Robust Biocatalyst Discovery and Applications

Nordic-Irish Process Chemistry Forum 2023

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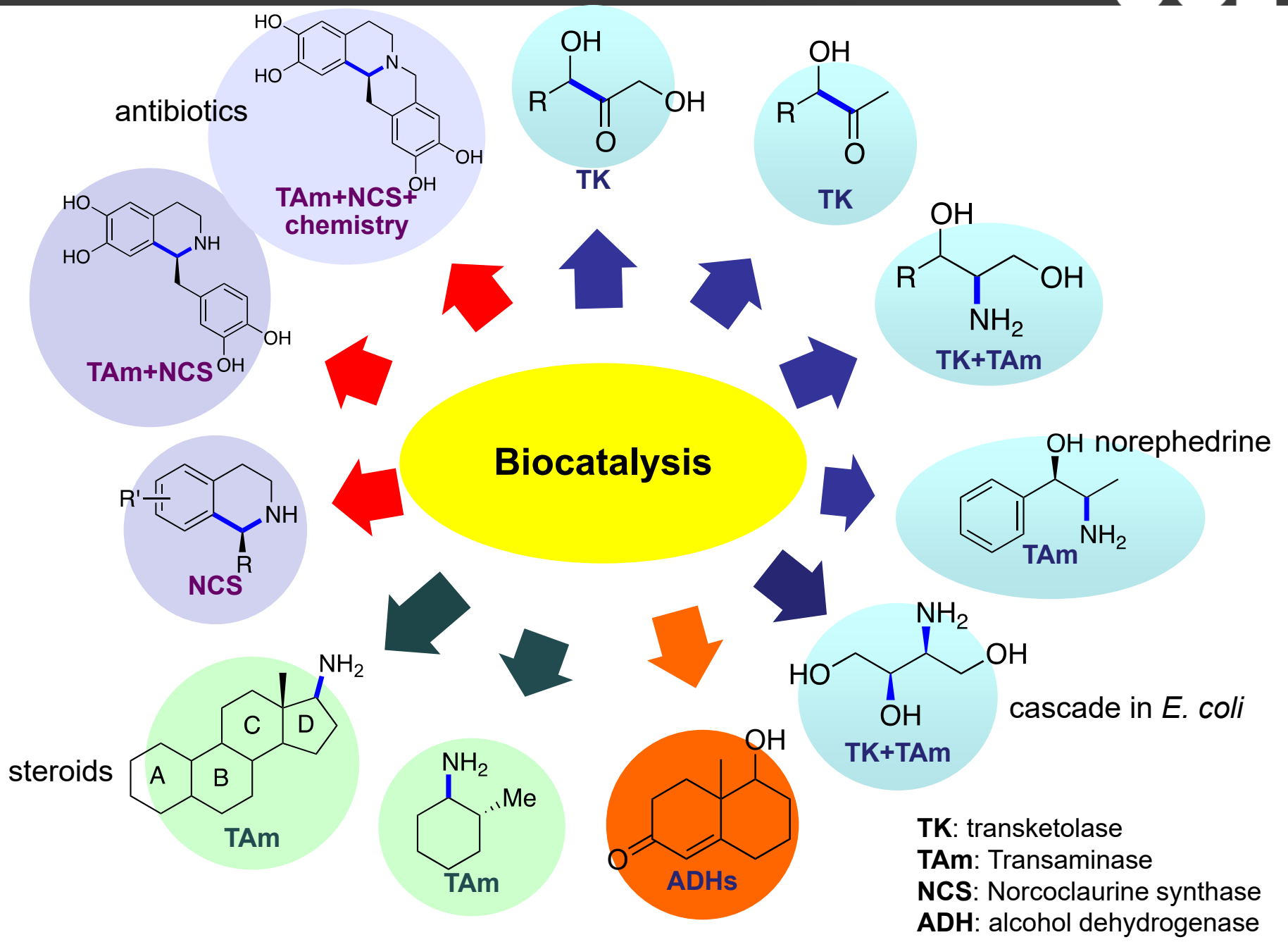
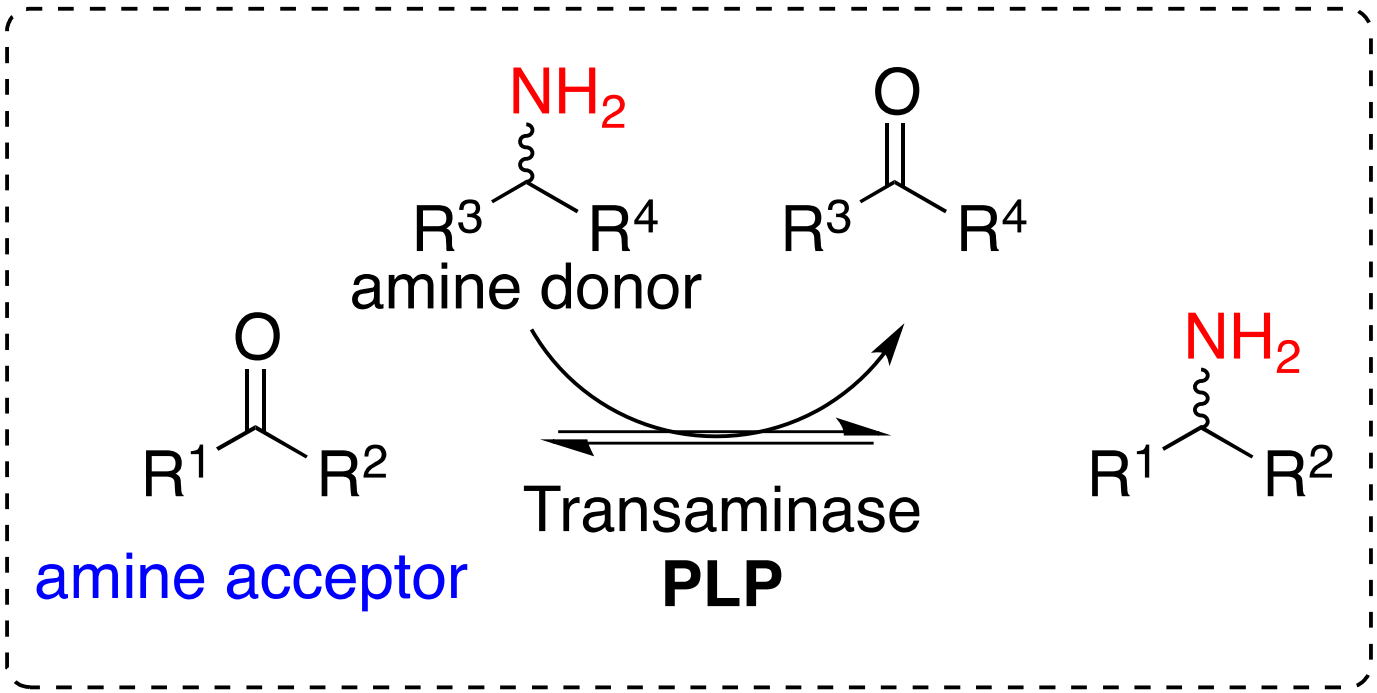
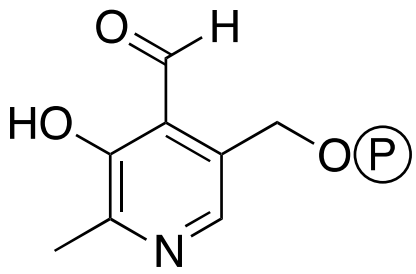
Biocatalysis



