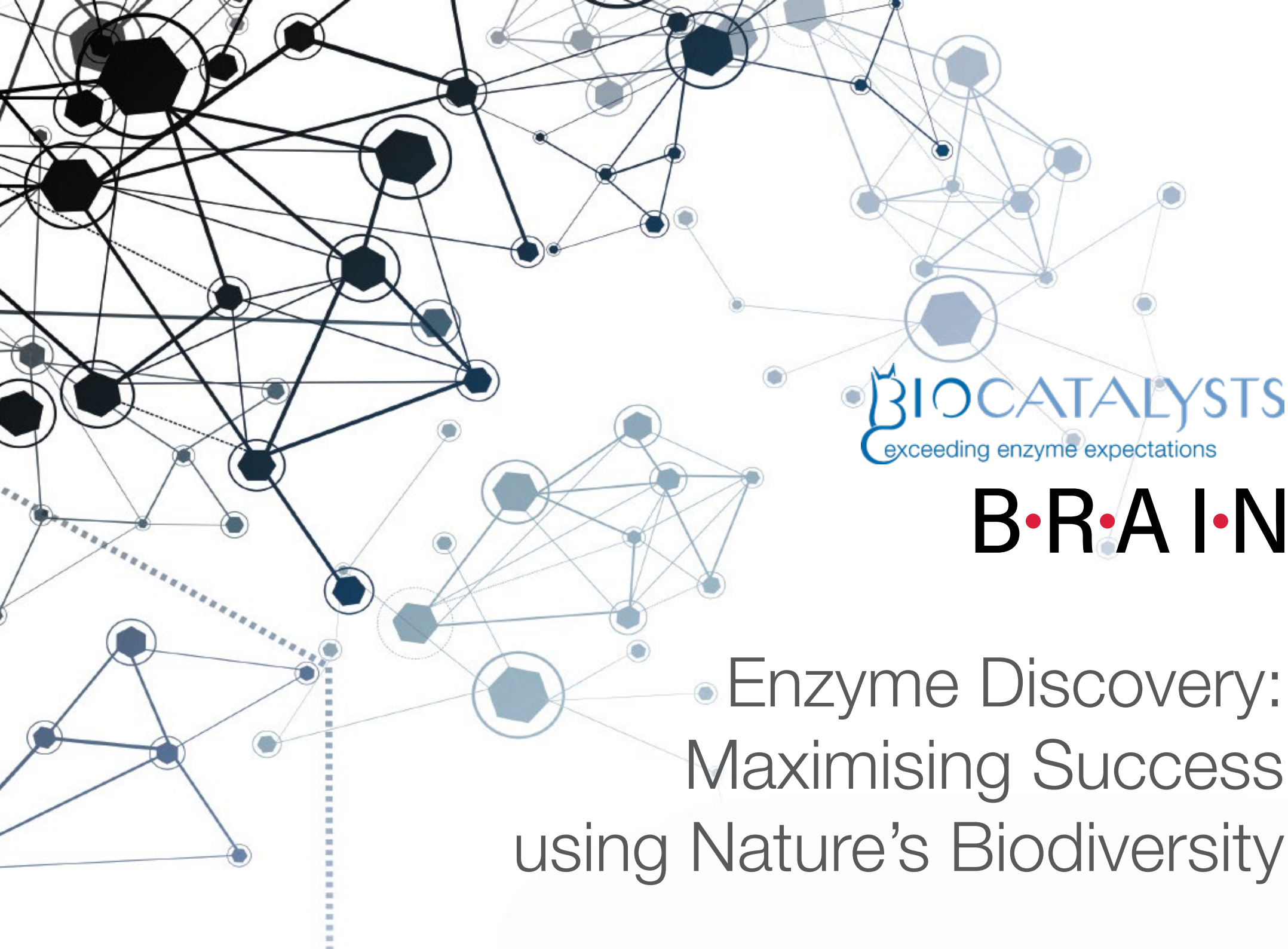

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Enzyme Discovery: Maximising Success using Nature's Biodiversity

Enzyme Discovery: Maximising Success using Nature's Biodiversity

Authors: Dr Mark Blight, Principle Scientist at Biocatalysts Ltd
Dr Alexander Pelzer, Unit Head Enzyme Technologies at BRAIN AG

Abstract

An introduction to Biocatalysts' and BRAIN's metagenomic based solutions to identify novel enzymes with specific functions to meet the requirements of our customers. We explain the differences and the benefits of 'Sequence-Driven' and 'Function-Driven Metagenomics' and why this powerful combination gives the best opportunity to access nature's biodiversity to select the right approach for your enzyme needs.

