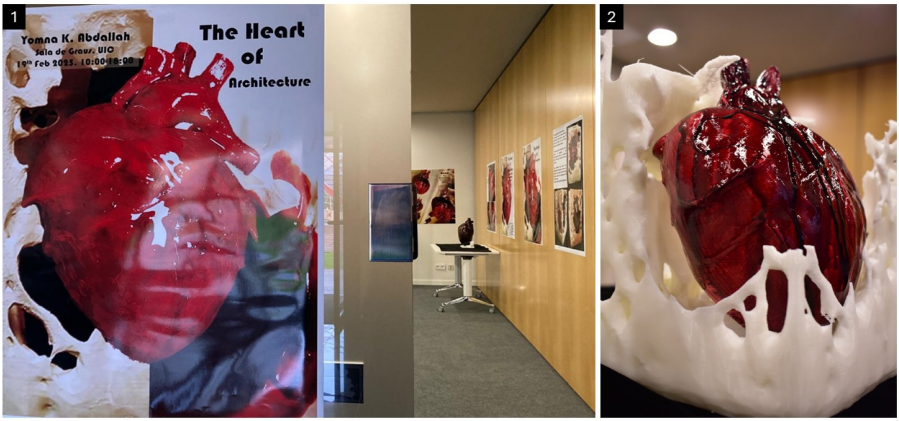


Figure 18. The Heart of Architecture Exhibition held at UIC Barcelona, February 2025, exhibiting the heart-shaped biocontainers and bone inspired biocontainers for liquid bioluminescent bacterial and algal cultures**.** By the authors.

3. Publication of research article: “BioLumCity: 3D-Printed Bioluminescent Urban Tiles Employing Aliivibrio *fischeri* Bioink as Passive Urban Light**”,** in *Appl. Microbiol.* 2025, 5(4), 105: https://doi.org/10.3390/applmicrobiol5040105. *Applied Microbiology* is an international, peer-reviewed, open access journal on application of microorganisms published monthly online by MDPI. Journal Rank: CiteScore - Q2 (Biochemistry, Genetics and Molecular Biology (miscellaneous)).

The article exhibited the results obtained from applying modified bioluminescent bacterial culture as sol-gel media on the developed 3D

printed bioreceptive tiles which acted as a scaffold to support the bioluminescent culture, allowing its application on architectural and urban scale and guarantying the ease of maintenance as explained above in the Results section (Results of Step 1 & 2).

The research article abstract and the keywords are as follow:

Integrating bioluminescent organisms as passive lighting sources in the built environment is currently a hot topic. However, there are several limitations facing the implementation and up-scaling of these naturally bioluminescent organisms in the built environment on architectural and urban scales, such as the scale, sensitivity, enclosure, and difficulty of maintenance. Moreover, there are complex technicalities and operational aspects of conventional bioreactors that host these bioluminescent agents, especially in terms of managing their recharge and effluent, not to mention their high maintenance cost. The current work offers a sustainable, stand-alone, bioluminescent urban screen system employing *Aliivibrio fischeri* CECT 524 bioink on 3D-printed customized scaffolds as bioreceptive panel design based on a field-diffusion pattern to host the bioluminescent bacterial bioink. The field-diffusion pattern was employed thanks to its proven efficiency in entrapment of the various microbial cultures. Three different growth media were tested for culturing *Aliivibrio fischeri* CECT 524, including Luria Bertani Broth (LB), the Tryptone Soy Broth (TSB), and the standard Marine Broth (MB). The results revealed that the Marine Broth (MB) media achieved the highest bioluminescent intensity and duration. The maximum light emission typically in range of ~490 nm of blue-green light captured by a conventional reflex camera (human eye vision) was observed for 10 consecutive days in complete darkness after 3-10 s, at a room temperature of 25°C. This was visible mainly at the thin curvilinear peaks of the 3D-printed field pattern. P1 achieved the highest performance in terms of visible blue-green light, and a duration of 10 days of active bioluminescence was achieved without the need for refilling, thanks to the high number of peaks and narrow wells at <0.5 cm of its field-diffusion pattern. This study proves the efficiency of this biomimetic pattern in terms of the bioreceptivity of the bioluminescent bacterial bioink. Furthermore, the proposed 3D-printed urban screens proved their economic sustainability in terms of affordability and their minimized production processes, in addition to their easy maintenance and recharge. These results qualify these 3D-printed bioluminescent urban screens for easy and decentralized adoption and application on an architectural and urban scale.

Keywords: *Aliivibrio fischeri*; bioluminescence; bioreceptive; bioactive facades; green buildings; urban screens; 3D-printed scaffolds; bioluminescent bioink; fields; decentralized production; biotechnology in architecture; biosystems.

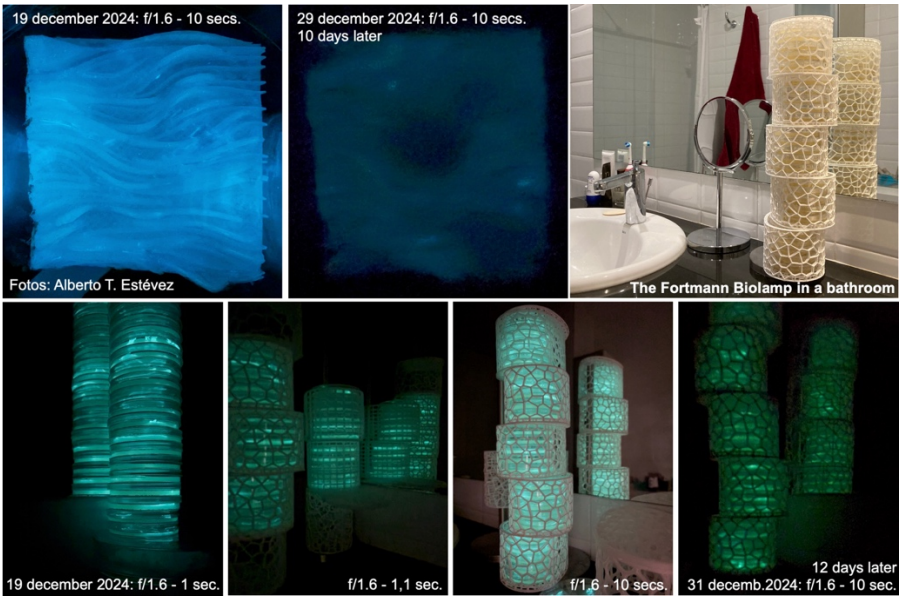
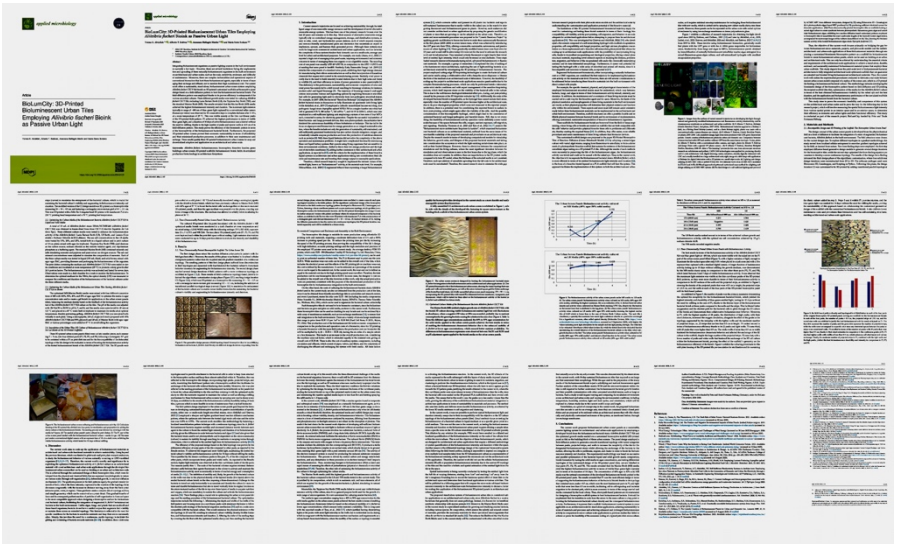


Figure 20. Above, left and center, bioluminescence on the panel. Right and below, bioluminescence, and the Fortmann-Bochum-BioLamp. Comparisons of durations over the days: photos taken with an Apple iPhone 16e digital camera, in total darkness, as an equivalent to how human eyes naturally see it. By the authors.

Below, pages of the article Abdallah, Y.K.; Estévez, A.T.; Balfagón Martin, A.; Serra Soriano, M., (2025), "BioLumCity: 3D-Printed Bioluminescent Urban Tiles Employing *Aliivibrio fischeri* Bioink as Passive Urban Light", *Appl. Microbiol., 5*(4), 105, ISSN: 2673-8007. https://doi.org/10.3390/applmicrobiol5040105.



BioLumCity Project
Bioluminescent Urban Screens 2023-2024
Alberto T. Estevez+ Yomna K. Abdallah
Funded by Fritz und Trude Fortman Stifung

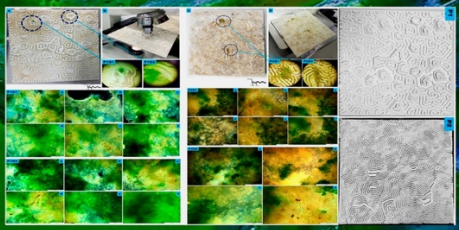
The Bioluminescent Urban Screens are an advanced design concept developed under the Biolumcity initiative, a funded project aimed at pioneering sustainable urban lighting through the integration of functional bioluminescent microbial strains. This approach harnesses biologically moderated, renewable light sources, establishing a new paradigm in eco-friendly urban illumination. At the current stage, the Bioluminescent Urban Screens utilize a passive immobilization system for the bioluminescent bacterial strain Aliivibrio fischeri. The design leverages a custom geometric structure based on Field Theory to enhance the chemotactic behavior of A. fischeri, optimizing conditions for maximal bioluminescent emission. These urban screens are engineered to support both liquid and solid media for bioluminescent bacterial and algal cultures, allowing flexible implementation of microbial light sources. Through precisely tailored microenvironments within the tiles' niches, the bioluminescent cultures are sustained and controlled, creating an innovative and self-sustaining light solution for urban applications.

Bioluminescent urban lighting has long captivated the interest of Professor Alberto T. Estevez, leading him and his team at the Institute for Biodigital Architecture & Genetics (iBAG) and the Genetic Architecture Office and Research Group to explore innovative ways of enhancing naturally occurring bioluminescence. By applying advanced genetic modification techniques, the group aims to amplify the brightness, duration, and color spectrum of luminescent emissions in select microbial strains. This research is directed towards integrating these enhanced bioluminescent organisms into urban infrastructure, creating a sustainable alternative to conventional lighting. The Genetic Barcelona Project exemplifies this vision, advancing through three progressive phases. The first phase involves the use of bioluminescent bacterial bioreactors as a form of natural, eco-friendly domestic lighting. The project's scope extends to the second phase, which aims to genetically modify the leaves of citrus limon (lemon trees) to emit a steady fluorescent glow, potentially transforming these trees into functional urban lighting fixtures. Through this work, Estevez's team is redefining urban illumination, demonstrating how genetic architecture can reshape our built environment by seamlessly integrating biological and technological innovation.

Previous Research and Background

Left: © Alberto T. Estévez, Genetic Barcelona Project, Barcelona, 2003-2008: genetic creation of bioluminescent plants for urban and domestic use. Right, photo of the iaac Genetic Architecture Laboratory, Barcelona, 2008: genetic research with Agusti Fontarnau, to obtain living elements, building materials and living spaces that can be useful to architecture (photo: Alberto T. Estévez).

Bioreceptive Topologies by (Abdallah and Estévez, 2023).



