Directed evolution of oxidoreductases using single-cell hydrogel capsules

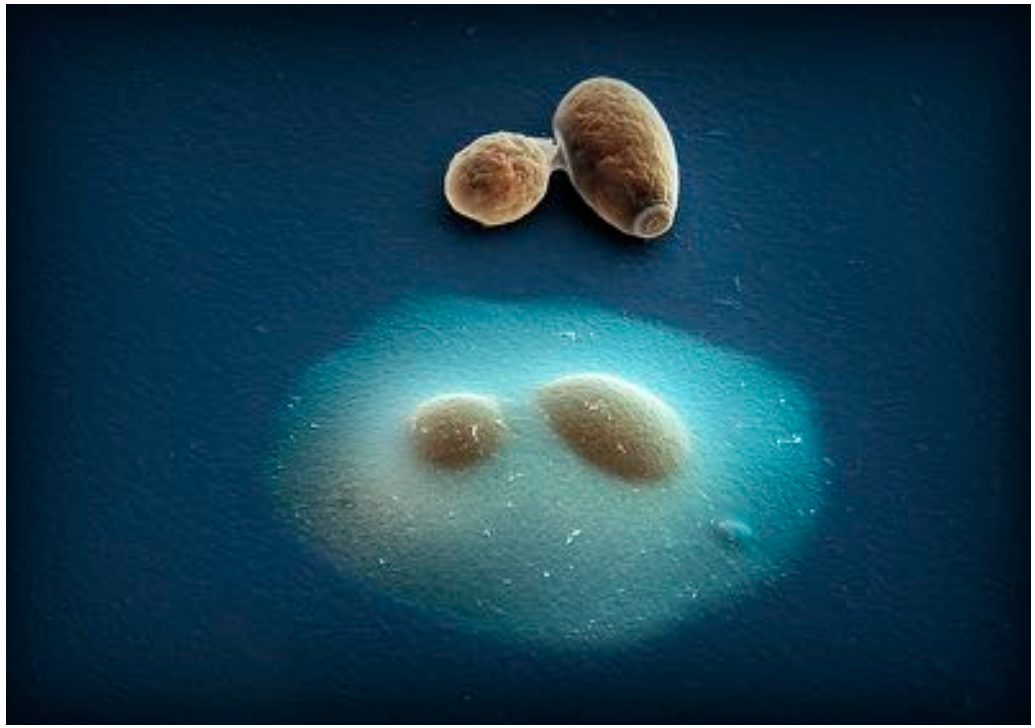

Image: Martin Oeggerli

Michael A. Nash

September 25, 2019

Catalysis Workshop Basel

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Department of Chemistry

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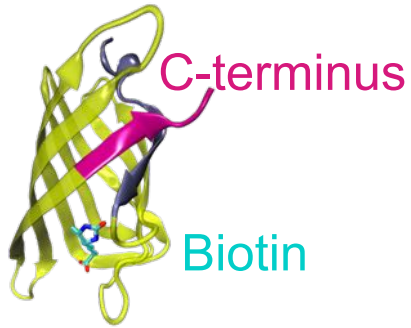

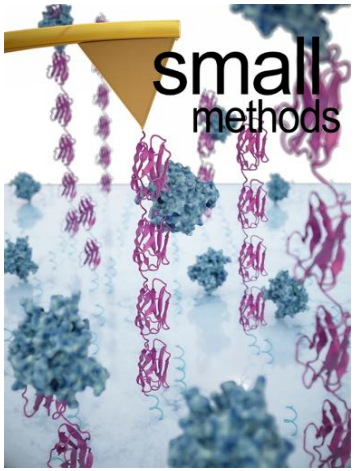

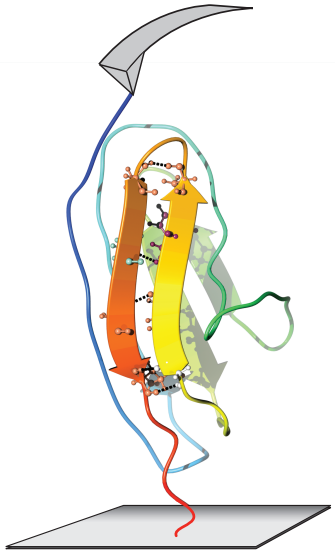

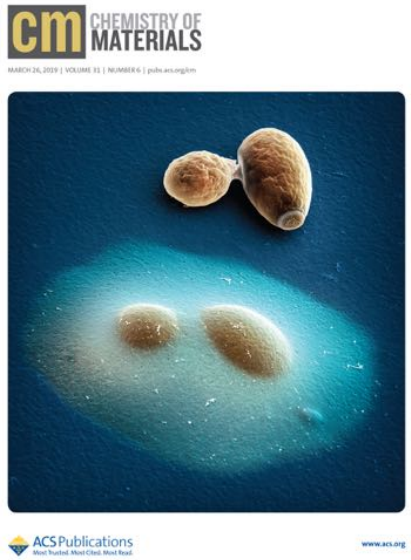

Biophysics, biotechnology, nanotechnology, bioengineering, protein engineering, directed evolution, artificial systems

Acknowledgments

<u>UniBasel/ETH:</u>
Mariana Santos
Ivan Urosev
Zhaowei Liu
Joanan Morales
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Haipei Liu
Byeongseon Yang
Fanny Risser
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Regina Dönen

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Sai Reddy (ETHZ)
Fabian Rudolf (ETHZ)
Florian Seebeck (Uni. Basel)
Cheemeng Tan (UC Davis)
Philip Tinnefeld (LMU München)

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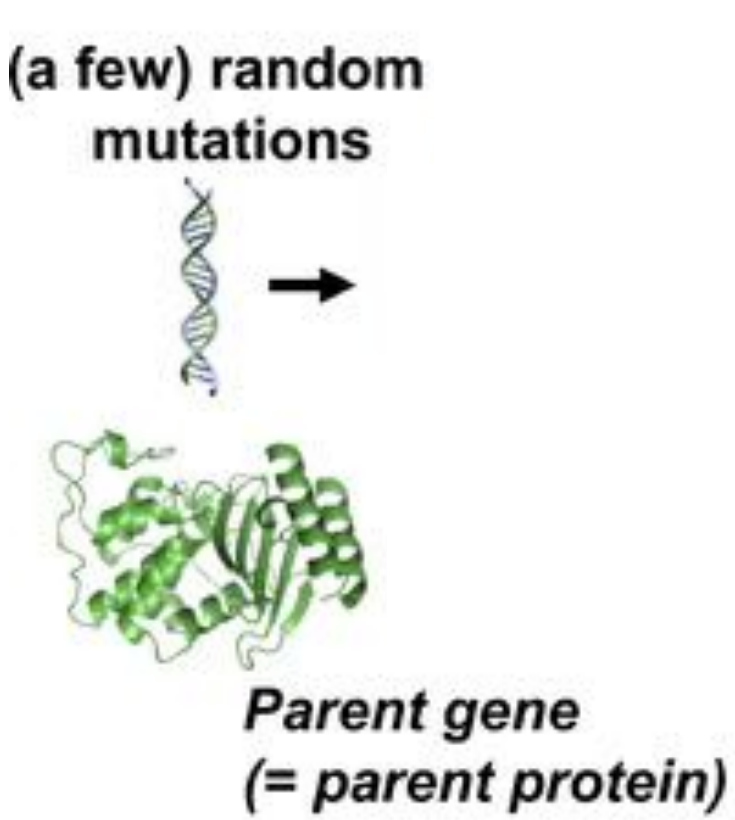
life science zurich graduate school

SNI Swiss Nanoscience Institute
Eine Initiative der Universität Basel und des Kantons Aargau

Using accelerated evolution to create new enzymes with improved properties



www.pnas.org cgi doi 10.1073 pnas.0901522106

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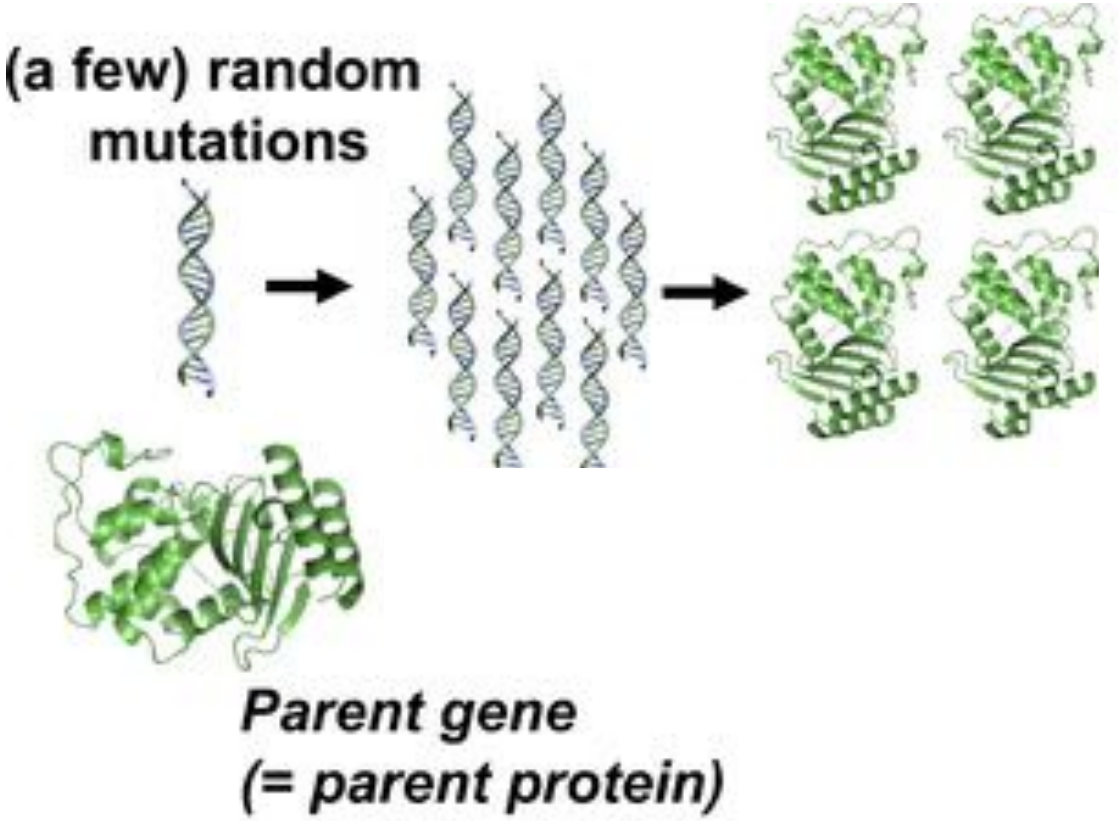

