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The Internet of Biofilm Living AI Devices

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Abstract

As the world searches for groundbreaking, unconventional computing technologies, especially for intelligent edge applications, biological AI is emerging as an energy-efficient, robust, and reliable alternative. Researchers have unveiled the immense computing capacity inherent in biocomputing elements such as bacterial cells. The computing power of bacteria can be harnessed through Gene Regulatory Neural Networks (GRNNs). Biofilms, acting as sophisticated collections of GRNNs, leverage the natural distributed computing architecture with capabilities like parallel processing and analog computing in individual cells while consuming very little energy relative to conventional computing systems. This study introduces the concept of Biofilm Living AI Devices (BLAIDs), which proposes engineering biofilms using optogenetics to function as self-sustaining AI edge devices that interface with modern telecommunications architectures. Our simulation-based analysis demonstrates the computing complexity and reliability of BLAID, establishing it as a compelling candidate for the next generation of low-energy computing and advanced AI technologies.

Introduction

Our lives are increasingly becoming more dependent on Artificial Intelligence (AI). We are witnessing this at the level of applications that support our everyday lives, as well as advancements in the development of systems and infrastructures that support our society in general. A particular area that is witnessing more integration of AI is edge computing, where AI capabilities are deployed closer to data sources, reducing latency and enhancing processing efficiency. Edge AI leverages the power of distributed devices to perform real-time data analysis, enabling faster, more context-aware decision-making without relying on centralized cloud infrastructures. This shift is particularly crucial as we encounter an explosion of data from numerous devices and sensors connected through the Internet of Things (IoT). With the introduction of molecular communications (MCs), where information is encoded into molecules rather than electromagnetic (EM) waves, we saw the introduction of the Internet of Bio-Nano Things (IoBNT) [1] that elevates IoT by interconnecting to engineered biological systems, expanding our paradigm of computing devices that are built from natural biological components.

With the pursuit of pushing the frontiers of AI, the computing and communication research communities are increasingly interested in incorporating biological systems with conventional computing systems to utilize their natural AI properties [2]. An excellent example is the concept of *Organoid Intelligence*, which is expected to transform AI by integrating living cells, such as brain organoids, into conventional computing systems, thereby creating new forms of bio-hybrid systems. This approach has shown that *in vitro* cultured neurons can be trained for computing applications and possibly function in synergy with silicon technologies. Although this sounds appealing, there are several complexities in using neurons to perform AI, which can lead to unreliable computing. These challenges include

- Culturing and maintaining these fragile and demanding cells over long periods
- Addressing ethical concerns
- Managing their unpredictable connectivity changes

This has led the research community to investigate other cell lines to create Living AI, with one promising candidate being bacteria. Bacteria have been leveraged to create AI machines that include single perceptron [3] as well as consortia population neural networks [4].

In this paper, we propose the **Internet of Biofilm Living AI**, where we aim to use bacteria as a source of Living AI and to interconnect them in the form of a biofilm to the wider Internet as shown in Fig. 1. Our aim is twofold:

- To harness the natural computing capabilities of bacteria, accessible via the Internet for computational tasks
- To propose biofilms as a platform for intelligent computation, enabling novel applications in healthcare and environmental monitoring, where bacteria naturally thrive.

To extract AI properties from bacteria to perform computing, we focus on their gene regulatory systems. Notably, Gene Regulatory Networks (GRNs) hold a critical role in this emerging paradigm as natural computing architectures, enabling cells to make decisions in response to their changing environment through a complex interplay of gene regulatory processes.

Transcriptomic data can be used to quantify the regulatory influence of one gene over another, assigning weights analogous to those in artificial neural networks (ANNs) [5]. Once these gene-

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gene interaction weights are inferred, the resulting GRN can be interpreted as a neural network with an inherently random architecture, which we refer to as the Gene Regulatory Neural Network (GRNN) (Fig. 1). In contrast to conventional biocomputing approaches that engineer fully synthetic genetic circuits for specific functions [4], our method utilizes the organism's native GRN as a pre-existing computational scaffold. In a separate study, we showed that the GRNN can be viewed as a repository of numerous sub-GRNNs. Using Network Architecture Search (NAS) algorithms, application-specific sub-GRNNs can be identified for general-purpose computing tasks, mitigating the need for extensive network training [6]. Further, bacterial cells, functioning as individual processing units with GRNNs, collectively form biofilms that exhibit intelligent behaviors with feedback loops, non-linear signal processing, and complex population-wide behaviors.

