Terrell et al. **Bioelectronic control of a microbial community using surface-assembled electrogenetic cells to route signals.** Nature Nanotechnol. (2021).

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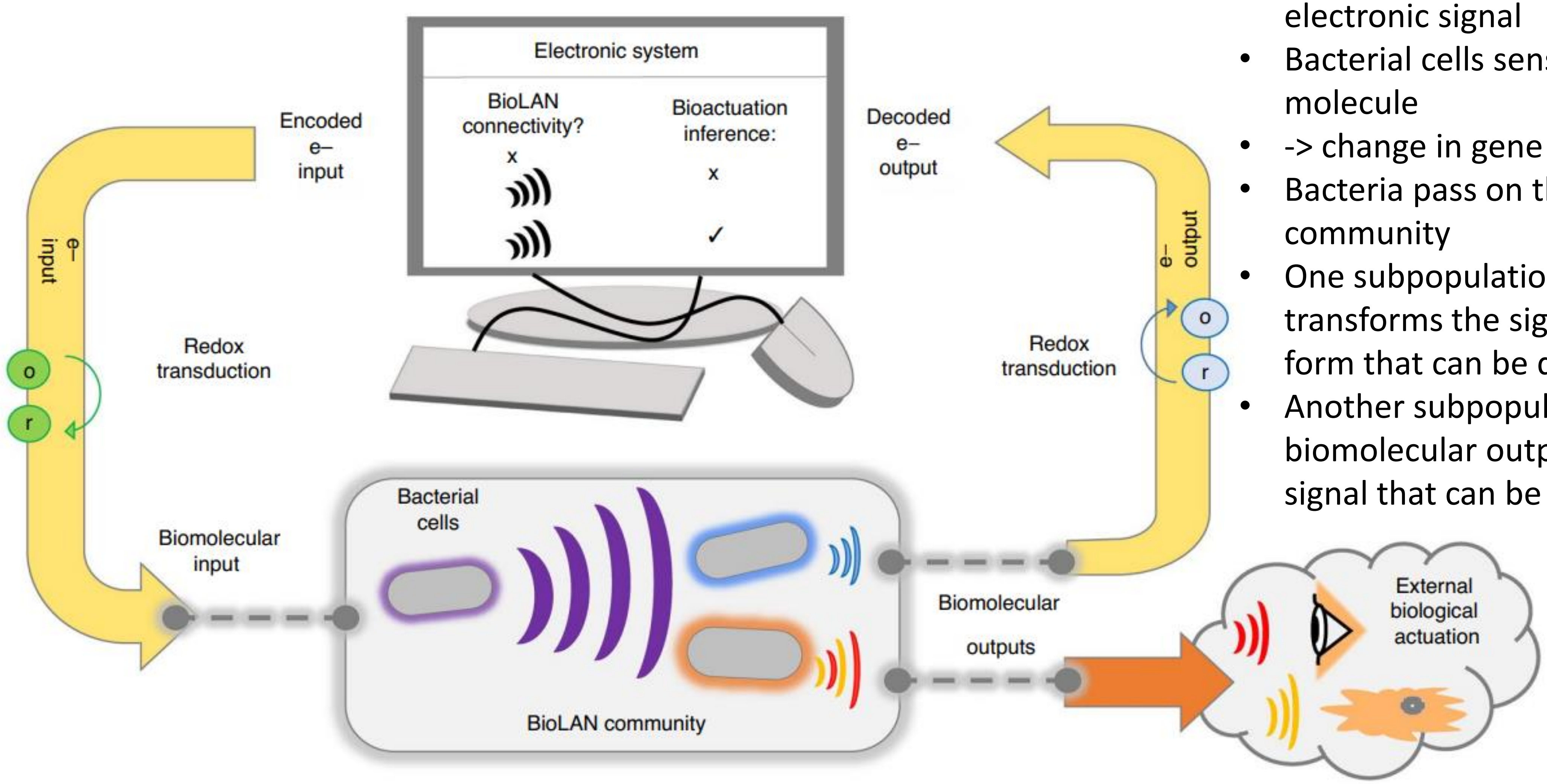
Introduction