Transaminases (TAms)



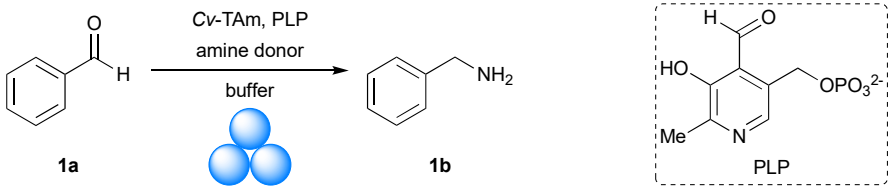


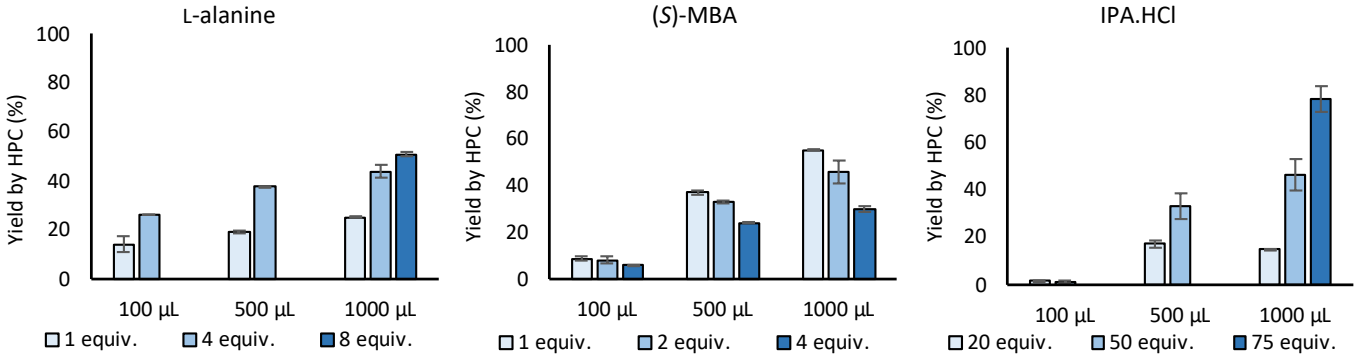
PLP = Pyridoxal-5-phosphate

- Co-factor **PLP** and amine donor required e.g. *S*-α-methylbenzylamine (MBA), isopropylamine, or alanine.
- Using them in single enzyme transformations and enzyme cascades.

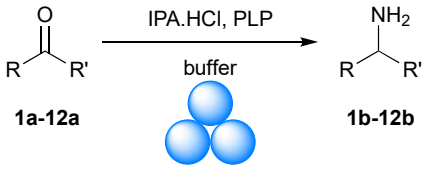
Mechanoenzymatic reactions

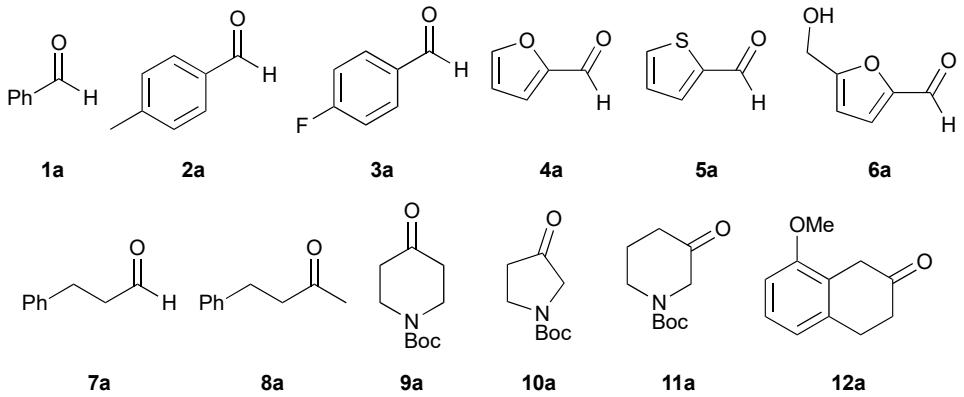
- Mechanochemical reactions have emerged as a green synthetic method as reactions can be performed more rapidly and using less solvent
- To date very few mechanoenzymatic reactions have been described – hydrolytic reactions
- These have been explored for the first time with TAms
- Reaction parameters to explore include: frequency of milling, milling time and aging period
- Also, what form the biocatalyst is in…..

