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How Do We Find New Functional Proteins in Sequence Space?

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PURPOSE OF THE ABSTRACT

Functional proteins for a variety of useful applications, as binders and catalysts, are required, but currently not known. Functional metagenomics and directed evolution promise access to such new proteins, but the chances of finding them are low. Therefore high-throughput technologies are crucial to beat the odds: screening in picoliter water-in?oil emulsion droplets produced in microfluidic devices allow screening of >10e7 clones and permit successful selections While potentially faster, the vastness of sequence space (and the scarcity of ?solutions' in it) require strategies for the identification and interconversion of enzymes. In this context the role of ?promiscuous' enzymes, sequencing of full length of genes at high throughput (UMIC-seq) and insertion/deletion mutagenesis (using the transposon-based method TRIAD) will be discussed. Together with a molecular and mechanistic understanding new routes to functional can be charted.

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FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

directed evolution | functional metagenomics | enzyme mechanism | droplet microfluidics

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Florian Hollfelder is Professor for Chemical and Synthetic Biology at the Biochemistry Department of the University of Cambridge/UK. Born in Berlin he was trained at TU Berlin, was a visiting fellow at Stanford (with D. Herschlag), obtained his MPhil and PhD Degrees at Cambridge (with AJ Kirby) and worked at as a postdoc at Harvard Medical School (with C. T. Walsh). His group was started in 2001 at Cambridge and employs a broad multi-disciplinary approach that combines methods and ideas ranging from physical-organic chemistry to biophysics, molecular biology and directed evolution. High- and low-throughput approaches are combined with classical kinetic and thermodynamic analysis. For directed evolution, the group has developed microfluidic devices to carry our screening of up to 108 clones via assays in emulsion droplets at a picolitre scale. Such high throughput experiments are used to gain insight into the process of protein evolution for binders and catalysts, into strategies to identify new enzymes from metagenomic sources and, on a fundamental level, to investigate the origins of enzymatic rate accelerations. The mechanistic principles that emerge from this work will form a basis for development of transferable, general rules to guide future enzyme evolution and protein engineering.
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