The PxDm chemiluminescent sensor for detection specific nucleic acid

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INTRODUCTION

Health care-associated infections (HAI) are infections acquired in healthcare settings that affect a large number of patients worldwide (Fig. 1), causing increased mortality and financial losses. The prevalence of these infections varies depending on the region, the type of infection, and the care setting [1].

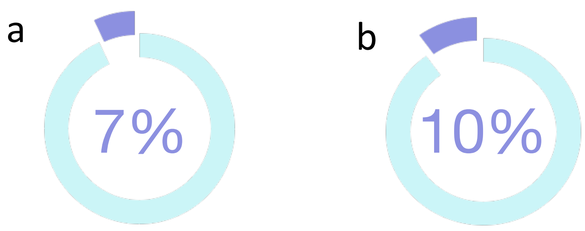

Fig. 1 Percentage of cases in developed countries (a) and in developing countries (b)

There are now several basic methods for detecting HAI pathogens. These methods include culture-based methods (Fig. 2a), serological tests (Fig. 2b), and molecular methods (Fig. 2c). However, these **methods** have some **limitations:**

- **culture-based** methods (long processing times);
- **serological** tests (low sensitivity);
- **molecular methods**, incl. PCR [2] (expensive).

Therefore, it is important to develop new approaches that can overcome these limitations.

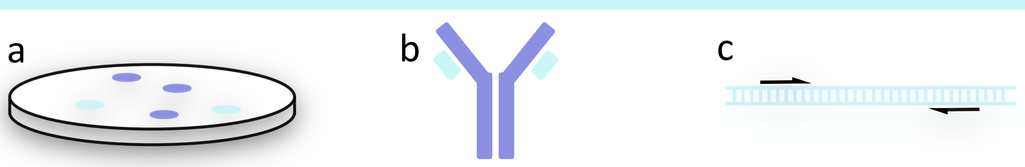

Fig. 2 Schematic representation of the main detection methods

One of the key developments in this field is the integration of **microfluidics** with **chemiluminescent** detection.

- **Microfluidics** allows for precise control of fluid flow and mixing, enabling efficient reaction kinetics and reducing sample consumption.
- **Chemiluminescent** reactions have proven to be a highly sensitive and specific detection method that require no external light sources or expensive labeling reagents [3].

This study presents a detection strategy based on **the peroxidase - like DNA-nanomachines (PxDm) sensor** for detecting HAI pathogens. The sensor is based on the G-4. These structures are able to form complexes with hemin that exhibit peroxidase activity. In the presence of such a G-4/hemin complex, hydrogen peroxide oxidizes luminol times faster, resulting in chemiluminescence (CL) [5], which can be detected by a photon counter. This principle is used in the system.

METHODS

The proposed detection system is based on **the PxDm sensor**. Figure 3 depicts the G-4DNM assembly process [6].

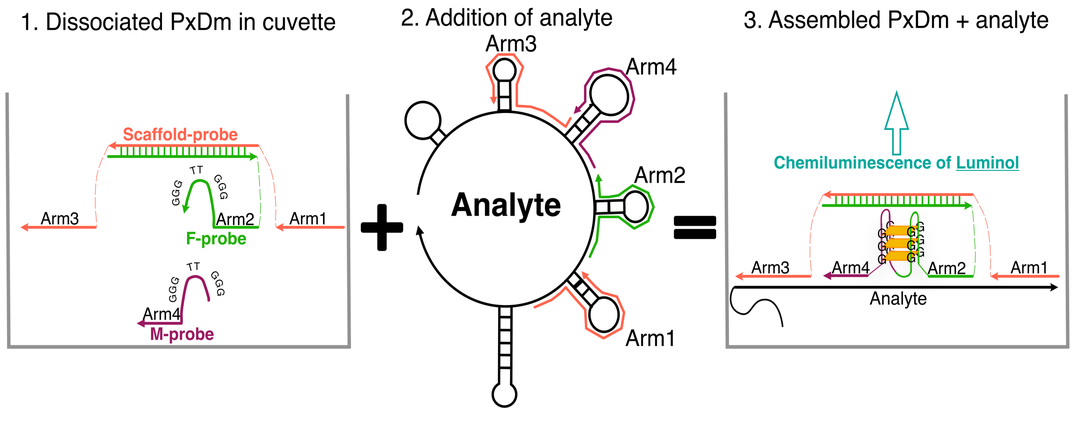

Figure 3. Schematic representation of the preparation of the PxDm.

The initial bacteriological strain for detection was ***S.aureus* NCTC 8325 (StAu).** StAu, a gram-positive facultative anaerobic bacterium, poses a serious danger to human health due to its virulence, invasiveness, and antibiotic resistance [7]. One of the main causes of HAI, for instance, is *StAu*, which may also cause profound endocarditis, abscesses, and bacteria that cause toxic and septic shock syndromes [8].

The target area of the genome was determined to be the ATP-dependent nuclease subunit A gene region. Even among closely related species, *StAu* may be identified by its distinctive sequence.

The CL-based detection system includes a special impermeable box that protects from external light. The quartz cuvette is the crucial component of the prototype, and the CL sensor, along with other necessary devices, indicated in Fig.4.