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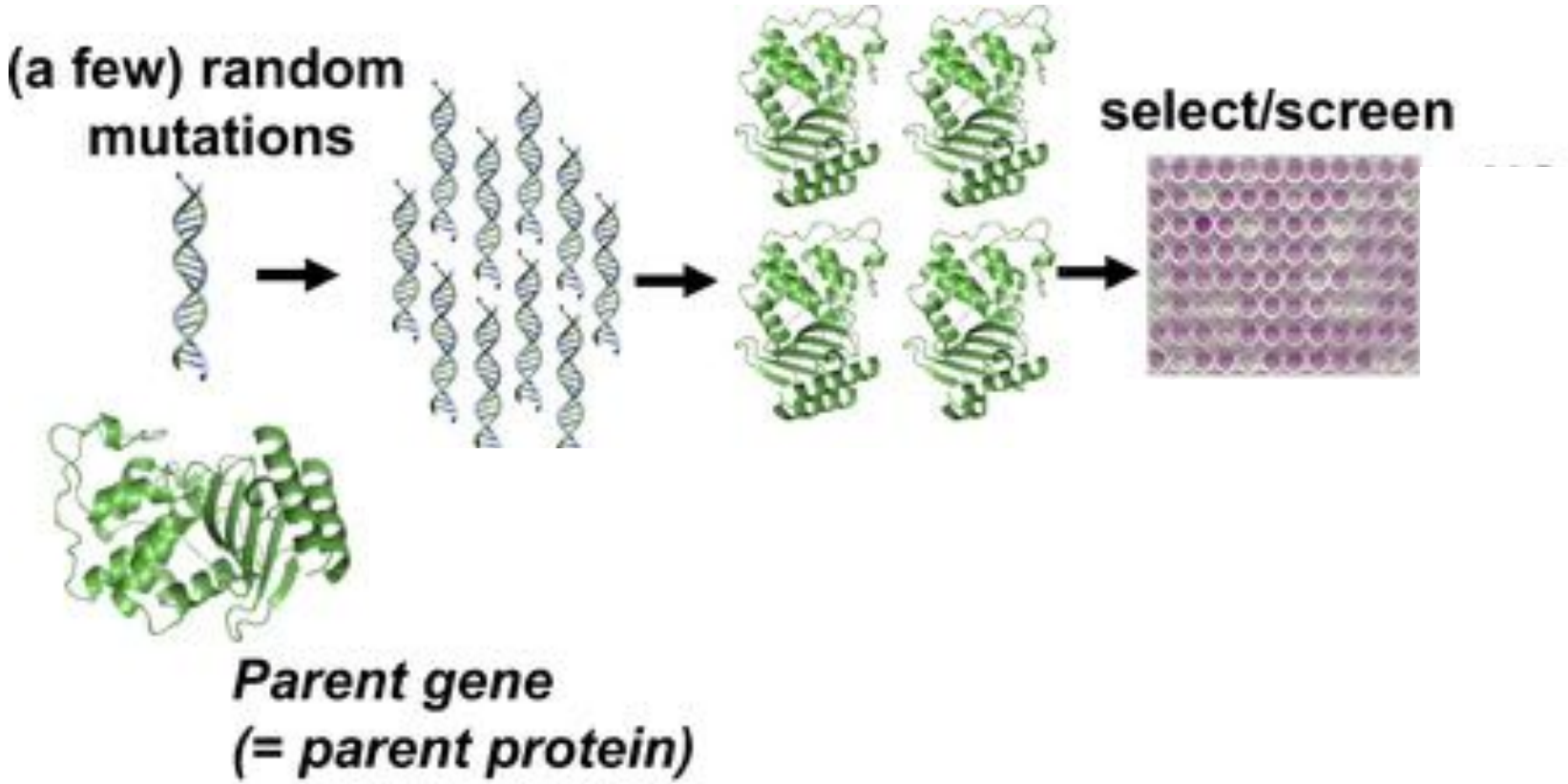

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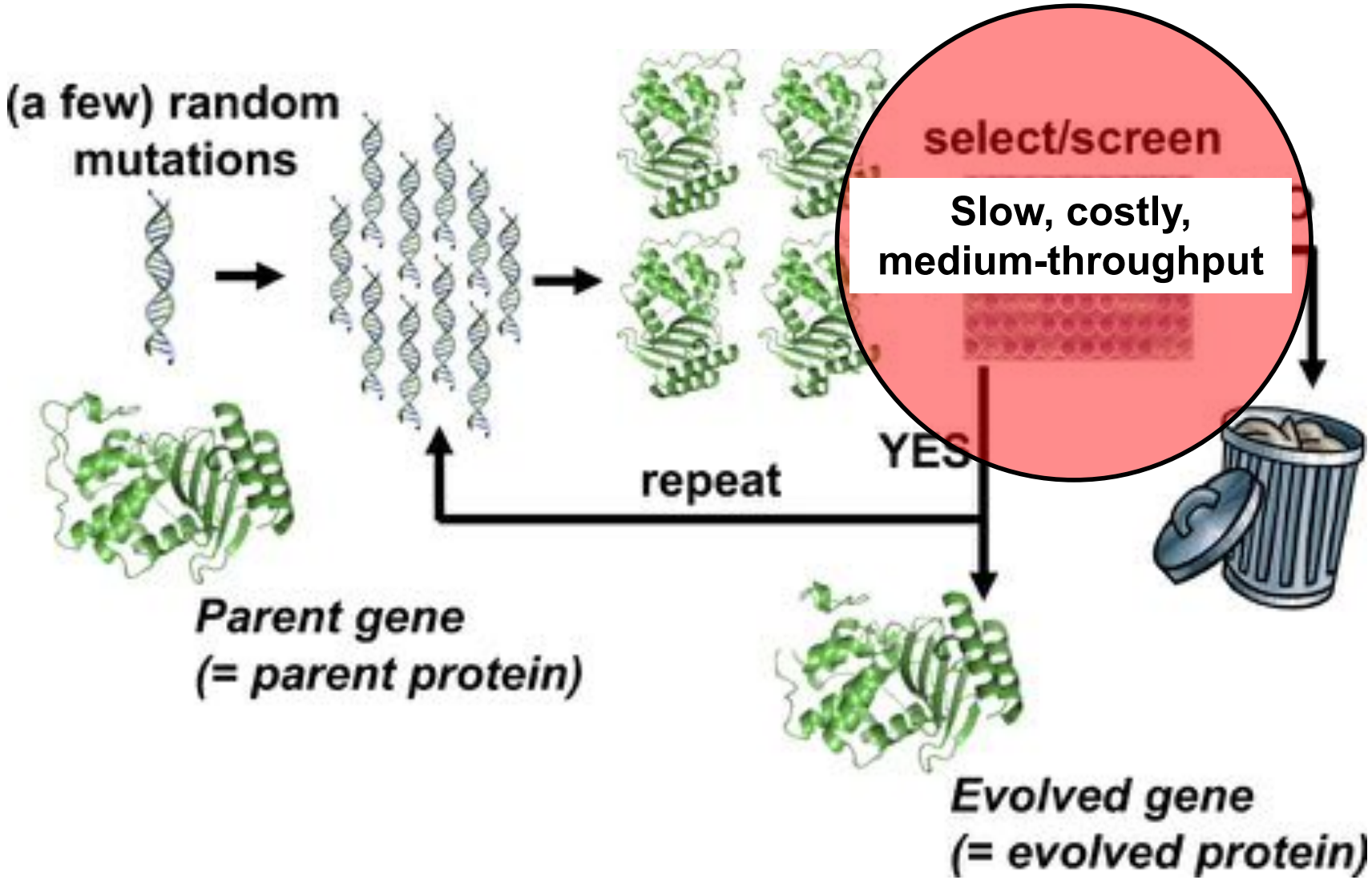
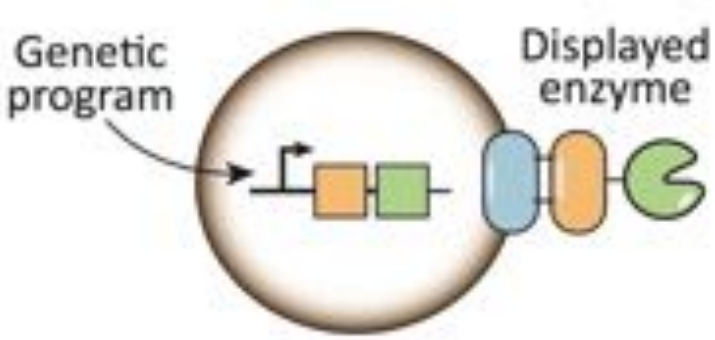


www.pnas.org cgi doi 10.1073 pnas.0901522106

Enzyme-mediated cell-gel encapsulation technology for ultrahigh throughput library screening



European priority patent application EP18184204
Chemistry of Materials, DOI: 10.1021/acs.chemmater.8b04348, 2019.
Biotechnology and Bioengineering, DOI: 10.1002/bit.27002, 2019.