- The system allows to exchange information between electronics and an engineered microbial network
- Biological information is often transmitted as gradients of molecules/ions
- Electrical information is transmitted by electron flow
- Redox-active molecules are a good link between electronics and cells because they work as electron carriers and biomolecules

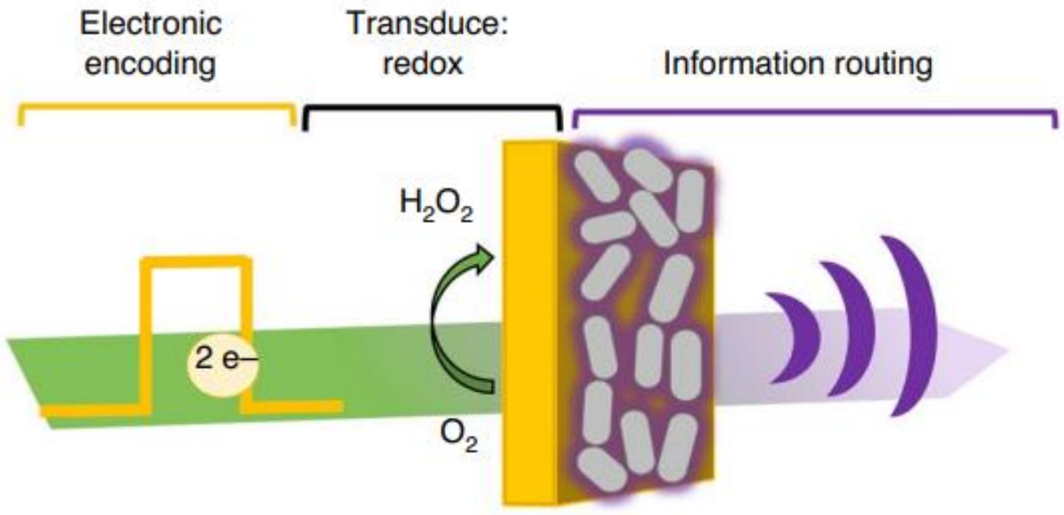
Main aim

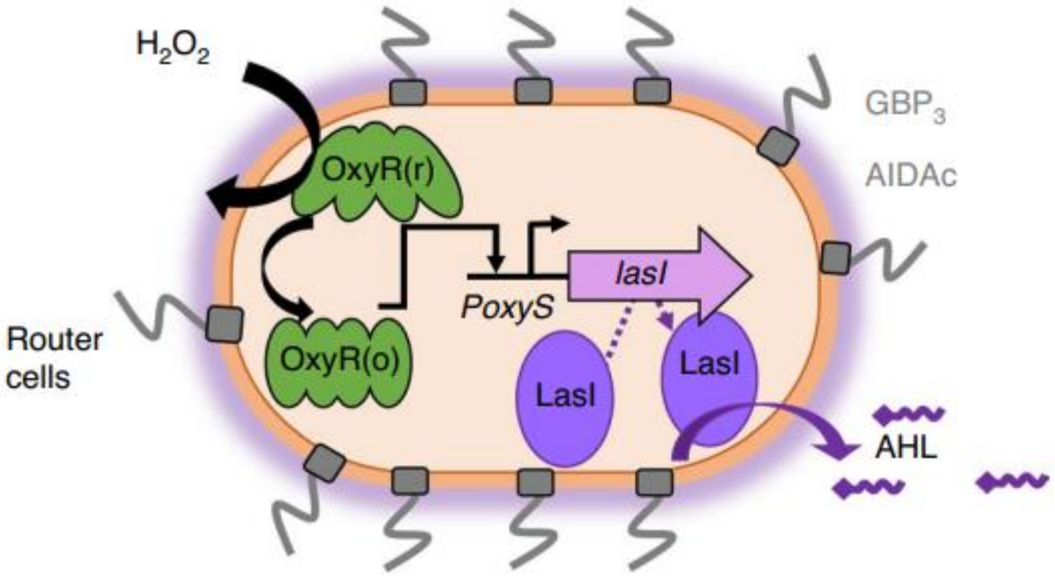
- The aim of the work was to create a bioelectronic communication system and investigate how bioelectronic techniques could be used for controlling and manipulating a microbial community
  - Surface-assembled electrogenetic cells were employed to route signals
- Exploring possibilities of bioelectronic control
  - Regulating microbial communities
- Developing a method for accurate control of microbial communities, possible significance to various other fields
  - Manipulating the behavior of microbial communities enables harnessing their potential in environmental engineering, biotechnology and other applications