In this study, we explore the integration of Biofilm Living AI into conventional fifth and sixth generation (5G/6G) telecommunication systems enabling them to be controlled for computing tasks using standard plug-and-play interfaces. As shown in Fig. 1, the Biofilm Living AI Device (BLAID) can be interconnected through massive Machine-Type Communication (mMTC) connectivity, where we extend the connection of machines to engineered biological systems. To interface EM waves with the biofilm, we propose utilizing optogenetics as a promising solution to activate specific target genes. Optogenetic systems have been successfully implemented in bacteria to control gene expression for diverse applications, ranging from driving metabolic flux and regulating the gut microbiome to controlling cell morphology and managing co-culture dynamics [7]. This allows precise, light-based control of gene expression as inputs to the GRNN. Integration of light as a key regulator not only enhances the functionality of GRNNs but also open new avenues for applications in biofilm computing, where traditional methods may fall short.

Once the target genes are triggered, internal biochemical-based communication within the cells performs computing similar to ANN, producing outputs ranging from mRNA to phenotypes. This approach merges two pillars of MC and EM- based nano-communications into a biofilm computing body.

To this end, this paper first investigates the computing architecture of Biofilm Living AI and the engineering of BLAID. Subsequently, we explore internet interfacing via optogenetics, examine computing across various MC scales, and conduct a simulation-based analysis of its computational properties. Finally, we discuss challenges, future directions, and conclusions.

Biofilm Living AI

While current AI research has predominantly focused on neuronal cultures and organoids, we propose leveraging the inherent computational intelligence of non-neuronal organisms to broaden the scope of AI. Bacteria exhibit sophisticated decision-making, adapting effectively to changing conditions through complex GRNs. This section subsequently dives deep into designing biofilm-based AI systems leveraging natural computational capabilities using GRNNs.

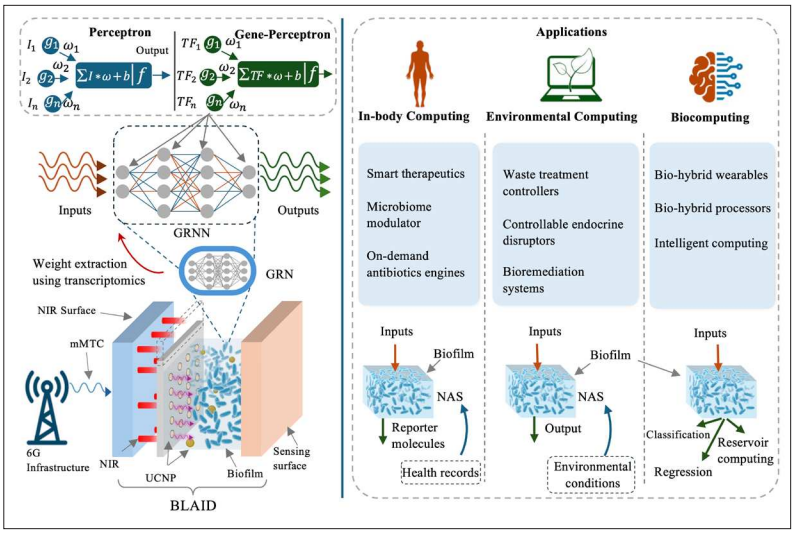

FIGURE 1. BLAID architecture utilizes biofilm-based GRNNs for bio-hybrid computing by integrating optogenetic control, MC, and near-infrared (NIR) interfaces for stimulating upconverting nanoparticles (UCNP). By converting bacterial GRNs into GRNNs, biofilms function as distributed, low-energy computing units capable of classification, regression and reservoir computing. Supported by 6G infrastructure, BLAID enables multi-functional applications in in-body computing, environmental computing, and biocomputing, bridging biological intelligence with next-generation AI and telecommunications networks.