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Introduction

Metagenomics is the study of genetic material directly isolated from environmental samples. This broad field of study can also be referred to as environmental genomics, community genomics or ecogenomics. From the perspective of enzyme discovery, there are two primary approaches to metagenomics. Firstly, “Sequence-Driven Metagenomics”, where DNA is isolated from a given environmental sample, directly sequenced with Next Generation DNA sequencing technology creating an ideally unbiased yet “silent” in silico image of all genetic information present, followed by computational analysis. Metagenomic sequences are compared with public database sequences and identified genes collected into groups with similar predicted functions. Inferred function and functional distributions can then be assessed. Secondly, in “Function-Driven Metagenomics” DNA is again isolated from an environmental sample but is then directly cloned into plasmids, cosmids or fosmids. A recombinant host micro-organism is transformed with the library of clones, the metagenomic DNA is expressed and a functional screen performed to identify the desired enzyme function. This approach usually requires high-throughput screening of 105 to 106 clones and, therefore, the enzymatic screen is critical to the throughput capacity and duration of the project. Ideally, a natural or synthetic substrate that confers a selective growth advantage to its host or produces a measurable change (e.g. colour development upon cleavage) provides simplified screening of colonies on agar plates.

Both the Sequence-driven and Function-driven approaches have advantages and limitations. In the former case the principle advantage is that large numbers of similar enzyme classes can be rapidly identified by similarity to a known enzyme and these novel sequences then filtered to provide sub-sets for functional studies. The principle limitation is that if a metagenomic DNA sequence has no known homologue in the public databases then no functional annotation can be provided, and a relevant gene may be missed. In the latter approach, the main advantage is that enzymes identified by function are not required to have any sequence or structural similarity, therefore enabling the identification of novel functional classes with yet unknown sequences. The main limitation is that if the metagenomic gene(s) in the library clones are not expressed in the recombinant host (e.g. requirement for a specific transcriptional activator absent in the host) and remain “silent” then the enzyme cannot be identified by the activity-based screen.

Metagenomics has led to a wide variety of applications including human and animal health and nutrition, preventative medicine and understanding of disease, drug discovery, bioenergy, bioremediation, ecology, biodefense and biotechnology. In the industrial biotechnology sector, a unique metagenomics enzyme discovery platform is provided by Biocatalysts Ltd and BRAIN AG that offers both sequence-driven and function-driven metagenomics platforms that are specifically designed for the discovery of novel enzymes for commercial applications.

“Tapping into the wealth of enzymatic diversity found in nature is a key aspect of the technological approach within the BRAIN Group to offer our customers the opportunity to obtain previously undiscovered enzymes.“