The Two Bio receptive tiles designed following two patterns of reaction diffusion for sharp and localized cellular immobilization for the multi-scale lengths mixed algal cultures, each tile is 3D printed with white opaque PLA, and is followed by the Microscopy study revealing the different hosted strains on each tile from the five strains of algae ranging from filamentous to unicellular in the mixed algal culture. (Abdallah and Estévez, 2023).

This robust foundation in research and design has been instrumental in developing the urban bioluminescent screens, serving as a critical basis for the creation of bio-receptive architectural elements intended for integration within the built environment. The bioreceptive tiles were meticulously designed through algorithmic modeling that simulated the chemotactic behavior of the bacterial strain Aliivibrio fischeri. These tiles were then domestically fabricated using iBAG's UIC 3D printers, employing various materials to assess their potential to enhance the bio receptivity of the surfaces. This approach not only refined the design parameters but also provided empirical insights into the material and structural properties most conducive to supporting and sustaining bioluminescent organisms on architectural surfaces.

Estevez's visionary approach has been synergized with the scientific rigor of Yomna K. Abdallah, whose work focuses on developing bioreceptive surfaces and materials for construction applications in the built environment. Together, their collaborative research, as demonstrated in numerous projects and published studies, has revealed how specific geometric designs and surface micromorphology significantly influence the adhesion and viability of hosted microorganisms. This effect is especially pronounced for unicellular microalgae and cyanobacteria, whose morphology and scale are well-suited to bacterial and algal strains intended for bioluminescent urban screens. Their findings open new avenues in creating biologically integrated architectural elements, where the morphology of construction surfaces is precisely tailored to support and sustain these bioluminescent organisms, enhancing both environmental compatibility and urban aesthetics.

Chemotactic-simulated Based Bioreceptive tiles Variations (Abdallah and Estévez, 2023).

Figures 21 and 22. On the page before and above, poster with the panel explanation and the 3D fabricated bioreceptive panel, both exhibited at the conference of the Sociedad Iberoamericana de Gráfica Digital, SIGraDi 2024 Biodigital Intelligent Systems, held at Universitat Internacional de Catalunya, Barcelona, November 2024.

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Entrance to the UIC Graduation Hall (seminar room) and to the exhibition room of the biolamps and the biopanels on 21st-23rd January 2026, with Pandora reference.

Here is the second part of this chapter, titled PANDORA Project, as a branch in itself of the BioLumCity Project, from the Alberto T. Estévez "Genetic Architectures" research group at iBAG-UIC Barcelona (Barcelona, Spain).

PANDORA Project: Bioluminescent Systems for Sustainable Architecture and Urban Design

1. Executive Overview

The PANDORA project explores bioluminescence as a radically sustainable alternative to conventional urban lighting systems. Grounded in microbiology, molecular biology, plant biotechnology, and architectural design, the project aims to integrate living systems capable of emitting visible light into urban and architectural environments.

During the reporting period, the project has reached an advanced experimental phase. Stable cultures of the marine bacterium *Aliivibrio fischeri* have been established and optimized for sustained bioluminescence. Parallel efforts have progressed in plant biotechnology, focusing on in vitro cultivation systems for model and ornamental plant species as a foundation for future transplastomic approaches. Molecular biology workflows have been implemented to enable multigene transfer, specifically targeting the Lux operon responsible for bacterial bioluminescence.

Collectively, the work completed to date validates the scientific feasibility of bioluminescent systems for architectural applications and establishes a robust experimental and infrastructural base for the next phase of translational development.

2. Scientific and Societal Context

The project aligns with the Convention on Biological Diversity and the European Biodiversity Strategy for 2030, emphasizing sustainability, responsible biotechnology, and urban-ecological integration. Lighting represents a critical intersection between energy consumption, urban liveability, and environmental impact. Bioluminescence offers a non-electrical, low-energy, and carbon-neutral alternative that could redefine how light is produced and distributed in cities.

By combining biotechnology with architectural research, PANDORA addresses both scientific challenges—such as stable light production and genetic regulation—and societal needs, including energy efficiency, aesthetic enhancement, and ecological responsibility.

3. Project Structure and Research Lines

Research activities are organized into three tightly integrated lines:

1. **Microbiology and Bioluminescence Optimization**
2. **Molecular Biology and Multigene Engineering**
3. **Plant Biotechnology and In Vitro Cultivation**

These lines converge toward the long-term objective of producing autonomous, biologically based lighting systems suitable for urban deployment.

4. Microbiology Research Line

4.1 Stable Cultivation of *Aliivibrio fischeri*

A stable, reproducible culture of *A. fischeri* exhibiting visible-spectrum bioluminescence has been successfully established. Bioluminescence is regulated via quorum sensing, making population density and environmental conditions critical variables.

4.2 Optimization of Culture Media

Three media were systematically evaluated:

- **Luria Broth (LB):** Suboptimal despite NaCl supplementation; insufficient ionic complexity.
- **Tryptic Soy Broth (TSB):** Supported growth but limited bioluminescence.
- **Marine Broth:** Provided optimal growth, intensity, and duration of bioluminescence.

Marine Broth was selected as the reference medium due to its seawater-mimicking ionic composition.

4.3 Environmental Parameters

- **Temperature:** Optimal growth at 26 °C; most stable bioluminescence at 24 °C ± 2 °C.
- **pH:** Optimal at pH 6.8.
- **Oxygenation:** Identified as a limiting factor for sustained light emission.

4.4 Autonomous Oxygenation Systems

Two systems are under development:

- A **fermenter-based bioreactor** for controlled aeration and parameter regulation.
- A **passive porous membrane system** enabling oxygen diffusion without electrical input.

These systems aim to extend culture lifespan and minimize contamination risks.

4.5 Growth Curve and Quorum Sensing

Growth curves were established to identify the exponential phase associated with peak bioluminescence. Optical density measurements in marine media required methodological adjustments due to salt consumption artifacts.

4.6 Clonal Selection of High-Bioluminescence Strains

Phenotypic screening enabled the isolation of *A. fischeri* clones with significantly enhanced light output and stability. Selected strains are being prepared for genomic sequencing to identify mutations or regulatory variations linked to improved performance.

4.7 Iron Availability Studies

Systematic studies are underway to assess the influence of iron concentration on:

- Bioluminescence intensity
- Siderophore production
- Biofilm formation

Preliminary hypotheses indicate optimal performance at intermediate iron concentrations, with inhibition at both deficient and excessive levels.

4.8 Hypothetical Symbiosis with *Candida albicans*

Exploratory work proposes potential mutualistic or competitive interactions between *C. albicans* and *A. fischeri* under iron-limited conditions. These studies open new avenues for understanding polymicrobial systems and resource-sharing mechanisms relevant to long-term bioluminescent stability.

4.9 Material Interaction Studies

- **Cotton (100% unbleached):** High moisture retention supports prolonged bioluminescence.
- **Polyester:** Promotes bacterial adhesion and biofilm formation but limits long-term activity due to low hygroscopicity.

These findings inform material selection for bioluminescent architectural surfaces.

5. Molecular Biology Research Line

5.1 Bacterial Transformation Infrastructure

Competent *E. coli* strains and transformation protocols have been standardized. A plasmid library (pUC18, pUC19, pXGAL) has been established.

5.2 Lux Operon Engineering

The bacterial LuxCDABEG operon (~6.8 kb) has been selected as the gene of interest. pUC19 was identified as the most suitable cloning backbone.

5.3 Shuttle Plasmid Design

A hybrid shuttle plasmid has been designed to enable expression in both prokaryotic and eukaryotic systems, incorporating:

- Chloroplast targeting sequences
- Strong constitutive promoters (CMV)
- Regulatory and selection elements

This design is a critical milestone toward chloroplast transformation.

6. Plant Biotechnology and *In Vitro* Culture Line

6.1 Model Species

In vitro cultivation protocols were successfully established for:

- *Nicotiana tabacum*
- *Arabidopsis thaliana*

Protocols include seed sterilization, germination, clonal propagation, rooting, and acclimatization.

6.2 Acclimatization to Ex Vitro Conditions

A gradual acclimatization protocol has been optimized, reducing transplant shock and ensuring plant viability outside sterile conditions.

6.3 Direct Organogenesis

Hormonal regimes using auxins and cytokinins are being optimized to induce direct shoot regeneration from leaf explants, a prerequisite for chloroplast transformation.

6.4 Ornamental Species

Protocols have been adapted for species of architectural interest, including *Ipomoea*, *Dianthus*, *Calendula*, and *Petunia*. Selection criteria included aesthetic value, biomass performance, ecological adaptability, and maternal chloroplast inheritance.

7. Architectural and Urban Applications

A major translational milestone is the publication of the article: “BioLumCity: 3D Printed Bioluminescent Urban Screens Employing *Aliivibrio fischeri* Bioink as Passive Urban Lights”. This work demonstrates the feasibility of integrating living bioluminescent systems into 3D-printed architectural elements, reinforcing the project’s applicability beyond the laboratory.

8. Current Status and Readiness Level

- Laboratory infrastructure fully operational.
- Stable microbial and plant systems established.
- Genetic engineering workflows designed and partially implemented.

- Proof-of-concept architectural applications validated.

The project is positioned at an advanced experimental stage, transitioning toward integrative prototypes.

9. Strategic Outlook

The next phase will focus on:

- Genetic optimization of bioluminescence intensity and duration.
- Chloroplast and cyanobacterial transformation.
- Long-term stability and biosafety validation.
- Scaled architectural demonstrators.

PANDORA demonstrates strong scientific coherence, interdisciplinary integration, and high potential impact, positioning UIC Barcelona as a leader in biodigital and bioluminescent architectural research.

10. Concluding Statement

The PANDORA project has investigated bioluminescence as a novel, biosustainable alternative to conventional lighting systems, with a particular focus on its potential integration into architectural and urban environments. Through an interdisciplinary approach combining microbiology, molecular biology, plant biotechnology, and architectural research, the project has established a solid scientific and technical foundation for the development of living, energy-autonomous light-emitting systems.

Throughout the project, a stable cultivation system for the marine bacterium *Aliivibrio fischeri* was successfully established, enabling consistent emission of visible-spectrum bioluminescence. Extensive optimization of culture conditions—including growth media, salinity, temperature, pH, and oxygen availability—demonstrated that Marine Broth, slightly acidic pH (≈6.8), and temperatures around 24-26°C provide optimal conditions for both bacterial growth and sustained light emission. The critical role of oxygen in the luciferase-mediated bioluminescent reaction led to the development of autonomous oxygenation strategies, including controlled bioreactor systems and passive porous membrane devices designed to function without external electrical input.

In parallel, growth curves were established to identify the exponential phase associated with maximum bioluminescence, and clonal selection strategies allowed the isolation of *A. fischeri* strains with enhanced and more stable light output. These strains represent a significant step toward biotechnological robustness and are currently being prepared for genomic analysis to identify the genetic determinants underlying improved bioluminescent performance.

The project also addressed the influence of environmental iron on bacterial physiology, bioluminescence, siderophore production, and biofilm formation. Experimental designs were established to explore iron as a key regulatory factor, revealing its dual role as an essential micronutrient and a potential source of oxidative stress at high concentrations. Additionally, exploratory research proposed and conceptually analyzed possible interactions between *A. fischeri* and *Candida albicans* in iron-limited environments, opening new perspectives on polymicrobial systems and metabolic cooperation or competition relevant to long-term bioluminescent stability.

Material-based studies further demonstrated that substrate selection plays a decisive role in bioluminescent performance. Hydrophilic, hygroscopic materials such as unbleached cotton were shown to support prolonged bacterial activity and light emission, while hydrophobic materials such as polyester promoted adhesion and biofilm formation but limited long-term viability. These findings provide essential criteria for the design of bioluminescent architectural surfaces.

