**Biological Applications of Droplet-Based Microfluidics**

Droplet-based (or two-phase) microfluidics has a variety of applications but has recently been used as a tool for the development of a wide range of biological assays, including protein crystallization, cell stimulation and the measurement of protein aggregation kinetics. This technique works on the basis of producing individually addressable picolitre sized aqueous droplets within a microfluidic device, separated by a fluorinated oil phase. Each droplet can be considered as a reaction vessel which can be monitored by a range of techniques both within the microfluidic device as well as “off-chip”. The technique has the advantages of being reproducible, highly controllable and utilizes only minute amounts of biological materials.

Two recent biological applications of droplet-based microfluidics will be discussed. The first is concerned with the improvement of membrane protein crystallization. It has been shown previously that membrane proteins can be crystallized in a droplet in a manner analogous to standard crystallization techniques. Here, a droplet method is used to screen additives which improve protein-protein contacts within the crystal, leading to protein crystals with different morphologies. When analysed using single crystal x-ray diffraction, this method shows that the protein packing within the crystal has been changed, leading to a potential method for screening membrane proteins which have not previously been crystallized.

Secondly, droplet-based microfluidics has been used to develop a method for screening protein aggregation with implications for a new diagnostic tool for Alzheimer’s disease. Amyloid beta peptides are implicated in neuronal cell damage in the Alzheimer’s disease pathway. However a bottleneck to working with these peptides in vitro is their relative instability to aggregation. By tuning the surface chemistry of the aqueous – oil interface of the droplet, increased stability has been achieved, leading to a viable method for testing the aggregation kinetics of these peptides against proteins found in murine cerebral spinal fluid (CSF), which are thought to retard peptide aggregation. The reproducible and high-throughput nature of this method points towards its potential as a screening technique for protein levels in CSF which could serve as an indicator for Alzheimer’s disease onset and progression.