Detecting Infectious Organisms: A Concerted Approach using Genomics, Molecular Engineering and Nano-enabled bio-MEMS Technologies

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ABSTRACT

Development of technology/workflows to detect target organisms within minutes, are being evaluated using Methicillin Resistant *Staphylococcus aureus* (MRSA) as a proof of principle test case. Bacterial genomics and powerful bioinformatics identify unique surface layer proteins, informing molecular engineers of a target sequence against which to produce peptide aptamers (lead time ~12 weeks). The peptide aptamers have engineered tethers within their sequence for site specific immobilisation at sensor interfaces and subsequent recognition of their target protein. In parallel microelectromechanical systems (MEMS) sensors are being fabricated with the aim of capture and detection of target bacteria with little or no sample processing.

Keywords: genomics, peptide aptamer (Scannin), bio-MEMS, bacteria, clinical diagnostics.

1 INTRODUCTION

Nosocomial and community-acquired infections are a major cause of morbidity and mortality in the developed world. While infections caused by *Staphylococcus aureus* (including MRSA) and *Clostridium difficile* tend to make the headlines, these simply represent the tip of an iceberg which includes, for example, pan-resistant *Stenotrophomonas maltophilia*, *Acinetobacter baumannii* and antibiotic-resistant bacteria which produce Extended-Spectrum Beta-Lactamase enzymes (ESBLs) *e.g. Escherichia coli*. Considerable attention has been given to identifying infection or carriage among elective hospital patients on arrival, with infective or carrier patients being isolated and subjected to a prescribed care pathway that is designed to reduce the effects on themselves, other patients or healthcare professionals. While comparatively rapid screening systems have been developed (primarily PCR-based), they are relatively expensive. Moreover, the results are not usually available, at best, for 5 to 6 hours or, more usually, until the following day. The primary aim of this work is to develop patient-side, nano-enabled devices capable of detecting target bacteria within minutes by using peptide aptamers specific for bacterial cell surface proteins. The devices will be designed to detect target organisms at sensor surfaces on the basis of a unique fingerprint derived from organism-specific surface layer proteins. Such potentially inexpensive devices could revolutionize screening procedures since elective patients could be screened at their primary healthcare centre or care home, and treated to remove the target organism prior to hospital admission. The devices could also ultimately be used to screen emergency-through-the-door patients prior to allocation to a ward or extended for other applications such as monitoring of infectious outbreaks in the environment, and water and food supplies.

2 BIOINFORMATICS

The raw material to identify unique surface layer proteins exists in currently available databases, with 6118 available genome sequences and roughly 7,000,000 bacterial proteins to investigate. In addition new genome sequences are becoming available with ever increasing regularity. However the effective harvesting of the relevant information from these sources is a computationally intensive task. MRSA was chosen as a proof of principle and investigated for the presence of unique surface layer proteins. To establish an effective pipeline E-Science Grid-based workflows [1], cloud computing and bioinformatics databases have been used to exploit data from the complete microbial genome sequences available. The vigorous and intensive interrogation of *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* (MRSA) genomes against all other available genome sequences has identified a unique surface layer protein fingerprint for MRSA (protein A and PBP2A). Figure 1 highlights this workflow, but the important feature is that once established, it can be used as a pipeline to identify unique fingerprints in any number of infectious organisms.

3 PEPTIDE APTAMER SCREENING

Peptide aptamers have recently been established as an alternative to antibody recognition molecules. The peptide aptamers developed herein are known as Scannins. They possess a scaffold that is 13 kDa in size, they are inexpensive to produce, and stable / robust when attached to a surface via oriented molecular tethers [2, 3]. Figure 1 shows a visual depiction of the Scannin scaffold. The target peptide sequences identified in the bioinformatics work

