Discovery and Development of Novel Enzyme Biotransformations

(1) Introduction to EMBL

(2) Transform-MinER

- discovery of novel valuable biotransformations

(3) PocketAnalyser

- modification of enzyme pocket towards novel biotransformation

We welcome enquiries regarding experimental collaborations to validate and develop the opportunities identified

Dr. Jonathan Tyzack
Thornton Group

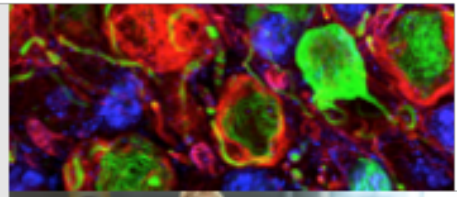
www.ebi.ac.uk

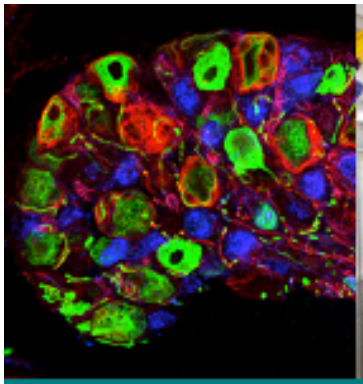
EMBL-EBI

EMBL: Europe's Centre of Excellence in Life Sciences

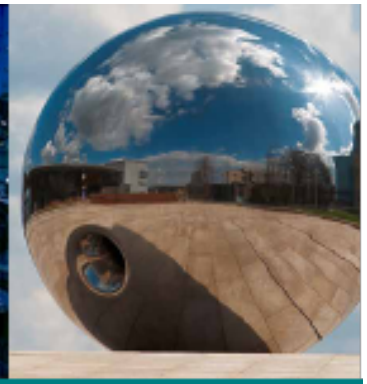
- Founded in 1974 as an intergovernmental organization to promote molecular life sciences in Europe
- 6 EMBL sites – over 1700 people and more than 80 nationalities
- Currently 26 member states and 2 associate member states (Australia and Argentina)

EMBL: principles and missions

EMBL	Scientific excellence	Collaboration	Staff turnover	
		Scientific freedom		
	Internationality and diversity		Young talent and early independence	







Basic research | Services | Advanced training | Technology development & transfer | Integration of life science research

