Experimental Polymersome-based Receiver Design for Molecular Communication

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***Abstract*—In this abstract, we present preliminary experimental results for a realistic receiver (RX) design based on functionalized polymersomes for molecular communication (MC) systems. The proposed design consists of an enzyme that catalyses a light-emitting reaction and is encapsulated into a polymersome with membrane porins. The porins allow to control the passive diffusion of signaling molecules over the membrane and therefore allow to influence the sensitivity of the proposed RX design.**

I. Introduction

One of the main applications of molecular communication (MC) is the Internet of Bio-Nano Things (IoBNT) where in-body communication networks shall facilitate continuous health monitoring and smart treatment of diseases [1]. Next to the theoretical development of communication concepts, one of the main challenges is the design of practical hardware serving as transmitters (TXs) and RXs [2].

In this work, we propose a realistic RX design based on functionalized polymersomes, and present inital results on its experimental development. The main building blocks for the RX are hollow polymer vesicles which can encapsulate molecules in their intravesicular space. The surrounding membrane can be functionalized, e.g., with membrane porins or ion pumps, to facilitate the translocation of molecules from the extra- to the intravesicular space and vice versa [3]–[5]. Due to the wide range of possible functionalizations, polymersomes with different properties can be tailored for a variety of applications such as controlled release of substrate [4], or as nanoreactors for enzymatic reactions [3]. For the proposed RX, we encapsulate enzymes into the intravesicular space which catalyze an externally observable light response upon the conversion of substrate. To control the sensitivity of the proposed RX, we insert different types of porins, i.e., membrane channels, into the polymeric membrane.

II. System Design

The proposed system design is shown in Fig. 1. As signaling molecules, released by a TX, we use coelenterazine which serves as substrate for the reaction inside the RX. The RX consists of polymersomes with encapsulated enzymes, i.e., luciferases. Moreover, membrane porins in the polymeric membrane facilitate the passive transport of substrate into the intravesicular space. In the following, we describe the components of the proposed RX design in detail.

A. Enzyme Mediated Light-Output

The employed light-producing reaction catalyzed by the enzyme is illustrated in the lower left part of Fig. 1. In particular, the released substrate coelenterazine is oxidized to the product coelenteramide by the encapsulated enzyme Gaussia luciferase (GLuc) [6], resulting in a bioluminescence signal by the emission of photons of blue light. We observe the produced light signal by an external measurement device.

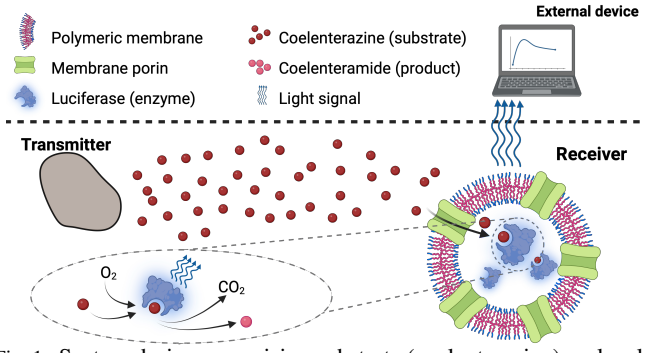


Fig. 1. System design comprising substrate (coelenterazine) molecules emitted by a TX, the polymersome RX with membrane porins, facilitating transport across the membrane, and encapsulated enzymes (luciferase) generating light upon a catalytic reaction.

B. Polymersomes

The employed polymersomes are spherical polymer vesicles whose membrane consists of a biocompatible triblock polymer [7]. During production, GLuc is encapsulated into the intravesicular space of the polymersomes. The polymeric membrane is semi-permeable, i.e., allows the translocation of some molecules between the extra- and intravesicular space. The permeability of the membrane to a specific molecule depends on various factors, including the size of the molecule, where smaller molecules have a higher membrane permeability. As the membrane permeability of the employed polymersomes is very low, and GLuc is a large molecular compound (protein), we assume full retention of GLuc in the intravesicular space of the polymersome [3].