Bioelectronic Communication System



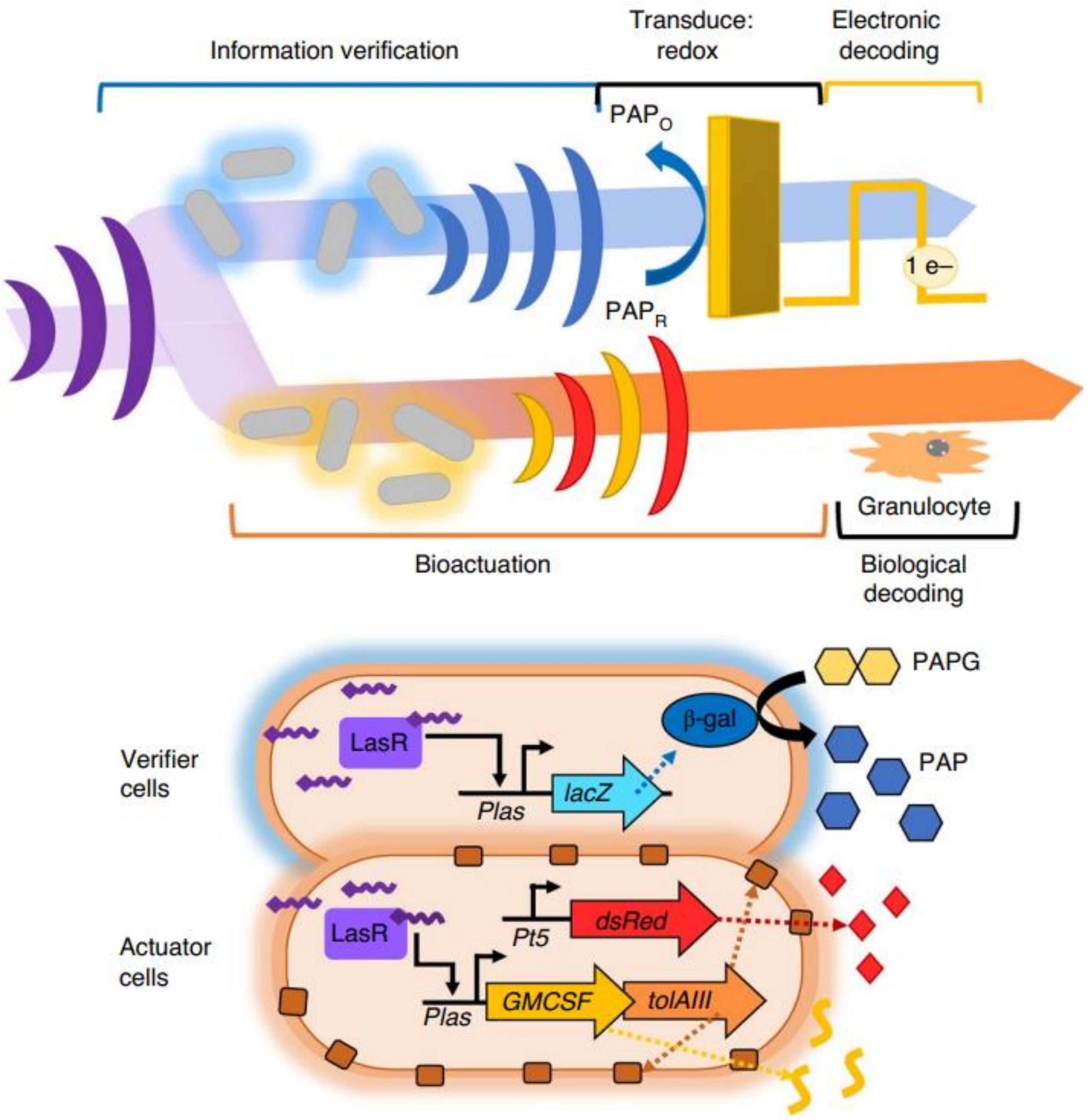
- Electronic signal from electronic system to electrode that is connected to bacterial community
- Reduction of a bioactive molecule as it receives electrons from the electronic signal
- Bacterial cells sense this reduced molecule
- -> change in gene expression
- Bacteria pass on the signal in the community
- One subpopulation of bacteria transforms the signal back to electric form that can be detected
- Another subpopulation has biomolecular output based on the signal that can be detected





