Droplet Microfluidics Accelerates Enzyme Discovery

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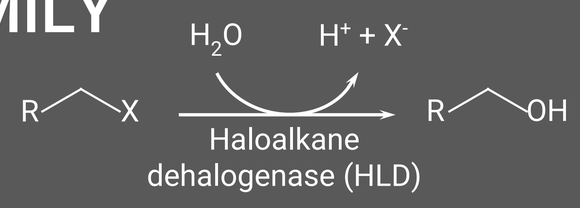
INTRODUCTION

- **New enzymes are needed** for industrial, medical and environmental applications.
- **Natural diversity** captured in the sequence databases is widely **underexplored** .[1]
  >200,000,000 uncharacterized sequences
- **Smart exploration** of this “treasure” is necessary!

WORKFLOW

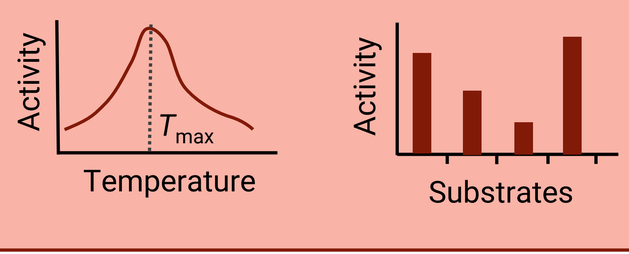
1. Sequence Bioinfromatics [2] (ENZYME MINER) >24K SEQUENCES → Structure Bioinfromatics >2.5K MODELS → Hits Prioritization 45 SELECTED GENES
2. Expression Analysis 40 EXPRESSED GENES → Biochemical Characterization 24 SOLUBLE ENZYMES → Mechanistic Insight 6 BEST ENZYMES

MODEL ENZYME FAMILY



MicroPEX

Microfluidic Profile EXplorer[3]



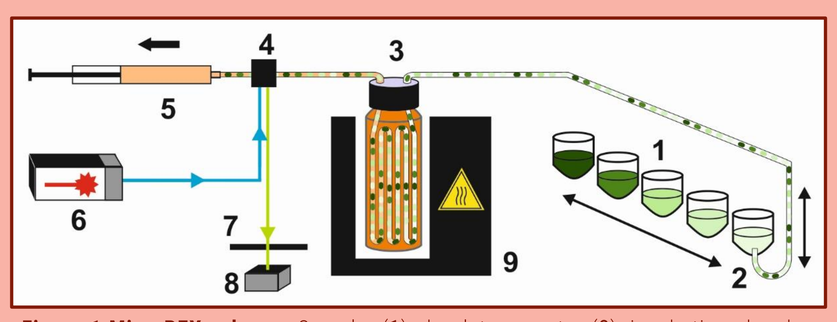
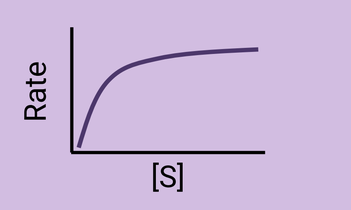


Figure 1 MicroPEX scheme. Sampler (1), droplet generator (2), incubation chamber containing substrate (3), detection box (4), syringe pump (5), laser source (6), kinematic cube with filter (7), detector (8), temperature controller (9), substrate (orange) and enzyme variants (green).

KinMAP

Kinetic Microfluidic Autonomous Platform[4]



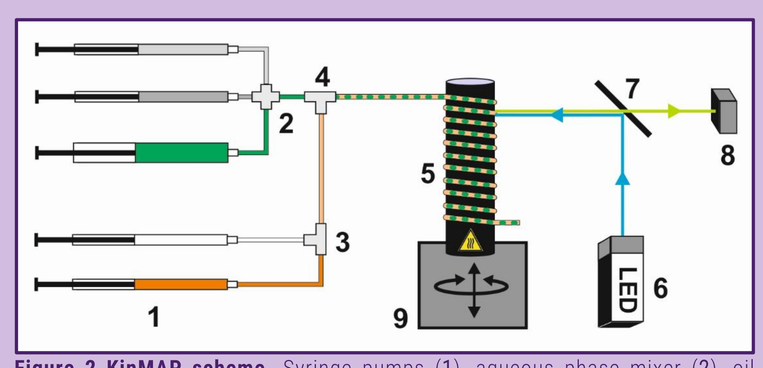


Figure 2 KinMAP scheme. Syringe pumps (1), aqueous phase mixer (2), oil phase mixer (3), droplet generator (4), incubation coil wrapped around heating rod (5), LED source (6), kinematic cube with dichroic mirror and filters (7), aspheric lens and optical fiber (8) connected with detector (not shown), motorized stages (9).

