

Towards Directed Evolution in Microfluidic Microdroplets on a Chip

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In vitro compartmentalisation, IVC, is a methodology that uses water-in-oil emulsions to form individual reactors for chemical or biological experiments. Each compartment is a microdroplet of water containing all the components to perform a unique experiment.⁽²⁾ The encapsulation of a single gene and an *in vitro* transcription/translation (IVTT) system in individual droplets allows *in vitro* expression of protein in a format that allows detection of its activity for directed evolution. IVC has been used successfully by the groups of Tawfik and Griffiths to generate novel biocatalysts.⁽³⁾

The IVC approach for directed evolution has the advantages of rapid generation and screening of novel catalyst on a small scale. But droplets formed by standard bulk emulsification protocols lack homogeneity and control over droplets is limited.

Microdroplets generated in microfluidic devices offer high level of control allowing rapid formation of homogeneous droplets of defined size and controlled contents, enabling quantitative high-throughput screening. Microdroplets generated on chip had been used as individual microreactors to perform chemical and biochemical reactions, but only on a very small time-scale (order of minutes).⁽⁴⁾ In order to study a slow enzyme, a low activity mutant protein, or protein expression the residence time of droplets within the microfluidic device need to be extended.

To this end we have designed an integrated device allowing precisely controlled formation of water-in-oil droplets, their storage for several hours in a reservoir, and individual analysis of droplets by laser detection.⁽¹⁾

As an example of typical assay reaction, we choose the *in vitro* expression of a model protein, a mutant of Green Fluorescence Protein (GFP). Plasmids coding for GFP and the IVTT mixture are mixed on chip and the produced droplets are stored in the reservoir for up to seven hours, using a suitable emulsion formulation. Kinetics of *in vitro* expression of GFP were measured by following the increase in fluorescence intensity after excitation at 488 nm of individual droplets. The high yield of GFP obtained made it possible to detect protein expression from a single copy of the DNA template.

Our ability to create monoclonal droplets in which genotype and phenotype are linked opens the way for directed evolution experiments in microfluidic devices. Protein expression in a microfluidic device is also key to a future directed evolution platform on a chip. This technology is currently applied to the *in vitro* expression of hydrolases whose activity is detected by measuring the accumulation of fluorescent product of the enzymatic reaction.

¹ Courtois F., Olguin L.F., Whyte G., Bratton D., Huck W.T.S., Abell C and Hollfelder F., *ChemBioChem*, 2008, 9, 439.

² Griffiths A.D. and Tawfik D.S. *Nat. Biotechnol.*, 1998, 16, 652.

³ Kelly BT, Baret JC, Taly V, Griffiths AD. *Chem. Commun. (Camb)*, 2007, 18, 1773.

⁴ Song H., Chen D.L., Ismagilov R.F., *Angew. Chem. Int. Ed. Engl.*, 2006, 45, 7336.