Gene Regulatory Neural Networks

The field of DNA computing has advanced as a promising alternative to traditional silicon-based computing. This concept gained momentum due to the functional similarities between a gene and a perceptron model. An expression of a gene results in the production of bio-molecules, including mRNAs, proteins, and small non-coding RNAs, and subsequently diffuse into the cytoplasm. These biomolecules can serve as transcription factors (TFs) and intracellular signaling molecules. These diffusible molecules accumulate in the cytoplasm, potentially contributing to cellular memory or noise, while a subset of TFs acts as inputs that bind to the promoter region of a target gene. The influence of TFs on gene expression depends on factors including TF-binding affinity, regulatory elements (such as enhancers and silencers), and the stability of the DNA-TF complex, which collectively act as weights. Our previous study introduced a framework to quantify these gene-gene interaction-based weights of a GRN using transcriptomic data and converting it into a GRNN [5]. The combined effect of multiple TF species, along with their associated weights, modulates the expression level of the target gene, analogous to the input-weighted summation mechanism in a perceptron. This analogy is illustrated in the top panel of Fig. 1.

The computation process of each gene results in non-linear behavior in the temporal domain, comparable to a perceptron with a Rectified Linear Unit (ReLU) activation function. Our previous investigations have demonstrated that GRNNs, due to their intricate structural complexity (e.g., the Escherichia coli GRNN comprising over 4,000 genes and 10,000 interactions), are capable of performing regression tasks ranging from simple linear to complex polynomial regressions. They can likewise perform pattern recognitions including image classifications, while exhibiting analog and parallel processing capabilities that are well-suited for complex, real-time computing applications [6].

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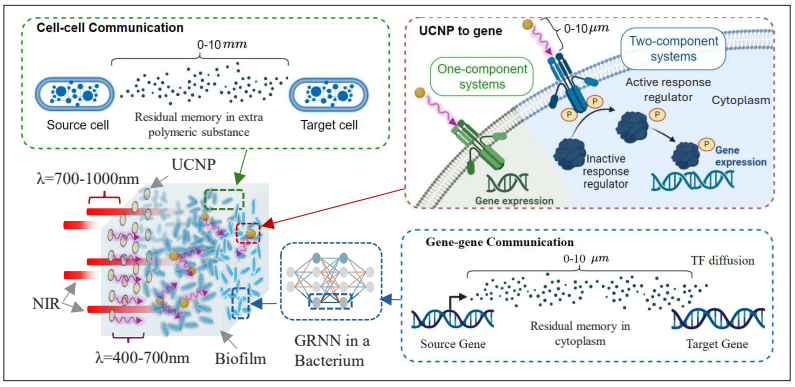

FIGURE 2. Illustration of multiscale communications within BLAID, showing multiscale interactions. NIR light activates UCNPs, emitting visible light to control gene expression via optogenetics. Cell-cell and gene-gene communication occurs through molecular and TF diffusion, driving GRNN dynamics. One- and two-component systems enable precise genetic control for bio-hybrid computation.

Distributed Computing with Molecular Communications

Bacteria, often live in biofilms typically containing 107 to 109 per square centimetre, where each bacterial cell functions as an individual processing unit equipped with a GRNN. Within biofilms, the GRNN computing capabilities can extend to multi-cellular communities that support multi-layered distributed GRNN computing architectures as depicted in Fig. 2. This is facilitated by MC via quorum sensing (QS) and other signaling pathways. Such decentralized computing architecture, in turn, enables parallel processing and real-time decision-making of biofilms, similar to distributed computing models found in silicon-based systems.

Unlike traditional computing, these computations are emergent, arising from stochastic molecular exchanges that enable adaptive responses to environmental changes. This form of emergent computation offers insights into creating more adaptive and robust systems for generic computing to process information and communicate efficiently in unpredictable and unstable conditions.

Metabolic cross-feeding and the division of labor within biofilms represent specialized computational tasks, wherein resource management is collectively optimized, analogous to task allocation protocols in distributed computing networks. The extracellular matrix serves not only as a physical scaffold but also as a medium for signal propagation, similar to a shared data bus in computer networks. Environmental factors such as nutrient availability and cell density act as either external inputs or as regulatory signals that modulate the computing state of the biofilm, thereby influencing its computational capacity. This integrated biological communication and computation enable biofilms to efficiently process inputs, resulting in sophisticated non-linear computing.

