Experimental Work on Media Modulation in Molecular Communications

Maike Scherer, Lukas Brand, Maximilian Schäfer, Sebastian Lotter, Kathrin Castiglione, and Robert Schober
Friedrich-Alexander University Erlangen-Nürnberg, Germany

***Abstract*—Experimental testbeds for molecular communication (MC) are crucial, as they allow to validate the envisioned theoretical MC systems. In this work, we present our interdisciplinary, on-going work towards a practical implementation of media modulation based MC with the green fluorescent protein variant "Dreiklang" (GFPD) as signaling molecule. We focus on practical aspects, providing insight into the manufacturing process of GFPD and the testbed setup, and show initial results.**

I. Problem Description

Molecular communication (MC) is a bio-inspired communication paradigm, in which signaling molecules are utilized to convey information. This concept is realized by encoding information in the type or concentration of signaling molecules that are transported from a transmitter (TX) to a receiver (RX) by diffusion, flow, or a combination thereof. Most MC systems developed so far consider TXs which release signaling molecules to transmit information. Such TXs imply some practical conditions which are challenging in long-term experiments. In particular, a storage unit for the signaling molecules at the TX is required, which is undesired, e.g., in medical applications. Furthermore, the TX should be (i): easily accessible to ease a regular replenishment of the signaling molecules, or (ii): functionalized such that autonomous replenishment is possible [1], which can in general not be guaranteed for MC systems.

To overcome these limitations, in [2], media modulation based MC was proposed. In media modulation, a TX is considered, which uses *switchable signaling molecules* already present in the channel for communication. In the simplest case, the signaling molecules can be switched between two states upon an appropriate stimulus at the TX, and a specialized RX can distinguish between the two states of the molecules. Hence, information is modulated into the state of the signaling molecules. Of course, the realisation of a practical media modulation system requires a careful TX and RX design, respectively, and the choice of signaling molecules with suitable properties. This highlights the need of experiments to prove the practical feasibility, and validate the theoretically deduced benefits of media modulation.

A promising signaling molecule for media modulation is the green fluorescent protein variant "Dreiklang" (GFPD), which was considered in [2]. There, theoretical results for the communication performance in terms of bit error rates (BERs) were shown. GFPD is a *reversibly* switchable signaling molecule, which is an important property, as this allows to use GFPD for consecutive information transmissions in closed loop MC experiments, cf. Fig. 1. To show that switching the states of GFPD is practically feasible, in this work, we present

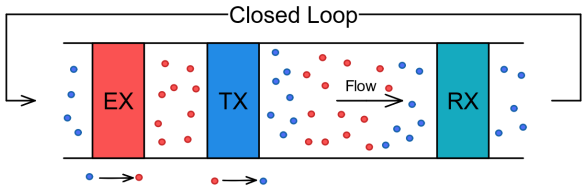

Figure 1. Media modulation based closed loop MC system: For transmission of bit 1, the TX modulates the signaling molecules (red and blue dots) by an appropriate stimulus, which results in their switching from default state B (red) to state A (blue). Here, TX and RX are complemented by an eraser (EX), which returns the switchable signaling molecules to their default state. Thus, the information from past transmissions is deleted to allow for another modulation by the TX.

the current status of our on-going *experimental work* on media modulation based MC using GFPD as a signaling molecule. Hereby, we complement the theoretical work in [2]. This article is structured as follows. In Section II, we briefly discuss the unique properties of GFPD. Next, in Section III, we provide insight into the synthesis and manufacturing process of GFPD. Finally, we show the experimental setup in Section IV and initial results in Section V, before concluding with Section VI.