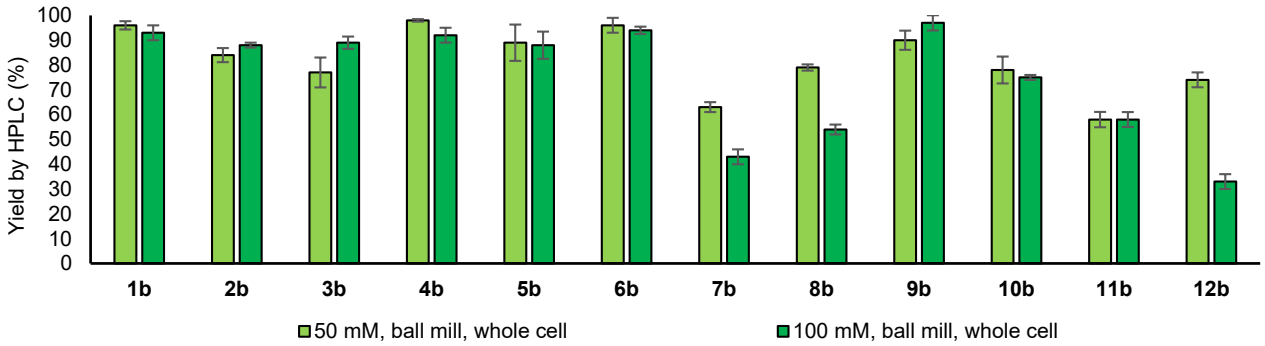




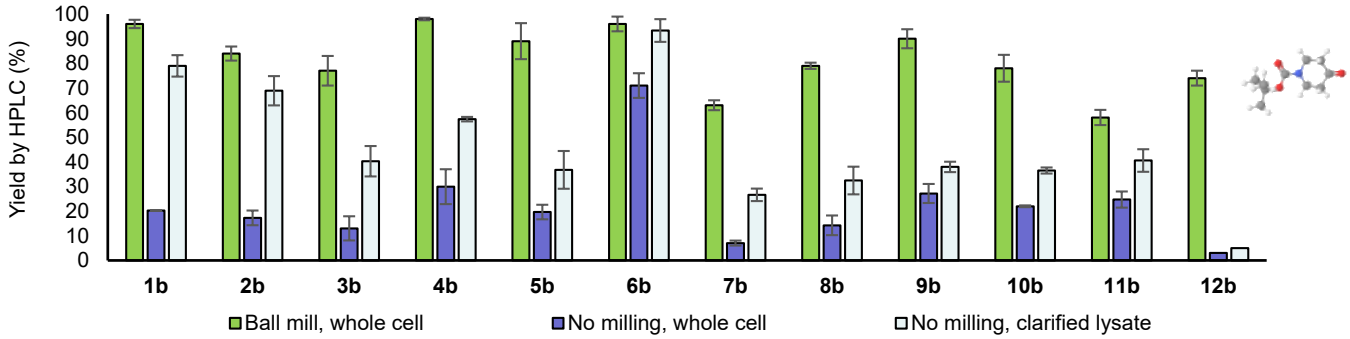
Mechanoenzymatic reactions







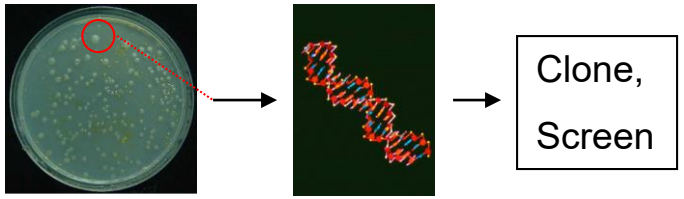
- Extended to other analogues
- **Shaken, not stirred** worth exploring more widely