Engineering BLAID

Effective interfacing with GRNNs is crucial for designing BLAID. To achieve this, we propose a three-tiered engineering approach focusing on input interfacing, spatial engineering of biofilm, and output interfacing.

1. **Input interfacing:** This study explores optogenetics as a promising approach for precise control over gene expression using light as an external input. Light-sensitive proteins like phytochromes, cryptochromes, and LOV domains are commonly employed to modulate cellular processes, facilitating GRNN engineering by incorporating optogenetic circuits to regulate specific genes and act as data input gateways, thereby enhancing precision and control. In one-component systems (Fig. 1), a single protein serves as both the light sensor and TF, whereas in two-component systems, these roles are divided between a light-sensing protein and a TF, enabling more nuanced and sophisticated gene regulation [7].
2. **Spatial engineering of biofilm:** Recent advances in synthetic biology have enabled the engineering of biofilms to enable fit-for-purpose fabrication of devices containing cellular components. Synthetic GRNs and QS circuits allow cells to self-regulate biofilm thickness [8], while optogenetics facilitate photolithographic deposition of biofilms onto material surfaces [9]. This approach allows precise exogenous control over biofilm spatial patterns and thickness (Fig. 3), enabling the biofilm to be shaped to fit Upconversion Nanoparticle (UCNP) and light-sensing substrates.
3. **Output interfacing:** Here, we propose using in-vivo mRNA sensors [10] to trigger expression redox active molecules in response to the mRNA signals. Redox active molecules can serve as electrochemical signals that can be detected using electrodes embedded in the second biofilm substrate to enable BLAIDs to be integrated into living electronic devices [11].

Interfacing to the Internet

This section explores the mechanisms through which engineered light-sensitive biofilms can interface with external systems, enabling real-time communication and interaction via Internet by modeling light propagation through biofilm.

Communicating with Biofilm AI

We first model the interaction between engineered light-sensitive cells and incident light as it propagates through biofilm substrates designed to support biofilm growth. This approach allows us to simulate how light interacts with bacterial cells embedded within the biofilm structure. To achieve effective light activation in this system, we use near-infrared (NIR) laser, emitting photons in the 700–1000 nm range. NIR light is advantageous due to its deeper penetration into biological tissues, minimized scattering, and reduced absorption within this frequency window, which collectively minimize photodamage, autofluorescence, as well as phototoxicity.

Despite these advantages, many biological transcriptional systems are responsive to visible (400–700 nm) or ultraviolet (< 400 nm) light rather than NIR wavelengths [12]. This mismatch presents a key challenge in leveraging the benefits of NIR light for effective biological activation. UCNPs are uniquely suited to address this issue. In BLAID, the first layer incorporates UCNPs that emit visible light upon NIR laser excitation. This emission occurs through a series of energy transfers among dopant ions within the nanoparticles,

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culminating in the release of higher-energy photons. The efficiency of this upconversion process relies on the use of NIR wavelengths that align with the UCNPs' absorption peaks, as deviations from these optimal wavelengths reduce absorption and photon yield.

The interaction between the NIR laser and UCNPs exemplifies EM nanocommunication, which plays a pivotal role in enabling precise and efficient interfaces across biological, physical, and digital domains, supported by advancements in miniature devices such as optical nanoantennas. In our system, NIR photons act as EM carriers of information, with the laser emitting at a wavelength of 820 nm. A Gaussian beam is used to model the NIR laser propagation through a region populated by *E. coli* cells (dark red, elliptical shape) and nanoparticles (gold points) as illustrated in Fig. 4. An interaction occurs at the substrate-biofilm interface, where the dynamics of NIR light propagation change as it travels through the biofilm. Using the same methodology as in [13], we observe that, in the initial stages, reflection and early spreading dominate, causing an intensity loss at the surface. As the light penetrates deeper into the biofilm, scattering becomes more prominent, impacting the power reaching the UCNPs in the biofilm.