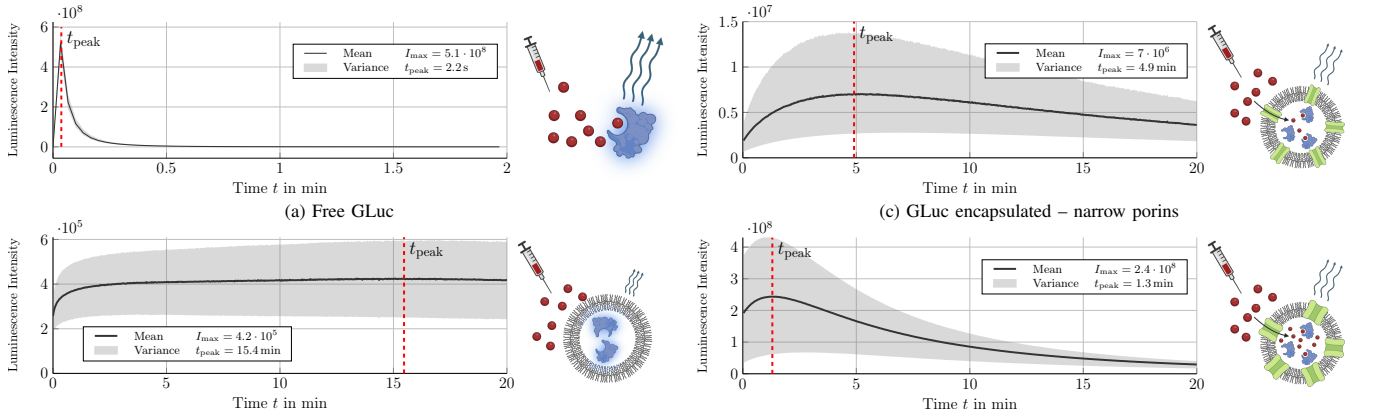
C. Membrane Porins

To enable and control the translocation of the substrate across the polymersome membrane, membrane porins of different channel diameters are inserted (shown in green in Fig. 1). As the polymersome membrane has a very low intrinsic permeability for the substrate molecules, the uptake rate of the substrate by the RX and thus the generated light response can be significantly influenced by the membrane porins, i.e., by the diameter of the opening they create and their number per vesicle.

III. Experimental Results

A. Experiment Description

All experiments were conducted in a microplate. Luminescence was detected by a microplate reader in combination with an injector-system. To evaluate the performance of the proposed RX design, we conducted triplicates of different experimental settings. First, we conducted two control experiments where the light response of free GLuc and of GLuc encapsulated into polymersomes without porins was measured. Then, two experiments with porins of different diameters



(a) Free GLuc

(c) GLuc encapsulated – narrow porins

(b) GLuc encapsulated without porins

(d) GLuc encapsulated – wide porins

Fig. 2. Measured luminescence intensity over time of the RX-nanodevice. (a) shows the result of the reaction using the free enzyme, while (b) - (d) depict the results of the encapsulated enzyme with no porin as the control group, and narrower and wider porins inserted into the polymeric membrane, respectively.

were performed. For each experiment, we measured the luminescence intensity over time and observed the maximum amplitude $I_{\max}$ and peak time t_{peak}.

B. Control Experiments

Figs. 2(a) and (b) show the mean and variance of the measured luminescence intensity triplet over time for free GLuc (a) and GLuc encapsulated into polymersomes without porin (b). For the free GLuc (Fig. 2(a)), we can observe an instant peak of luminescence intensity that has completely faded until $t = 0.5\,\text{min}$. The fast increase of luminescence is due to the fast reaction kinetics of GLuc [8]. After $t = 0.5\,\text{min}$ no luminescence signal can be observed, as, presumably, substrate molecules are oxidized and the enzyme is partly degraded [9]. For GLuc encapsulated without porin (Fig. 2(b)), we can observe a small rise and then an almost constant luminescence signal, which is three orders of magnitudes smaller compared to Fig. 2(a). This is due to very slow diffusion of the substrate over the polymeric membrane. However, as the luminescence intensity compared to the other three scenarios is very low, we refer to this very slow diffusion of substrate over the membrane as background permeability.

The control experiments provide lower and upper sensitivity bounds for the proposed RX design, e.g., using free GLuc as RX very fast and short responses can be produced, but the RX might be unstable due to enzyme degradation.

C. Influence of Membrane Channels

In the following, we investigate, how the RX sensitity, i.e., the shape of the produced light response upon substrate reception, can be influenced by different membrane porins. Figs. 2(c) and (d) show mean and variance of the luminescence intensity for GLuc encapsulated into polymersomes with narrow (c) and wide membrane porins (d).

First, we observe for both types of porins that the flash-kinetic of free GLuc (see Fig. 2(a)) is modulated to an extended luminescence signal over a longer period of time. Moreover, the maximum amplitude $I_{\max}$ and peak time t_{peak} of the received signal is shifted. For narrow porins, (Fig. 2(c)) the luminescence response increases more slowly and no isolated peak is observable. In comparison, for wide porins (Fig. 2(d)), a clear peak can be observed, which decreases significantly for $t > t_{\text{peak}}$. The reason for the stronger and more rapid response is the higher influx of substrate molecules facilitated by the wider porins.

For practical RX design, the porin diameter and number can be chosen depending on the application. For example, if a prolonged signal at the RX is required, e.g., due to large sampling intervals at the measurement unit, narrower porins are preferable, while wider porins can be used to produce a clear peak in the observed signal.

IV. Conclusions and Future Work

In this abstract, we presented a novel practical RX design for MC systems based on functionalized polymersomes, which can produce a light response upon signaling molecule reception. Our preliminary experimental results show the potential of the proposed RX design. Especially, the introduction of different membrane porins allows for the fine tuning of the RX sensitivity depending on the application.

For our future work, we plan to further investigate the influence of membrane porins (diameter and number) on the received luminescence signal, the development of suitable simulation models, and finally the development of an experimental end-to-end system employing the envisioned RX.

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