II. GFPD as On-off Switchable Signaling Molecule for an MC System

GFPD is a photochromic molecule, i.e., a molecule which can be reversibly interconverted between two states, while the transition between the states is induced by a light stimulus. Employing GFPD as signaling molecule, the states, which we denote as state A and state B, reflect the embedded information. In particular, the states differ in their fluorescence properties, i.e., GFPD is only fluorescent in state A. The fluorescence can be switched on and off by light stimuli of *mutually different* wavelengths [3], i.e., optical sources emitting light at different wavelengths can be used as TX unit to modulate information (B $\rightarrow$ A) and as eraser (EX) unit to delete the information (A $\rightarrow$ B), cf. Fig. 1. Moreover, fluorescence can be detected by a fluorescence-stimulating light source (RX). Furthermore, GFPD has been shown to be stable over several photo-switching cycles [3], which allows the long-term usage of GFPD as signaling molecule.

III. Expression and purification of GPFD

Microorganisms such as the intestinal bacterium *Escherichia coli* can be used for the production of synthetic proteins like GFPD in large quantities. The gene encoding the protein is introduced into the bacterial cell using a vector, such as a ring-shaped DNA plasmid, that also contains an antibiotic resistance gene. GFPD production is under the control

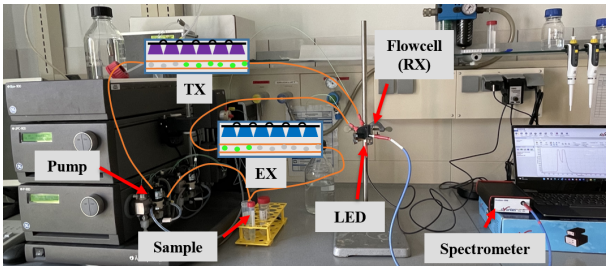

Figure 2. The proposed setup to implement a first testbed for media modulation based MC. The testbed consists of a sample reservoir of GFPD, a piston pump, connecting tubes, and a fluorescence flow cell with an LED to detect GFPD (RX). Furthermore, LED stripes will constitute the TX and EX, respectively. These LED stripes are currently built and enable the radiation of light of wavelengths $\lambda = 365\,\mathrm{nm}$ and $\lambda = 405\,\mathrm{nm}$ to triggering the switching of GFPD.

of a promoter that can be induced by IPTG (isopropyl-β-D-thiogalactopyranoside) to start transcription and thus the protein expression. To facilitate the subsequent purification of GFPD, a small peptide of six histidines, a so-called His_6-tag, is added to the beginning of the protein that can bind to nickel-containing chromatography materials. After the protein synthesis, the cells are harvested by centrifugation, lysed with ultrasound, followed by another centrifugation step that separates cell debris and intact cells. Next, immobilized metal affinity chromatography is conducted as central purification step. In this step, the cleared cell lysate is pumped through a chromatography column, where the His_6-tag interacts with the column matrix and retains GFPD. To elute GFPD from the column, the interaction between tag and matrix is disturbed with a suitable buffer solution. Thus, the protein is recovered in purified form and can be used for experiments under defined conditions.

IV. Experimental Setup

The proposed testbed for media modulation based MC is shown in Fig. 2. It comprises a sample reservoir of GFPD diluted in storage buffer, a piston pump, connecting tubes, and a fluorescence flow cell (Ocean Insight) that is connected to the initial sample reservoir, whereby GFPD is circulating through the testbed. To measure the fluorescence intensity, GFPD is excited by a *light-emitting diode* (LED) of $\lambda = 500\,\mathrm{nm}$ (serial number: NSPE310S), and the emitted light due to fluorescence is measured by a spectrometer (Avantes) connected to the flow cell via a *sub-miniature version A* (SMA) coupled fiber optic cable of $400\,\mu\mathrm{m}$ (Ocean Optics). To complete the MC system, a TX and an EX will be implemented as UV-LED stripe radiating light of wavelengths $\lambda = 365\,\mathrm{nm}$ and $\lambda = 405\,\mathrm{nm}$, respectively, which trigger the switching of GFPD.

V. Initial Experimental Results

First experiments were conducted to validate the controlled switching of GFPD, which is the key element of the experimental setup shown in Fig. 2. The switching was tested in an isolated experiment (not within the setup).