Assuming a distance of 2 mm between the NIR laser and the biofilm and a propagation distance of 30 μm within the biofilm, we demonstrate two cases: high and low power. In the high power scenario, with an input power of 50 mW, the intensity reaching the nanoparticles in the biofilm is 100 mW/mm^2. Considering an upconversion efficiency of η = 10%, the visible light intensity emitted by the nanoparticles is 10 mW/mm^2. This intensity level is in excess of literature values required to activate light-responsive proteins, enabling targeted manipulation of biological processes within the biofilm, as demonstrated in optogenetic studies [14]. In the low power scenario, with an input power of 10 mW, the intensity reaching the nanoparticles in the biofilm is 20 mW/mm^2. Considering the same η, the visible light intensity emitted by the nanoparticles is only 2 mW/mm^2. Light penetration depth critically influences cell-to-cell communication in the biofilm, impacting its computational properties. Deeper penetration enables more cells to receive input and engage with external signals, while limited penetration confines activation to surface layers. This phenomenon is systematically analyzed through the in silico experiments described later.

mMTC and BLAID Connectivity

This study further proposes using mMTC to integrate BLAID into networks, facilitating seamless interaction with digital infrastructures for enhanced real-time data exchange in intelligent edge computing. By harnessing 5G/6G frequency bands, along with the advanced capabilities of mMTC, this architecture enables ultra-dense device connectivity and low-latency, real-time data processing.

In particular, 6G wireless technology promises a significant leap in connectivity, offering data rates up to 1 terabit-per-second (Tbps), 1 ms latency, and spectral efficiency of 100 bps/Hz. A key feature of 6G is mMTC, designed for dense IoT networks. With mMTC, the base station can transmit data to the NIR laser in our bio-integrated system in real-time, controlling intensity, frequency, and duration with sub-millisecond precision. This low-latency, high-reliability link allows for instantaneous adjustments to laser parameters based on continuous feedback from biofilm sensors. The base station can monitor feedback signals, such as visible emissions or molecular markers from the biofilm, and send precise data to the laser as inputs or regulatory signals to maintain or modify the biofilm's computing properties as needed. In addition, mMTC's high device density capabilities support continuous data exchange, enabling the 6G network to interpret rapid changes in the biofilm environment and adjust laser settings without delay. This efficient communication loop ensures that the laser operates in close alignment with the computing needs, optimizing control over nanoparticle activation and the overall capabilities of BLAID.

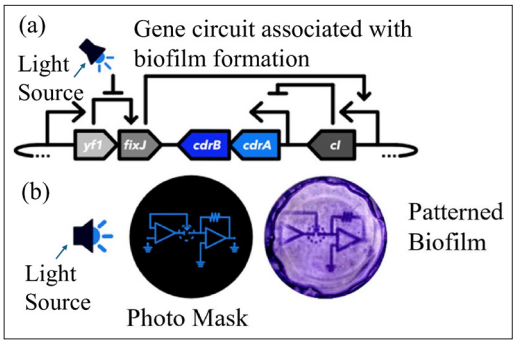

FIGURE 3. An optogenetic gene circuit for spatial biofilm patterning: a) A blue light-activated gene circuit involving *yf1* (light-sensitive kinase), *fixJ* (response regulator), and *cI* (transcriptional repressor) control expression of *cdrA* and *cdrB* (biofilm matrix proteins) [9]; b) Biofilm patterns are controlled using a photo mask for selective gene expression. The bottom shows a *Shewanella oneidensis* biofilm stained with crystal violet, where light guides biofilm formation. *S. oneidensis* biofilms are well-suited for bioelectronic applications due to the ability to perform extracellular electron transfer.

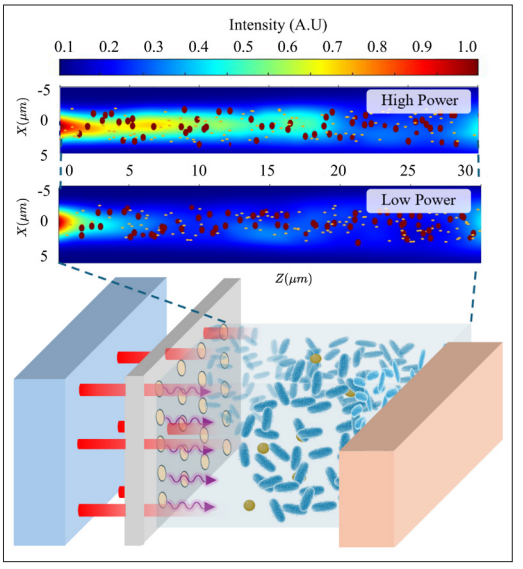

FIGURE 4. Simulation of laser interaction with a UCNP-embedded biofilm under high- and low-power NIR light. NIR light (red arrows) excits UCNPs to emit visible light (purple waves), modulating gene expression in the GRNN. Higher power enables deeper penetration and stronger UCNP activation.