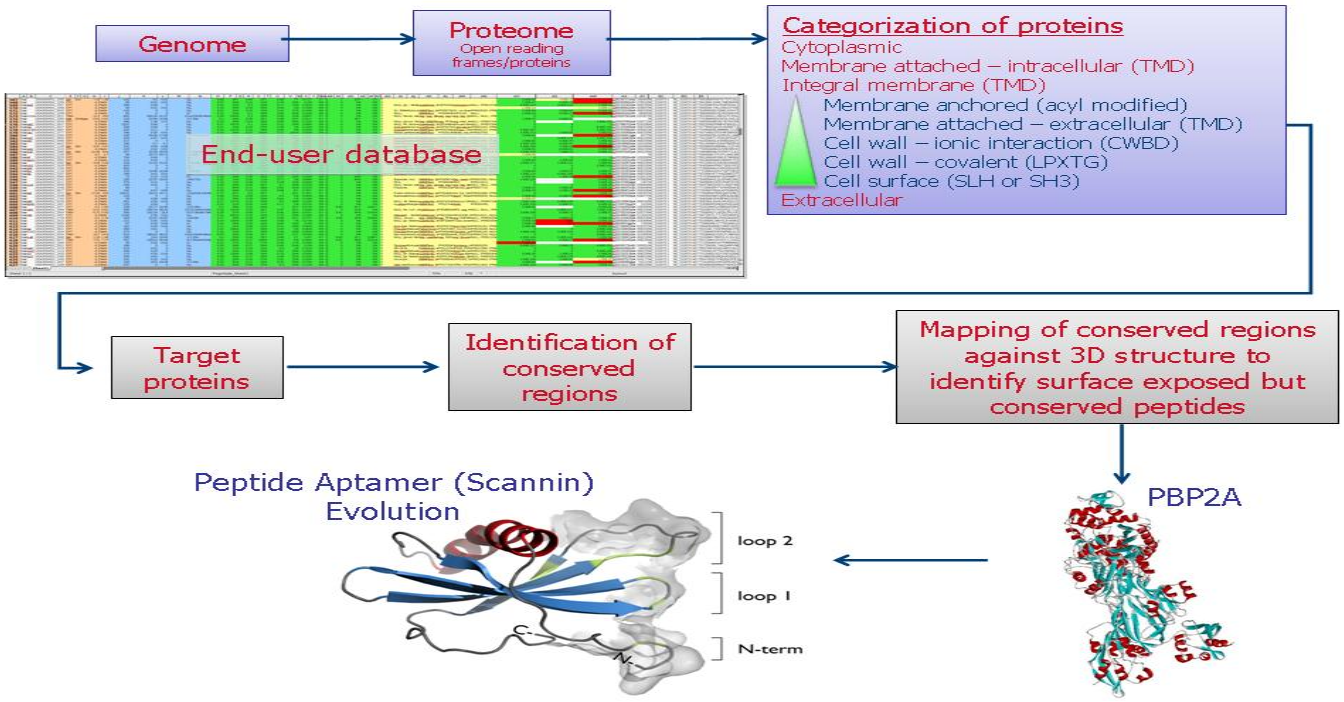

Figure. 1. Bioinformatics pipeline for an infectious organism. Bacterial proteins identified and investigated using Microbase job management system, bioinformatics database searches and cloud computing provide an end user database of potential surface layer proteins. Blast searches against all other genome sequences can then be used to select any unique proteins or tokens that will identify the infectious organism. Finally, multiple alignments across all MRSA strain PBP2A sequences revealed conserved surface exposed regions of the protein of interest. Scannin recognition molecule generation. The identified peptide sequences are used in a yeast 2 hybrid screen to find Scannins (artificial antibodies) against the target. The protein illustrations were created in WeblabViewer using the PDB files.

were screened against randomised libraries of peptides (inserted into the loop regions of the Scannin protein), using a yeast two hybrid screen. The technique allowed the x-gal reporter genes to identify a number of Scannins which may interact with the target sequence in PBP2A (results not shown). The Scannins against PBP2A have been expressed and purified using standard *E. coli* expression systems as previously described [2]. In addition a soluble form of PBP2A has been expressed in *E. coli.* The Scannin recognition molecules against PBP2A have been site specifically orientated and immobilised at gold surfaces to assess whether they have a true binding affinity for the target protein. This validation has taken place using Surface Plasmon Resonance as the biosensor technique. Figure 2 shows the Scannin immobilisation procedure and Figure 3 shows PBP2A binding to a Scannin baselayer. The preliminary results indicate that the Scannin shown has a binding affinity in the order of a tens to a few hundred nanomolar, however further experiments need to be carried out to verify the Kd in absolute terms.

