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| 14. ABSTRACT<br><b>A Q-sense E4 QCM system was employed to study bacteria-phage interactions.</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                    |                                     |                                        |                                                        |                                 |
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## Specific recognition and detection of MRSA based on molecular probes comprised of lytic phage and antibody

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Methicillin resistant strains of *Staphylococcus aureus* (MRSA) are implicated in serious infections and nosocomial outbreaks. MRSA show resistance to a wide range of antibiotics thus limiting the treatment options. Therefore, rapid detection of MRSA is of key importance in prevention and diagnosis of infections caused by antibiotic resistant *S. aureus*. Currently existing methods of MRSA detection have some limitations and lack sensitivity or specificity. We examined a new approach in specific recognition and detection of MRSA, including identification of bacteria together with conformation of MRSA in real time. For this purpose we use a newly isolated *S. aureus* bacteriophage with a wide spectrum of hosts (including MRSA strains) together with monoclonal antibody against a penicillin-binding protein (PBP 2a). PBP 2a is a cell wall protein and it is responsible for antibiotic resistivity of MRSA. We showed that simultaneous recognition of *Staphylococcus* bacteria and PBP 2a protein increases specificity and reliability of MRSA detection.

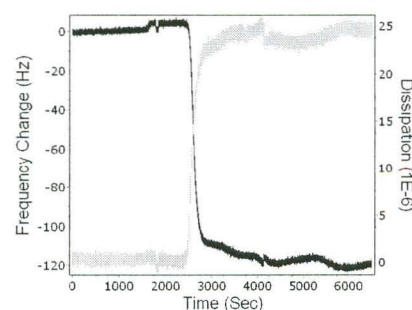
A Q-sense E4 QCM-D system (Sweden) was employed to study bacteria-phage interactions. Lytic phages were constructed into hollow spherical particles upon exposure to a chloroform-water interface. These particles were converted into monolayers and deposited onto QCM-D crystals using Langmuir-Blodgett technique [1]. Binding of MRSA has been manifested by frequency and dissipation changes (Fig.1). Similar results were obtained with antibiotic sensitive *S. aureus*.

An agglutination test was carried out using a latex reagent sensitized with antibody against PBP 2a (Denka Seiken Co., Ltd, Tokyo, Japan). The bacterial cells were mixed with the latex reagent on a test card. A visible agglutination occurred in about three minutes for MRSA. For the antibiotic sensitive *S. aureus* strains no agglutination occurred, even after one hour.

The interaction between PBP 2a protein and antibodies is not specific for *Staphylococcus aureus* because other bacteria have antibiotic binding proteins with large sequence similarity to PBP 2a. Therefore in our work we used *S. aureus* bacteriophage with wide spectrum of hosts together with antibodies against PBP 2a protein. In order to build a biosensor to specifically detect and identify MRSA we will employ a device with two parallel channels. One channel will have a *S. aureus* bacteriophage monolayer as a sensor probe, while the sensor of another channel will be covered with PBP 2a specific antibodies. Consequently, one channel will identify *S. aureus* bacteria, while another one will be sensitive to the antibiotic-binding protein. When signals coming from two channels are positive it would indicate the specific detection of MRSA.

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[1] Guntupalli, R, Sorokulova, I, Long, R, Olsen, E, Neely, W, Vodyanoy, V (2010) Phage Langmuir monolayers and Langmuir-Blodgett films. *Colloids and Surfaces B-Biointerfaces* 82: 182-189.



**Figure 1. Time change in frequency and dissipation energy during MRSA binding**