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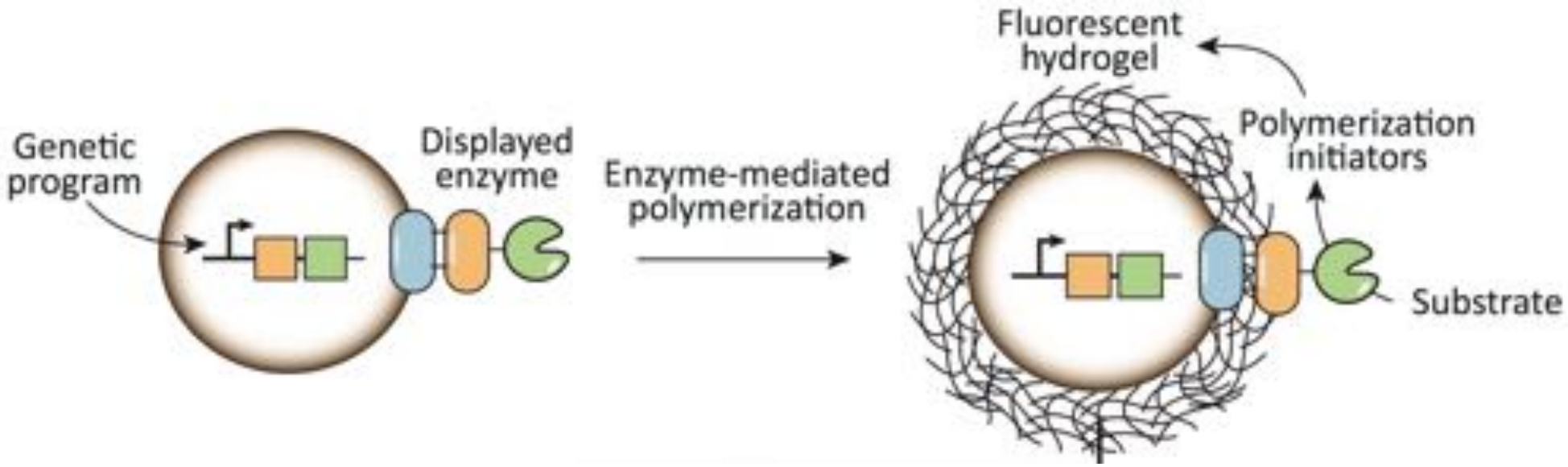

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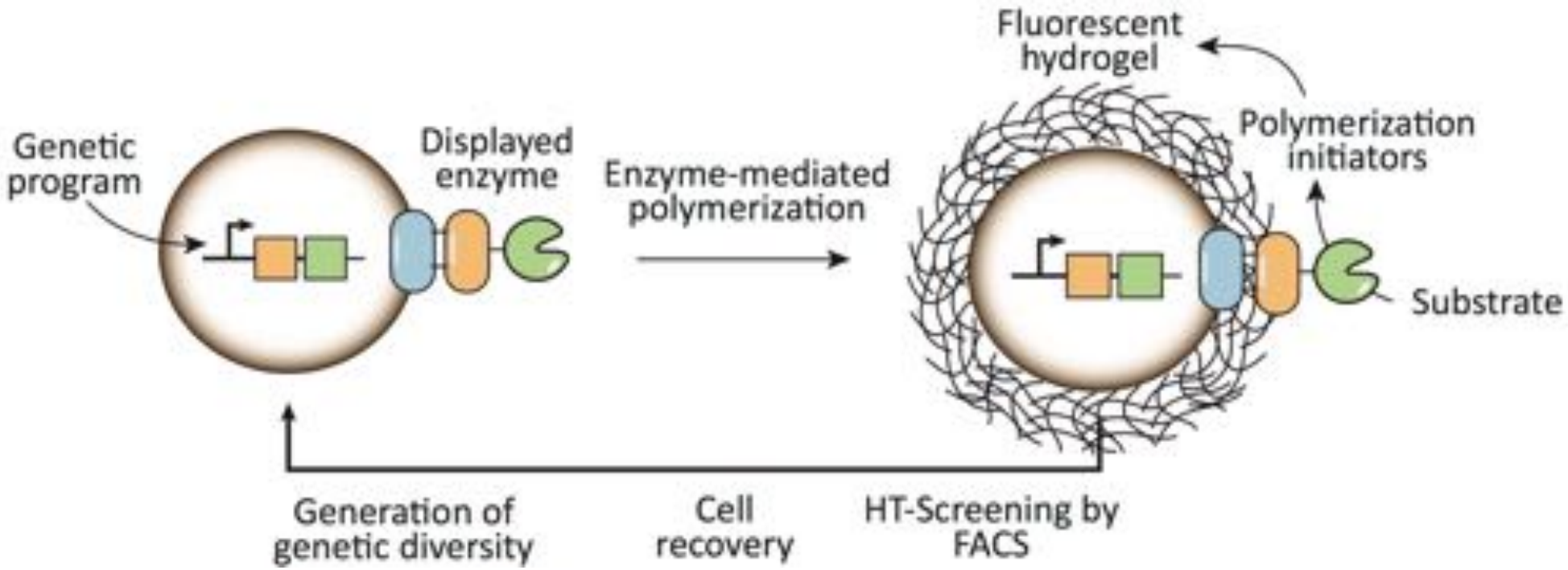

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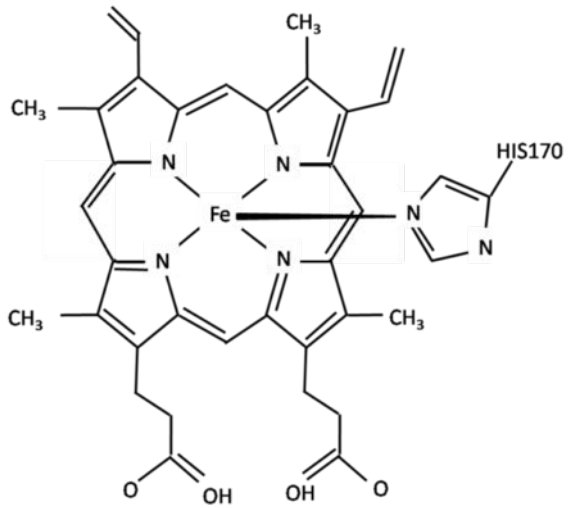
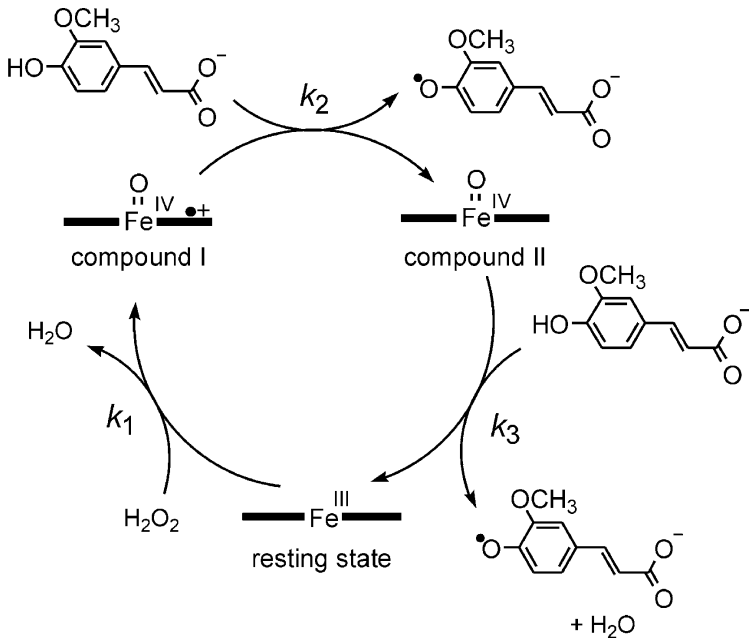
Biotechnology and Bioengineering, DOI: 10.1002/bit.27002, 2019.

Peroxidase enzymes can initiate radical polymerization

- Heme-enzyme that catalyzes the reactions of H_2O_2
- Oxidizes a wide range of organic and inorganic substrates (promiscuous) according to: $\mathrm{H_2O_2 + AH_2 \rightarrow 2\,H_2O + A}$



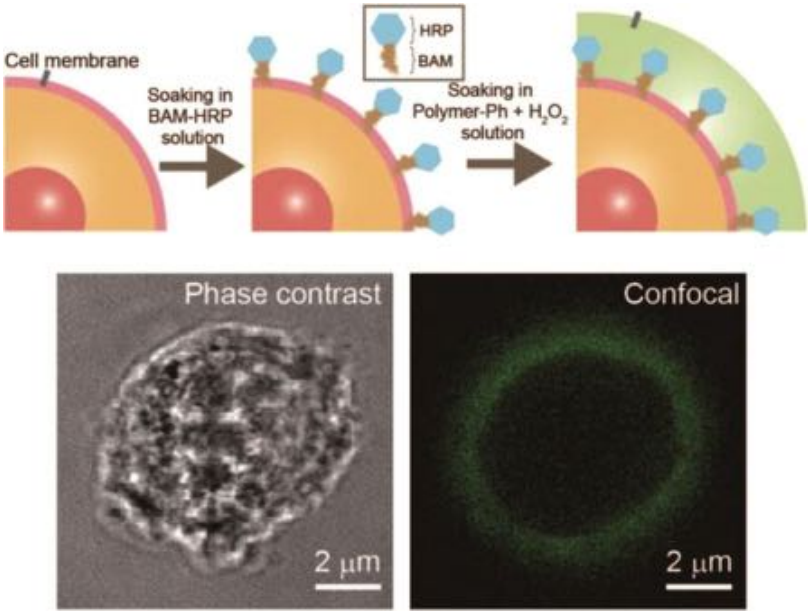
Structure of the porphyrin heme group in horseradish peroxidase.