EMBL: principles and missions

EMBL

Scientific excellence ✓

Internationality and diversity

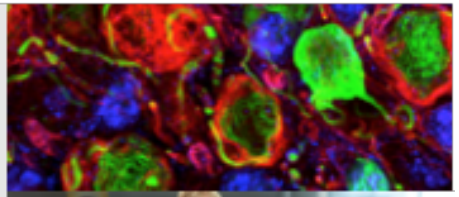
Collaboration ✓

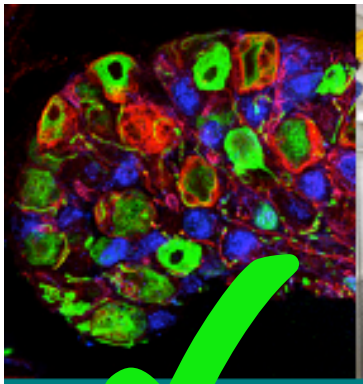
Scientific freedom

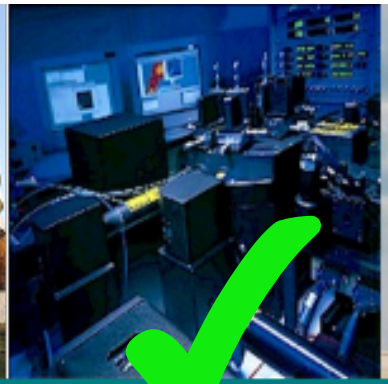
Cutting-edge infrastructure

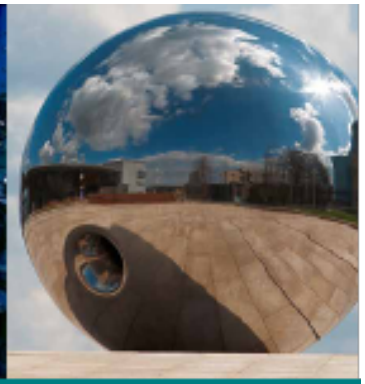
Staff turnover

Young talent and early independence









Basic research ✓

Services

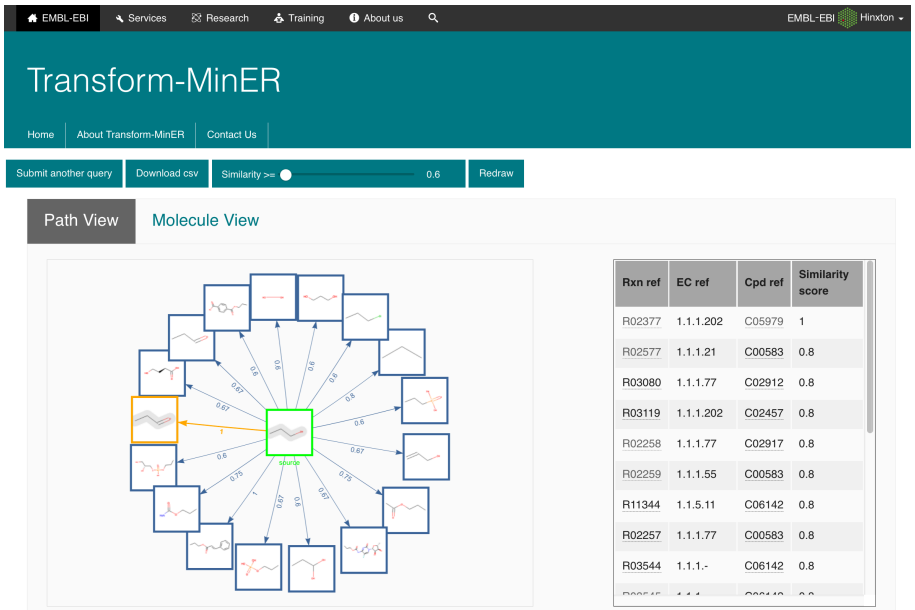
Advanced training

Technology development & transfer ✓

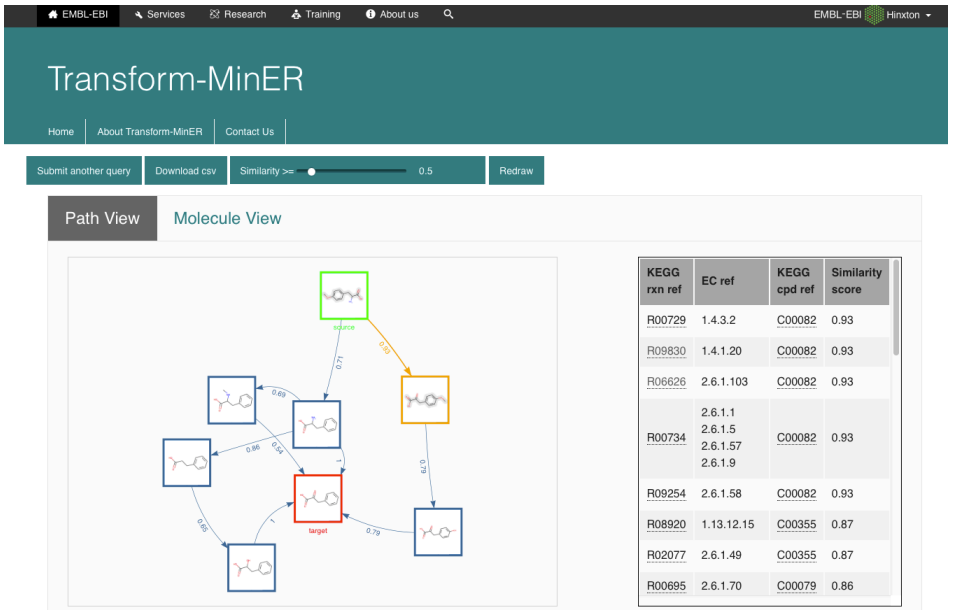
Integration of life science research

1) Transform-MinER overview

Transform-MinER performs data mining of enzyme transformations to propose novel enzymatic transformations with similarity to known transformations