This low-latency, high-reliability link allows for instantaneous adjustments to laser parameters based on continuous feedback from biofilm sensors.

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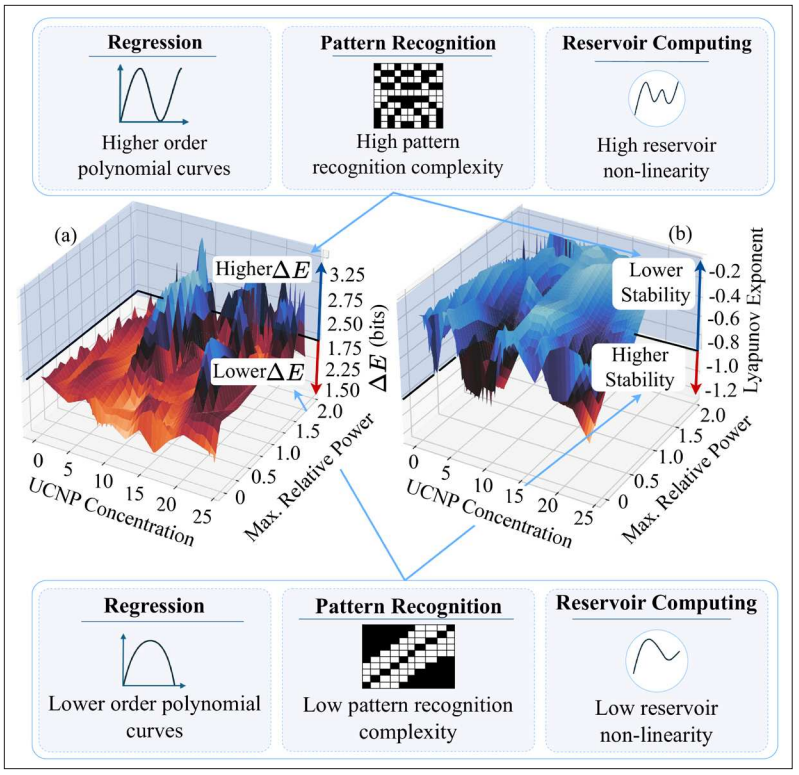

FIGURE 5. Computational analysis of BLAID showing: a) entropy difference (ΔE) and; b) Lyapunov exponent across UCNP concentrations and laser powers. Higher ΔE indicates greater computational complexity, supporting tasks like regression and pattern recognition. Negative Lyapunov values ensure stability, while near-zero values suggest flexible dynamics.

Computational Complexity and Stability of BLAID

In this study, we first utilize the entropy difference of input and output signals (ΔE) as a key metric to understand non-linear transformations during internal computational processes BLAID. The magnitude of non-linear transformations reveals the capacity of a system to process complex data, enabling pattern recognition, adaptability, and generalization of key traits of intelligent behavior while assessing its potential for decision-making and handling intricate data relationships.

Lyapunov exponents quantify the chaotic behavior of processes by measuring the divergence or convergence of trajectories relative to relevant system variables [15]. By employing the Lyapunov exponent, the stability of these computational processes can then be assessed, revealing how perturbations evolve over time and whether the system maintains computational coherence or diverges chaotically. This approach further helps differentiate whether the observed nonlinear transformations result from intrinsic computational dynamics or from random noise interference.