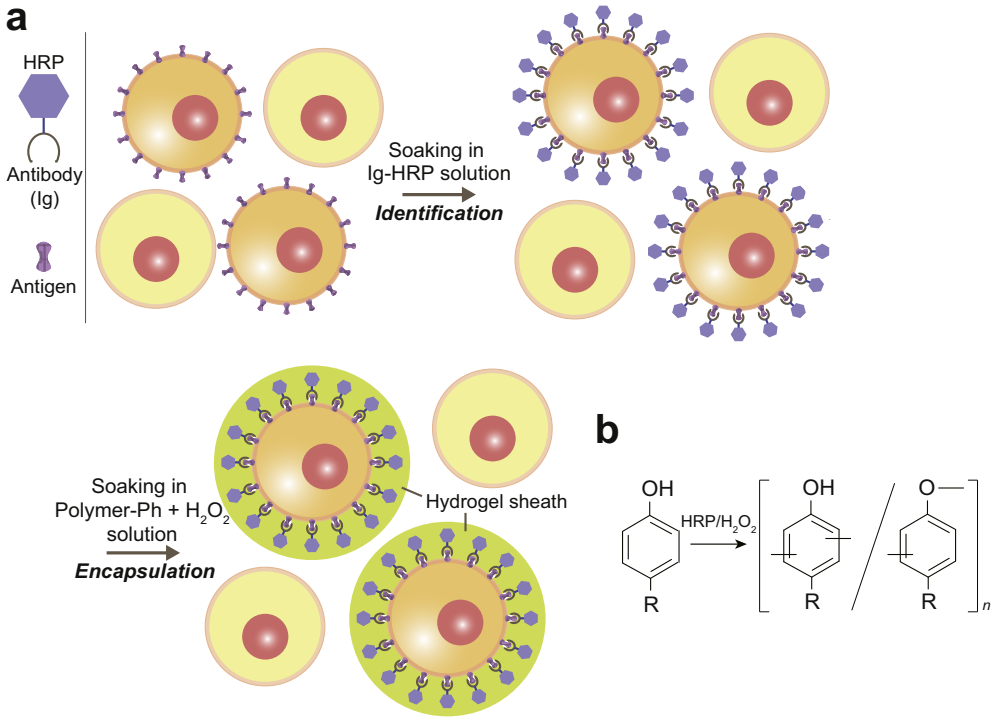
Catalytic cycle of horseradish peroxidase.

Peroxidase-mediated cell encapsulation using bio-specific recognition

- Biospecific cell labelling followed by *in situ* hydrogel polymerization



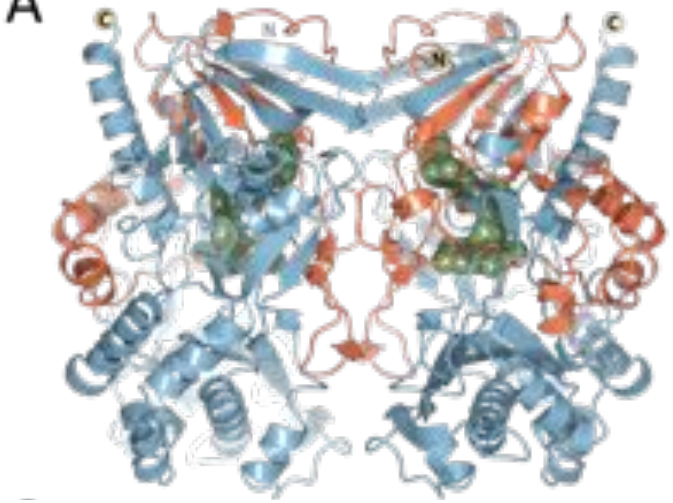
ACS Macro Lett., 3(10): 972–975, 2014.



Biomaterials 53, 494e501, 2015.

Evolving GOX activity and stability using gel-encapsulation system

A



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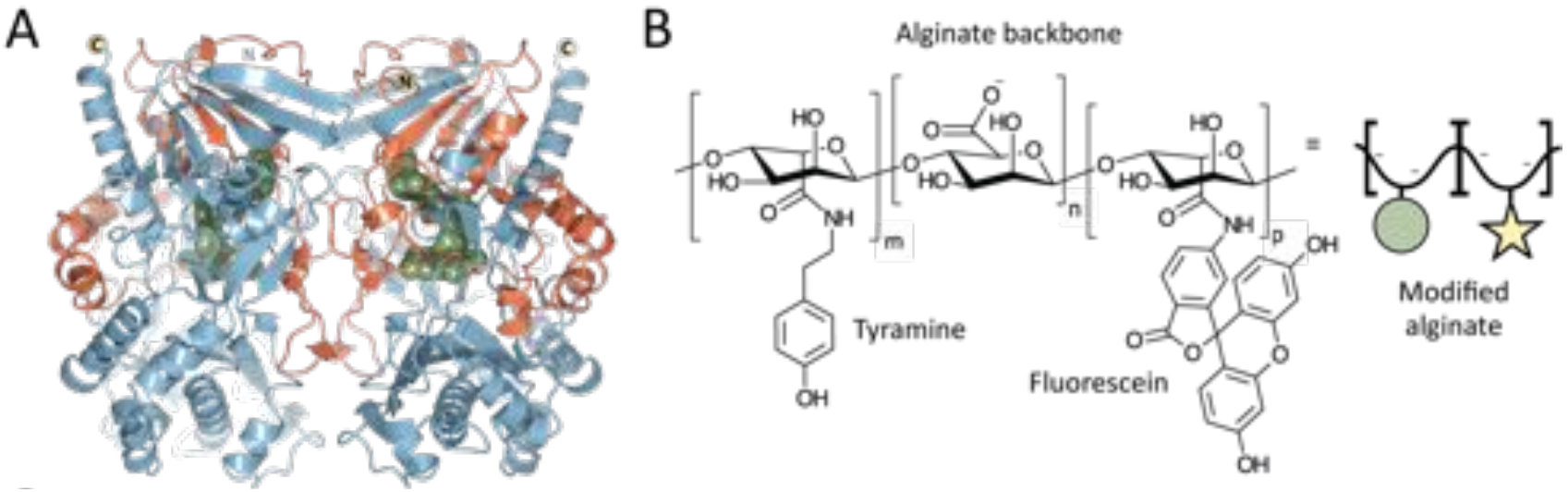