Molecule Search - propose enzyme transformations for a submitted molecule

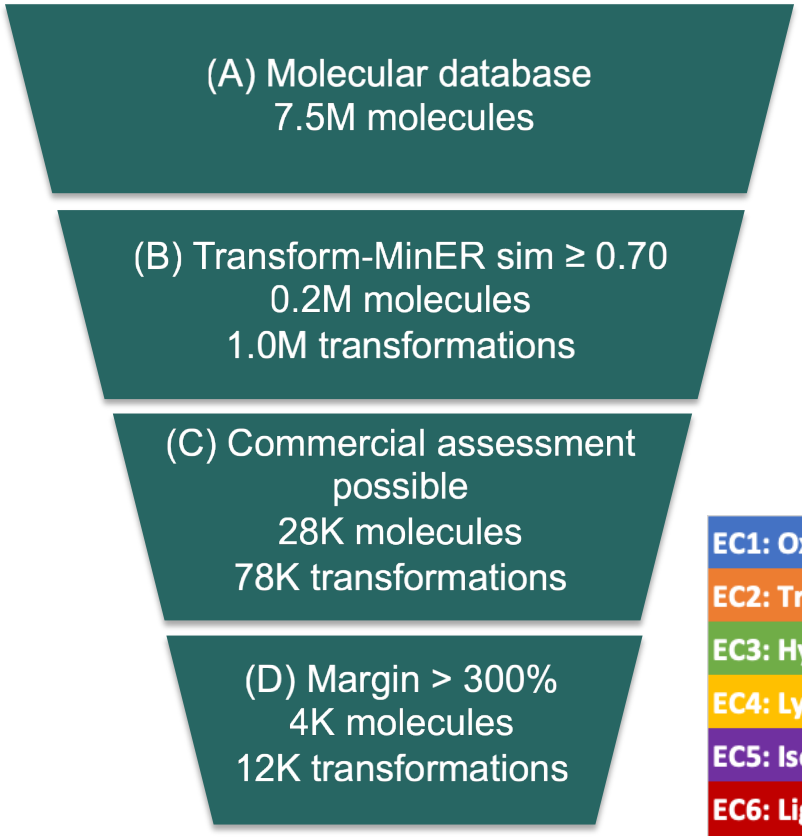


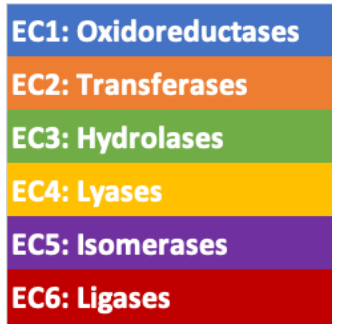
Path Search - link a substrate and product molecule via multiple enzyme transformations

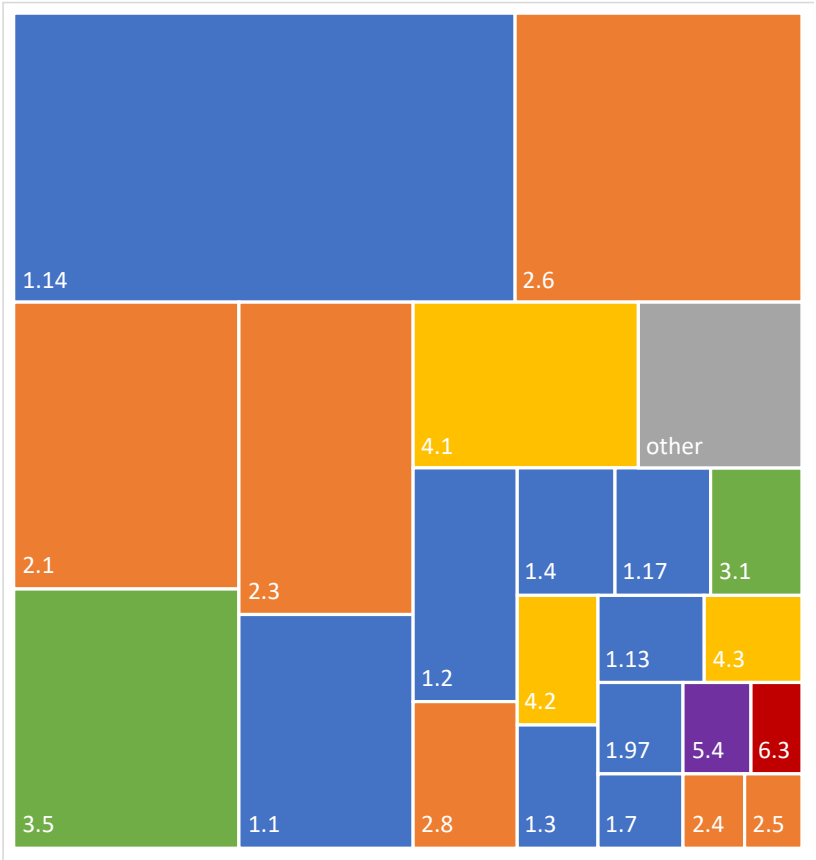
A restricted version of Transform-MinER is publicly available (analysis of single query substrates, no high-throughput function): https://www.ebi.ac.uk/thornton-srv/transform-miner/