To induce photoswitching, two *ultra-violet light-emitting diodes* (UV-LEDs) with wavelengths of $\lambda = 365\,\mathrm{nm}$ (serial number: NCSU276AT) and $\lambda = 405\,\mathrm{nm}$ (serial number: NVSU119CT) with power of $0.78\,\mathrm{W}$ and $2.2\,\mathrm{W}$ respectively, were deployed. The experiments are performed at room temperature while the protein is diluted in storage buffer. The GFPD probe is measured in a 96-well plate (black with clear bottom, Greiner Bio-One GmbH) up to a volume of $100\,\mu\mathrm{L}$ and irradiated by the UV-LEDs directly attached to the plate. Fluorescence of GFPD was measured with an Infinite M200 pro microplate reader (Tecan) measured at $\lambda = 500\,\mathrm{nm}$ excitation and $\lambda = 529\,\mathrm{nm}$ emission.

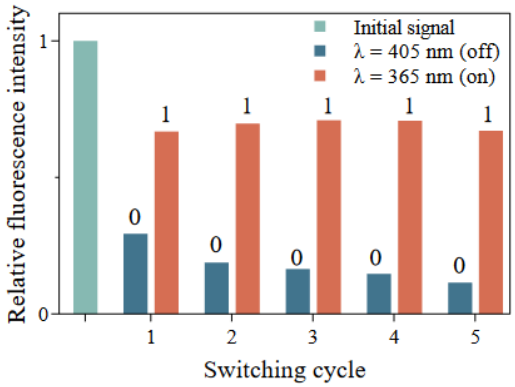

Figure 3. Experimental data of repeated switching cycles. Fluorescence intensity is normalised to the initial signal. Here, bit 1 and bit 0 can be conveyed by the fluorescence intensity of GFPD, which is low (blue bar) and high (red bar) after triggering the off-switching, i.e., A $\rightarrow$ B, and on-switching, i.e., B $\rightarrow$ A, respectively.

In order to examine the switching behaviour of GFPD, an initial experiment with five switching cycles is performed. The obtained fluorescence intensities of the on- (red bar) and off-state (blue bar), which represent the two states A and B, respectively, are shown in Fig. 3. After detecting the initial signal (cyan bar), GFPD is switched off (A $\rightarrow$ B) for $30\,\mathrm{s}$ with $\lambda = 405\,\mathrm{nm}$, followed by an immediate detection of the off-signal. Upon irradiating GFPD with $\lambda = 365\,\mathrm{nm}$ for $30\,\mathrm{s}$, photoswitching to the on-state (B $\rightarrow$ A) could be detected while the initial signal intensity could not be regained. Nevertheless, the two states can be clearly distinguished from each other, which allows to convey binary information in the states of GFPD and consequently to modulate information (A $\rightarrow$ B) as well as to delete it (B $\rightarrow$ A).

VI. Conclusion and Future Work

In this work, we showed promising initial experimental results, which motivate the application of GFPD as signaling molecules in media modulation based MC systems.

Hence, as a next step, the experimental setup is put into operation, which will allow us to conduct the first long-term closed-loop MC experiment.

References

[1] M. Schäfer, L. Brand, S. Lotter, A. Büyükoglu, F. Enzenhofer, W. Haselmayr, K. Castiglione, D. Appelhans, and R. Schober, "Controlled signaling and transmitter replenishment for MC with functionalized nanoparticles," *Proc. 9th. Int. Conf. Nanosc. Comp. Commun. (NANOCOM)*, 2022.

[2] L. Brand *et al.*, "Media modulation based molecular communication," *IEEE Trans. Commun.*, pp. 1–1, Sep. 2022.

[3] T. Brakemann *et al.*, "A reversibly photoswitchable GFP-like protein with fluorescence excitation decoupled from switching," *Nature Biotechnology*, vol. 29, no. 10, pp. 942–947, Sep. 2011.