A Biological Pulse Generator Circuit for Bacteria-based Molecular Communication

Massimiliano Pierobon, J. Patrick Bardill, Brian K. Hammer, and Ian F. Akyildiz

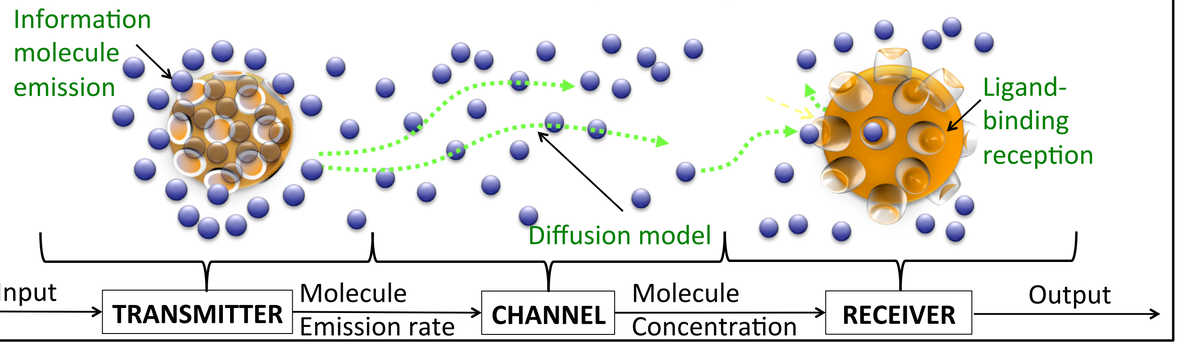
Molecular Communication

I. F. Akyildiz, F. Brunetti, and C. Blázquez, "NanoNetworking: A New Communication Paradigm," Computer Networks Journal, (Elsevier), June 2008.

Transmission and reception of information encoded in molecules

- Natural intra- and inter-cellular communication
- Candidate for nanoscale machine communication
- Tool for nanoscale machines to interact with biological processes

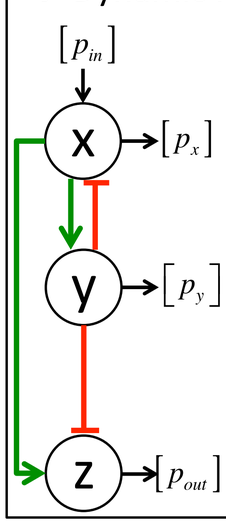
Bio-inspired End-to-end Molecular Communication System Design

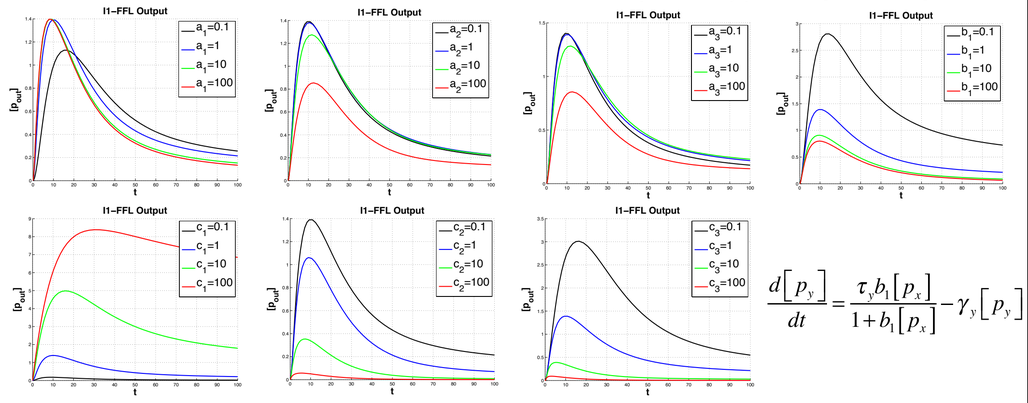


Biological Pulse Generator Design

Tunable pulse shape in emission of molecules at transmitter bacterium

→ Dynamic response of Incoherent Feed-forward Loop of Type 1 (I1-FFL)





$$\frac{d[p_y]}{dt} = \frac{\tau_y b_1 [p_x]}{1 + b_1 [p_x]} - \gamma_y [p_y]$$

$$\frac{d[p_x]}{dt} = \frac{\tau_x a_1 [p_{in}]}{1 + a_1 [p_{in}] + a_2 [p_y] + a_3 [p_{in}][p_y]} - \gamma_x [p_x]$$

$$\frac{d[p_{out}]}{dt} = \frac{\tau_z c_1 [p_x]}{1 + c_1 [p_x] + c_2 [p_y] + c_3 [p_x][p_y]} - \gamma_z [p_{out}]$$