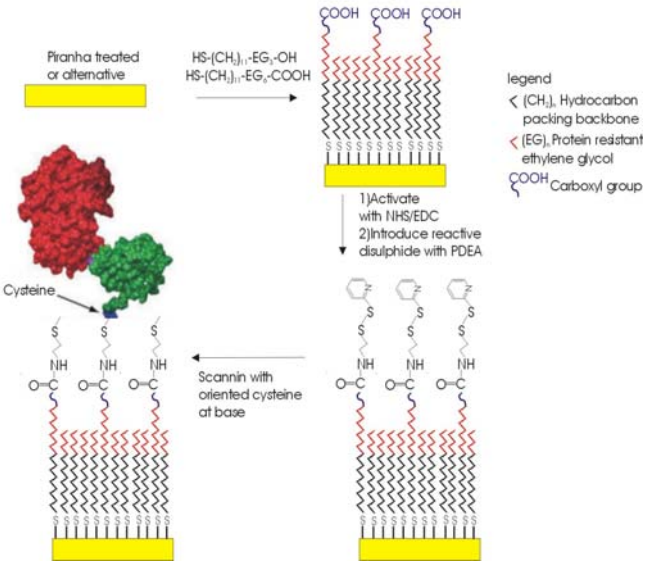

Figure 2. Oriented Scannin Immobilisation. A mixed thiol terminated monolayer of polyethylene glycol molecules that present a protein resistant group, or a carboxyl group, are immobilised at the surface. Further standard chemistries introduce a reactive disulphide that can bind the cysteine tether on the aptamer in a site specific manner.

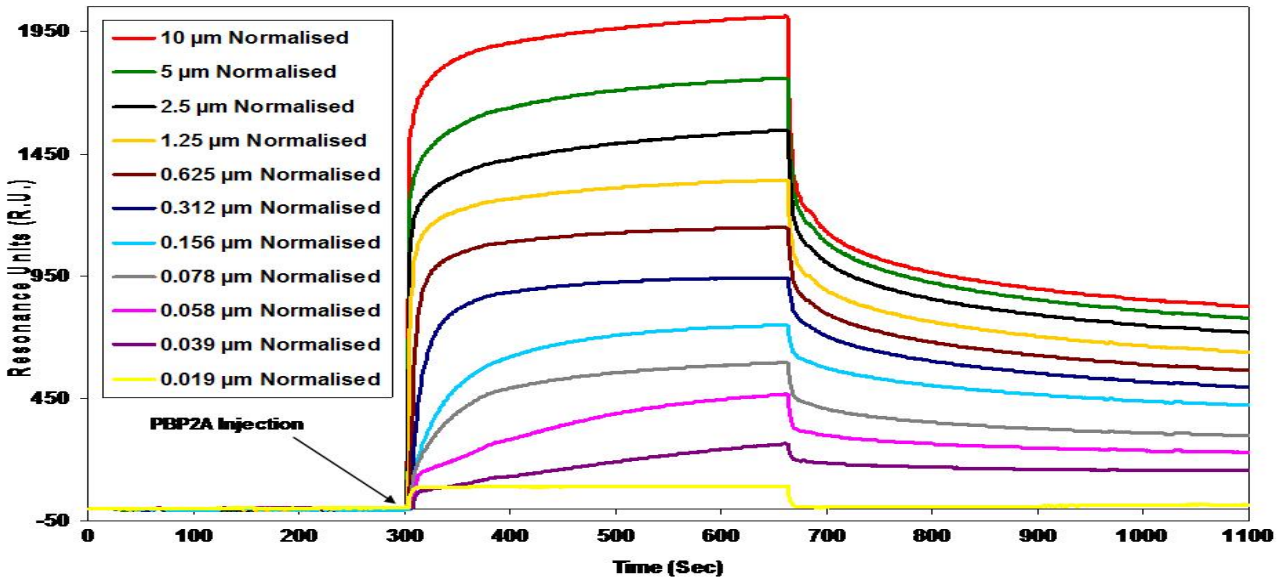

Figure 3. A Scannin against PBP2A was immobilised at gold SPR interfaces and shown to have an affinity for recombinant PBP2A, which is likely to be tens to a few hundred nM. The preliminary experiments are being extended so a definitive Kd can be assigned.