Complementing the microbiological work, a comprehensive in vitro plant biotechnology platform was established using *Nicotiana tabacum* and *Arabidopsis thaliana* as model species. Protocols for seed sterilization, germination, clonal propagation, rooting, direct organogenesis, and ex vitro acclimatization were optimized, ensuring reproducible and healthy plant material. These systems form the basis for future chloroplast (transplastomic) transformation strategies aimed at expressing bacterial bioluminescence pathways in higher plants. In addition, cultivation protocols were adapted for selected ornamental species of architectural interest, ensuring their suitability for future urban and landscape applications.

From an applied perspective, the project culminated in the publication of the research article “BioLumCity: 3D Printed Bioluminescent Urban Screens Employing *Aliivibrio fischeri* Bioink as Passive Urban Lights” in *Applied Microbiology* (2025), which demonstrates the feasibility of integrating bioluminescent organisms into 3D-printed architectural elements as passive lighting systems. This work represents a key translational milestone, bridging laboratory research and architectural implementation.

In conclusion, the PANDORA project has successfully validated the scientific feasibility of bioluminescent systems as sustainable light sources and has established the experimental, technological, and conceptual groundwork necessary for their future development. The results achieved position bioluminescence not as a speculative concept, but as a viable biological technology with clear potential to contribute to energy-efficient, ecologically integrated, and aesthetically innovative urban environments.

The work completed to date confirms that bioluminescent systems are no longer speculative but experimentally viable. PANDORA establishes the scientific, technical, and conceptual foundations necessary to transform living light into a functional component of future sustainable cities.

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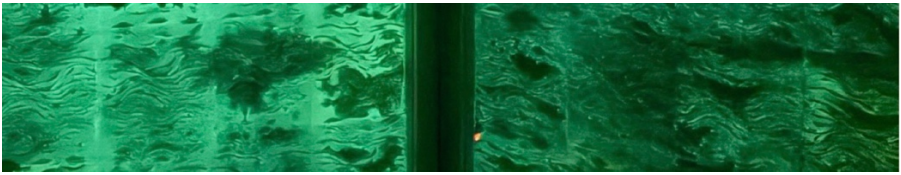
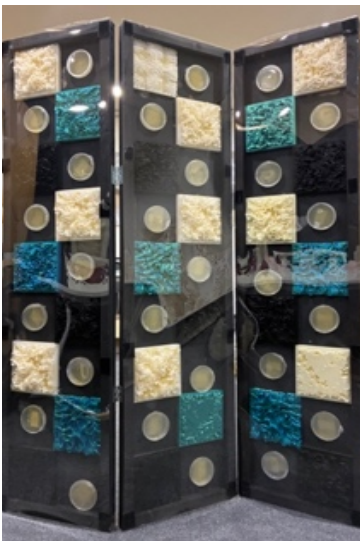
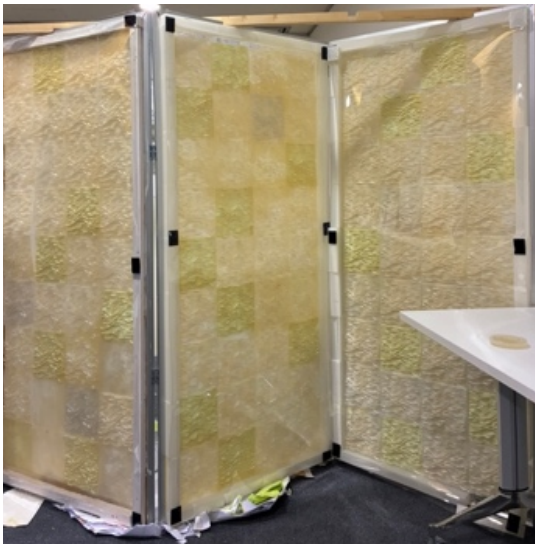
Posters and seminar with the results presentation by the members of the teams of the BioLumCity Project in the UIC Graduation Hall of UIC Barcelona, 21st January 2026.

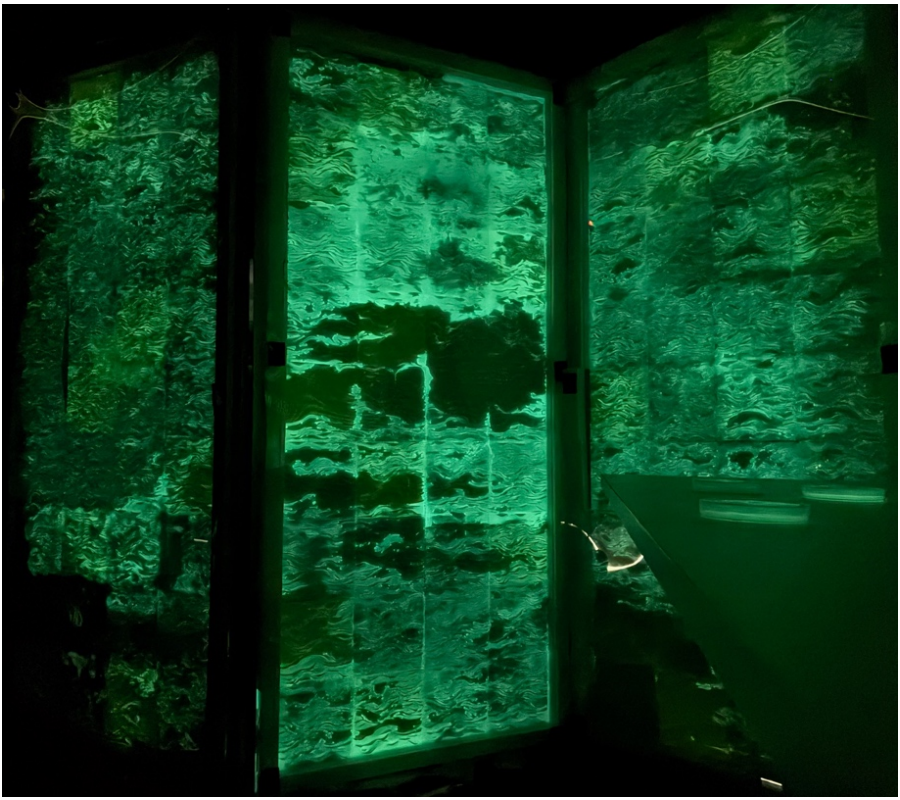
Below: detail of bioreceptive bioluminescent 3D printed panels in the exhibition, UIC Barcelona, 21st-23rd January 2026 (photo: Alberto T. Estévez, Apple iPhone 16e, ISO 2500-5000, f 1,64, 3,4 sec., equivalent to how the human eye naturally sees it).



Two different designs for bioreceptive bioluminescent 3D printed panels in the exhibition, UIC Barcelona, 21st-23rd January 2026 (photo: Alberto T. Estévez, Apple iPhone 16e, ISO 2500-5000, f 1,64, 3,4 sec., equivalent to how the human eye naturally sees it). By the authors.







Above, bioreceptive bioluminescent 3D printed panels, and **below,** biocontainers (left) and biolamps (right), in the exhibition, UIC Barcelona, 21st-23rd January 2026 (photo: Alberto T. Estévez, Apple iPhone 16e, ISO 2500-5000, f 1,64, 3,4 sec., equivalent to how the human eye naturally sees it). By the authors.

CRAG group
BioLumCity
Project Report

Jae-Seong Yang

Here is the final report for BioLumCity from the Jae-Seong Yang group at CRAG (Barcelona, Spain). This version includes only newly updated information and does not repeat content from the previous report.

Final report of the Project

Objective 1. Setup of a bioluminescent system in microalgae

In the previous report, we successfully tested the activity of luciferase (Luz**)** gene in *Chlamydomonas* with obtained luciferin from LightBio. This substrate is oxidized by enzyme Luz to emit light while produces caffeoyl pyruvate (**Figure 1**). In order to complete the bioluminescent reaction cycle, during the last year we have cloned and tested other three remaining enzymes H3H, HispS, and CPH with the same strategy of to clone the Luz gene. One strategy involved using a codon optimized for plants such as *Arabidopsis*, and the other for *Chlamydomonas*. The version optimized for plants was obtained from LightBio company, and the one for *Chlamydomonas* was synthesized. We have obtained genetic parts from the *C. reinhardtii* MoClo toolkit for cloning of luciferase gene into universal Golden Gate backbone vectors. The typical assembly reaction condition was 100 fmol of each insert and 50 fmol of the backbone, in a 10 µl reaction in rCutSmart buffer with 0.5 units/µl restriction enzyme, 20 units/µl T4 ligase and 1 mM ATP. Transformation of TOP10 cells (ThermoFisher scientific) was performed by heat-shocking for 90s at 42 °C. For all genes, we generated the level 2 vector, containing CDS under the control of pAR promoter (HSP70-RBCS2 hybrid constitutive promoter) and RPL23 terminator. The antibiotic resistance gene is included in the vector in a separate expression cassette.

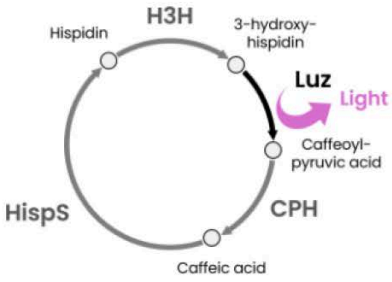


Figure 1. Simplified caffeic acid cycle from *N. nambi*. 3-hydroxyhispidin is oxidized to caffeoylpyruvic acid under light emission. The intermediate is first converted to caffeic acid and then to hispidin, before being recovered as the original substrate of the cycle. CPH, Caffeoylpyruvate hydrolase; HispS, Hispidin synthase; H3H, Hispidin-3-hydroxylase; Luz, Luciferase.

As we already know that Luz from *N. nambi* is functional in *Chlamydomonas*, we decided to test the activity of hispidin 3-hydroxylase (**H3H**), which converts hispidin into 3-hydroxyhispidin, the fungal luciferin. Instead of directly supplying luciferin, we provided hispidin as the substrate together with the H3H extract and the Luz microalgae extract. If H3H is active, light emission should be observed.

With the same transformation protocols, we selected the best transformants that express H3H enzyme (data not shown here). Then we mixed the extract of H3H transformants and nnLuz expressing transformants with hispidin. However, we found that nnH3H (from *N. nambi*) is not active in *Chlamydomonas*. Taking into account that the pCM2_nnH3H-F2A-

NL (HygR) vector yielded colonies with high reported NL activity it seems to be a matter of protein activity, maybe due to incorrect folding or post-translational modifications, but expression concerns cannot be completely ruled out without further proof (qPCR). In any case, nnH3H is not suitable for the incorporation of the FBP pathway in *Chlamydomonas* and other synthetic genes must be designed in its place.

To do this we checked the literature to see if some other H3H was used before for bioluminescent cycle. Current literature only describes the use of nnH3H (*Neonothopanus nambi*) and mcH3H (*Mycena chlorophos*), but there are more protein sequences available at UniProt. In the phylogenetic analysis presented by Kotlobay et al. (2018), several distinct clades within the order *Agaricales* exhibit bioluminescence (BL) (**Figure 2**). To maximize the likelihood of successful H3H expression, we selected two homologs from phylogenetically distant lineages: one from the *mycenoid* lineage (mcH3H) and one from the *Armillaria* lineage (amH3H, derived from *Armillaria mellea*). This strategy increases sequence diversity and thereby enhances the probability that at least one construct will express efficiently. In contrast, selecting closely related proteins may lead to similar expression or functional limitations, as observed for nnH3H.