Dr Jürgen Eck, Chief Executive Officer BRAIN AG

Biocatalysts' Sequence-Based Metagenomics Platform; MetXtra™

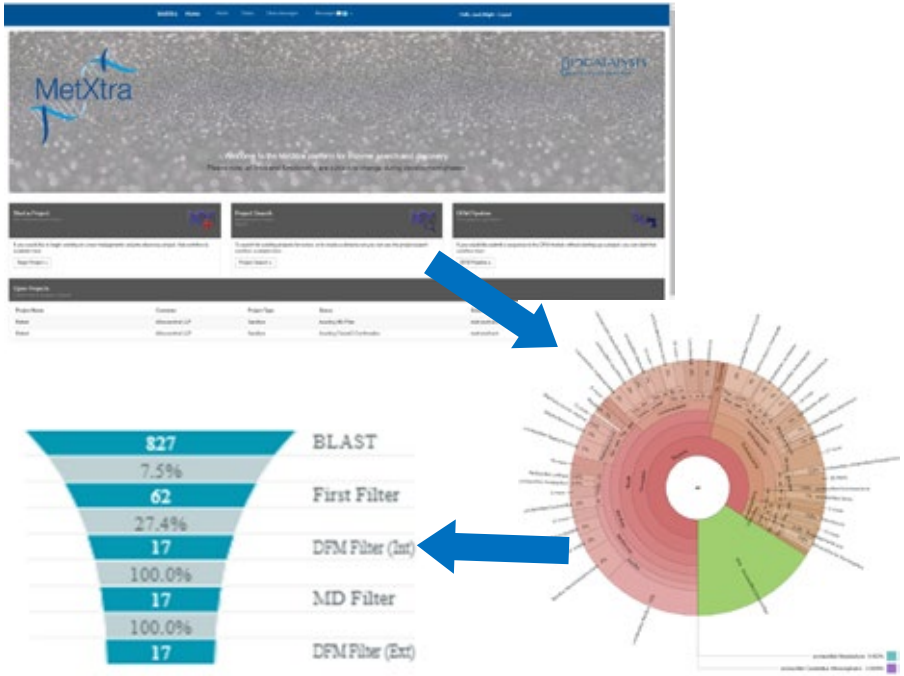
In September 2017, Biocatalysts Ltd. launched its metagenomics platform for novel enzyme discovery; MetXtra™. This platform comprises a unique software suite together with Biocatalysts metagenomic libraries, providing access to over 335 million non-redundant protein coding sequences. 98% of these proteins are unique to Biocatalysts and not found in the public domain. MetXtra™ enables the identification, analysis and RATIONAL selection of novel enzymes from these metagenomic libraries. The final output from a MetXtra™ search is typically 20 to 60 rationally selected enzyme sequences that are then produced using gene synthesis and expressed in vivo in one of Biocatalysts recombinant microbial hosts. Enzyme samples of approximately 1g of lyophilised powder are then produced in 3 days using Biocatalysts' bespoke Research Grade Sample (RGS) production platform. Packaged samples with appropriate documentation are then dispatched to the customer. The entire process from project inception to sample shipment is achieved in approximately 4 weeks, with much of this time being required for gene synthesis.

MetXtra™ is a Sequence-Based metagenomics platform that can screen large metagenomic libraries based on homology to a known sequence or by domain functionality using Interpro Pfam matrices. An important unique feature are the more than 20 automated bioinformatics tools that provide simultaneous analyses of these sequences. The analysis of the selected enzyme sequences is aligned with Biocatalysts' Design for Manufacture (DFM) principles, whereby enzyme candidates are selected based upon predictive algorithm outputs that maximise the probability that the enzyme can be not only highly expressed in vivo but also be a robust candidate for manufacturing process scale-up.

How do customers benefit from MetXtra™?

- Process optimisation – find a better enzyme solution to the current one.
- Freedom to operate – identify enzymes outside of existing IP restrictions
- Rational not random, selection of metagenome enzyme panels.
- Bioinformatics tools developed based around DFM principles.
- Output feeds directly into Research Grade Sample (RGS) pipeline – fast, efficient, cost-effective production of 1g enzyme samples.
- Unique product offering – unavailable elsewhere

The overall benefit to customers is an increased chance of successfully obtaining the desired enzyme for their application. Since the launch of MetXtra™ Biocatalysts has completed over 10 customer and 2 internal MetXtra™ enzyme discovery projects with a 100% success rate in delivering highly expressed, active soluble enzyme sample panels and a 90% success rate at delivering at least one candidate per RGS enzyme panel that met the required customer specifications. Customer applications for MetXtra™ enzymes include; pre-pro-biotic development, animal feed production, beverage market, dairy industry, pharmaceutical API production and artificial sweetener development.



BRAIN's Function-Based Metagenomics Platform; ABEL® and LIL®

BRAIN's metagenomic libraries are Activity-Based Expression Libraries (ABEL®) and Large Insert Libraries (LIL®). ABEL® libraries are made using plasmids with clones expressing mostly single genes, while LIL® libraries contain cosmids or fosmids with inserts that can carry large genes or small operons (gene clusters). LIL® libraries can be transferred to multiple host's, which broadens the range of enzyme encoding genes and even complete pathways that are functionally expressed.