E. M. Carter, E. Ambrose-Dempster, J. M. Ward, T. D. Sheppard, H. C. Hailes, *Green Chem.*, **2022**, *24*, 3662

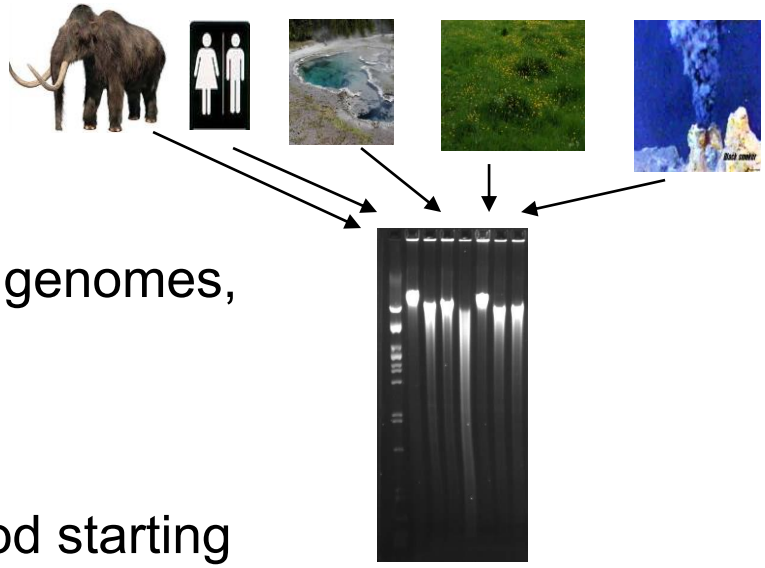
How to get a new enzyme

- Enrich or select wild type bacteria



- Synthetic genes: plants, fungi, extremophiles

- Metagenomics (culture independent)

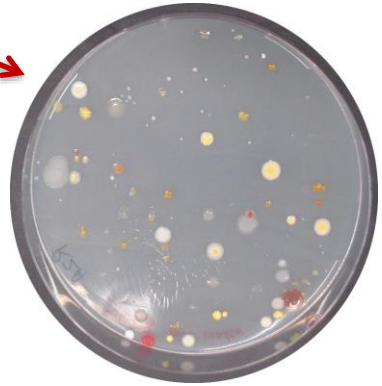


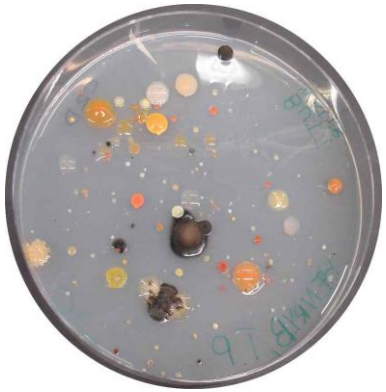
- Genome mining – Bioinformatics: bacterial genomes, archaeal genomes, eukaryotic genomes.

- Enzyme mutagenesis – but you need a good starting point

Why use metagenomics?

Direct isolation from soil only 0.1–1%



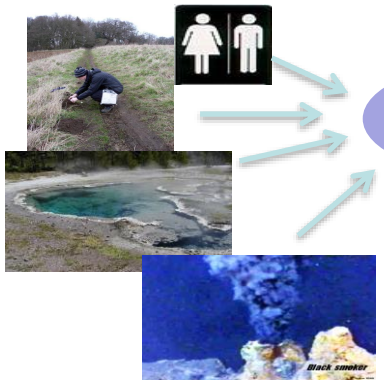


Isolate DNA directly from soil.

All organisms are represented.