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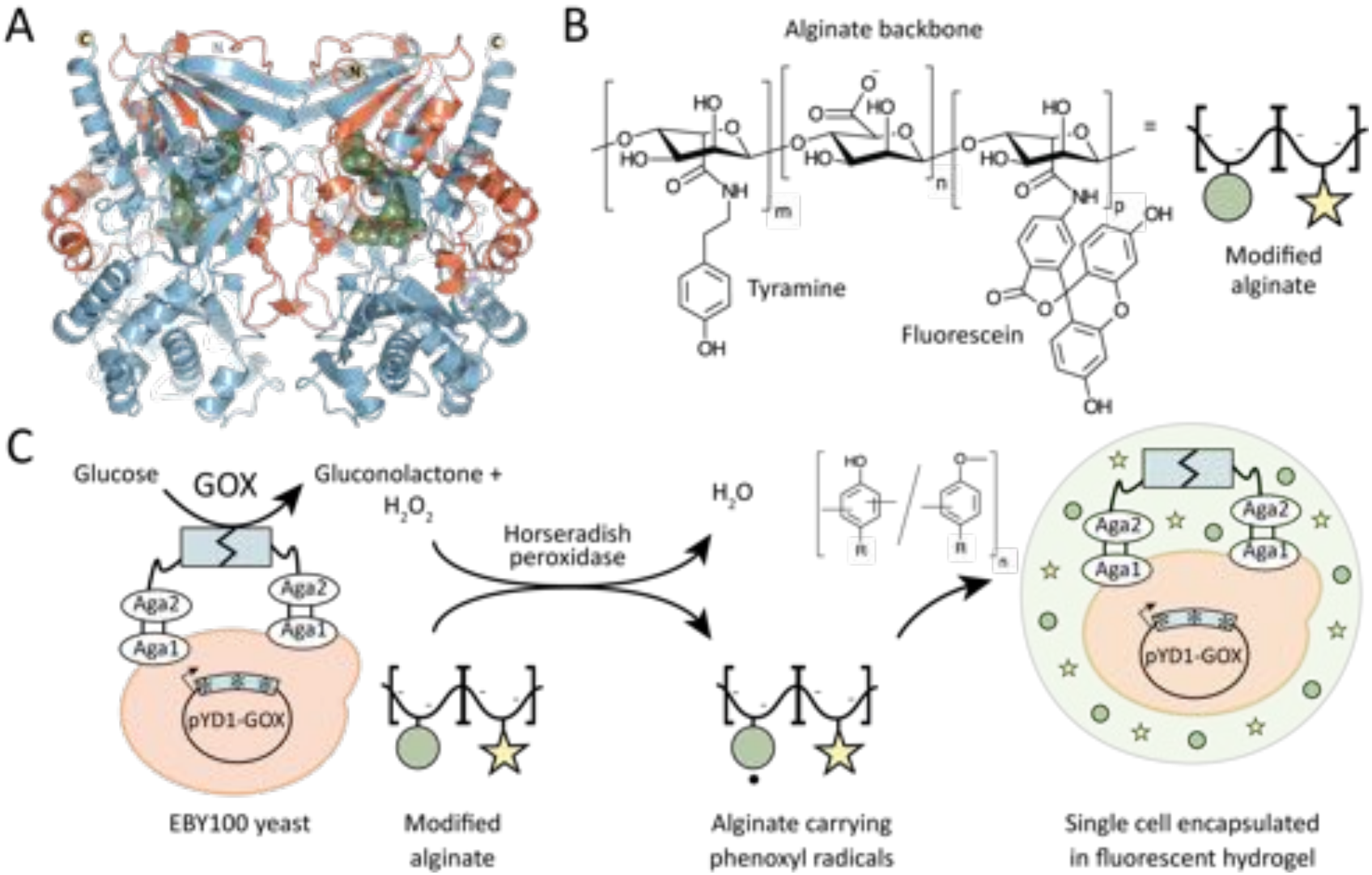
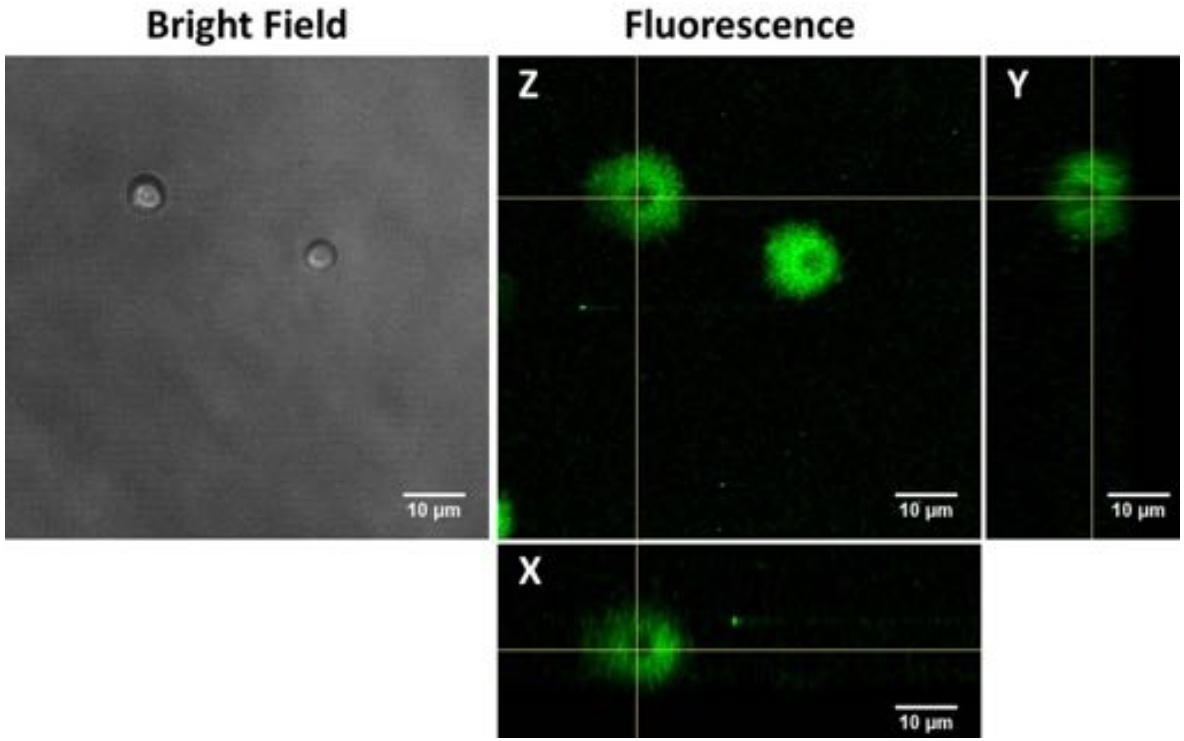
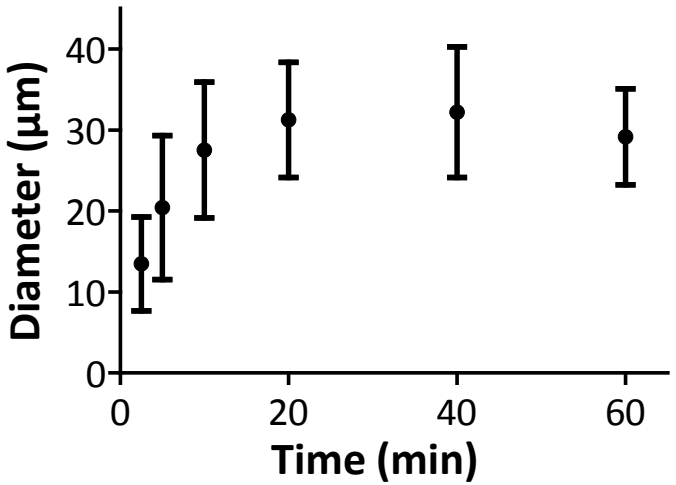


Confocal microscopy shows singe cells encapsulated in conformal hydrogel

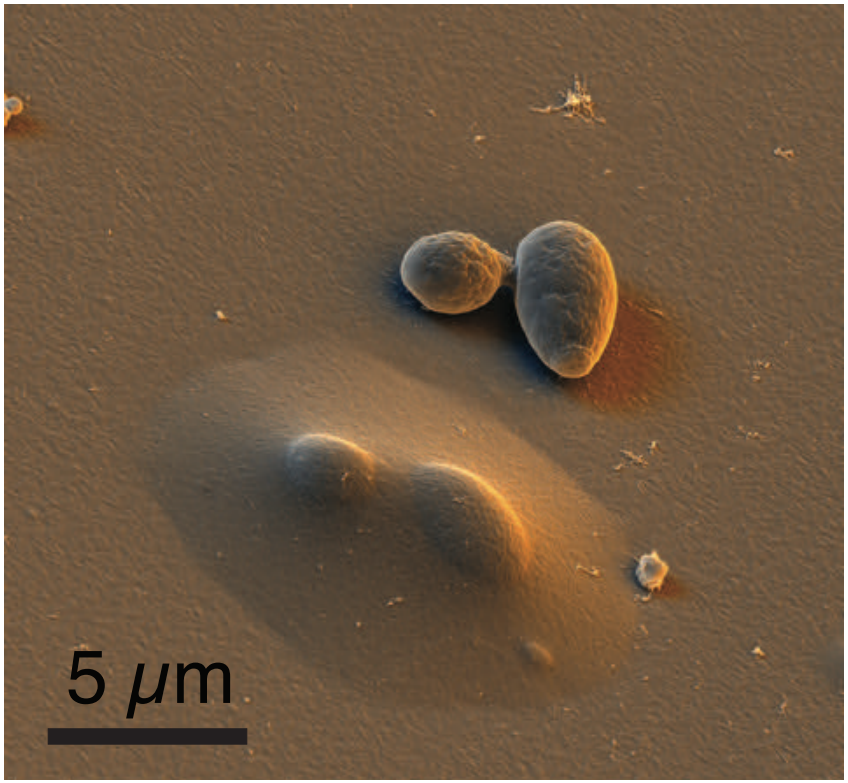




Hydrogel coating is conformal and uniform with diameter <30 µm (adequate for flow cytometry)

SEM Imaging confirms single encapsulated yeast cells or budding yeasts

A



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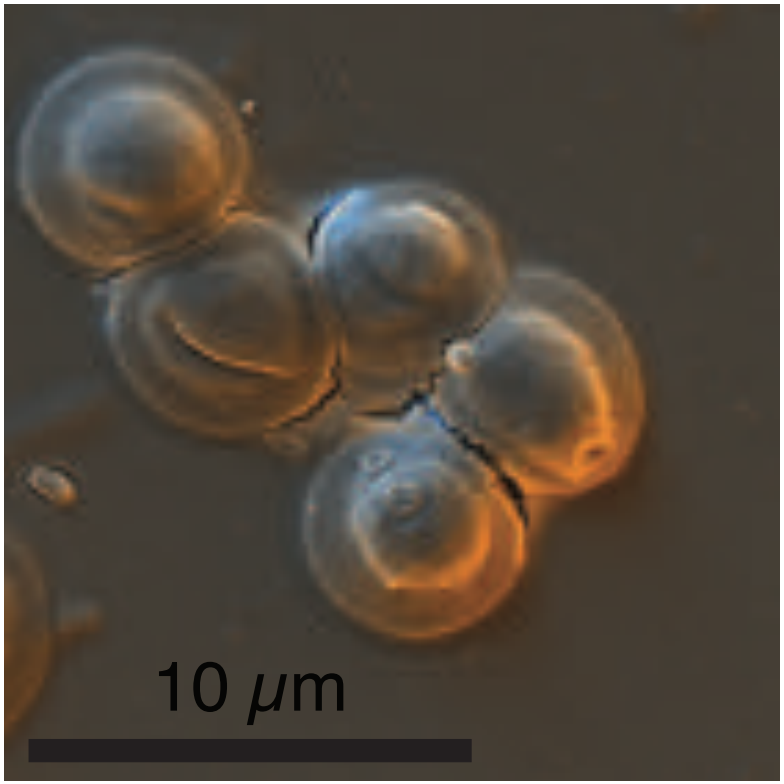
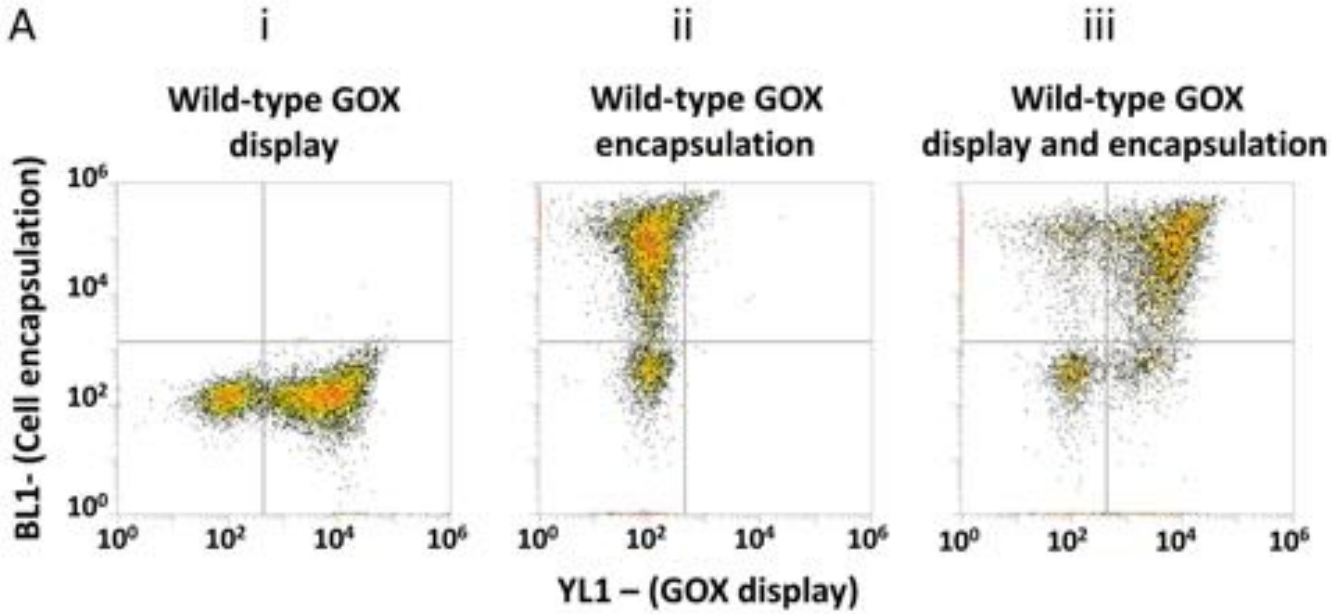


Optimization of expression, encapsulation, and screening conditions

Co-localization of fluorescence signals for GOx display and hydrogel capsules.