BRAIN provides an extensive ready-to-screen resource of ABEL® and LIL® libraries, the former comprising the equivalent of ~ 148 million genes, the latter representing more than ~ 66 million genes. Insert DNA mostly originates from diverse soil samples, but was also recovered from marine habitats, microbiomes as well as specific collections of microorganisms from the BRAIN BioArchive. According to the customer's requirements, tailor-made ABEL® and LIL® libraries can be constructed from rationally selected and optimal substrate conditioned microbial habitats which greatly enhances the likelihood of tapping into an enriched pool of biodiversity containing the relevant enzyme activity. Total extracted metagenomic DNA can be randomly cloned and introduced into the screening host. Alternatively, genes encoding variants of the customer's enzyme of interest can be directly recovered and transferred to a targeted ABEL® library, making use of the functional microdiversity present in highly diverse habitats. Targeted gene libraries increase the hit rate of functional enzyme variants with distinct enzymatic properties to >90%.

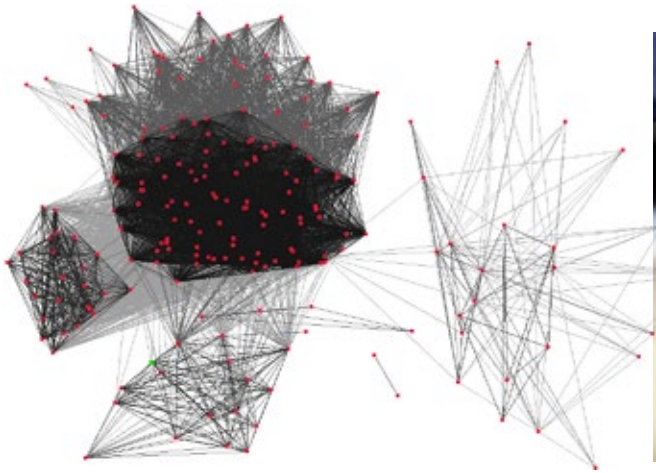
Functional screening for a given enzyme activity preferably involves a substrate that provides a growth advantage to the screening host upon conversion or a colour change, both efficiently detectable on agar plates. Screening in microtiter plates (96-well, 384-well) is also feasible as well as in FACS format. In the absence of substrates for growth selection or colorimetric detection, more complex screens can also be used, based on chromatographic methods and other techniques (GC/MS, GC/FID, LC/UV, LC/MS, LC/ELSD, HPAEC, ICP/MS, TLC) all are available as part of B.R.A.I.N.'s analytical platforms. Depending on the complexity of the screening assay, typically between 10,000 and > 1,000,000 clones are screened per project.

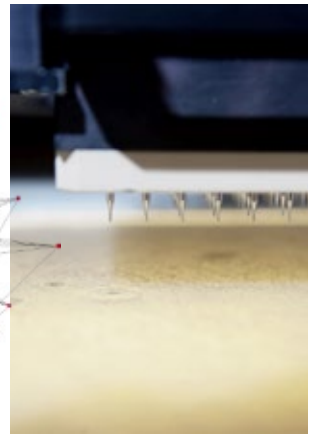
The typical time taken for a customer project is between 4 to 12 months for the generation and screening of a customer specific tailor-made library. The customer receives the enzyme encoding gene sequence, a plasmid containing the novel sequence and a data package summarizing the activity screening.

How do customers benefit from ABEL® and LIL®?

- Find novel enzymatic solutions for catalysing a given chemistry.
- Freedom to operate – identify enzymes outside of existing IP restrictions.
- 100% of all identified enzymes are active.
- No artificial activity – enzyme identification using application relevant conditions possible.
- High success rate by creating tailor-made libraries according to the customer's requirements.

