# Chromatographic Purification of Bacteriophages for Improved Surface Capture of Bacteria – Towards a General Approach

R. Naidoo, A. Singh, N. Glass, S. Arya, S. Evoy*

*Department of Electrical and Computer Engineering and National Institute for Nanotechnonology, University of Alberta, Edmonton, T6G 2V4 Canada, naidoo@ualberta.ca

## ABSTRACT

We report in this study the largest surface capture density of E Coli using the wild-type T4 bacteriophage; whereby higher surface capture density can enable higher sensitivities for surface-based biosensors. This was possible due to our purification of the phage lysate, which significantly improved phage surface density, achieving maximum (jamming) surface coverage. Our methods could be generalized - to be applicable to a large set of phage biodiversity. We can develop a new screening method to select for the best bacteriophage, for bacterial biosensor application, out a set of candidate phages.

***Keywords***: bacterial biosensor, wild-type bacteriophage, biointerfaces, chromatography, biodiversity.

## 1 INTRODUCTION

The capture of bacteria to a surface is a critical aspect in the development of rapid biosensor platforms for their detection. Our objective in this study is to find a general method to achieve the highest surface capture density of bacteria, using wild-type bacteriophages.

Most of the current literature presenting the use of bacteriophages for bacterial biosensor development implements M13 filamentous phage display technology; the M13 phage requires genetic modification of a high-repeat coat protein to typically display an antibody fragment. This study employs *wild-type* bacteriophages to quickly develop surfaces that exhibit highly specific affinity to pathogens of interest, as has been demonstrated recently [1]. Bacteriophages are probably the most widely distributed biological entity in the biosphere, with an estimated population density of ~10 million per cubic centimeter in any environmental niche where prokaryotes reside [2]. We believe this incredible biodiversity is a major strength of the wild-type phage approach - necessitating general methods applicable to a large set of phage diversity.

We report in this study the largest surface capture density of *E Coli* using the model T4 bacteriophage; whereby higher surface capture density can enable higher sensitivities for surface-based biosensors. This was possible due to our purification of the phage lysate, which significantly improved phage surface density, achieving maximum (jamming) surface coverage. To our knowledge this has never been reported before. Additionally we purified 2 other bacteriophages: P22 and Campy P1, and also show that their phage surface densities improved significantly relative to unpurified suspensions. Previous work with unpurified T4 suspensions and Au surfaces demonstrates a poor phage surface density by physisorption at (0.49 ± 0.15) phages/$\mu m^2$; covalent attachment by cysteamine-glutaraldehyde improves this to reach (18 ± 0.15) phages/$\mu m^2$ [1]. One would expect that by attaining the jamming coverage of phages on the surface that this would correlate with the highest possible bacterial capture density – however it is not the case. We have instead determined an *optimal phage surface density* for the model T4 system.

We also apply these improvements to demonstrate the real-time detection of *E. Coli* using surface plasmon resonance along a T4 immobilized surface.

## 2 EXPERIMENTAL METHODS

### 2.1 Bacterial Culture and Bacteriophage Amplification

Bacterial enumeration was done by plate count method and was expressed in cfu/ml while the phage count was performed using the soft agar overlay technique and expressed in pfu/ml (Sambrook and Russell, 1989). For growing P22, the *Salmonella enterica* serovar Typhimurium culture was prepared by streaking onto a Nutrient agar plate and incubated overnight at 37°C. Two single colonies from the plate were inoculated into 3 ml Nutrient broth (NB) and were grown overnight at 37°C in a shaker to obtain an overnight bacterial culture. Then, 900 µl of $10^7$ pfu/ml P22 phages were mixed with 3.6 ml of *Salmonella enterica* serovar Typhimurium culture and incubated at room temperature for 10 min. This mixture was added to 630 ml of LB and was incubated at 37°C, while shaking at 150 rpm for 15h. The amplified phages were then centrifuged at 2500 rpm for 20 min to remove bacterial cells. The supernatant was vacuum filtered and ultracentrifuged at 55,000 rpm for 1 h. The pellet was resuspended in 1ml SM buffer, vacuum filtered and titrated. The phage solution was purified to remove bacterial contaminant proteins. The phages were used for

immobilization on the gold surfaces and bacterial binding studies.

## 2.2 Surface Modification of Gold Substrates

The gold substrates were fabricated using piranha cleaned 3" silicon (100) wafer by sputtering 5 nm thick chrome adhesion layer followed by 20 nm thick layer of gold. The gold coated wafers were diced into 5 mm x 7 mm rectangular pieces using a diamond tip pen. The substrates were then sonicated for 5 min in acetone followed by cleaning in isopropanol and ethanol for 5 min each. They were finally rinsed in Milli Q water for 5 min prior to their surface functionalization.

Phages were activated with 5 mg/ml of EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) and 6 mg/ml of NHS (N-hydroxysuccinimide) prior to overnight immobilization onto cysteamine functionalized gold substrates. All surfaces were kept in the shaker during immobilization. Further, the surfaces were blocked in 1 µg/ml solution of bovine serum albumin in PBS for 30 min to check non-specific binding of the bacteria onto the surface. The surfaces were cleaned in 0.05% PBS-tween20 solution followed by PBS buffer for 5 min each prior to bacterial capture. The density of the phages on the surface was calculated from the scanning electron microscopic (SEM) images.