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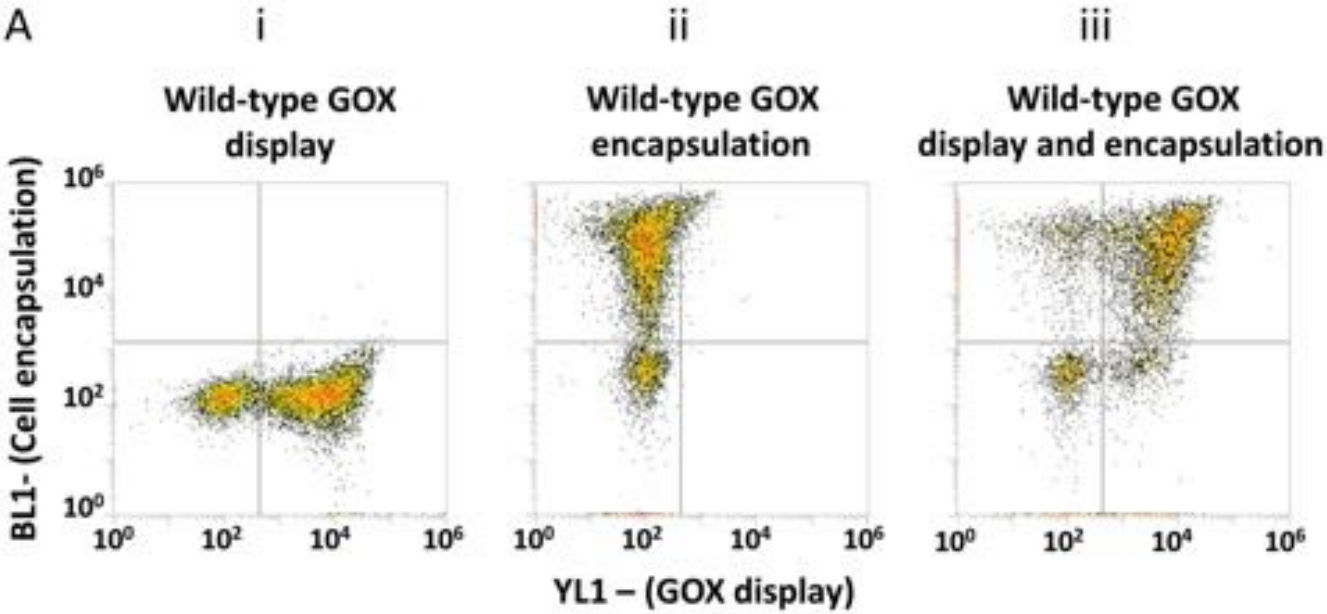
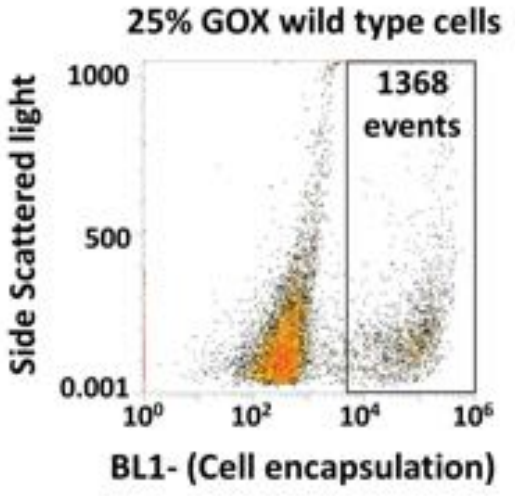


Drop-in experiments of positive cells into large background confirms specificity.

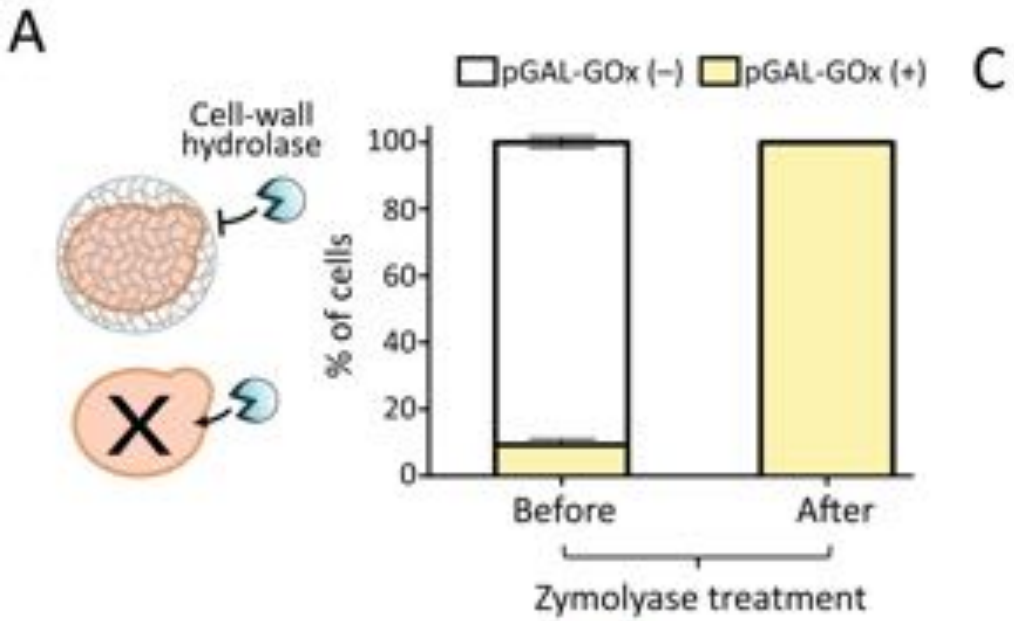
B

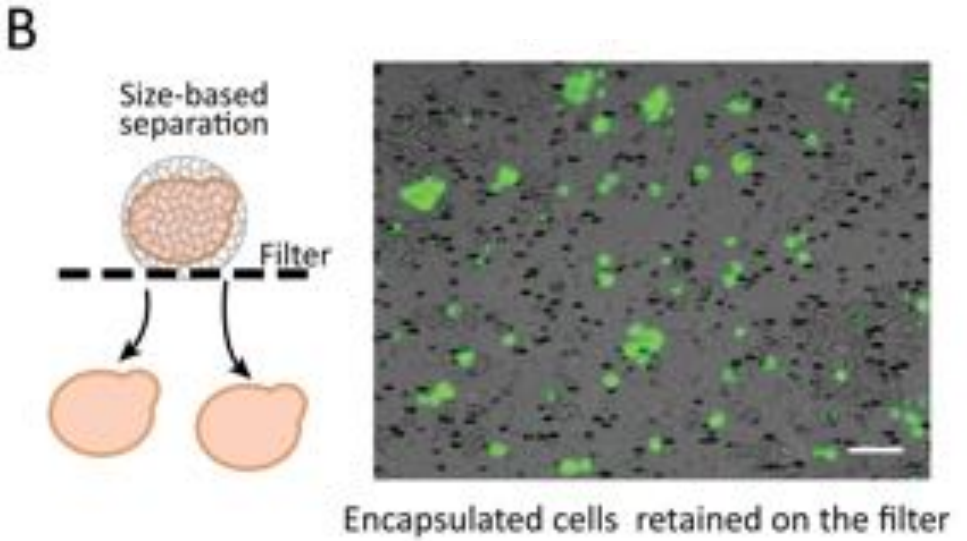
Yeast mixtures (Percent of GOX cells)	Encapsulated cells (out of 10'000)
100%	5923
75%	3980
50%	2890
25%	1368
10%	463
1%	65
0%	15