2) Novel high margin opportunities by virtual HTS

Transform-MinER can run high-throughput, in-parallel on a cluster







Molecule Search enables:

- Expansion of substrate source and product space
- To prioritise high similarity hits for value, identification of high margin opportunities across diverse EC areas *(examples in the next slide)*

3) Novel high margin opportunity examples

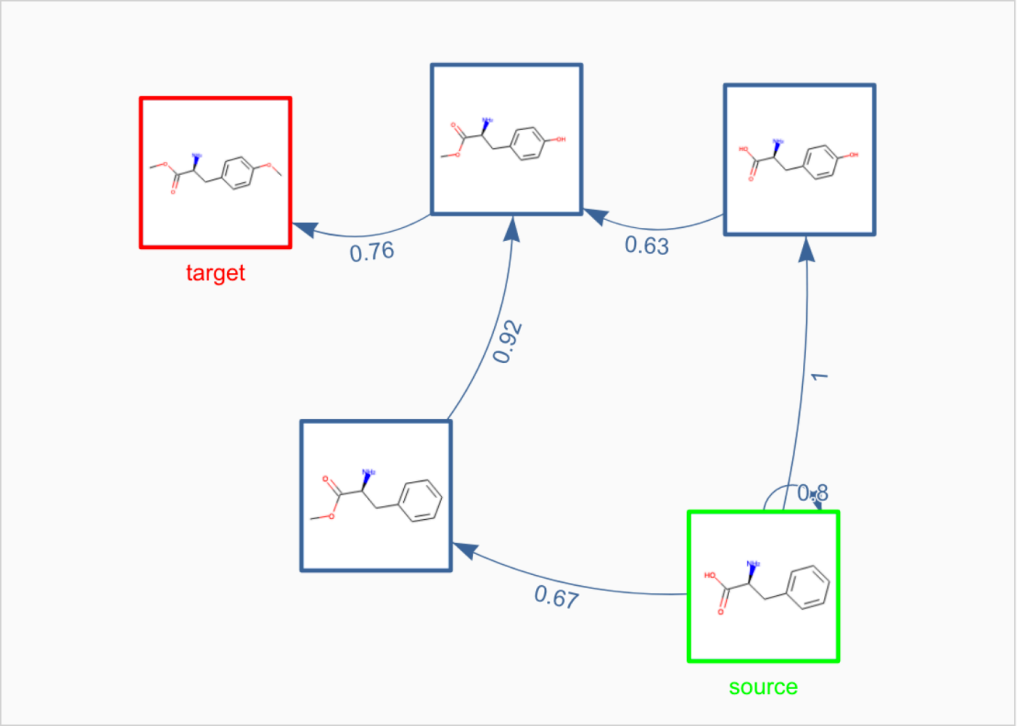
Examples of novel biotransformations with the highest margin:

Ref	EC subclass	Substrate Value $	Product Value $	Factor x	Margin % %
1	1.1	28	1056	**38**	**3768**
2	1.14	28	1312	**47**	**5204**
3	2.1	26	1443	**56**	**6105**
4	2.3	16	855	**52**	**6148**
5	2.6	15	484	**32**	**2363**
6	3.1	6	673	**112**	**12863**
7	3.5	18	2369	**134**	**11470**
8	4.1	61	3056	**50**	**6529**