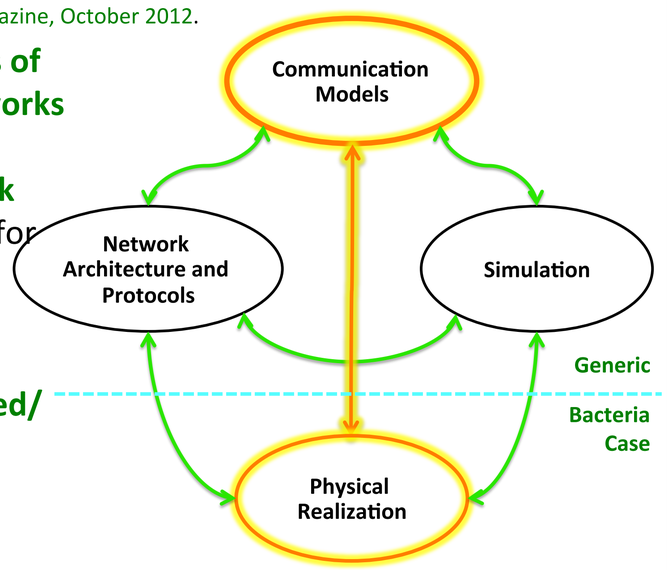
NSF MoNaCo Project

I. F. Akyildiz, F. Fekri, C. R. Forest, B. K. Hammer, and R. Sivakumar, "MONACO: Fundamentals of Molecular Nano-Communication Networks," IEEE Wireless Comm. Magazine, October 2012.

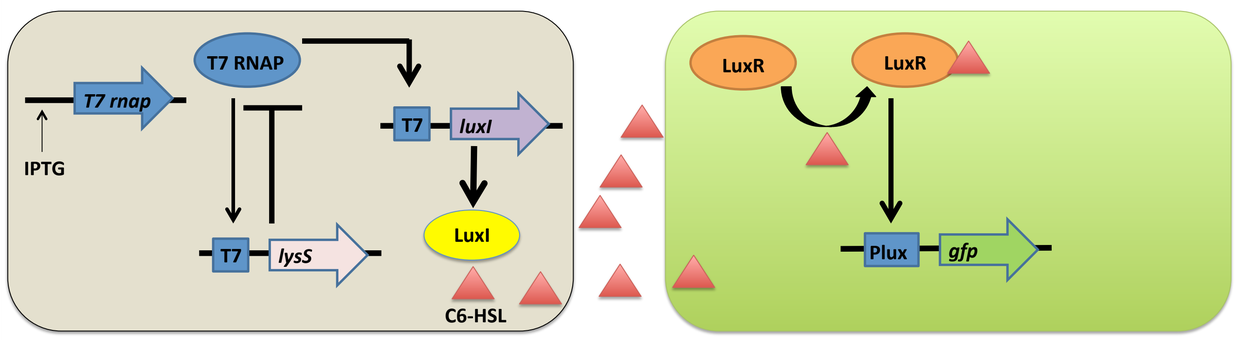
Establish the **theoretical foundations of diffusion-based molecular nanonetworks**

Design **modulation schemes, network architectures and protocols** suitable for molecular nanonetwork applications

Develop a molecular communication network based on **genetically modified/ engineered bacteria in a microfluidic device**