4 BIO-NANO-PATTERNING AND SENSOR DEVELOPMENT

The circular diaphragm resonator (CDR) device [4, 5], shown schematically in Figure 4, lies at the core of the detection system.

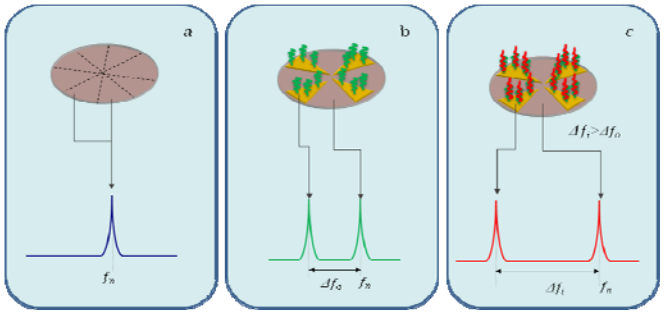

Figure 4. CDR MEMS sensor. A) A homogeneous diaphragm clamped at the edges presents two spatially independent modes of vibration which share a common natural frequency. B)/C) A frequency split is created between the two modes by site specific mass addition during functionalisation. By patterning the CDR sense sectors with Scannin molecules specific for the target organism the CDR will capture and identify samples with little or no processing.

In the current CDR fabrication run improvements are are being made over our previous attempts, namely the use of PZT for both actuation and sensing. This has two main drivers, it makes the fabrication simpler and more robust and provides a route to simpler electronic readout solutions, thus potentially improving the signal to noise ratio. Figure 5 depicts the major CDR design features and the images in Figure 6 show the current fabrication progress.

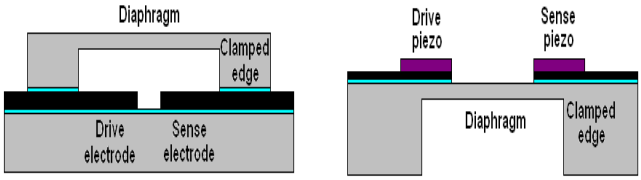

Figure 5. Schematic representation of current CDR design features.

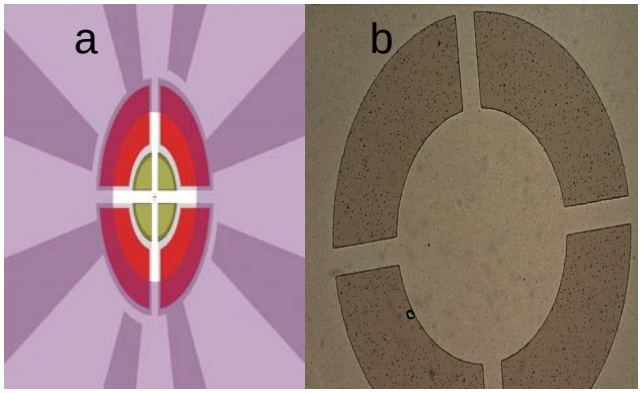

Figure 6. *Shows a design mask and image of the CDR MEMS sensor at the current stage of fabrication.*

Proposed methods of Scannin immobilisation have been developed in parallel to the device fabrication and indeed must be agreed to instruct the fabrication design. Two routes to functionalistion have been pursued. Firstly the fabrication will finish with a lithography step, which allows a polymer to break the axisymettry of the CDR, site specifically load biomolecules at the interface and perform requisite assays. This has been described in detail elsewhere [6]. In a second strategy the CDR interface will be patterned with gold sense and reference sectors that act as individually addressable electrodes and allow patterning with nm resolution as described previously [7]. This is shown as a schematic in Figure 7.