- Electronic signal is electron flow
- This electron flow reduces oxygen to hydrogen peroxide at electrode
- Peroxide activates OxyR transcriptional activator of bacterial cells
- The engineered bacteria have a synthetic genetic circuit that is regulated by OxyR
- -> activation of the circuit when peroxide is present
- Expression of LasI enzyme is activated by OxyR
- LasI synthesizes AHL (acyl homoserine lactone) that is a biological signaling molecule

- -> activation of biological signaling can be controlled by electronic signal



- Two subpopulations of cells in the down-stream of biological signalling: verifier cells and actuator cells

- Verifier cells detect AHL via LasR
- -> expression of beta-galactosidase is activated
- -> beta-gal cleaves PAPG to PAP
- -> PAP oxidizes on the electrode releasing electrons
- -> electron flow (biological signal can also be transformed to electronic!)
- -> the electronic signal is detected

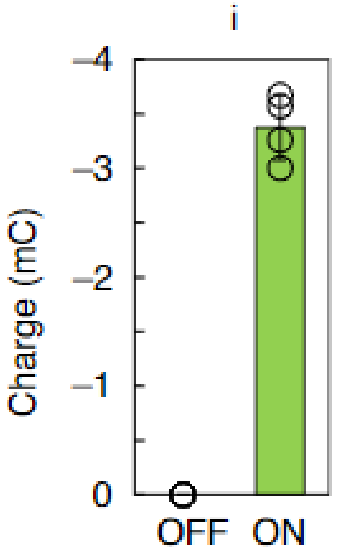
- Actuator cells detect AHL via LasR
- -> secretion of GMCSF which is a therapeutic factor that activates granulocytes
- -> secretion of a fluorescent marker that can be optically detected

- -> fluorescence and electronic signal confirm that the electronic signal have been successfully transformed to biological

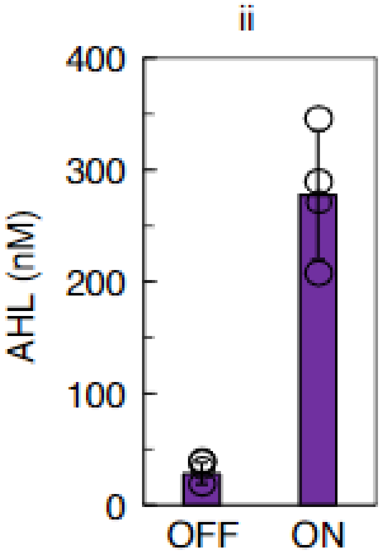
Methods

- Several different methods were utilized
  - Surface assembly
    - A pattern of microelectrodes was created, and genetic circuits were integrated into the cells
  - Genetic engineering
    - Genetic circuits were engineered to enable communication and control
  - Signal routing
    - The electrogenetic cells were arranged strategically, determining the electronic signals' flow and direction to enable targeted communication and manipulation
  - Microscopy and imaging
    - To visualize and analyze the electrogenetic cells' behavior and arrangement to learn about the effectiveness of signal routing