RESULTS

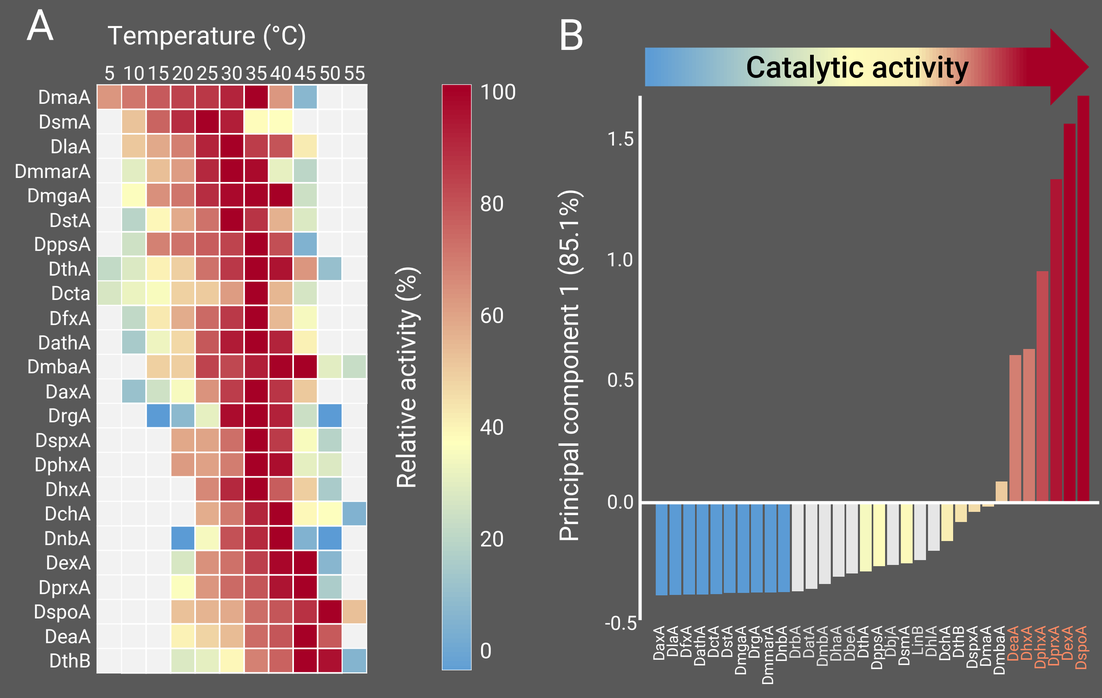


Figure 3 MicroPEX characterization. (A) Temperature profiles. The heat map represents the relative activity of individual enzymes. (B) Multivariate analysis of catalytic activity. The score plot of the principal component analysis compares the enzymes in terms of their overall activity with 27 substrates and explains 85.1% of the data variance. The previously characterized HLDs are colored grey. The heat maps (A) and bars (B) are color-coded by enzymatic activity from low activity (blue) to medium activity (yellow) and high activity (red).

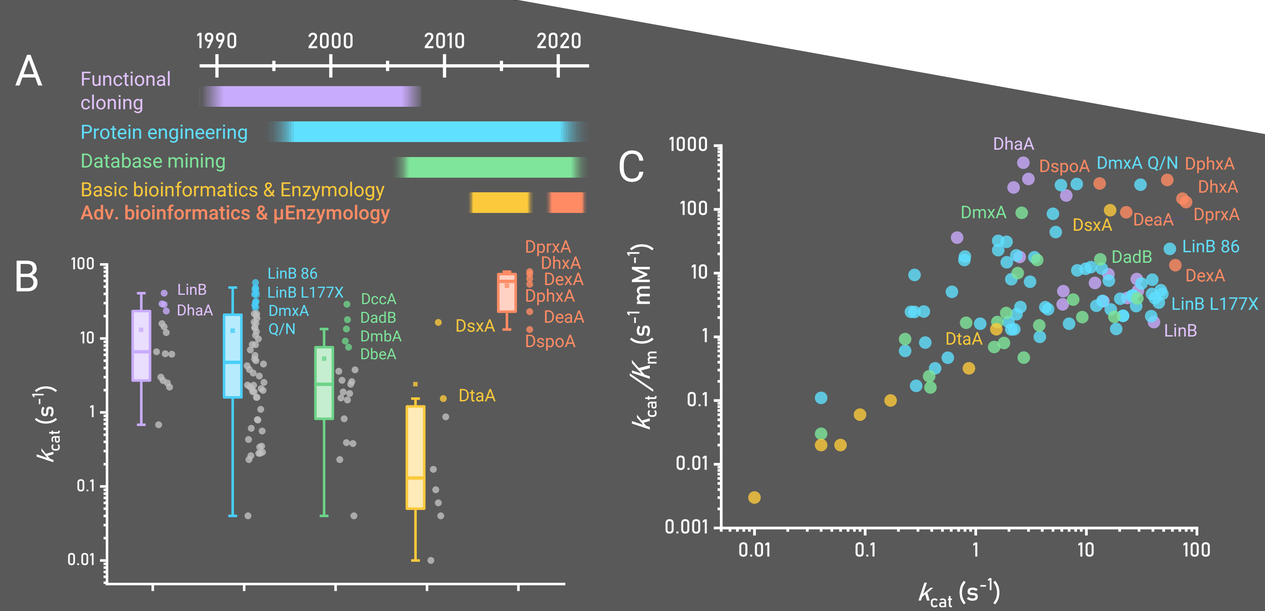


Figure 4 Functional characteristics of the HLD family members. (A) Functional cloning (purple), protein engineering (blue), database mining (green), basic bioinformatics & enzymology (yellow), and advanced bioinformatics & µEnzymology (red, present study, KinMAP). (B) Box chart comparing turnover numbers for enzyme variants obtained by respective strategies. The box shows median (line), mean (small square), quartiles, minima, and maxima. (C) The dependence of catalytic efficiency on turnover numbers provides the complex catalytic evaluation of enzymes.

CONCLUSIONS

- Adv. Bioinformatics & µEnzymology accelerate the discovery of new enzymes.[4]
- Obtained biocatalysts outperform previously-known wild-type & engineered HLDs.

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