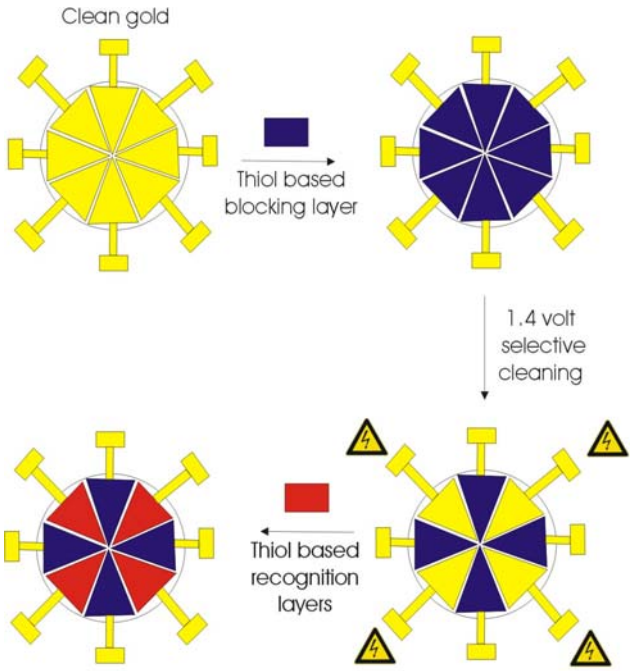

Figure 7. Patterning of biomolecules at a CDR diaphragm using discrete gold layers. Thiol base layers combined with a sequentially applied potential on the discrete gold electrodes (CDR diaphragm) allows biomolecules to be site-specifically immobilised with nm resolution and hence break the axisymmetry of the CDR diaphragm into a sense (recognition region) and a reference. (blocked region).

The strategy has been used to capture bacteria at a specific location, providing a suitable surface exposed marker and recognition molecule are available. In a model pull down assay, dummy gold CDR interfaces were site specifically patterned with an anti protein A antibody and used to capture *Staphylococcus aureus,* but not other bacteria that do not contain protein A at the cell surface. The fluorescent image in the positive *Staphylococcus aureus* assay can be seen in Figure 8. The experiments are currently being extended to capture assays on intact or partially processed bacteria using Scannins as the recognition molecule.

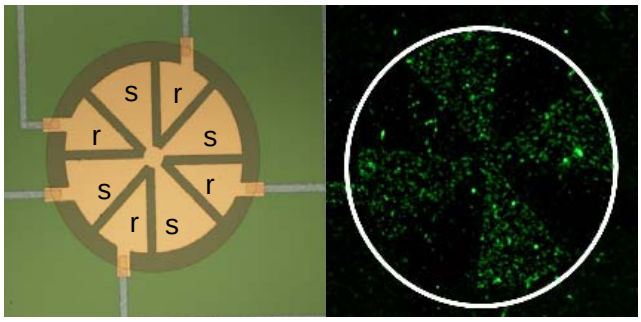

Figure 8. The image on the left shows a dummy CDR interface with electrically addressable sense and reference sectors (gold). Using the methods described herein the reference sectors (r) have been blocked with a 100 % Polyethylene glycol layer while a mixed monolayer on the sense sector (s) has allowed anti protein A immobilisation and site specific pull down of the Staphylococcus aureus target strain. This is visualised in the right hand panel using fluorescent microscopy and bacteria stained with acridine orange.

5 CONCLUSIONS

The bioinformatics strategy has demonstrated the potential to identify unique proteins in target organisms and is now setup as a pipeline to investigate further examples. In the case of MRSA, PBP2A has been shown to be unique to resistant strains and Scannins against PBP2A sequences have been screened for using a yeast two hybrid system. One Scannin, to date, has shown a good affinity for recombinant PBP2A, when used as a recognition molecule in SPR experiments. On the hardware front, the CDR sensor, which is central to the detection platform, is still in fabrication, while the site specific patterning strategy has pulled down bacteria using a model protein A assay. The next step is to show the novel reagents pulling down intact, or partially processed bacteria, on dummy patterns and ultimately on CDR sensors in a label free system.

6 REFERENCES

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