Biological Pulse Generator Engineering



Transmitter Receiver

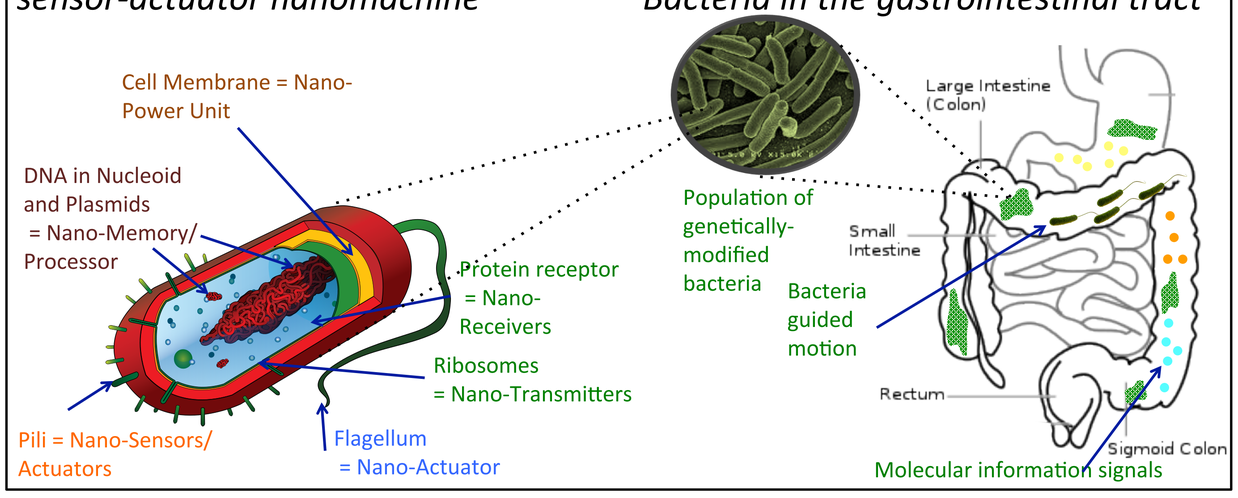
Experimental setup: The LuxIR quorum sensing system of *Vibrio fischeri* was divided into Transmitter and Receiver components and expressed in *E. coli*. In the transmitter bacterium, addition of IPTG activates transcription of T7 RNA polymerase (T7 RNAP). T7 RNAP then activates the production of LuxI, which chemically generates the signaling molecule *N*-(3-Oxohexanoyl)-L-homoserine lactone (C6-HSL) which freely diffuses through the bacterial membrane. T7 RNAP also activates production of T7 lysosyme (*lysS*) which binds to T7 RNAP and inhibits its activity. T7 RNAP is encoded on the genome while the T7 controlled LuxI and LysS are encoded on two separate plasmids. The plasmid expressing LysS also carries an inhibitor of T7 RNAP transcription. Production of C6-HSL can be measured using a receiver bacterium that expresses LuxR. Upon binding of C6-HSL, LuxR activates production of a reporter green fluorescent protein (GFP).

MoNaCo Applications - Nanomedicine

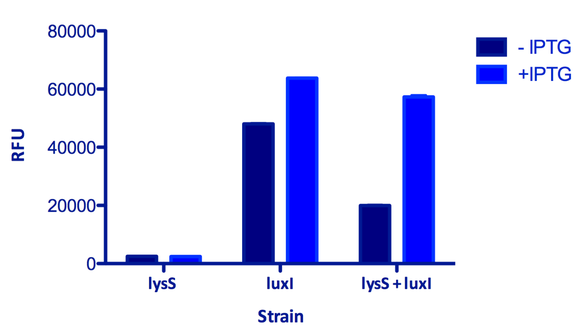
Intrabody sensor/actuator nanonetworks for advanced healthcare

A genetically-modified bacterium sensor-actuator nanomachine

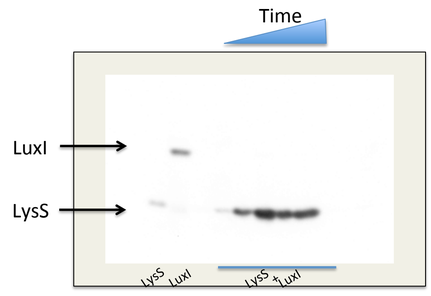
Networking of genetically-modified Bacteria in the gastrointestinal tract



Signal Production from Pulse Generator



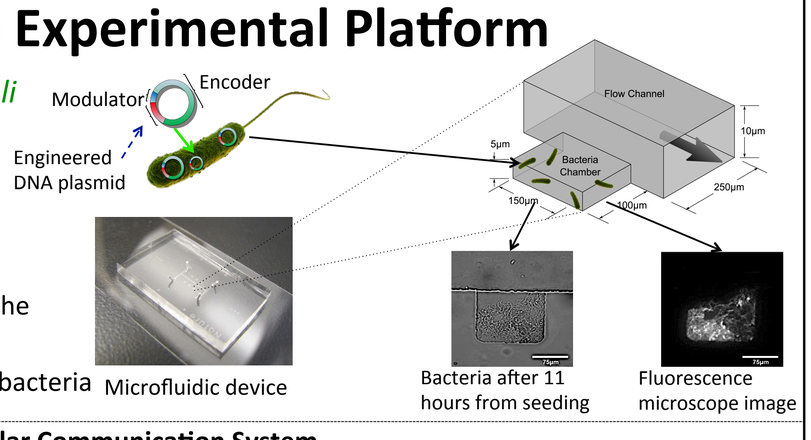
The transmitter bacteria produce C6-HSL. Transmitter bacteria with the indicated genes were grown overnight in the presence of absence of IPTG. Following growth the bacteria was removed and the growth medium was filter sterilized. These cultured supernatants were diluted 1:4 with fresh medium and used to grow cultures of receiver bacteria. GFP levels were measured on a fluorescence plate reader after 10 hrs.