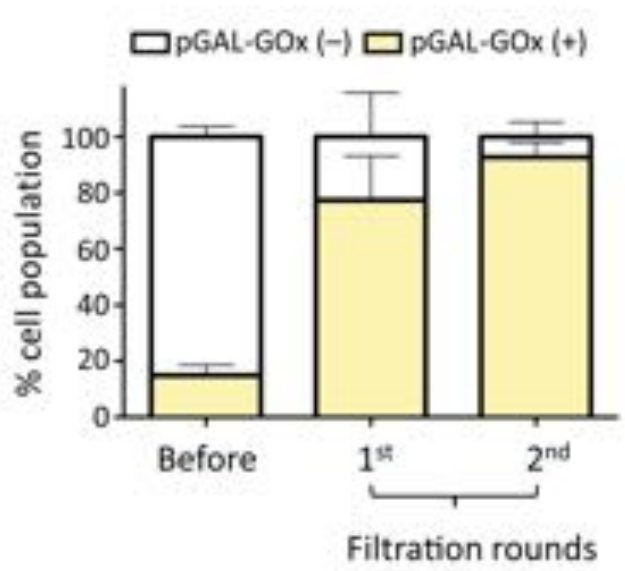


Methods for isolating/enriching encapsulated cells

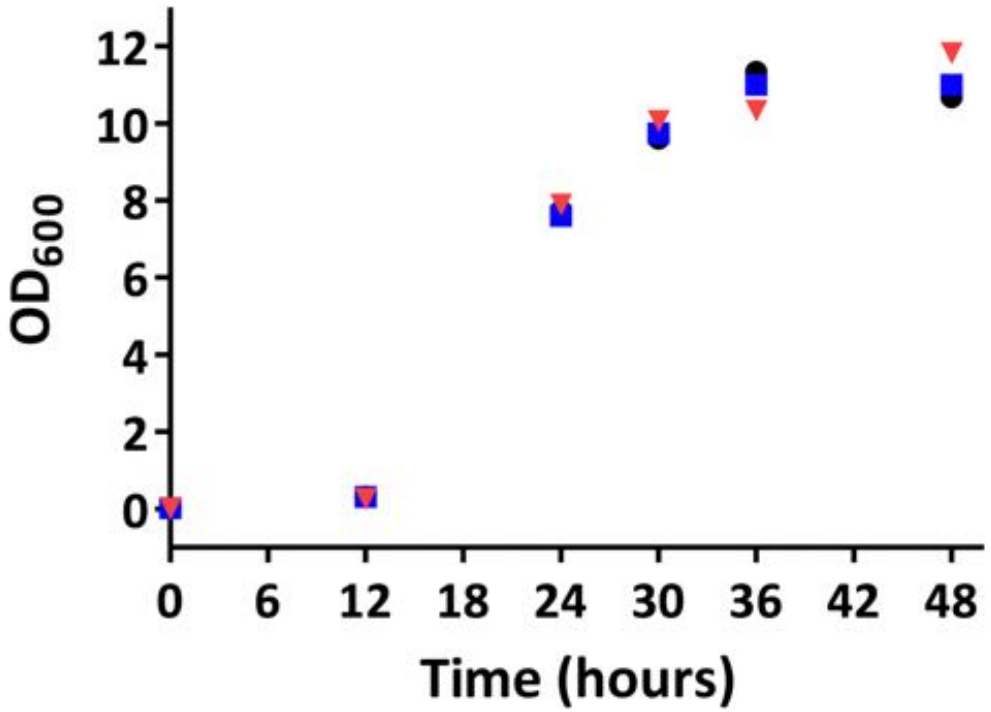
- Cell sorting/flow cytometry
- Cell survival under selective pressure
- Size-based filtration





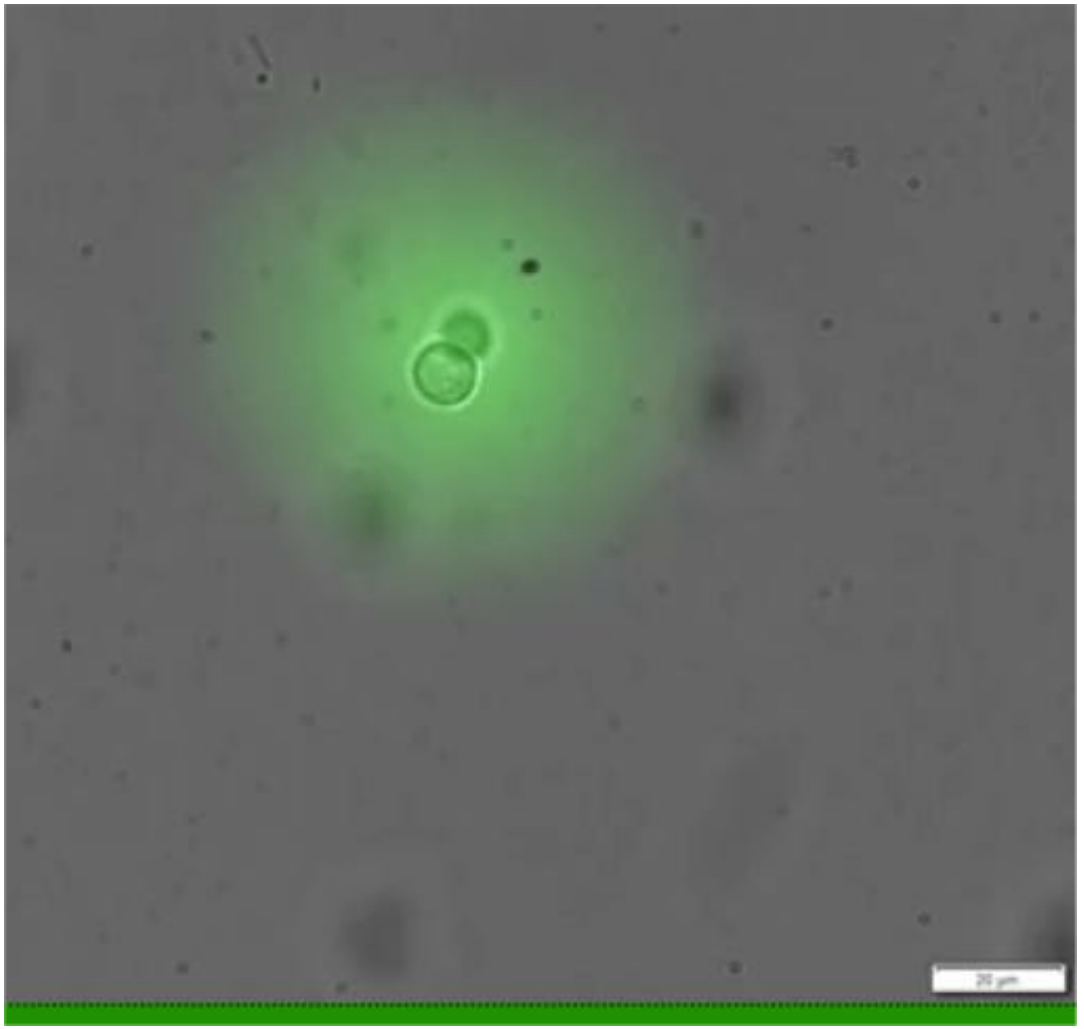


Alginate shells do not inhibit cell growth



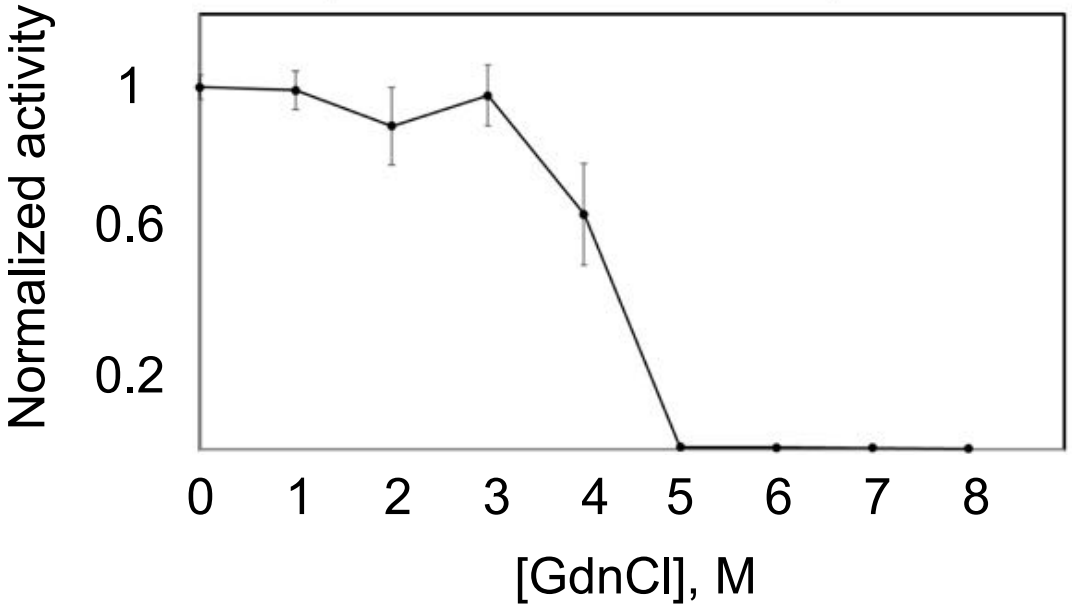
- Healthy EBY100 cells
- EBY100 (+) pGAL-GOx cells in reaction mixture lacking HRP
- EBY100 (+) pGAL-Gox encapsulated

