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Enzyme Mechanisms and Evolution

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Enzymes are biological catalysts with remarkable properties. They perform all the vital tasks of life - process food, produce energy, replicate genes and provide the building blocks for the formation of new cells and organisms. They perform reactions that would otherwise take millions of years in seconds or even milliseconds; they select their specific target molecule from billions of other, often quite similar ones; and they produce a single product out of many possible alternatives.

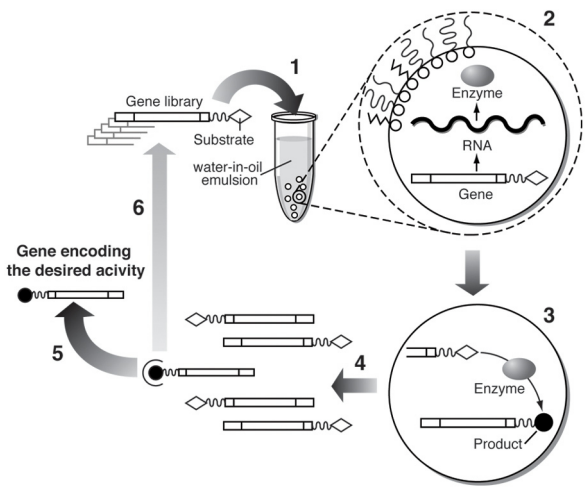

Fig.1 *Gene selection by in vitro compartmentalisation (IVC). In Step 1, a cell-free translation extract containing a library of genes is dispersed in a water-in-oil emulsion The genes, that are all linked to a substrate for the reaction being selected, are transcribed and translated within their individual compartments (Step 2). Subsequently (Step 3), proteins with enzymatic activities convert the substrate into a product that remains linked to the gene. Next (Step 4), the emulsion is broken, and genes linked to the product are selectively enriched, then amplified, and either characterised (Step 5), or subjected to further rounds of selection (Step 6). More recent IVC formats allow the direct sorting of emulsion droplets that carry a fluorescent product by FACS (fluorescent activated cell sorter).*

Not much is known as to how these remarkable molecules evolved - we lack the fossils and the dinosaurs that have been so useful in studying the evolution of organisms. Nevertheless, biology is united by the rules of Darwinian evolution that explain how organisms as complicated as humans evolved by natural selection operating on wide and genetically diverse populations. Can we perhaps, in the absence of fossils and dinosaurs, reproduce the evolution of biological molecules including enzymes in the test tube and in real time? This, however, requires the development of new technologies that can apply the principles of Darwinian evolution to genes and enzymes.

We are applying and developing a variety of such technologies, aimed at the creation of genetic diversity (gene libraries) and their selection for the desired enzymatic activity. With these technologies in hand, we can reproduce the evolution of enzymes and thus get a glimpse of the 'dinosaurs of the protein world' - the inefficient, primitive intermediates that may have led the way to the highly proficient enzymes known to us today. One of our interesting findings regards the role of functional and structural diversity of proteins. Our work indicates that the promiscuous and moonlighting activities of proteins, and their structural plasticity, greatly facilitate the evolution of new functions.

We have also developed a new technology (in collaboration with Andrew Griffiths, Camberidge, UK) dubbed IVC (In vitro Compartmentalization). IVC allows billions of different genes to be placed individually in artificial, cell-like compartments where they are translated to give many copies of the enzyme they encode. The substrates for the

enzyme to be selected and the products of its activity all remain within the same compartment. A selection is applied for the 'survival of the fittest' - only genes that encode an enzyme which produces the desired activity survive. We have applied IVC, and a variety of other technologies developed by us and by others, for the evolution of a variety of different enzymes, including DNA-methyltransferases, and phosphotriesterases (enzymes that degrade organophosphates, such as pesticides and nerve gasses). Most recently, we have applied directed evolution to dissect the structure, the mechanism and evolution of the serum paraoxonase (PON) family of detoxifying and anti-atherosclerotic enzymes.

Selected Publications

Tawfik, D.S. and Griffiths, A.D. (1998) Man-made cell-like compartments for molecular evolution. *Nature Biotechnology* **16**, 652-656.

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Harel, M., Aharoni, A., Gaidukov, L., Brumshtein, B., Khersonsky, O., Meged, R., Dvir, H., Ravelli, R.B.G., McCarthy, A., Toker, L., Silman, I., Sussman, J.L. and Dan S. Tawfik (2004) Structure and evolution of the serum paraoxonase family of detoxifying and anti-atherosclerotc enzymes. *Nature Struct. Mol. Biol.* **11**, 412-419.

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