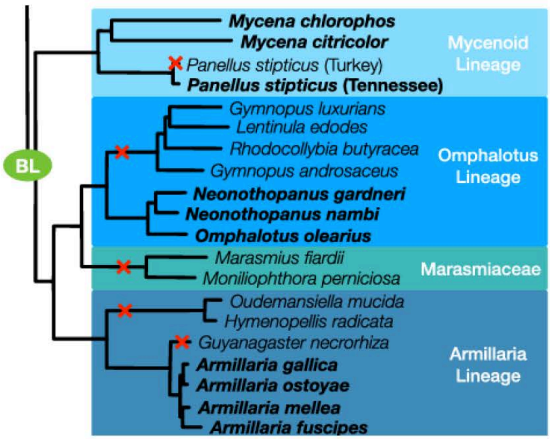


Figure 2. Phylogeny of Agaricales species. The rectangles with the gene names indicate where luz, h3h, and hisps genes emerged as a result of duplication. An oval in the bioluminescence (BL) clade indicates the common ancestor of all bioluminescent species. (The figure and text were from the original paper Kotlobay et al. (2018)).

To test H3H from other species, we cloned these genes using synthetic DNA fragments designed to generate intronized and codon-optimized versions of the amH3H and mcH3H genes. Lyophilized gene fragments (1,000 ng) were resuspended in 20 µL of nuclease-free water to obtain a final concentration of 50 ng/µL. Samples were briefly vortexed, collected by short centrifugation, and incubated at 37 °C for 1 h to ensure complete dissolution. Level 0 assemblies were performed to generate the pCM0_amH3H (B3) and pCM0_mcH3H (B3) constructs (**Figure 3**).

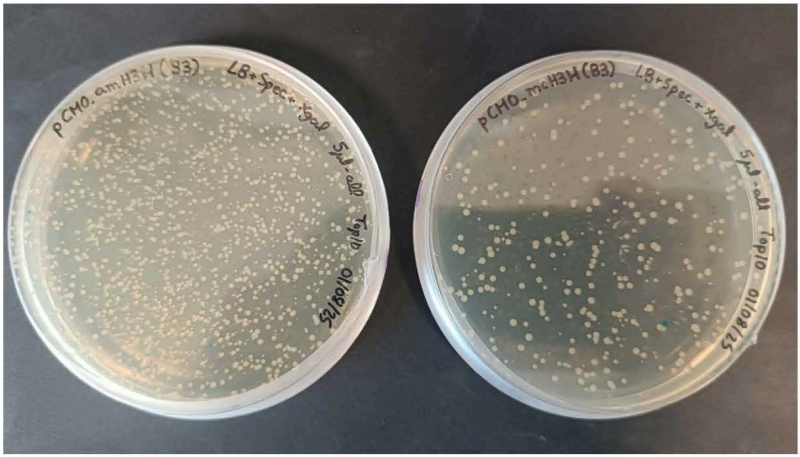


Figure 3. Successful Transformation of pCM0_amH3H (B3) and pCM0_mcH3H (B3) in *E. coli* Top10.

Two colonies from each plate were picked and inoculated into 5 mL of LB supplemented with spectinomycin, followed by overnight incubation at 37 °C. To confirm successful cloning, plasmids were extracted by miniprep and digested with BbsI, which yields characteristic restriction patterns. The expected digestion patterns were simulated using SnapGene, allowing identification of the correct clones (**Figure 4**).

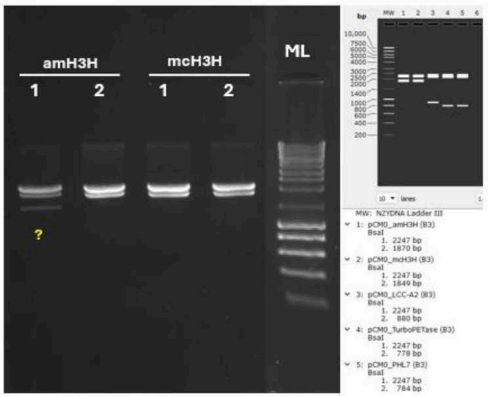


Figure 4. Restriction digestion analysis of pCM0_amH3H (B3) and pCM0_mcH3H (B3).

To generate an expression cassette for *Chlamydomonas*, the pCM2_amH3H-F2A-NL (HygR) and pCM2_mcH3H-F2A-NL (HygR) constructs were assembled. Gene expression was driven by the pAR promoter and terminated by the RPL23 terminator, with NL serving as the selectable marker gene.

The assembled plasmids were transformed into ***E. coli*** Top10 competent cells. Five microliters of the assembly reaction were added to the cells and incubated on ice for 20

min, followed by heat shock at 42 °C for 90 s. Cells were immediately transferred to ice for 2 min, after which 250 µL of SOC medium was added. The cultures were incubated at 37 °C for 1 h and then plated on LB agar supplemented with kanamycin. Plates were incubated overnight at 37 °C, and white, kanamycin-resistant colonies were obtained (**Figure 5**).

Two colonies from each transformation plate were selected and inoculated into 5 mL of LB medium containing spectinomycin, followed by overnight incubation at 37 °C. Plasmids were isolated the following day using an MN miniprep kit and eluted in 50 µL of nuclease-free water. DNA concentrations were measured using a NanoDrop spectrophotometer with 1.5 µL of each sample. To verify correct assembly, restriction digestion was performed in a total reaction volume of 10 µL, and the resulting digestion patterns were analyzed to identify correctly assembled constructs.

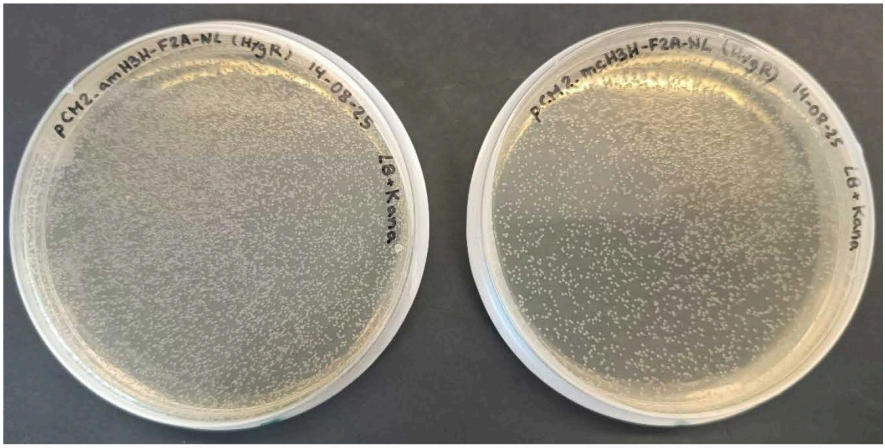


Figure 5. Successful Transformation pCM2_amH3H-F2A-NL (HygR) and pCM2_mcH3H-F2A-NL (HygR) in *E. coli* Top10.

For *Chlamydomonas* transformation, 5 mL of an LM8523 colony 2 culture were inoculated into 95 mL of TAP medium and incubated overnight at 23 °C under light level 3. Following transformation, 48 colonies for each construct (**Figure 6**) were picked into 96-well plates containing 200 µL of TAP medium per well and grown in a laboratory incubator at 23 °C under light level 3.

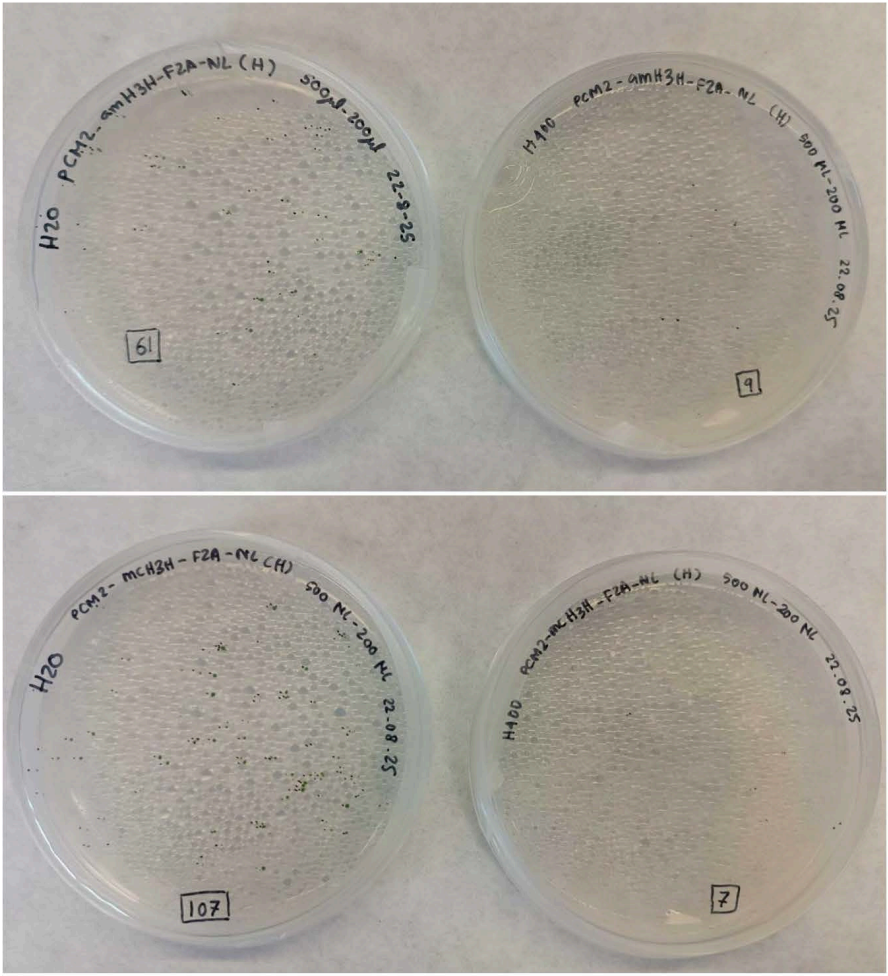


Figure 6. Successful Transformation pCM2_amH3H-F2A-NL (HygR) and pCM2_mcH3H-F2A-NL (HygR) in *Chlamydomonas*.

More than 50 microalgal transformants were selected, and gene expression was assessed using a NanoLuc reporter assay. The highest-expressing transformant colonies were then selected and inoculated into 6 mL of TAP medium. Specifically, amH3H strains A2 and E1, mcH3H strains A2 and E11, and the Luz control strain G6 were cultured under continuous illumination (light intensity level 3) at 23 °C for 72 h.

For samples expressing both Luz and H3H, 20 µL of Luz protein extract and 20 µL of H3H protein extract were combined per well in a black, clear-bottom 96-well plate, followed by the addition of 5 µL of hispidin (1:10 dilution) and 5 µL of NADPH (1:10 dilution). For Luz-

only control samples, 40 µL of Luz protein extract was aliquoted per well and supplemented with 10 µL of 3′-hydroxyhispidin (1:20 dilution). Luminescence was measured immediately using a Victor Nivo plate reader with a 530 nm emission filter and an integration time of 1,000 ms.

Figure 7. Luminescence activity of amH3H- and mcH3H-expressing *Chlamydomonas* colonies (A2, E1, E11) in combination with Luz-expressing *Chlamydomonas*.

Luminescence measurements at 520 nm revealed marked differences in H3H-dependent Luz activity between the amH3H and mcH3H constructs. In strains expressing amH3H (clones A2 and E1), luminescence signals were low and comparable to those observed in the corresponding negative controls lacking H3H activity, indicating minimal or no functional contribution of amH3H under the tested conditions.

In contrast, strains expressing mcH3H exhibited substantially higher luminescence levels (**Figure 7**). The mcH3H A2 clone showed a clear increase in Lum520 signal relative to its negative control, while the mcH3H E11 clone displayed the strongest luminescence among all tested samples. In both cases, the negative control samples showed only background-level signals, confirming that the observed luminescence was dependent on functional H3H activity.

Overall, these results demonstrate that mcH3H, but not amH3H, supports efficient production of the Luz substrate under the experimental conditions used, with notable variability in activity among independent mcH3H transformants.