- Valuations for substrate and product from same supplier and quantity
- Margin calculated on a per mole basis using valuations for all substrates and products

4) Identification of novel reaction paths

Transform-MinER is capable to find novel transformation paths



Green: Substrate

Red: Product

Path Search:

- Links a query substrate with a query product over a number of intermediate enzymatic reactions
- Identifies novel paths of enzymatic reactions

5) Pocket Analyser

Pocket analysis:

- Identify potential substrate/protein clashes
- Propose residues for mutation

Requirements:

- Protein structure (or homology modelling)
- Bound native substrate in a catalytically relevant pose (or from docking)

Prototype available for testing.

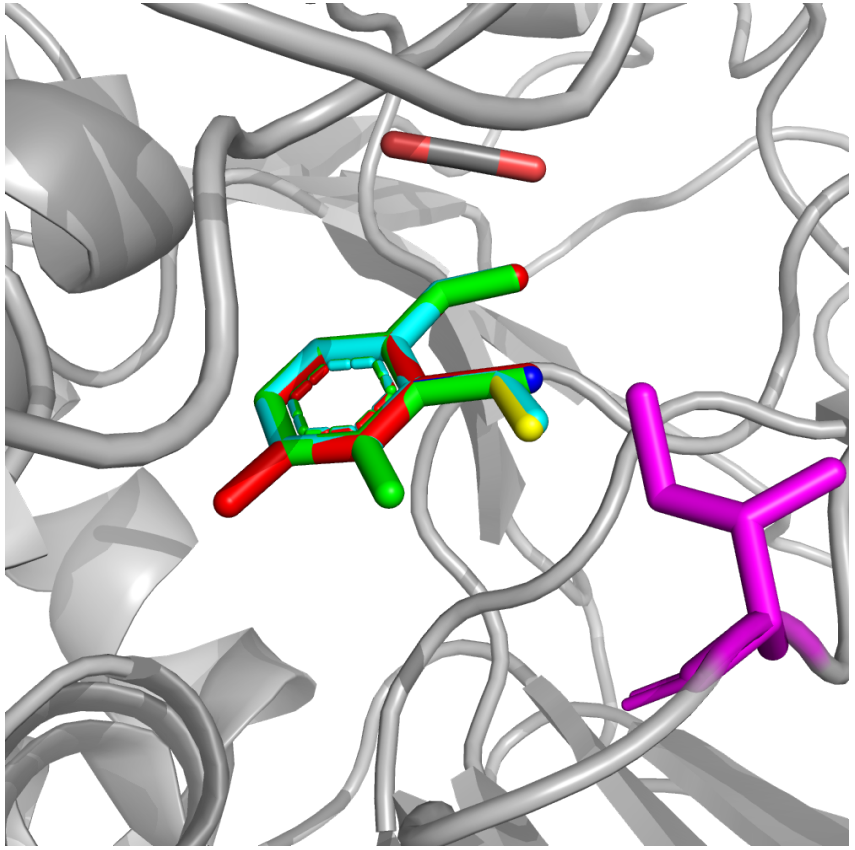


Fig1: Pocket Analyser Example.

Potential clashes identified between ortho substituents and isoleucine (magenta). Mutation to alanine predicted to relieve steric clashes.

