

On-site Methods within DECATHLON – towards point-of-use molecular analysis and next generation sequencing

DJ Kinahan^a; G. Schneider^b, Y. Cesa^b, D. Chung^a and J Ducreé^{a*}

^a*School of Physical Sciences, Dublin City University; Glasnevin, Dublin*

^b*Leiden University, Leiden Institute of Chemistry, Leiden, The Netherlands*

Corresponding Author: jens.ducree@dcu.ie

There is an increasing need for on-site applications of DNA-based methods, both, for inspection services by public authorities, as well as for companies that wish to monitor their supply chain. The capability to apply DNA-based methods in the field, or at ‘point-of-use’, is critical where centralised laboratories are inadequate to meet the demand of prompt answers for early intervention. The application fields addressed by DECATHLON are food analysis with specific emphasis on pathogen detection for food safety, GMO identification and customs issues.

In recent years much progress has been made in the development of different types of on-site devices that can accommodate DNA-based methods. Within the DECATHLON project, two primary approaches for on-site use of DNA-based methods are developed in parallel; sample preparation and nucleic acid identification using a centrifugal microfluidic platform, and development of a novel graphene nano-gap sensor for next generation sequencing.

Centrifugal Platform

The centrifugal microfluidic platform is of increasing interest in the microfluidics community, particularly for low-cost, rugged point-of-use applications such as medical diagnostics and environmental monitoring. This lab-on-a-disc (LoaD) concept uses the rotationally induced inertial field to pump and manipulate on-board liquids, e.g. for sample preparation and reagent management. The actuation by a simple spindle motor can be implemented by low complexity instrumentation without the need for error-prone fluidic interfaces.

Here we present early work towards a ‘Sample-to-Answer’ cartridge for DNA based detection of pathogens in food. This cartridge will wash a sample (pre-lysed and concentrated off-chip) through silica beads to purify DNA. This purified DNA will then be eluted and discretised into up to 12 individual wells to enable spatial multiplex analysis of samples. In a flow control scheme providing LAMP reagents and primers, this disc cartridge is intended to address 12 different gene targets towards identification of Shiga Toxin producing *E. Coli* (STEC) serotypes. Our approach enables a fully automated, closed process to be implemented at both ‘point-of-use’ and in the laboratory. Compared with fluorescent multiplexing, spatial multiplexing greatly simplifies the reagent management and instrumentation for fluorescence detection common in nucleic acid testing protocols.

Graphene based sensors

DNA sequencing is very rapidly growing into an industry of major interest. A variety of techniques exist, each with their own pros and cons. The use of nanopores – nanoscale holes in a membrane – for DNA sequencing was proposed more than 20 years ago. The idea is straightforward: pass a DNA molecule through the pore from head to tail, and read off each base when it is located at the narrowest constriction of the pore, using the ion current passing through the pore as the probe for detecting the identity of the base. While biological pores were investigated for quite some time, solid-state nanopores are now emerging. They have tunable pore size, are more stable than biological membranes, offer re-usability upon cleaning, and allow for scaling and device integration. But DNA sequencing has so far not been demonstrated with these devices: conventional silicon-based nanopore membranes are relatively thick, typically ~30 nm, which corresponds to ~60 bases along a single-stranded DNA molecule. While solid-state nanopores are excellent new tools for biophysical studies, they are therefore not directly useful as-is in DNA sequencing applications. Recently however, graphene nanopores were introduced. Graphene forms the ultimate nanopore membrane since it is a carbon sheet with a thickness of only a single atom. Furthermore it is electrically conductive, which opens up new modalities of measuring the traversing nucleotides, for example by running a tunneling current through the DNA molecule that is traversing a graphene gap, to directly probe the chemical nature of the bases.

In this talk, we will describe our latest work on studying DNA using graphene nanopores to ultimately design nano gaps. We will also highlight what we think are the most important chemical and physical questions to answer in the next few years in this field where graphene is used for biomolecular detection and sequencing.

Word Count: 646

Acknowledgements

This work is funded by Grant No. FP7-KBBE-2013-7-613908-Decathlon under the Seventh Framework Program