In addition, *Chlamydomonas* strains expressing HispS and CPH were generated in order to reconstitute a more complete luminescence pathway. Although luminescence

measurements were performed using these integrated components, no substantial light emission was detected under the tested conditions. This suggests that further optimization of expression levels, protein interactions, or reaction conditions may be required to achieve functional activity of the full pathway.

Despite these limitations, the identification of a functional mcH3H enzyme represents a critical step toward establishing a fungal bioluminescence system in *Chlamydomonas*. Based on this finding, we are currently preparing a manuscript that focuses on the successful expression and activity of mcH3H as a foundation for future pathway reconstruction.

In addition to testing the caffeic acid cycle derived from *N. nambi*, NanoLuc expression was also evaluated independently in *Chlamydomonas*. Cultures were grown from agar plates in 20 mL of TAP medium for 10 days. Cells were harvested by centrifugation at 1,000 × g for 5 min, resuspended in 2.5 mL of fresh TAP medium, and transferred to 5 mL Falcon tubes. To induce luminescence, 2.5 mL of NanoGlo buffer–substrate mixture (98% buffer and 2% substrate) was added to each sample according to the manufacturer's instructions.

In this system, visible bioluminescence was clearly observed by the naked eye upon addition of the substrate, confirming functional NanoLuc activity in *Chlamydomonas* (**Figure 8**). Although the biochemical pathway responsible for the endogenous synthesis of the natural NanoLuc substrate has not yet been fully elucidated, these results suggest that this pathway could potentially be exploited for future bioluminescent applications.

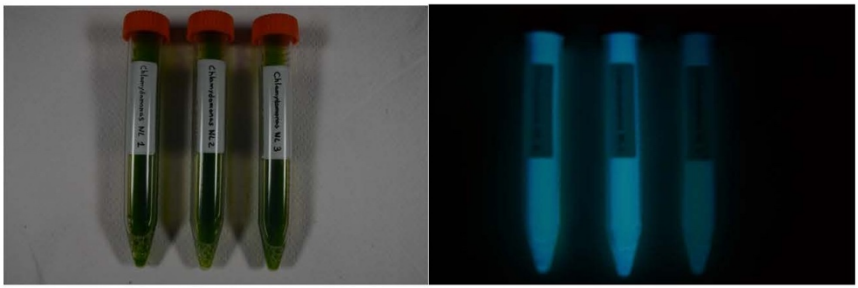


Figure 8. Substrate-Induced Visible Bioluminescence from NanoLuc-Expressing *Chlamydomonas*

Bioluminescent signals were recorded using a Nikon D7000 camera. Images under light conditions were acquired with an exposure time of 1/20 s and an aperture of f/13, while images under dark conditions were captured with an exposure time of 15 s and an aperture of f/4.5. The light-condition images were adjusted for brightness (+20), exposure (+20), and contrast (−20) and subsequently cropped. Dark-condition images were not modified and were only cropped for presentation.

Objective 2. Setup of a co-culture system

Task 2.1. Setup bioluminescent *E. coli*

To establish bioluminescent *E. coli*, we attempted to clone the luxCDABEG operon using modular cloning (MoClo) strategies. Currently, two major MoClo toolkits are available for use in *E. coli*: the CIDAR MoClo system (Iverson et al., 2016) and the EcoFlex toolkit (Lai et al., 2018).

A defining feature of any MoClo toolkit is its assembly syntax, which specifies the nucleotide sequences generated after digestion by type IIS restriction enzymes. This syntax dictates the order of part assembly, the overall design capacity of the system, and its compatibility with other Golden Gate–based cloning frameworks. The CIDAR MoClo system enables combinatorial assembly and provides an improved part library; however, its specific syntax limits assemblies to a maximum of four transcriptional units (TUs). In addition, promoters and terminators must be cloned in four distinct syntaxes, which restricts flexibility for more complex genetic designs (Iverson et al., 2016).

In contrast, the EcoFlex toolkit extends assembly capacity to up to 20 TUs at level 3 and offers a broad collection of modular parts, including promoters, ribosome binding sites, and fluorescent proteins, making it well suited for gene circuit construction (Lai et al., 2018). Nevertheless, EcoFlex does not follow the CIDAR MoClo syntax, limiting compatibility between toolkits. Furthermore, level 0 assembly in EcoFlex relies on conventional restriction–ligation rather than Golden Gate cloning, necessitating additional domestication steps that are labor-intensive and may reduce sequence diversity available for cloning (Iverson et al., 2016; Lee et al., 2015).

To overcome these limitations, our group is developing a new modular cloning toolkit for *E. coli*, termed SynColi. This system fully adheres to the MoClo standard syntax, allowing direct use of existing backbone vectors for level 0 assembly (**Figure 9**). As a result, SynColi is compatible with the CIDAR toolkit while extending its assembly capacity and addressing its previously described constraints.

During cloning attempts using this modular system, we observed that several constructs, particularly those containing the iLuxAB operon, could not be successfully obtained. We hypothesized that uncontrolled overexpression of the iLuxAB operon imposes a substantial metabolic burden on host cells, thereby reducing cell viability. Because the original MoClo backbone vectors lack a lacI repressor, expression from inducible promoters cannot be effectively regulated during cloning.

The absence of lacI-mediated repression may therefore explain the failure to recover correct iLuxAB-containing plasmids, as cells harboring fully assembled constructs may not survive long enough to form colonies following transformation. To test this hypothesis and enable successful cloning of iLuxAB, we proposed the construction of a new backbone vector incorporating a lacI transcriptional unit, thereby allowing tighter control of operon expression during cloning and propagation.

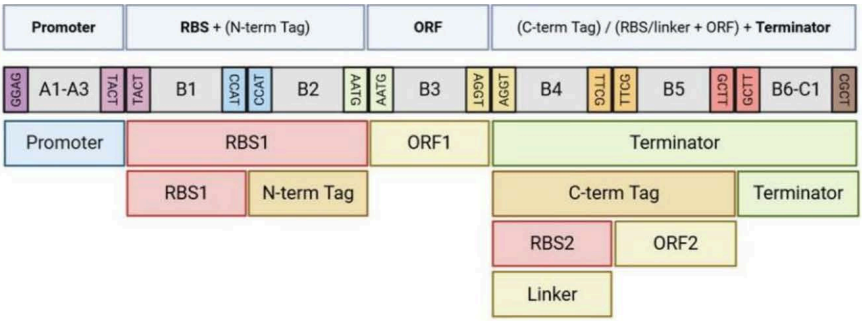


Figure 9. SynColi syntax and assembly capabilities during level 1 assembly. Simple transcription units (TUs) containing a promoter, an RBS, a single ORF and a terminator can be assembled. However, it's also possible to add an N-terminal or a C-terminal tag or to perform protein fusions between two ORFs through the use of a linker sequence. Additionally, small operons containing two RBS sites and two ORFs can be also assembled. Abbreviations: RBS: Ribosome Binding Site, ORF: Open Reading Frame, N-term Tag: N-terminal Tag and C-term Tag: C-terminal Tag.

Additionally, SynColi supports operon-based designs. For instance, in the diagram, A1 -A3 corresponds to the promoter region, while module B1-B2 contains first ribosome binding site (RBS1), when applicable of N-terminal tag. Module B4-B5 can either contain C-terminal tags and serve as a second ribosome binding site (RBS2) as well as second open reading frame (ORF2). The kit also allows for the addition of both N-terminal and C-terminal tags and includes a newly developed lvl0 backbone vector for cloning terminator parts in B4-C1 position (**Figure 9**). Moreover, the kit is expected to contain lvl1 and lvl2 backbone vectors bearing a lacI TU for controlled protein expression. The successful generation of the first of these backbone vectors is described in this work.

SynColi kit was further developed and used to assemble different constructions containing the iLuxAB genes and the YFP and cpYFP protein to test their bioluminescent output.

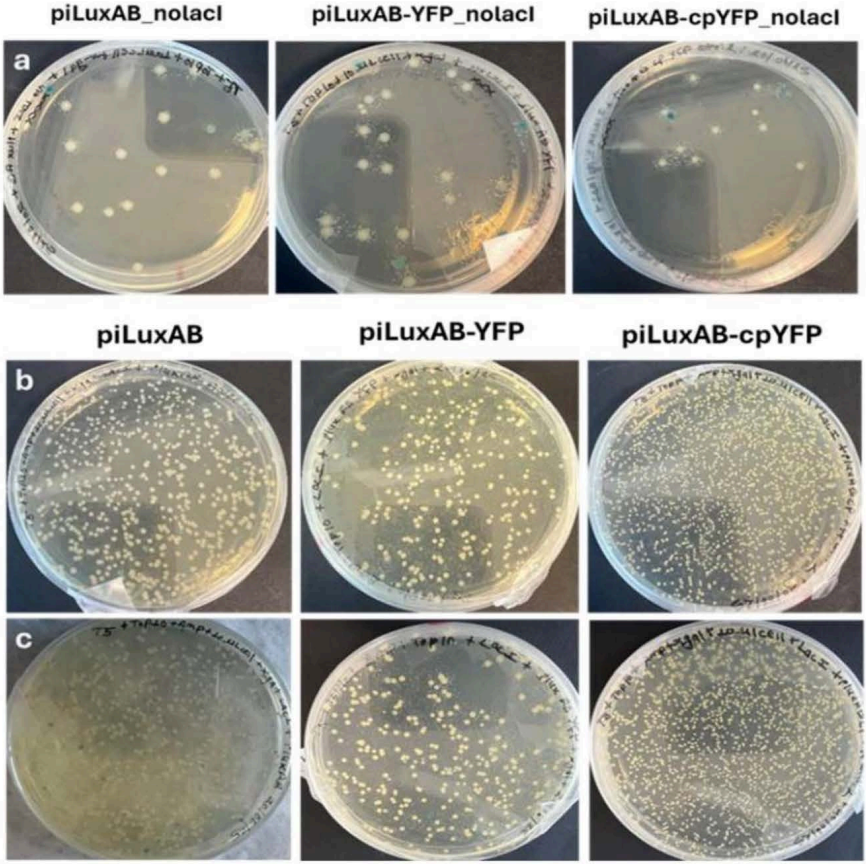


Figure 10. Comparison of the three different iLuxAB constructs assembled using the pICH47751 backbone vector with and without lacI (a) Transformation plating the of the lacI-lacking constructs after O/N incubation (b) Transformation plates showing the transformation results of the lacI-containing constructs after O/N incubation (c) Transformation plates showing the transformation results of the lacI-containing constructs after an additional 48h at 4°C.

To actually confirm that the absence of lacI repression leads to toxicity problems manifested through the survival of only those colonies that incorporated failed assembly products, we compared the outcome of cloning using either lacI-containing or lacI-lacking backbone vectors. Although transformation plates of the lacI-lacking constructs yielded fewer colonies overall, some white colonies were still observed. To determine whether these colonies contained correctly assembled constructs or not, we performed a diagnostic restriction digest. Three colonies from each construct assembled using the lacI-containing backbone (piLuxAB, piLuxAB- YFP, and piLuxAB-cpYFP) along with three colonies from

the same constructs assembled using the lacI-lacking backbone (piLuxAB_nolacI, piLuxAB-YFP_nolacI, and piLuxABcpYFP_nolacI) were picked, miniprepped and digested with BbsI. Digestion with this enzyme was predicted to yield two bands: one corresponding to the backbone and one to the insert. Results were clear and definitive. All 9 lacI-containing constructs displayed the expected restriction pattern, confirming successful and accurate assembly. In contrast, all 9 lacI-lacking constructs yielded unexpected banding patterns, consistent with incorrect assembly or insert rearrangement (**Figure 11**).