In Silico BLAID Setup

To effectively harness the capabilities of BLAID, an *in silico* 3D biofilm is constructed using Python, carefully designed to replicate the natural characteristics of microbial biofilms. Cells are randomly distributed within a $30 \times 30 \times 30$ μm^3 volume to emulate realistic spatial heterogeneity. Cell-to-cell communication is modeled using a Graph Neural Network (GNN), where each node represents an individual *E. coli* cell and edges capture the diffusion-based QS interactions. The selected model organism is E. coli, and each GNN node is embedded with an E. coli GRNN, implemented as a randomly structured ANN that governs the node's update dynamics.

The *E. coli* GRNN incorporates four key QS components: three signaling molecules: autoinducer-1 (AI-1), autoinducer-3 (AI-3), and indole — and the transcription factor SdiA, which detects acyl-homoserine lactones from other Gram-negative species. To better align the *in silico* model with natural biofilm behavior, the GNN's message-passing layer simulates diffusion-based signaling using AI-2, AI-3, and indole, which are well-characterized in *E. coli* communication. The gene **b3067** is selected as the GRNN input gateway due to its high regulatory connectivity, with 1703 outgoing edges, allowing broad influence on gene expression. The simulation framework, including the GNN architecture and diffusion modeling, is described in detail in [5].

Next, UCNPs are positioned within the biofilm and the substrate. GRNNs of cells near the UCNPs receive upconverted signals, which are computed within the GRNN and transmitted via QS signaling molecules to neighboring cells. This compute-and-communicate process ripples through the biofilm, creating a distributed computing architecture. The in silico experiment includes 169 setups covering 13 different UCNP concentrations and 13 maximum NIR input power levels. UCNP concentration ranges from 0 to 3.0×10^{-2} µm in increments of 2.5×10^{-3}µm, while NIR input power ranges from 0 to 120 mW in increments of 10 mW.

Entropy of input and output signals is then calculated using a histogram-based binning method, with the entropy difference ΔE derived by subtracting input entropy from output entropy. This metric provides insight into computing complexity and the system's non-linear signal transformation characteristics. Additionally, the Lyapunov exponent of the output signal was computed to evaluate the system's sensitivity to initial conditions. A negative Lyapunov exponent suggests converging trajectories, indicating stable and predictable behavior.

Results Analysis

The 3D surface in Fig. 5a, representing ΔE as a function of varying UCNP concentrations and maximum input laser power, exhibits no discernible trend. Instead, it displays highly irregular and fluctuating patterns, suggesting a complex and nonlinear dependence on these parameters. Regions of high entropy drift suggest significant information transformation or computational complexity introduced by BLAID under these conditions. The results indicate that changes in UCNP concentration and laser intensity have a non-linear effect on entropy difference, highlighting specific parameter combinations that either maximize or minimize information transformation. According to Fig. 4, light penetration depth influences the biofilm's ability to receive and process input signals, affecting cellular response at different depths. Despite a linear increase in laser power, system behavior remains non-linear due to complex interactions like feedback loops, saturation effects, and varying sensitivities in gene and cell communication. From a computational perspective, understanding this relationship is crucial for optimizing processes, enabling efficient information extraction or enhancing complexity, depending on the intended application.

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Figure 5b illustrates the Lyapunov exponent values for different UCNP concentrations and maximum input laser power, exhibiting no regular pattern similar to Fig. 5a. Rather, the surface displays irregular fluctuations, suggesting a complex and nonlinear dependence on these parameters. The Lyapunov exponent is used to evaluate the BLAID's sensitivity to initial conditions, providing insight into the stability and predictability of the output. In this figure, negative Lyapunov exponent values across the parameter space suggest that the system predominantly exhibits convergent behavior, indicating stability in most conditions. However, variations in the Lyapunov exponent, particularly regions approaching zero, imply a transition zone where the system becomes more sensitive and potentially exhibits more complex or chaotic dynamics. These insights are important for understanding how specific combinations of UCNP concentration and laser intensity influence the stability of the BLAID, allowing for fine-tuning to achieve desired levels of stability or complexity.

It is important to note that, each unique combination of UCNP concentration and maximum NIR power represents a distinct computational state. A computational state with a larger ΔE suggests that, given the specific UCNP concentration and NIR power, the BLAID can achieve enhanced non-linear computational capabilities. Conversely, a state with a smaller ΔE would be sufficient for tasks with lower computational complexity. Furthermore, with fixed UCNP concentration and maximum NIR power, BLAID is expected to maintain computational complexity with sufficient stability, as indicated by negative Lyapunov exponent values.

