



WIDEnzymes

Widening Synergies
for Novel Enzymes Development

Directed evolution of enzymes

Workshop #2 of WIDEnzymes

02.06.2025 – 06.06.2025

Department of Chemistry, Heraklion, Greece



Funded by
the European Union

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University of Crete



1973

Establishment in two cities
Seat of the University in Rethymnon

1977

Acceptance of first students
(Philosophy and Mathematics)



1998

Gallos Campus in
Rethymnon inauguration

2013

Completion of Voutes Campus
Heraklion

Department of Chemistry



1985

Dept. of Chemistry was founded
Acceptance of MSc students

1987

Acceptance of first
BSc students



2006

Moving of the Dept. in the new
building @ Voutes Campus

2018

Enzyme Technology Lab
was established

WIDEnzymes



- Propel the enzyme technology ecosystem forward.
- Bridge the gap between widening and non-widening EU countries.
- Elevate Europe's position as a global leader in biotechnology and bioeconomy.

Activities:

- Workshops
- Online seminars
- Journal Club
- Mentoring programme

teamit.



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WIDEnzymes' workshops



1

Computational methods
for enzyme design

Milan, Italy

27-31 JANUARY
2025



POLITECNICO
MILANO 1863

POLITECNICO
DI MILANO

2

Directed evolution
of enzymes

Crete, Greece

2-6 JUNE
2025



UNIVERSITY
OF CRETE

3

Bioreactor production of
recombinant enzymes

Bratislava, Slovakia

WINTER 2025
Dates TBC



SLOVAK UNIVERSITY OF
TECHNOLOGY IN BRATISLAVA

SLOVAK UNIVERSITY OF TECHNOLOGY
IN BRATISLAVA (STUBA)

4

Structural, biochemical and
biophysical characterization
of enzymes

Riga, Latvia

SUMMER 2026
Dates TBC



Latvian Institute of
Organic Synthesis

LATVIAN INSTITUTE OF ORGANIC
SYNTHESIS (LIOS)



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Workshop #2: Directed evolution of enzymes



RATIONAL DESIGN

DIRECTED EVOLUTION

1. Computer aided design



2. Site-directed mutagenesis



Individual mutated gene

3. Transformation

4. Protein expression

5. Protein purification

6. not applied



Constructed mutant enzyme

IMPROVED
ENZYME

7. Biochemical testing

1. not applied

2. Random mutagenesis



Library of mutated genes
(>10,000 clones)

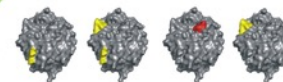
3. Transformation

4. Protein expression

5. not applied

6. Screening and selection

- stability
- selectivity
- affinity
- activity



Selected mutant enzymes



Nobel Prize in Chemistry

2018

*“for the directed evolution
of enzymes”*



Nobel Prize in Chemistry

2024

*“for computational protein
design and for
protein structure
prediction”*

©Z. Prokop & J. Damborský, Loschmidt Laboratories, Masaryk University, Brno, Czech Republic



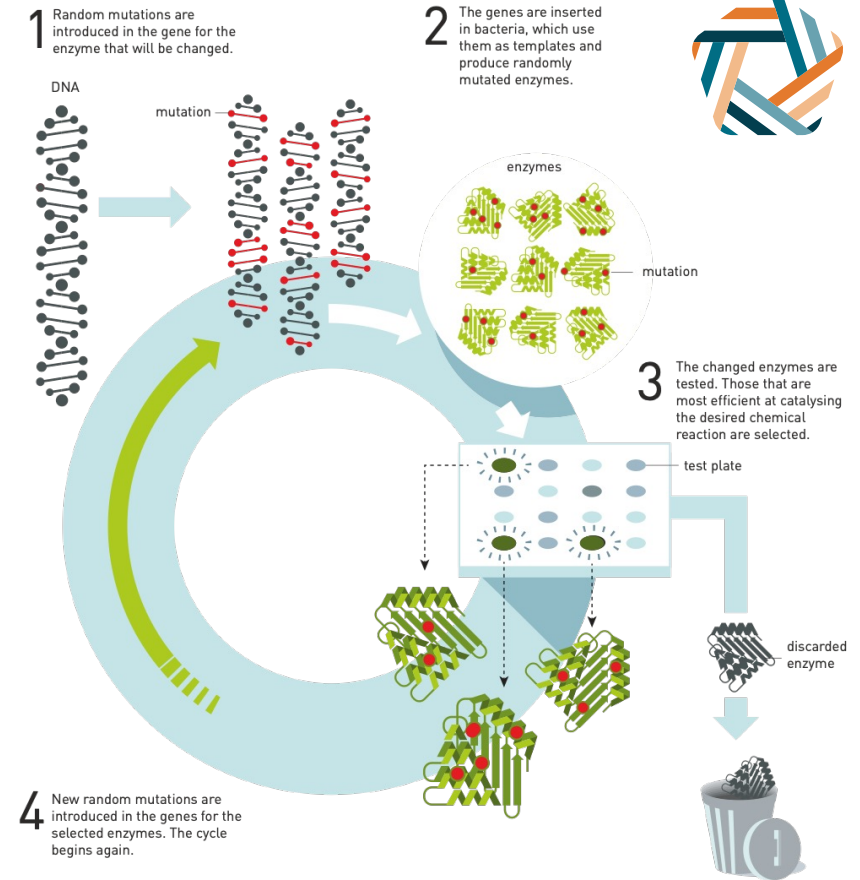
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Overview of workshop



1. Create libraries of mutated genes *with error-prone PCR*
2. Clone the library of genes back to the plasmid *with MEGAWHOP*
3. Digest the template *with DpnI*
4. Transform *E. coli* with the library
5. Protein expression in solid cultures and assay activity
6. Pick the best hits and transfer to liquid cultures
7. Express proteins on a small scale
8. Confirm the improved biochemical behavior
9. Normalize – if possible – the results on the expression level
10. Sequence the most interesting mutants



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Overview of workshop – Do not forget!



- Welcome drink tonight @ 18:00!
- Short presentation of your projects (Tuesday and Wednesday)
- Invited lectures on Wednesday
- Social event on Wednesday
- Short presentation on the results of your team (Friday)



**Beyond nature's blueprint:
Artificial enzymes and
unnatural building blocks**

Assist. Prof. Dr. Ivana Drienovská



**Functional screening for
enzyme discovery
and evolution**

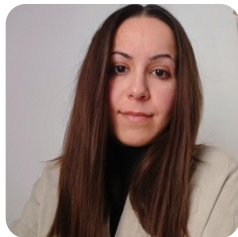
Prof. Dr. Aurelio Hidalgo



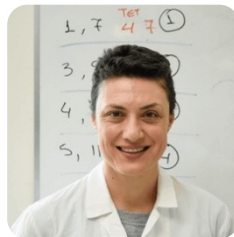
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VIPs of the workshop



*Dr. Renia
Fotiadou*



Maria Fouskaki



*Dr. Spyridoula
Charova*



*Chara
Rakopoulou*

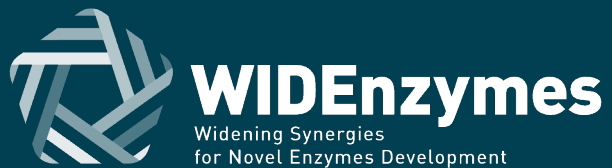


Eva Polenta



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Thank you!



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