## 2.3 Bacterial Capture & Surface Characterization

The modified surfaces were incubated with the target bacteria or bacterial controls for 20 min to facilitate bacterial capture. All SEM imaging was performed using a Hitachi S-4800 (Tokyo, Japan) scanning electron microscope (SEM). For SEM measurements of bacterial capture imaging, the samples were fixed in 2% aqueous solution of gluteraldehyde for 1h followed by washing them twice with de-ionized water for 5 min on an orbital shaker. The samples were dried under $N_2$ flow prior to analysis.

## 2.4 Real-time Bacterial Detection by Surface Plasmon Resonance

SPR measurements were carried out to record online binding of T4 phages and to quantify their binding. The prepared T4/DTSP/Au bio-electrode was also used to study the interaction with *E.coli* EC12 and to check the specificity against similar strains of *E.coli* such as NP10 and NP30. A baseline was first established for the bio-electrode flowing PBS for 5 min. A particular concentration of *E. coli* solution was injected and allowed to interact with the bio-electrode for 20 min. The system was then flushed by injecting PBS buffer for 5 min (flow rate of 200 µl/min) to wash off uncoordinated bacteria from the surface.

# 3 RESULTS & DISCUSSION

Since the simple physisorption of bacteriophages onto surfaces was first tried (P22 = 2.3 phages/µm$^2$), covalent attachment of our *purified* phages is now allowing us to approach the jamming (maximum) coverage of phages on the surface.

Despite achieving jamming coverage of phages on the surface for P22 and T4, an optimal phage surface density was determined that would be lower than jamming coverage.

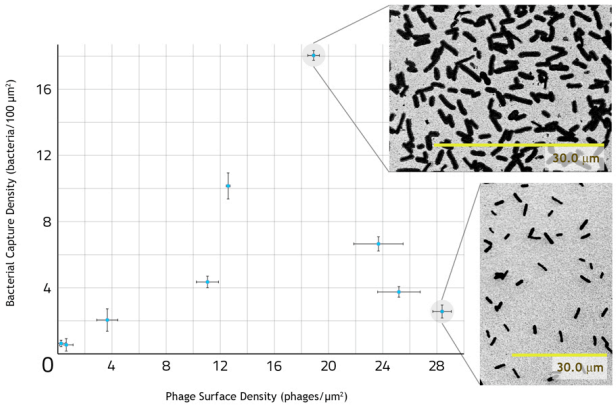


**Figure 1:** For the model T4 phage system, an optimal range for phage surface density was determined. Higher phage surface densities, approaching jamming coverage, causes a significant drop in EC12 bacterial capture density, possibly due to the excessive aggregation of phages on the surface.

This also correlates to our SPR data demonstrating real-time, specific detection of bacteria.

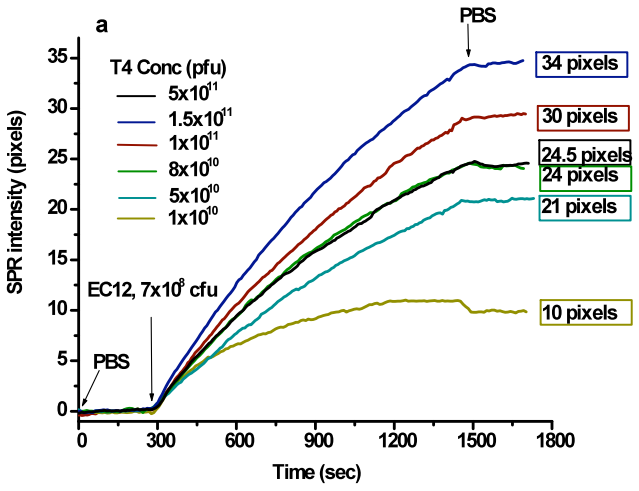


**Figure 2:** SPR response curve for real-time detection of bacteria by surfaces treated with different bulk concentrations of purified T4 phage.

## 4 CONCLUSION

Much of the details and discussion will be presented elsewhere. However, it is important to note that our purification method combined with our surface attachment procedures should be relevant for the vast majority of phage diversity.

This opens the way towards developing a generalized scheme that tests the efficiency of a certain phage to specifically capture the bacteria of interest to a surface. A list of candidate bacteriophages could be compiled from a phage library and tested in parallel, to then select the best bacteriophage suitable for biosensor application. In this way, it is the incredible biodiversity of bacteriophages that can be screened for the rapid development of better bacterial biosensors - instead of relying on the more complex genetic manipulation and proteomic study of a handful of well-known bacteriophages.

## REFERENCES

[1] A. Singh et al, Immobilization of bacteriophages on gold surfaces for the specific capture of pathogens, Biosensors and Bioelectronics. 24 (2009) 3645-3651.

[2] J. Karam, Bacteriophages: The viruses for all seasons of molecular biology, Virology Journal. 2 (2005) 19.