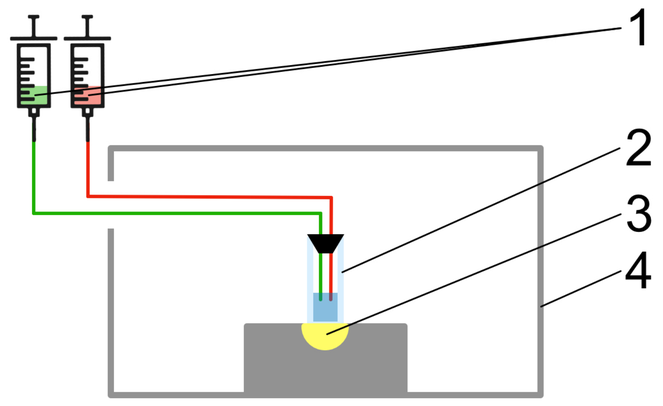

Figure 4. Schematic illustration of the system used: Syringes with tubes (1) inject reagents into a quartz cuvette (2) which is located above a photon counter (3) that detects the CL intensity. To minimize the influence of the environment the system is placed in a special impermeable box (4).

RESULTS

It was demonstrated that the PxDm does not assemble in the presence of a non-target analyte (Fig. 5), and thus the chemiluminescent signal is detected at a level comparable to the negative control. Furthermore, it was demonstrated that the proposed system successfully distinguishes the target analyte from closely related strains.

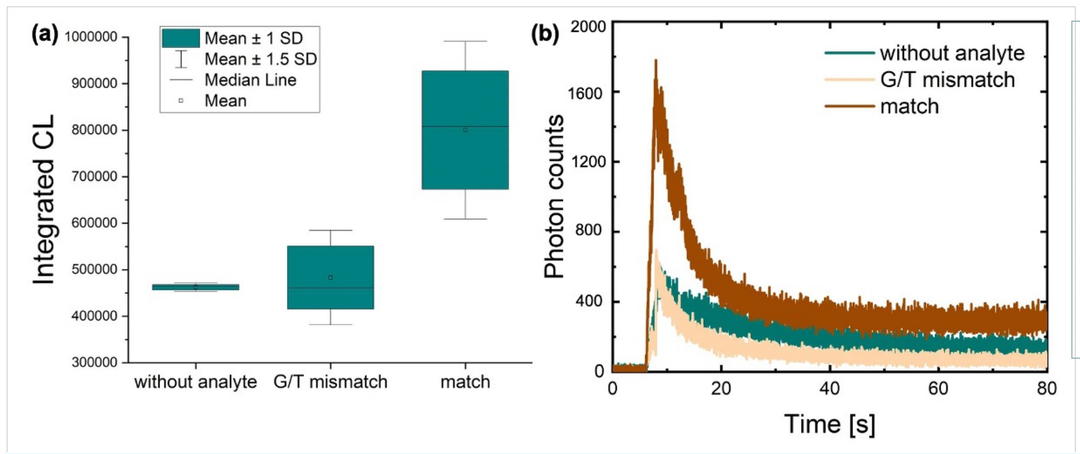

Figure 5. Discrimination of analyte *S.aur* from analyte *S.aur* G/T. (a) - Integrated CL with SD based on 3 independent experiments. (b) - Kinetics of CL registered during 15 minutes. Detection was done at 10 zeptoM concentration.

It was found that at an analyte concentration of 0.12 zM, the ratio of the maximum photon count in reaction with the analyte to the negative control became non-statistically significant. Therefore, it can be stated that the detection limit of the proposed PxDm sensor is 0,12 zM. Fig. 6 shows intensity curves as a function of time.

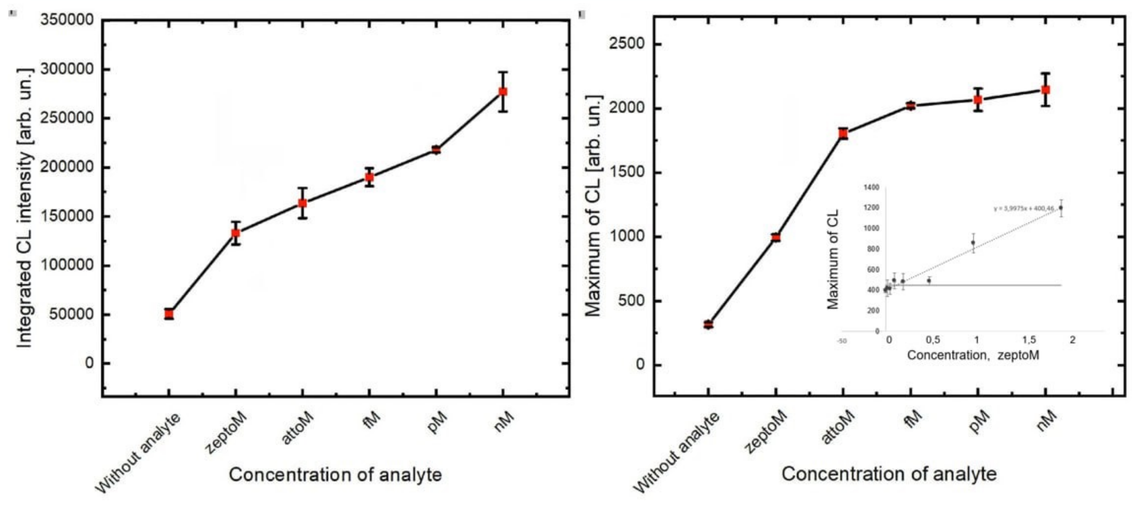

Figure 6. Sensitivity of PxDm. The integrated CL (a) and maximum of CL (b) of the PxDm with different analyte concentrations (1zeptoM - 1 nM) is shown.

CONCLUSIONS

The PxDm CL sensor was created and tested to detect *StAu*, one of the leading causes of HAI. As a result, it can be stated that the suggested detection approach is highly promising and may eventually lead to the development of a full-fledged lab-on-chip system.

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