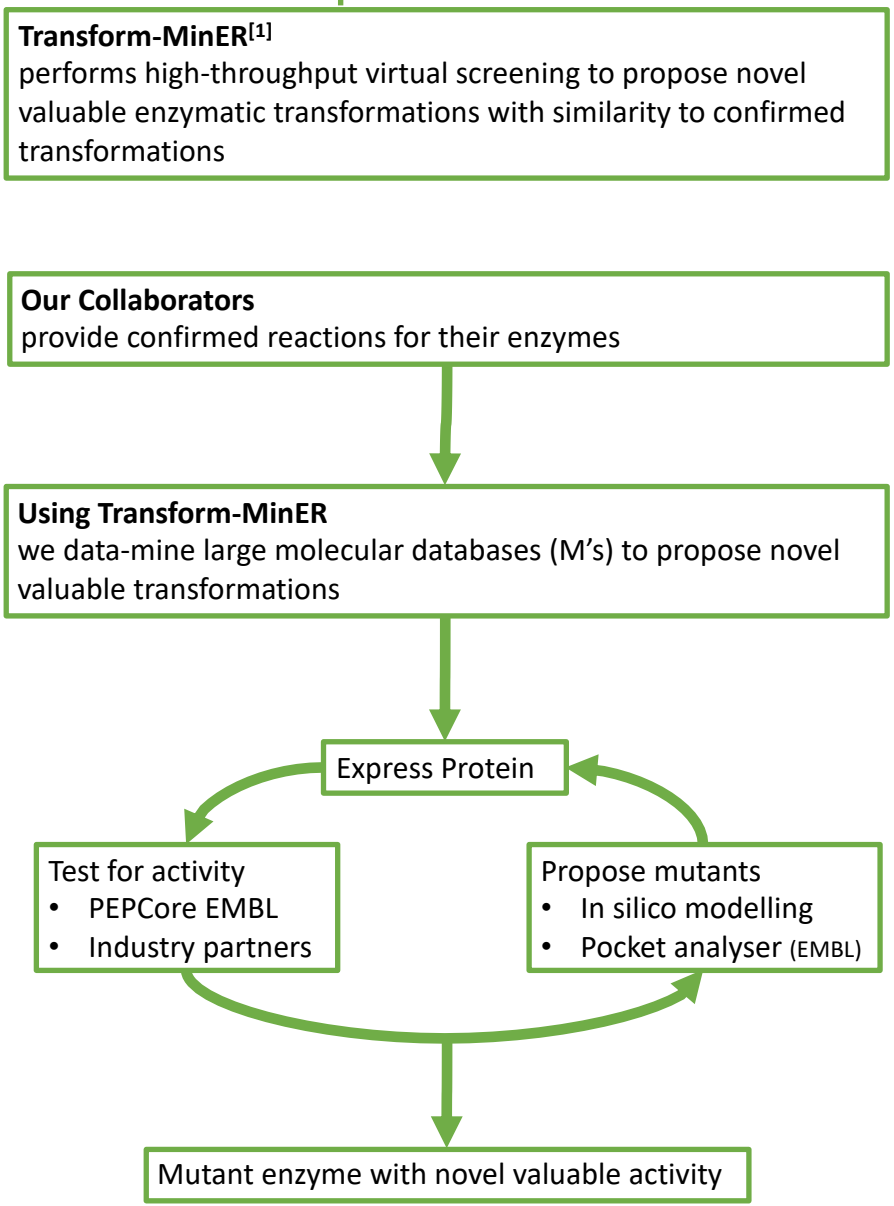
6) Bespoke versions for partners

Transform-MinER has been applied e.g. in the agrochemical arena to explore biosynthetic space.

Benefits:

- Identification of novel, valuable transformations that increase substrate and product space
- Development of enzymes with related and commercially lucrative activity
- Considerable time savings by high-throughput computational analysis (not available on public version)
- Possibility to test activity at EMBL Protein Expression and Purification Core Facility (PEP core)
- Optional use of EMBL Pocket Analyser tool to predict enzyme mutations

7) Work flow for bespoke versions



8) Summary

Transform-MinER molecule search:

- guide enzyme development into lucrative areas

Transform-MinER path search

- identify novel paths of enzyme reactions

Pocket Analyser

- propose clash-relieving mutations to optimize novel substrate binding

Partnerships

- To add valuable new functionality for commercial enzymes
- To develop mutant enzymes with novel activity

9) Further reading and contact

Paper:

[1] Exploring Chemical Biosynthetic Design Space with Transform-MinER, *ACS Synth Biol,* 2019, 8, 11, 2494-2506

[2] Transform-MinER: transforming molecules in enzyme reactions, *Bioinformatics,* 34(20), 2018, 3557-3599

Contact:

- Dr Jonathan Tyzack **tyzack@ebi.ac.uk**
- Dr Cornelia Brosi **brosi@embl-em.de**