Cells easily divide after being encapsulated in the alginate capsules

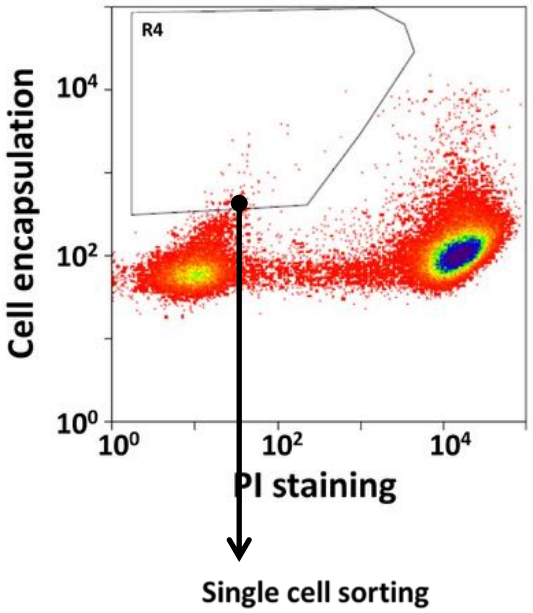


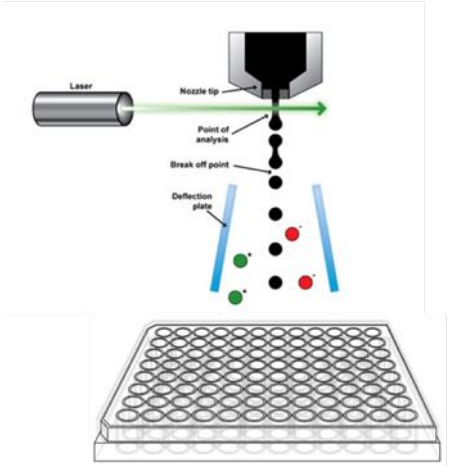
GOx mutant library screening

WT-GOx is inactive under selection pressure of 5M GdnCl



PI staining reveals viable cell fraction

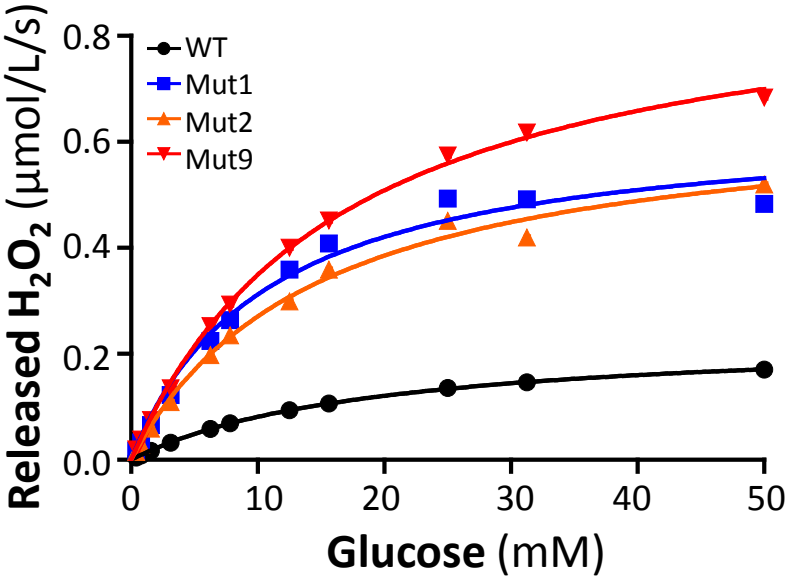




- Stress condition denatured WT-GOx but maintained partial cell viability
- Single-cell sorting of active mutants following stress condition provided new stabilized enzymes

Evolved GOx mutants have up to 4-fold faster k_{cat} and are more thermostable

A



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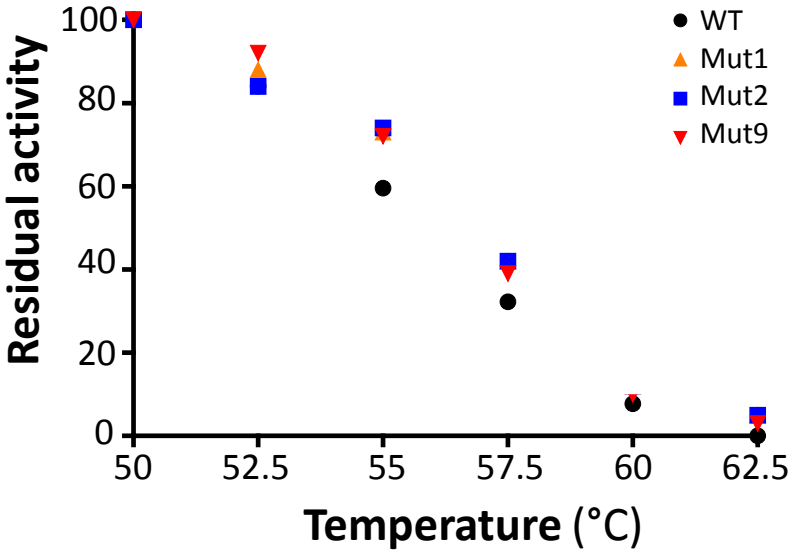
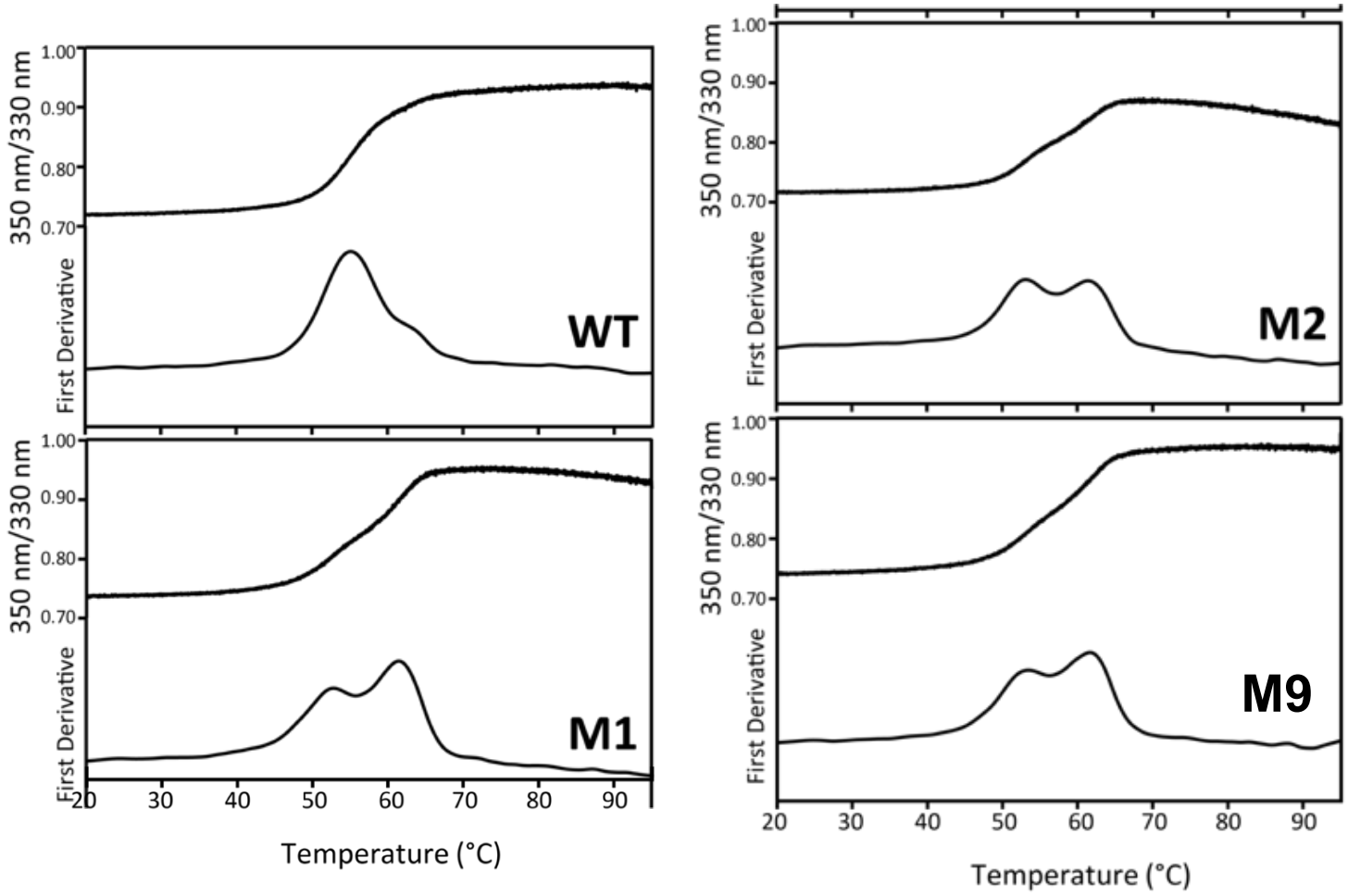


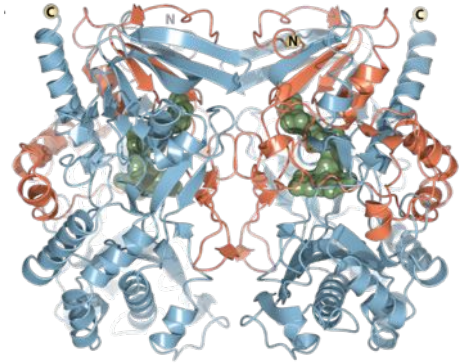
Sample	Mutation	Km (mM)	Kcat (s^{-1})	Kcat/Km (s^{-1}·mM^{-1})
Wt ●	-	19.08 (±0.34)	99.64 (±0.83)	5.22
Mut 1 ■	L9P	10.68 (±1.67)	272.30 (±15.94)	25.49
Mut 2 ▲	L13P-A16T	14.67 (±1.62)	281.80 (±13.02)	19.20
Mut 9 ▼	L13P	16.76 (±0.77)	394.30 (±7.99)	23.52

Thermostability by differential scanning fluorescence



Higher temperature denaturation peak is more prominent for evolved mutants. Attributed to dimer form.

Conclusions & outlook

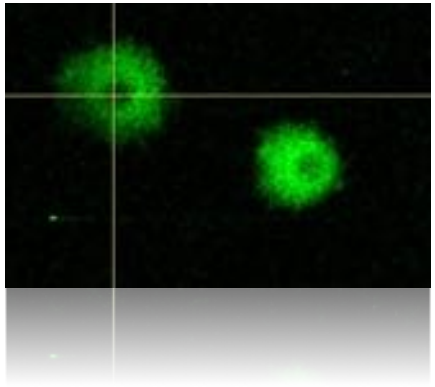


Conclusions

- Enzyme screening is a major bottleneck in the development of biocatalysts.
- A new high throughput screening method based on genetically encoded localized hydrogel formation is under development which is faster, cheaper, and relevant for industry.
- Valid for screening of enzymes that produce H_2O_2

Outlook

- Other enzymes to evolve: catalase, dehydrogenase, other oxidoreductases,
- Applications of cell-gel encapsulation for mammalian cells (top down)



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END