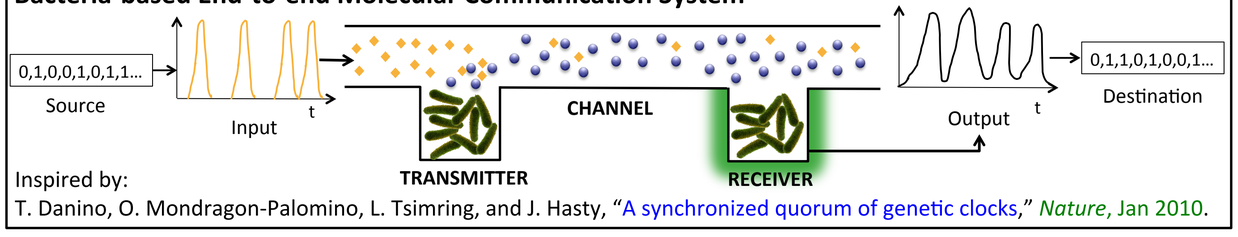
Some components of the transmitter create a wave shaped pulse. *E. coli* transmitter bacteria expressing both LysS and LuxI were grown to exponential phase and then induced with IPTG. Samples were taken every hour and subjected to SDS-PAGE analysis followed by western blot with an antibody that recognizes and epitope tag on both LuxI and LysS. Strains expressing individual components are indicated at left as controls.

MoNaCo Experimental Platform

- Genetically engineered *E. Coli* bacteria
  - Molecular transmitters
  - Molecular receivers
- Microfluidic device
  - Incubates, confines, controls the bacteria number
  - To stimulate and observe the bacteria



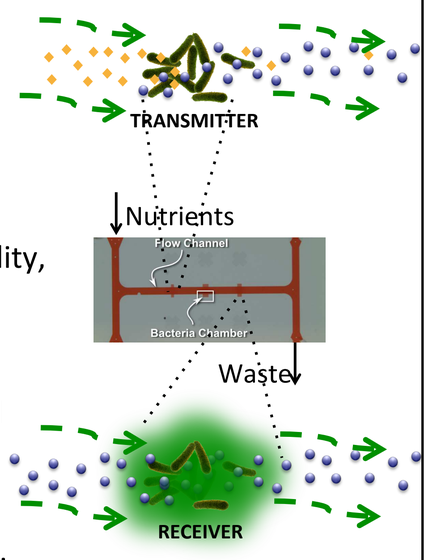
Bacteria-based End-to-end Molecular Communication System



Inspired by:
T. Danino, O. Mondragon-Palomino, L. Tsimring, and J. Hasty, "A synchronized quorum of genetic clocks," *Nature*, Jan 2010.

Future Directions

- Directly detect C6-HSL production over time by sampling media from bacteria in continuous flow conditions
- Implement sender and receiver communication in microfluidic device
- Shape curve by altering parameters such as protein stability, translation, and affinity between proteins and promoter regions (a_1, a_2, a_3, b_1, c_1, c_2, c_3)
- Engineer further levels of control of both transmitter and receiver
- Design more complex biological circuits for a complete **molecular signal processing** both at transmitter and receiver



This material is based upon work supported by the National Science Foundation
Grant No. 1110947

Disclaimer: Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation

MoNaCo Project Participants:
Faculties: Dr. Ian F. Akyildiz, Dr. Faramarz Fekri, Dr. Craig R. Forest, Dr. Brian K. Hammer, and Dr. Raghupathy Sivakumar
Post-Docs & Graduate Students: Dr. J. Patrick Bardill, Massimiliano Pierobon, Josep Miquel Jornet, Youssef Chahibi, A. Ozan Bicen, Kamal Shadi, Mohsen Sardari, Arash Einolghozati, Caitlin Henegar, Gregory L. Holst, Bhuvana Krishnaswamy, Shruti Sanadhya