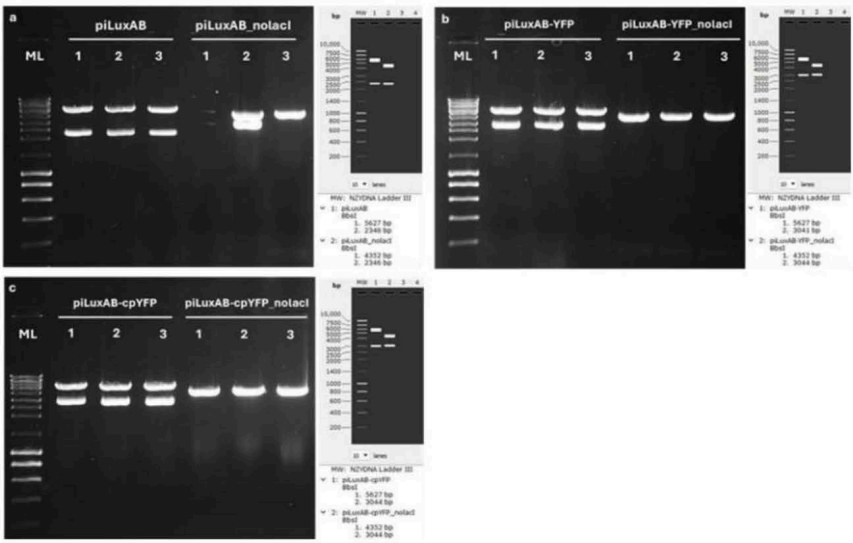


Figure 11. Restriction digestion analysis of constructs assembled with and without lacI in the backbone. (a) Digestion gel of iLuxAB constructs assembled with (lacI) and without (nolacI) repression backbones. (b) Digestion gel of iLuxAB-YFP constructs assembled with and without lacI. (c) Digestion gel of iLuxAB-cpYFP constructs assembled with and without lacI. All plasmids were digested with BbsI, and restriction patterns were compared to predicted fragment sizes based on SnapGene simulations.

Once the different assemblies were validated and the role of lac repressor was confirmed, we next focused on the second objective of this project: measuring and comparing the bioluminescence output of the different constructs. Concentrated liquid cultures of each of the confirmed piLuxAB, piLuxAB-YFP and piLuxAB-cpYFP transformants were imaged under dark and light conditions after IPTG induction and decanal addition. Under ambient light, no visible differences were observed between the different transformant strains and with the WT (**Figure 12a**). However, in the dark, bioluminescence was clearly detectable in all three transformant strains. Both the pGEX-iLux construct, used as positive control, and piLuxAB displayed strong light emission, confirming the functionality of the iLux syste m under the tested conditions. Conversely, both piLuxAB-YFP and piLuxAB- cpYFP cultures showed a comparatively weaker signal. Lastly, the untransformed Top10 strain, used as a

negative control, displayed no visible light emission (**Figure 12b**).

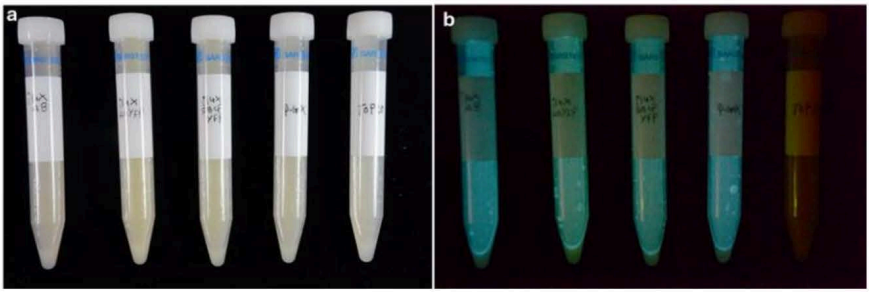


Figure 12. Visualization of the different bioluminescent stains. (a) Cultures under white light, revealing no differences in appearance. (b) Cultures under dark conditions following induction and decanal addition showing visible light emission confirming functional bioluminescence from all three constructs. Abbreviations: iLuxAB: piLuxAB, iLuxAB-YFP: piLuxAB-YFP, iLuxAB-cpYFP: piLuxAB-cpYFP, pGEX (C+): pGEX-iLux used as positive control of luminescence, and Top10 (C-): untransformed Top10 cells used as negative control of luminescence.

Following the imaging, the bioluminescence values of the cultures were quantified in triplicate, piLuxAB consistently gave the highest luminescence (even higher than previous published pGEX-iLux), which is consistent with the directly observed luminescence output. Despite fluorescence protein fusion to iLuxB, the piLuxAB-YFP and piLuxAB-cpYFP fusion constructs (YFP and cpYFP) displayed lower luminescence, suggesting suboptimal protein folding or inefficient BRET (**Figure 13**).

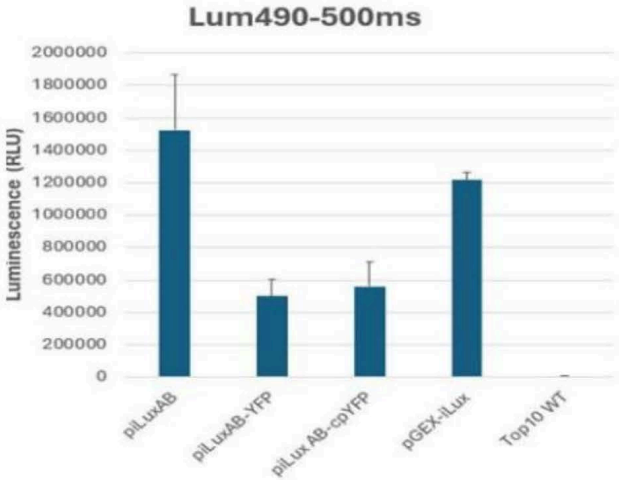


Figure 13. Quantification of the light emission of the different iLuxAB constructs. Luminescence was quantified using a victor plate reader. Bar chart displays raw luminescence values after decanal addition, with piLuxAB showing the highest signal.

Moreover, emission spectra analysis was performed to evaluate potential BRET resulting from the fusion of iLuxB with fluorescent proteins. Previous studies have shown that direct fusion with fluorescent proteins such as Venus or cpYFP produces a characteristic red-shift in emission, typically around 528 nm, due to efficient energy transfer (Kaku et al., 2021). Accordingly, we expected that our piLuxAB-YFP and piLuxAB-cpYFP constructs would display shifted emission spectra compared to unfused iLuxAB. The spectrum of the unfused piLuxAB exhibited a clear and strong peak centered at 490 nm, consistent with the expected emission profile (**Figure 14a**). In contrast, both fusion constructs (piLuxAB-YFP and piLuxAB-cpYFP) displayed nearly identical emission curves, with no detectable red-shift. Despite the presence of fluorescent protein fusions, the observed peak remained at 490 nm, indicating a lack of effective energy transfer and suggesting that BRET was not achieved under these conditions (**Figure 14b-c**).

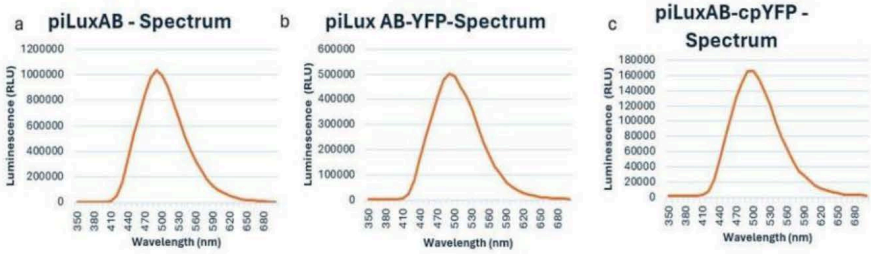


Fig 14. Emission spectra of bioluminescent constructs. (a) piLuxAB emission spectrum displayed a strong peak at 490 nm, consistent with its expected output. (b) piLuxAB-YFP emission spectrum showed no red-shift and a clear peak at 490 nm. (c) piLuxAB-cpYFP emission spectrum also showed no red-shift and a clear peak at 490 nm.

To determine whether the reduced brightness and lack of spectral shift observed in the piLuxAB-YFP and piLuxAB-cpYFP fusion constructs were due to a short 6-nucleotide spacer sequence coming from a cloning scar between iLuxB and the fluorescent protein coding regions, a scarless version of each construct was created. This approach ensured that iLuxB was directly fused to either YFP or cpYFP with no sequences that could affect the distance between the two fused proteins and affect BRET. The resulting scarless (sl) constructs were named pCM0_iLuxAB-YFPsl and pCM0_iLuxAB-cpYFPsl. After transformation one colony was sent for Sanger sequencing. Sequencing confirmed proper ligation and removal of the scar sequence in pCM0_iLuxAB-YFPsland pCM0_iLuxAB-cpYFPsl.

To assess the effect of scar removal in BRET efficiency and bioluminescent output, imaging and quantification of the bioluminescent signal were carried out. The scarless constructs were also compared to piLuxAB. Visual inspection under white light revealed no difference in appearance between the construct or to the untransformed Top10 control

(**Figure 15a**). In the dark, luminescence was clearly visible in all constructs, but no perceptible increase in brightness was observed in the scarless versions (**Figure 15b**).

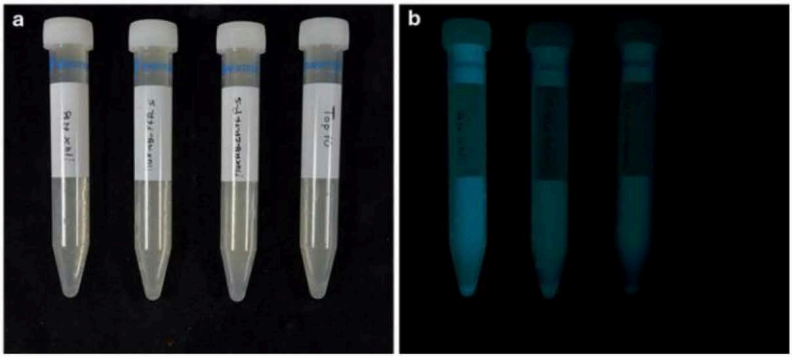


Figure 15. Visualization of the different bioluminescent stains. (a) Cultures under white light, revealing no differences in appearance. (b) Cultures under dark conditions following decanal addition showing visible light emission confirms functional bioluminescence from all three constructs. Abbreviations: iLuxAB: piLuxAB, iLuxABYFP: piLuxAB-YFPsl, iLuxAB-cpYFP: piLuxAB-cpYFPsl, used as positive control of luminescence, and Top10: untransformed Top10 cells used as negative control of luminescence.

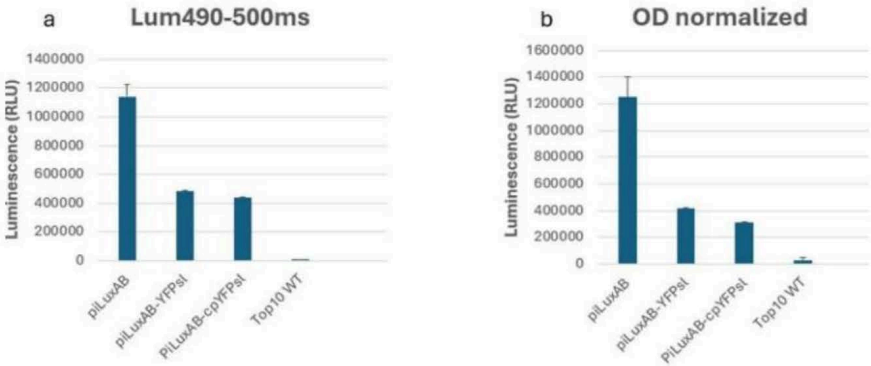


Figure 16. Quantification and emission spectra of scarless constructs. (a) Bar chart showing raw luminescence values, with piLuxAB exhibiting the highest signal. (b) Luminescence values normalized to OD_{600}, confirming that the observed differences in signal are not attributable to variations in cell density.