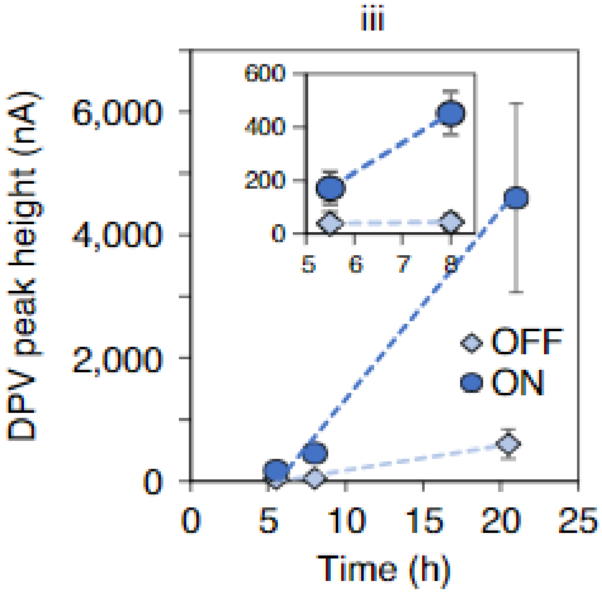
BioLAN measurements



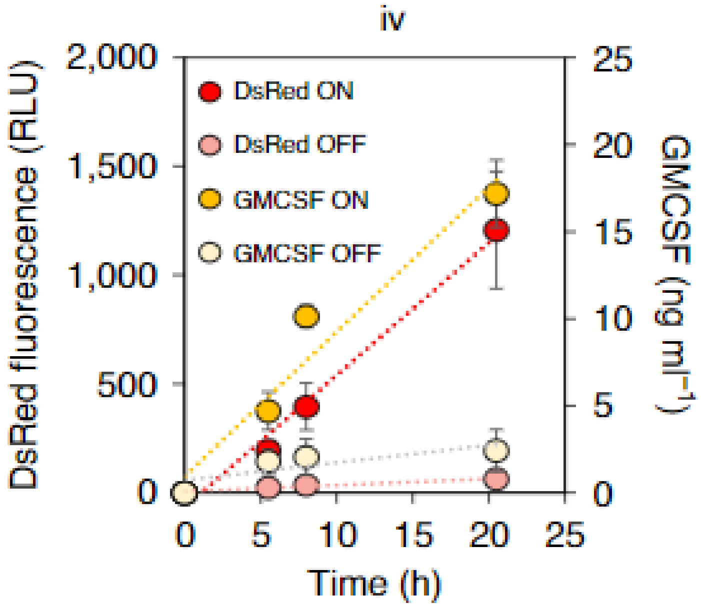
Applied charge dose



AHL production levels



Electronic output from verifier cells



GMCF and DsRed production by actuator cells

What was achieved? Were they successful?

- Successful integration of electrogenetic cells
  - Cells were able to communicate electronically and transmission of signals within the microbial community was possible
- Control over the routing of signals
  - Electronic signals could be directed to specific groups of bacteria and affect their interactions and behavior
- Microbial behavior could be manipulated
  - Influencing metabolic activity, communication, and behavior
- Future applications
  - In fields where accurate control over microbial communities is wanted
  - Control over microbial activity could be used in production of valuable substances

Why and how is this important?

- Achieving precise control over microbial behavior
    - Possibilities in optimizing microbial processes
- Biotechnology applications
    - Design of synthetic microbial consortia to perform biosynthesis or bioremediation
- Environmental engineering
    - Usage in enhancing the degradation of environmental contaminants, improving nutrient recycling, or promoting ecological restoration
- Fundamental understanding
    - Insights into underlying mechanisms of microbial communication and cooperation
- Potential paths forward
    - Optimizing and refining bioelectronic systems
    - Integration with other technologies and applications
    - Functional diversification of microbial communities
    - Ethical and safety considerations

References

- Terrell, J. L., Tschirhart, T., Jahnke, J. P., Stephens, K., Liu, Y., Dong, H., Hurley, M. M., Pozo, M., McKay, R., Tsao, C. Y., Wu, H.-C., Vora, G., Payne, G. F., Stratis-Cullum, D. N. & Bentley, W. E., Bioelectronic control of a microbial community using surface-assembled electrogenetic cells to route signals, *Nat. Nanotechnol.* **16** (2021) 688–697. https://doi.org/10.1038/s41565-021-00878-4