

**Hamilton Undergraduate Scholarship Programme 2010**  
**PROJECT NO. 4**

---

**Supervisor:** Dr. Barry Byrne  
**Co-supervisors:** Prof. Harry Holthofer

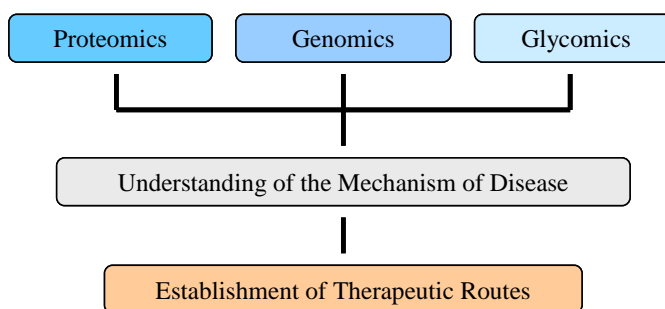
**Project Title:** Isolation and characterisation of urinary exosomes using Free-Flow Electrophoresis (FFE).

**Duration of Project**                      **From:** 7 June 2010                      **To:** 13 August 2010

---

**Project Description:**

Chronic kidney disease (CKD) and diabetes mellitus are major causes of mortality, and place a considerable financial burden on European healthcare systems. A key consideration in understanding the mechanism of disease is the identification of novel protein, carbohydrate and nucleic acid-based biomarkers, and identifying novel therapeutic routes based on these observations. Furthermore, one of the major challenges here is obtaining biological samples in sufficient quantities to permit this research to be completed.

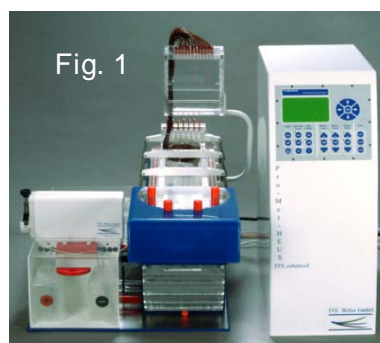


Exosomes are small (20-100nm) membrane vesicles that are released by a variety of cells into plasma and other bodily fluids. These include urine [1], an analytical matrix that can be obtained non-invasively in large volumes. With reference to renal disease, there has been significant recent interest in the biological importance of urinary exosomes, particularly in relation to how their genomic (DNA), glycomic (carbohydrates) and proteomic (protein) content provides key information about the mechanisms of disease associated with diabetes [2]. More specifically, exosomes contain an array of microRNA (miRNA) species whose functions have yet to be fully elucidated, but have been predicted to contain key information pertinent to the onset of disease. Furthermore, the proteinaceous markers found in exosomes that have been characterised to date have been demonstrated to be indicative of renal dysfunction and structural impairment [3]. Hence, these entities are invaluable sources of biological information and provide a key insight into diabetes-related disease mechanisms. Hence, they warrant immediate investigation.

Traditional methods of exosome isolation involve time-consuming multiple centrifugation steps, and yields are typically low. In order to extract sufficient quantities of exosomes from urine, it is therefore necessary to employ a high-throughput and sensitive method. Here, we propose the use of a novel methodology,

termed Free-Flow Electrophoresis (FFE), which is already in-house, for permitting the isolation of considerable quantities of exosomes from urine to allow the aforementioned studies to be continued.

FFE (Figure 1) permits the rapid fractionation and enrichment of proteins in simple or complex sample matrices. Liquid-based fractionation is facilitated through the separation of charged or chargeable entities under both native and denaturing conditions [4,5], and this exciting new methodology is ideally suited for the purification of proteins from serum [6]. Here, this methodology will be applied to urine.



### **About the project:**

The primary aim of this project is the isolation of urinary exosomes from urine. During this experimental work, the student will gain expertise in a number of different methodologies, including on how the FFE is operated and how proteomic and glycomic analysis of purified exosomes (using standard proteomic techniques and lectin-blotting) can be used for biomarker characterisation. This research will contribute heavily towards the non-invasive purification of these invaluable vesicles and the production of sufficient biological material for performing more rigorous characterisation at the genomic, proteomic and glycomic levels. Therefore, this experimental work will contribute heavily to an imminent key publication by the Glomerular Nephropathy Group in the National Centre for Sensor Research (NCSR).

### **References:**

- [1] Pisitkun, T *et al.* 2004. Identification and proteomic profiling of exosomes in human urine. *Proc. Natl. Acad. Sci. USA* **101**: 13368-13373.
- [2] Fernandez-Llama, P. *et al.* 2010. Tamm-Horsfall protein and urinary exosome isolation. *Kidney Int.* In Press.
- [3] Gonzales, P.A. *et al.* 2009. Large-scale proteomics and phosphoproteomics of urinary exosomes. *J. Am. Soc. Nephrol.* **20**: 363-379.
- [4] Wen, J *et al.* 2010. Icrofluidic preparative free-flow isoelectric focusing: system optimisation for protein complex separation. *Anal. Chem.* **82**: 1253-1260.
- [5] Song, Y.A. *et al.* 2010. Free-flow zone electrophoresis of peptides and proteins in PDMS microchip for narrow pI range sample prefractionation Coupled with Mass Spectrometry. *Anal. Chem.* In Press.
- [6] Wildgruber, R. *et al.* 2008. Free-flow electrophoresis system for plasma proteomic applications. *Methods Mol. Biol.* **424**: 287-300.