In conclusion, combined results from Figs. 5a and 5b reveal how UCNP concentration and input laser intensity influence the system's information processing and stability. The entropy analysis quantifies the system's information transformation capacity, identifying conditions for optimal computational efficiency. Concurrently, the Lyapunov exponent analysis reveals regions of stability versus chaotic behavior, indicating the system's long-term predictability. Together, these metrics provide a robust framework for optimizing experimental parameters with a tailored trade-off between computational complexity and stability for specific applications.

Challenges and Future Directions

Challenges for Internet of Biofilm Living AI

Developing Novel Application Services: The objective of BLAID is to pursue new opportunities and capabilities of AI that can go beyond algorithms or devices inspired from neural systems. This can result in novel applications that interface directly to chemical-based AI. However, this will require

- Discovery of novel mMTC services that can allow BLAID to be controlled from 6G
- Developing service directories that allow applications to dynamically match and map to GRNNs for deployment.

Security for BLAID: While security is a major challenge for conventional AI, it will be even more pronounced when considering Living AI. First and foremost, we are working with living cells to perform AI tasks. This also means that attackers could

- Exploit numerous inherent natural functionalities for malicious purposes (e.g., activating toxin production),
- Manipulate plasticity in a way that results in GRNNs with weights unsuitable for their intended applications.

Addressing this will require novel nanoscale sensing techniques within the culture to ensure that these functionalities are not triggered. Ethical guidelines must also be established to ensure that BLAID is used for legitimate applications, and properly disposed of after use.

Challenges in Controlling and Managing BLAID

Efficient Light Penetration: NIR light generally penetrates biological tissues effectively, but within biofilms, structural density affects light scattering, limiting targeted region access. Achieving stable beam propagation and sufficient penetration is challenging, especially since adequate intensity is needed for nanoparticle activation. Furthermore, UPCNs often exhibit limited efficiency, particularly at lower power intensities safer for biological systems, necessitating optimization of nanoparticle composition and size. In addition, NIR lasers and upconversion processes can cause localized heating, potentially damaging cells or altering biofilm integrity. Balancing sufficient energy for gene activation while minimizing thermal effects is crucial, especially for long-term use.

Non-linear Dynamics and Environmental Influence: The computational capabilities of BLAID depend on non-linear transformations within the GRNN, which are sensitive to environmental variations and MC limitations. Signal degradation, diffusion variability, and interference from the extracellular matrix complicate consistent biofilm-based computing. Furthermore, maintaining communication stability and fidelity over extended periods is difficult, as prolonged activity can alter biofilm structure, affecting signal transmission.

Training Biofilm Living AI for Computing: Bacterial cells exhibit adaptive plasticity in response to environmental changes, which can be directed similarly to training AI/ML models to fine-tune computation for specific applications. Directed plasticity could enable biofilms to adapt, advancing bio-computing, environmental sensing and precision medicine. Future research may explore using light as a teaching signal to directly regulate GRNN weights for more precise cellular responses. This will require software to compile transcriptomic data and algorithms to determine the appropriate light signals for adjusting GRNN weights.

Conclusion

In conclusion, this study presents the Biofilm Living AI Device (BLAID), an innovative, energy-efficient biocomputing platform that utilizes bacterial biofilms with Gene Regulatory Neural Networks (GRNNs) for distributed, analog computation. BLAID's adaptability, computational complexity, and integration with 5G/6G networks make it a promising solution for intelligent edge computing. This study further showed the computing capacity and the reliability of BLAID using series of *in silico* experiments. However, challenges such as light penetration, security, and environmental sensitivity still need to be addressed.

Future research should focus on hybrid interfacing systems that combine molecular and optical inputs, along with advanced methods to regulate GRNN weights for predictable, determin-

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istic outcomes. These advancements could enable scalable, programmable biofilm-based computing, driving progress in bio-nano technologies and synthetic biology.

Overall, BLAID has the potential to transform energy-efficient AI by integrating biological systems into novel computational applications in environmental monitoring, bioremediation, and healthcare, bridging the gap between synthetic and biological intelligence.

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