The emission spectra were collected for piLuxAB and the piLuxAB-YFPsl and piLuxAB-cpYFPsl scarless constructs using the SpectraMax M3 reader. The piLuxAB construct displayed again a single emission peak at 490 nm, consistent with prior observations (**Figure 16**). The scarless piLuxAB-YFPsl and piLuxAB-cpYFPsl constructs also exhibited

the same emission peak with no detectable red-shift, which would have indicated energy transfer to the fluorescent proteins (**Figure 17**). This result suggests that even when iLuxB was directly fused to the fluorescent domains, the BRET effect remained inexistant.

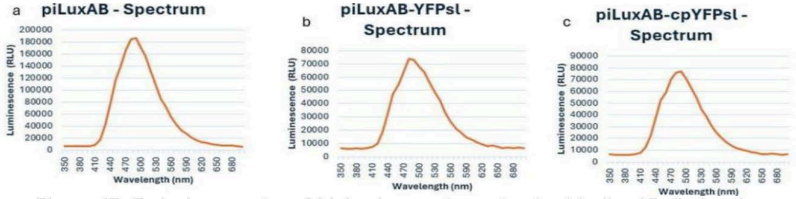


Figure 17. Emission spectra of bioluminescent constructs. (a) piLuxAB displayed a strong emission peak at ~490 nm, consistent with its expected native output. (b) piLuxAB-YFP and. (c) piLuxAB-cpYFPsl showed no redshift in emission compared to piLuxAB, suggesting inefficient energy transfer.

In this study, we aimed to establish a robust strategy for generating bioluminescent *E. coli* by cloning and expressing the luxCDABEG operon using modular cloning (MoClo) approaches. Existing MoClo toolkits for *E. coli*, including CIDAR MoClo and EcoFlex, each offer distinct advantages but also present limitations in terms of assembly capacity, syntax compatibility, and cloning flexibility. To address these constraints, we developed a new MoClo-compatible toolkit, SynColi, which adheres to the MoClo standard syntax, ensures compatibility with CIDAR parts, and expands design flexibility for operon-based constructs. During cloning attempts, we identified a critical limitation in existing MoClo backbones: the absence of lacI-mediated repression. Constructs containing the iLuxAB operon could not be reliably obtained, likely due to uncontrolled operon expression imposing a strong metabolic burden on host cells and reducing viability. By introducing a lacI transcriptional unit into the backbone vector, we successfully restored cloning efficiency, demonstrating that repression during cloning is essential for handling energetically demanding bioluminescent pathways.

Using the lacI-containing SynColi backbones, multiple iLuxAB-based constructs were successfully assembled and validated, including unfused iLuxAB and iLuxAB fusions with YFP and cpYFP. Functional assays confirmed that all validated constructs produced detectable bioluminescence following induction and substrate addition, with unfused iLuxAB consistently yielding the strongest signal. Fusion constructs exhibited reduced luminescence output, suggesting compromised enzyme performance or suboptimal energy transfer.

Emission spectrum analyses further revealed that neither YFP nor cpYFP fusion constructs exhibited the expected red-shift associated with bioluminescence resonance energy transfer (BRET). Removal of the cloning scar between iLuxB and the fluorescent proteins did not improve luminescence intensity or induce spectral shifts, indicating that direct fusion alone was insufficient to achieve efficient BRET under the tested conditions.

This work establishes SynColi as a flexible and reliable modular cloning framework for bioluminescent pathway engineering in *E. coli*. It highlights the critical importance of transcriptional regulation during cloning of metabolically burdensome systems and provides a validated platform for future optimization of bacterial bioluminescence and BRET-based sensor designs.

Furthermore, our research revealed that while the well-known H3H from *N. nambi* remains inactive in *Chlamydomonas*, the distantly related mcH3H is functional. This discovery provides critical insights for the future engineering of functional bioluminescent microalgae.

Towards a Bioluminescent Future

Alberto T. Estévez

Background

In this life, events gradually intertwine, forging a path as we move forward, the fog of time dissipates, leading to an unknown end, thus shaping the history of things as we look back. And so, it was with what we have before us here and now: pioneering research into the application of bioluminescence in our homes and cities.

More than 30 years have passed since that December of 1995 when the author of these lines proposed something to the not yet created Universitat Internacional de Catalunya (UIC) that they hadn't considered: founding a School of Architecture, ESARQ, within its walls. In fact, he had been thinking about it since August 1978, when he first heard about the proposed creation of UIC Barcelona. He was then tasked with establishing it as its first director, and he did so with a very clear vision: he wanted it to be a cutting-edge school of undisputed international prestige. Therefore, he filled it with the most pioneering figures from the local, national, and international scene (not without effort, nor without facing obstacles). This led to rapid worldwide recognition. Through this dedication, he discovered individuals scattered across the globe, initially inviting them to conferences, lectures and presentations, and later hiring them as undergraduate and postgraduate professors. In 2000, he even collaborated with them to create a systematic, advanced, and groundbreaking program for its time: the current Master's in Biodigital Architecture. These figures, instead of beginning their talks with the usual references among architects—the world of the Modern Movement—started with references to nature. Some integrated it into their work in unconventional and not “conservationist” ways, while others explored it using digital tools: Bernard Cache, Karl S. Chu, Marcos Cruz, Dennis L. Dollens, Evan Douglis, Mark Goulthorpe, Greg Lynn, Marcos Novak, Kas Oosterhuis, François Roche, Lars Spuybroek, among others.

Thus, the shift toward biology in architecture was already prophesied. Meanwhile, in December 1999, came to an “explosion” on the media about the spectacular advances in genetics. Seeing that genetics was beginning to be applied to two vital human needs—health and food—but not to those related to habitat (housing, light, and heat), the undersigned, as architect, decided to begin applying genetics to architecture, creating the “Genetic Architectures” Research Group & Office in January 2000, in ESARQ-UIC

Barcelona, which also launched a master's and a doctoral program. Then, with this, founding the first real genetic laboratory dedicated to architectural objectives in the world, with the collaboration of geneticists for first time in architectural history, in 2003 he initiated the Genetic Barcelona Project, which concludes its sixth phase (2023-2026) with this book, focusing on the application of bioluminescence to our spaces.

Bioluminescence and Architecture

The integration of bioluminescence into architecture—the emission of light by living organisms—as an architectural resource, represents a paradigm shift from energy-consuming systems to biologically embedded light production, a radical shift in the conception of energy, materiality, and environment in design. Traditionally, architecture has relied on external energy systems (fire, electricity), but bioluminescence proposes a self-producing, living light, embedded within biological systems themselves. This pioneering research was developed since 2003 under the name of Genetic Barcelona Project at the ESARQ-UIC Barcelona. Situating this work within the historical evolution of bioluminescence research and the rise of genetic engineering, with conceptual, technical, and ecological implications of bioluminescent systems in architecture.

The history of architecture has been deeply intertwined with the control and production of light, from fire and oil lamps to electricity. However, the emergence of bioluminescence as an architectural medium signals a radical transformation: light is no longer externally generated but biologically produced within living systems.

Since 2003, the Genetic Barcelona Project has explored this possibility through experimental applications of genetic engineering and living beings to architecture. The project proposes a shift from industrial fabrication to biological growth, aligning architecture with processes of life itself: science has beaten fiction, and today’s utopia is tomorrow’s reality.

Bioluminescence: Scientific Origins and Architectural Imagination

Bioluminescence—the production and emission of light by living organisms—has been observed across marine and terrestrial ecosystems.

Bioluminescence has long fascinated science and culture. Observed in organisms such as jellyfish, fireflies, deep-sea fish, worms, fungi, plankton, bacteria, etc., it has been studied since the 19th century, but only with the rise of molecular biology and genetic engineering in the late 20th century did it become possible to transfer luminescent properties between species.

The discovery and application of Green Fluorescent Protein (GFP)—originally isolated from the jellyfish *Aequorea victoria*—was a decisive turning point. GFP became a fundamental tool in biotechnology, allowing scientists to visualize genetic processes and, eventually, to imagine transferring light-emitting capabilities into plants and other organisms. Its biochemical basis typically involves the reaction between luciferin and luciferase, producing light without heat. So, the late 20th century marked a decisive turning point with the isolation of Green Fluorescent Protein (GFP), enabling the transfer of luminescent traits across species. This discovery transformed bioluminescence from a natural curiosity into a programmable biological function.

Architecture, meanwhile, was undergoing its own transformation. The ecological turn of the late 20th century, combined with advances in computation, led to new paradigms emphasizing sustainability, adaptability, and integration with natural systems. Within this context, genetics can be identified as a new frontier. The real revolution is not only digital, but also biological, with the possibility of “designing life”. Thus, bioluminescent architecture emerges at the intersection of biotechnology and design speculation, but also as a concrete research agenda.

The Genetic Barcelona Project: Research Phases and Experiments

Phase I (2003-2006). Transgenic Plants, Bioluminescent Trees. The initial phase of the project focused on introducing GFP into plants, particularly citrus species: the gen of the GFP was successful integrated in the DNA of seven lemon trees (type “fino”) that lives during decades. The goal was to produce autonomously glowing vegetation capable of functioning as urban lighting systems. The objective is the genetic creation of plants with natural light-emitting abilities for urban and domestic use. This phase is significant not only for its technical experimentation but also for its conceptual reframing of architecture. Light is no longer installed but cultivated, shifting design

toward agricultural and biological practices. (With the collaboration of Leandro Peña, Instituto Valenciano de Investigaciones Agrarias, and Agustí Fontarnau, UIC Barcelona).

Phase II (2007-2010): Bioluminescent Systems and Biolamps. The second phase expanded into the use of bioluminescent bacteria, enabling more controlled and intense light production. This led to the development of bioluminescent lamps (biolamps), experimental interiors illuminated without electricity, and prototypes for fully bioluminescent domestic environments. For the first time, a complete house was illuminated through bioluminescence, without cables or conventional energy systems. Here, the project moves from proof-of-concept biology to architectural application, demonstrating feasibility at the scale of inhabitable space. (With the collaboration of Agustí Fontarnau and Aránzazu Balfagón, UIC Barcelona. Founded by the Spanish Science Ministry and the Institut Català del Sòl, Generalitat de Catalunya).

Phase III (2011-2014): Hybridization and Design Integration. The third phase focused on increasing efficiency through genetic hybridization, combining the bacteria gens responsible of bioluminescence into plants DNA. The importance of this stage is that the closer the processes are to nature, the less obsolete and more “eternal” the results will be. This phase aligns biological experimentation with architectural design, producing systems that are not only functional but also aesthetically and environmentally integrated. (With the collaboration of Agustí Fontarnau and Aránzazu Balfagón, UIC Barcelona. Founded by the Spanish Science Ministry and the Generalitat de Catalunya).

Phase IV (2015-2018): Bioluminescent Fungi. Working on the growth of fungi, with the most bioluminescent species. (With the collaboration of Enric Gràcia, Universitat de Barcelona, and Agustí Fontarnau, UIC Barcelona).

Phase V (2019-2022): Bioluminescent Algae. Working on the growth of algae, in bioreceptive digital designed and 3D printed panels. (With the collaboration of Yomna K. Abdallah, UIC Barcelona).

Phase VI (2023-2026): Bacteria, Fungi, Algae and Plants. Improvement of bioluminescence and durability in bacteria. Introduction of genes responsible

for fungal bioluminescence into algae. Introduction of genes responsible for bacterial bioluminescence into ornamental plants. (With the collaboration of Yomna K. Abdallah, UIC Barcelona, Aránzazu Balfagón and Marta Serra, UIC Barcelona and XTART, and Jae-Seong Yang, CRAG. Founded by Fritz und Trude Fortmann Stiftung).