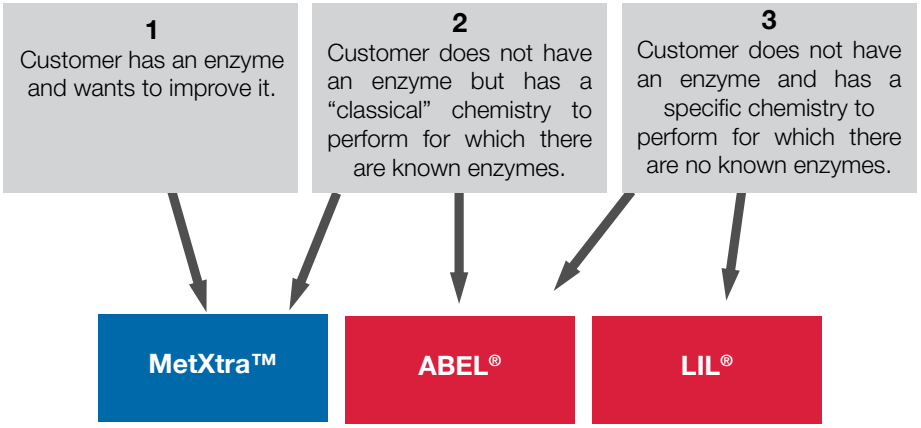
The benefit of BRAIN´s activity based screening approach is that a customer has the chance to identify candidates performing like no known model enzyme and has the greatly increased likelihood of obtaining active candidates outside of IP restricted sequence space. Rationally selecting microbial communities prior to activity-based metagenomics analysis is a powerful approach to obtain relevant enzyme hits. Utilising customized functional screens BRAIN has successfully identified and recovered numerous active, soluble enzymes from its proprietary LIL® and ABEL® metagenomic libraries. These have been used by customers for applications in chemical synthesis, detergent, textile and food industries, some of the enzyme classes recovered include proteases, oxygenases, transferases and amylases.





Guide to Selecting the Right Approach for your Enzyme Project

The diagram below illustrates a logic flow for determining the most appropriate metagenomics project approach for a given enzyme discovery situation. Essentially, the selection of the appropriate metagenomics solution depends upon the initial requirements. Although there will be a wide variety of specific customer requirements, typical projects fall into 3 main categories.



In case 1 the most appropriate solution is to use Biocatalysts' MetXtra™ platform to identify similar enzymes based upon amino acid sequence homology. This can be further refined to produce a panel of candidate enzymes that are either closely or distantly related to the customer's current enzyme. Closely related sequences enable the identification of candidates that have a high probability of performing the same enzymatic bioconversion but may have other attributes such as altered substrate specificity, altered Km Kcat or an altered activity profile with respect to factors such as temperature and pH. Selecting a panel of distantly related candidates can be used to identify much broader characteristics within the same set of parameters but with the potential of a wider spectrum of choice. The risk with the latter scenario is that some candidates may no longer provide the desired bioconversion for the customer application. MetXtra™ can be used to refine these two approaches further by selecting enzyme candidates with origins in metagenomes isolated from extreme environments. For example, if you are looking for enzymes that are active at low or high temperatures, MetXtra™ can filter candidates based upon the metagenome metadata (i.e. data that indicates the environmental parameters from where the metagenome sample was isolated) to look only for enzymes from cold or hot environments. This can be extended to other environments that have extremes of pH (e.g. alkaline soda flats, acid peat bogs) or salinity, for example. The initial filtered candidate set is then passed through the MetXtra™ DFM algorithms to further refine the list to candidates with a high probability of achieving in vivo production levels commensurate with commercial targets.

In case 2, the possibility exists to either use MetXtra™ and, rather than a homology-based search, the candidate set is identified using a Pfam functional domain search. Alternatively, a direct screen for candidates based upon actual enzymatic activity can be performed using BRAIN's ABEL® libraries.

