Physics for Medicine PARIS

**1-YEAR POSTDOC POSITION**

# Ultrasound imaging of gas vesicles for SonoGenetic Bacterial Diagnosis

One of the goals of **Synthetic Biology** is to build therapeutic bacteria that can target and treat pathologies *in vivo*, such as cancer, directly at the right body location. A recent example comes from the use of a non-pathogenic *E. Coli* (*Nissle*) engineered to perform quorum sensing and to release an anticancer molecule when a high enough bacterial density is reached. In Mice, such bacteria inoculated directly inside tumors, grew, activated a synthetic quorum sensing circuit and released a cytotoxic compound, leading to partial tumor size reduction. Similar such innovations building on **therapeutic bacteria** are on their way: they empower the design of advanced genetic circuit to transform bacteria as therapeutic sensors and effectors in complex, physiologically relevant, biological contexts.

Yet, such approaches are limited by the difficulty to ensure the robust functioning (and therefore the safety) of "autonomous" synthetic bacteria *in vivo*. Researchers in synthetic biology are taking inspiration from physics and control theory to design and build robust synthetic circuits, notably using logic circuits (see below) and feedback loop control methods. Yet, another limitation of bacterial therapeutic development is the difficulty to observe the growth and behaviors of bacteria *in vivo,* limiting the possibility to monitor their actions in real time. Indeed, **Biological tissues are not light transparent and optical methods cannot be used to image deep inside tissues.** In turn, we do not know how therapeutics bacteria behave after being injected *in vivo*. We cannot measure quantitatively and in real time their growth rate, their density, the expression of reporter genes and the (proper) functioning of logic based synthetic circuits as time goes. It is even harder to act on them from a distance, and recent, trendy strategies such as optogenetics are hardly usable in practice. Yet, being able to induce gene circuits or to control their functioning externally would greatly facilitate and amplify the potential of bacterial therapeutics. There is a need for an alternative imaging method.

**In contrast, ultrasound imaging has demonstrated its superior capacity to image deeply inside living tissues** albeit at the expense of a lower spatial resolution (typically 100 µm to 1 mm). Ultrasonic waves are routinely used to explore functionally the interior of animal bodies, from the shape of organs to the circulation of fluids. With the addition of acoustic contrast agents (such as tiny air microbubble), it is possible to obtain higher resolution and dynamical features. For example, we recently showed (Errico 2015, Maresca 2020*)* that ultrafast ultrasound, combined with gaz microbubbles (~ 1-3 µm), can achieve non-invasive imaging of the vascular system up to microscopic resolution at several centimeter depth (Figure 1-a). This super-resolution ultrasound approach was even recently translated to clinics (Demene 2021). **Interestingly, it was also demonstrated that smaller gaz vesicles (~100 nm) produced by bacteria could be used as contrast agent for acoustic imaging.** Gaz vesicles (GVs) have first been reported in the 1960s in *Cyanobacteria*. They are cylindrical or spindle shaped nanostructures built inside bacteria by combining different structural proteins. They are around 100 nm to 2 µm long and 45-200nm wide, with a thin (2 nm) amphiphilic shell that allows fast gaz permeation and accumulation. **In recent seminal works by the group of M. Shapiro, the genetic operon responsible for GVs production was engineered and expressed in *E. Coli* Nissle to make them produce hundreds of GVs per cells, turning bacteria into acoustic contrast agent.** Once injected in the mouse gut, it was possible to acoustically image the bacteria *in vivo* (Figure 1b). Additional demonstrations of sonogenetic

bacteria for acoustic imaging were proposed by the same group in the past 2 years, evidencing the existence of a rising field, with many unexplored scientific questions and applications.

Here, we would like to use "acoustic bacteria" as live reporters of the micro environment of tumors and other complex, hard to image, ecosystems. We aim at designing bacteria that can trigger the production of GVs only when and where desired thanks to specific sensing and logic based synthetic circuits. Ultrasonic imaging will then be used to "see" these cells, turned into smart, active, contrast agents for acoustic imaging.

Several commercial or research ultrasound scanners can be used in linear or non-linear mode to image acoustic bacteria, as demonstrated by the group of M. Shapiro. However, performances of such systems can be improved significantly by designing signal processing methods to improve the imaging sensitivity of Gas Vesicle imaging. Ultrafast nonlinear imaging sequences combining with coded excitations will be studied in order to improve the imaging of gas vesicles by imaging at ultrafast frame rate their buckling after a high amplitude activating ultrasonic beam. Moreover the ability of these sequences to perform Ultrasound localization of Gas vesicles at microscopic scale will be studied and implemented in a commercially available scanner (Iconeus One, Iconeus, France).

The candidate will be working in a physics lab expert in the field of Biomedical ultrasound. He will implement new ultrasonic sequences and beamforming processing for real time imaging of gas vesicles. An experience in the field of Biomedical Ultrasound is highly recommended. Good programming skills are mandatory, we mostly use matlab, C or C++. Given the duration of the project and the need for a quick set up of the candidate, it is best to be readily familiar with these tools.

## SKILLS

Matlab programming, ultrasound imaging, data processing, signal and image processing, experimental research, relational skills.

## DURATION & LOCATION

12 months, Paris

## CONTACT

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