Phase VII (2027-2030): Open Objectives, yet. Open to new collaborations, but for sure trying to finally obtain bioluminescent ornamental plants with a real effective bioluminescence and trying to obtain bioluminescence yeast. (With the collaboration of Yomna K. Abdallah, UIC Barcelona, Aránzazu Balfagón and Marta Serra, UIC Barcelona and XTART).

Biodigital Architecture: A Theoretical Framework

Light as Living Matter. The Genetic Barcelona Project is embedded within the broader concept of biodigital architecture, a term developed to describe the convergence of biological systems, digital technologies, and architectural design. This convergence has been extensively demonstrated in the exhibition, precisely in the biolamps, biocontainers and bioreceptive panels, “Towards a Bioluminescent Future: Art, Architecture & Design”, UIC Barcelona, 21st-3rd January 2026: a fusion between bits and genes, where digital and biological processes converge in design and production. This framework implies several key transformations:

From Construction to Growth: Buildings are no longer assembled but grown, shifting architecture toward biomanufacturing, in a hybridization and coexistence of biological and digital entities.

From Materials to Organisms: Materials become living entities, capable of adaptation, repair, and reproduction.

From Energy Consumption to Metabolism: Energy systems are internalized within biological processes, reducing external consumption.

Apart from all that, of course, the technical feasibility needs to be improved, and it can be only within the framework of specific companies interested in a mass production. Knowing that, because the current artificial urban night

light contamination in our cities make that the proposed prototypes are not enough useful or competitive outside, and only useful in dark interiors.

Taking in account also the ethical considerations about the genetic modification of organisms, in this case for architectural purposes. Because every manipulation of life forms, and the possible ecological risks, and questions of biosecurity needs to be taked carefully, and requires regulations and right knowledge. No one would think of performing heart surgery on a subway platform. So, of course, the same sense of responsibility that any professional scientist possesses should also be present in these cases. It is not about a caprice or a curiosity; it is about ecological sustainability.

Anyway, what it is here proposed have also aesthetic and cultural implications. Bioluminescent architecture challenges traditional aesthetics. It introduces dynamic, living environments, non-standardized forms, and temporal variability. And also, an important consideration is about the ecological potential vs. the technological dependency. Biodigital systems depend on advanced biotechnology infrastructures, but only the first exemplar. Like fire, which once lit you can give away for free to everyone. Or plants, which once you have a specimen, you can give away cuttings of. All of this being recyclable and renewable, like nature itself: learning from it, in a process of biolearning. Not only from its forms, but from its systems.

Toward an Integral Ecology

All this work needs to be understood and justify within the framework of integral ecology, where environmental, social, technological, and cultural dimensions are unified. The future of architecture lies in learning from nature, and bioluminescent architecture embodies this principle by reducing energy consumption, integrating with ecosystems, and rethinking the relationship between humans and the built environment. However, its success depends on thinking nature in a contemporary and open way, as it really is, without imposed prejudices by humans.

Anyway, the Genetic Barcelona Project represents the first in history and most sustained efforts to integrate bioluminescence into architectural practice. By combining genetic engineering with design, it proposes a new paradigm in which architecture becomes a form of living, metabolic system.

By merging genetics, ecology, and design, it can come a future where cities glow organically, energy is embedded in life itself, and architecture becomes part of nature's metabolic processes. Le Corbusier's idea of "The Radiant City" ("Ville Radieuse") in the 20th century is transformed into "The Glowing City" in the 21st century: a new alive sustainable architecture. (Figures 1-5).

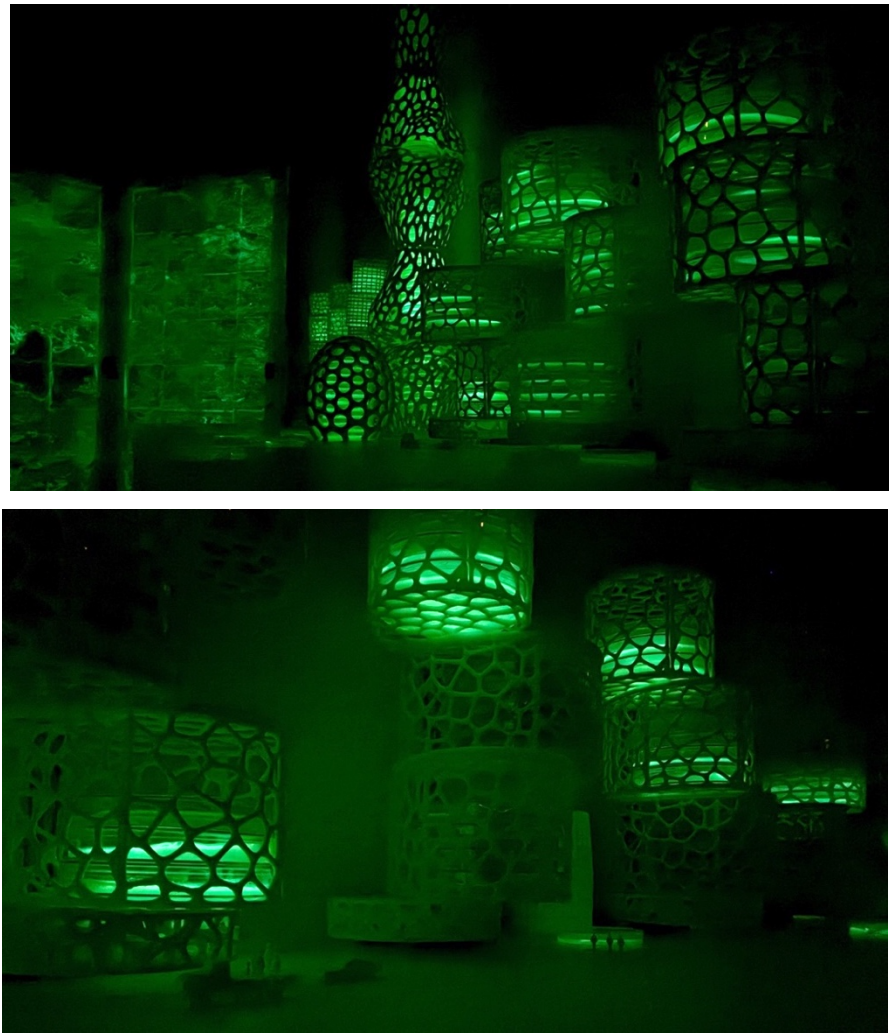


Figure 1-2. "The Glowing City". Different biolamps designs in the exhibition "Towards a Bioluminescent Future: Art, Architecture and Design, UIC Barcelona, 21st-23rd January 2026 (photo: Alberto T. Estévez, Apple iPhone 16e, ISO 2500-5000, f 1,64, 3,4 sec., equivalent to how the human eye naturally sees it). By the authors.

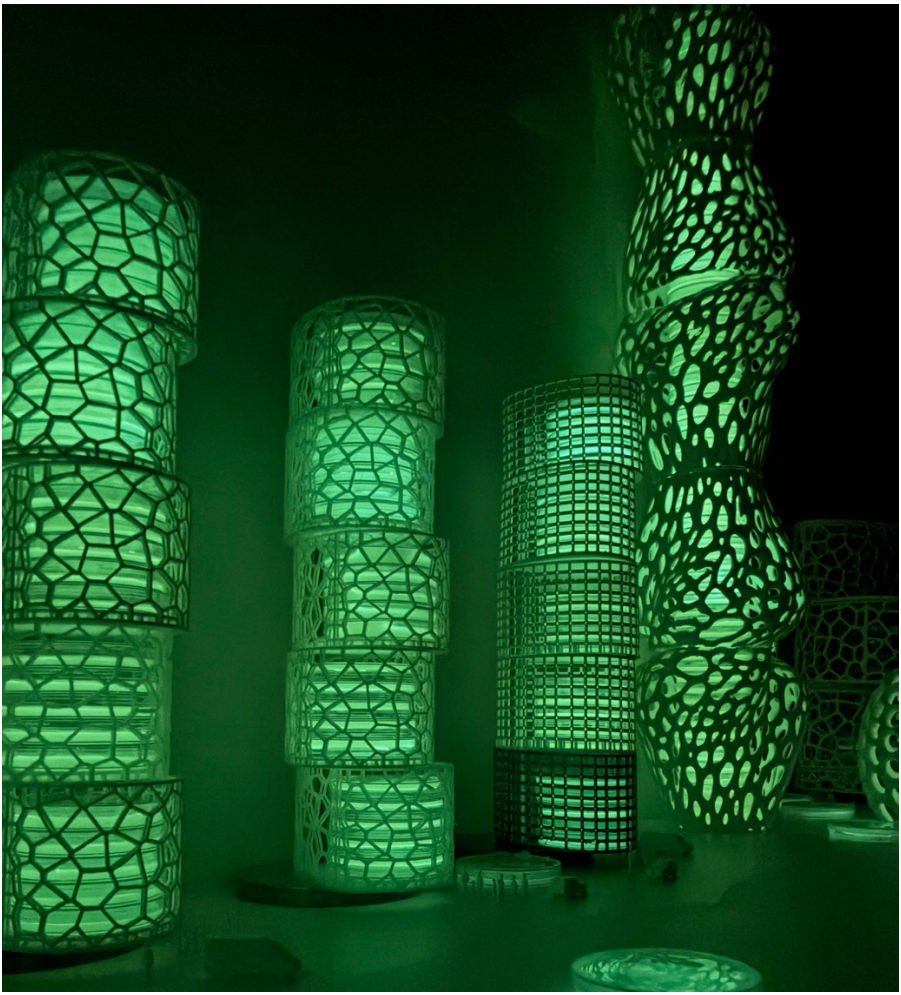
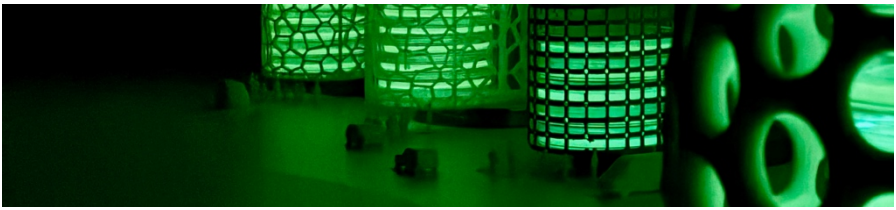


Figure 3-4. “The Glowing City”. Different biolamps designs (inspired in the Fortmann Foundation building, in Bochum), and a whole biocolumn in the exhibition “Towards a Bioluminescent Future: Art, Architecture and Design, UIC Barcelona, 21st-23rd January 2026 (photo: Alberto T. Estévez, Apple iPhone 16e, ISO 2500-5000, f 1,64, 3,4 sec., equivalent to how the human eye naturally sees it). By the authors.



In short, all the work carried out over these years on bioluminescence, whose relevance is obvious, has significantly influenced the debate on biodigital architecture and opened new horizons of research. Its actual implementation will now depend solely on the boldness of those who have the financial means to do so, as the Fritz und Trude Fortmann Foundation has demonstrated in this case with its generosity and open-mindedness. Clearly, it is not yet known for certain whether bioluminescent cities will become a widespread reality, but the conceptual shift they represent is already transforming architectural thinking. A true manifesto to open people's eyes to the new realities that we must build in our times: cities will be 50% biological and 50% digital, or they will not exist.

Alberto T. Estévez

Figure 5. "The Glowing City". Biolamps designs, biocolumn and bioreceptive panels in the exhibition "Towards a Bioluminescent Future: Art, Architecture and Design, UIC Barcelona, 21st-23rd January 2026 (photo: Alberto T. Estévez, Apple iPhone 16e, ISO 2500-5000, f 1,64, 3,4 sec., equivalent to human eye natural view). By the authors.