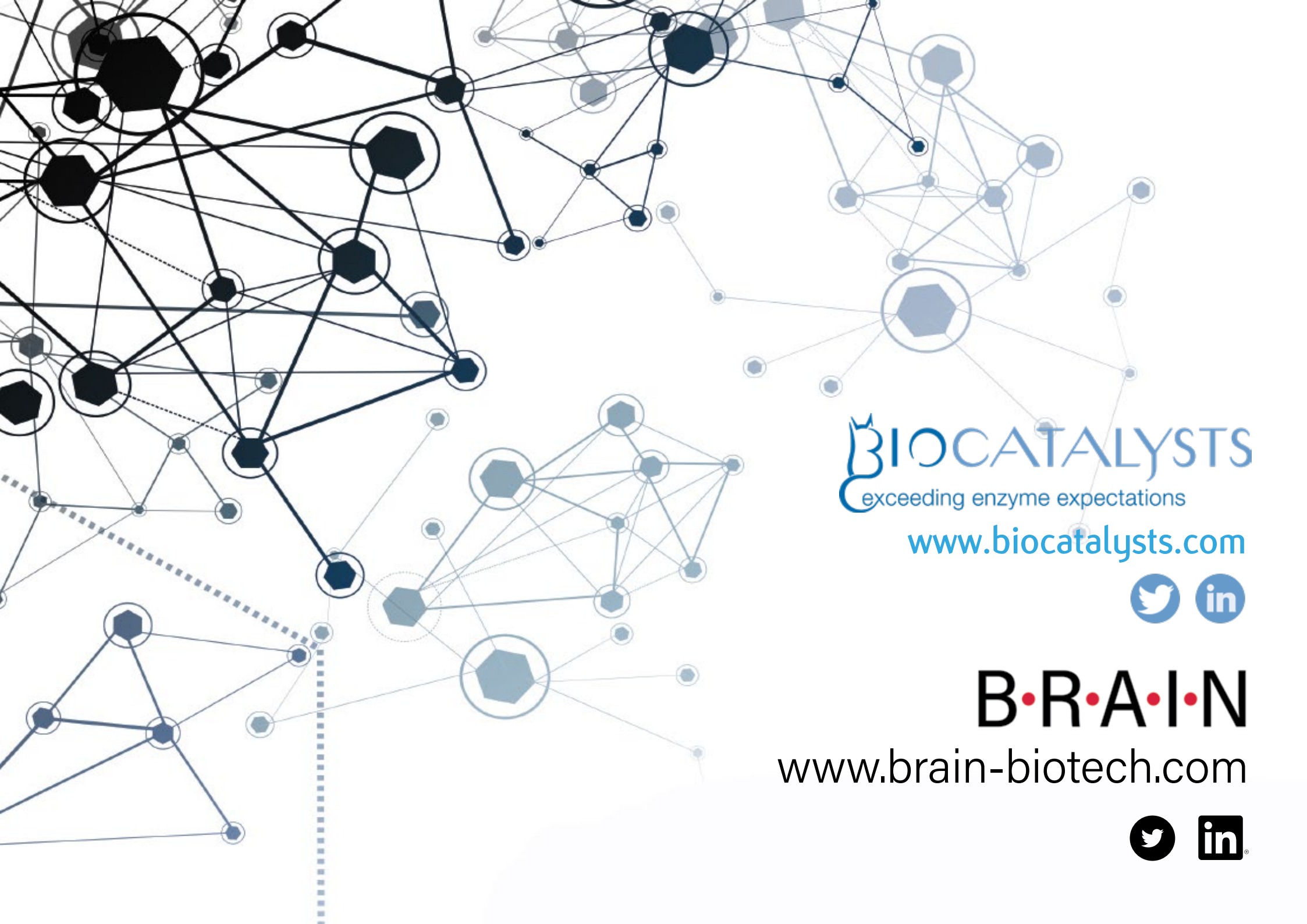
Finally, in case 3 the most appropriate choice would be to use BRAIN's ABEL® libraries to identify single enzymes that perform the desired chemistry or the LIL® libraries to identify potential multiple enzymes required to perform the desired chemistry that may be in encoded in operons.

As described above, each approach has its advantages and limitations, as one would expect. However, the overall potential output of this powerful combined offering is clear Jürgen Eck explains that "Together Biocatalysts and BRAIN offer a winning combination of technical solutions to directly extract identify and produce the right enzyme candidates for any application needs. Both activity-based strategies and the MetXtra platform offer a fast and efficient way of bringing metagenomic enzymes into application for our customers."

Biocatalysts and BRAIN –
Maximising Success Using Nature's Biodiversity for Enzyme Discovery

Contact us today to discuss the possibilities for your application!

enquiries@biocats.com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