New Enzymes: Functional Metagenomics

- Isolation of genes from organisms in an environment



Roche 454, Illumina

Sampling → Sequencing → Library creation → Library → Sequence retrieval → Assay → Functional enzyme

Contig assembly

Driver sequence Blast

Pfam, FunFam

In silico

PCR/cloning/ expression/ purification

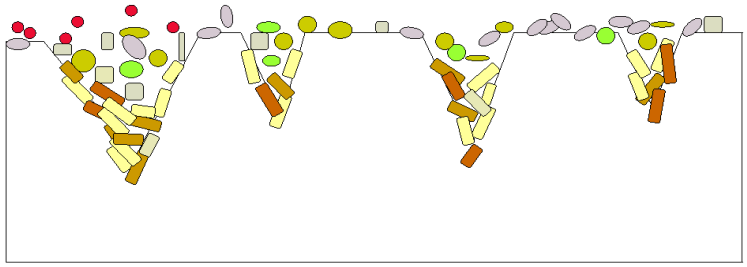
In vivo

- New functional enzymes for biocatalysis:

- Transaminases; Cyt P450; CRED; Transketolases; Decarboxylases; Ene-reductases, imine reductases; etc

- Once created for a metagenomic sample, the database can be searched for other enzymes

Tongue metagenome library



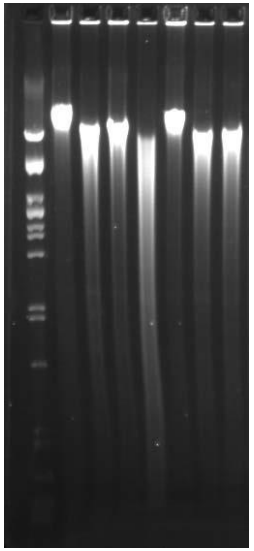
Tongue contains >100 bacterial species but over 50% of these are unculturable

Total DNA extraction from tongue scrapes: 9 individuals

Remove human DNA

Extract bacterial DNA

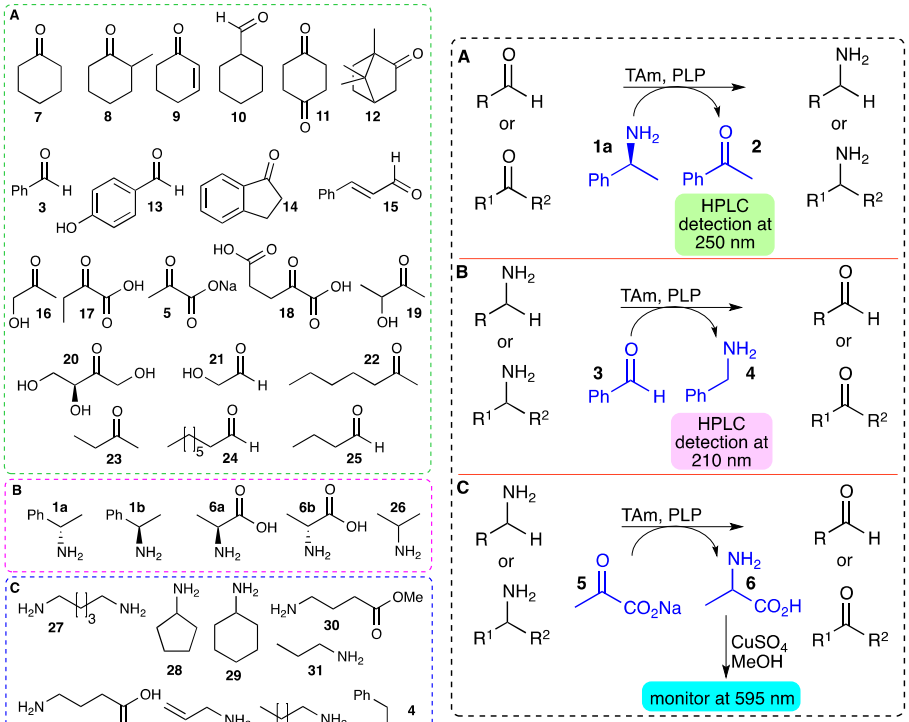
Sequence and clone genes

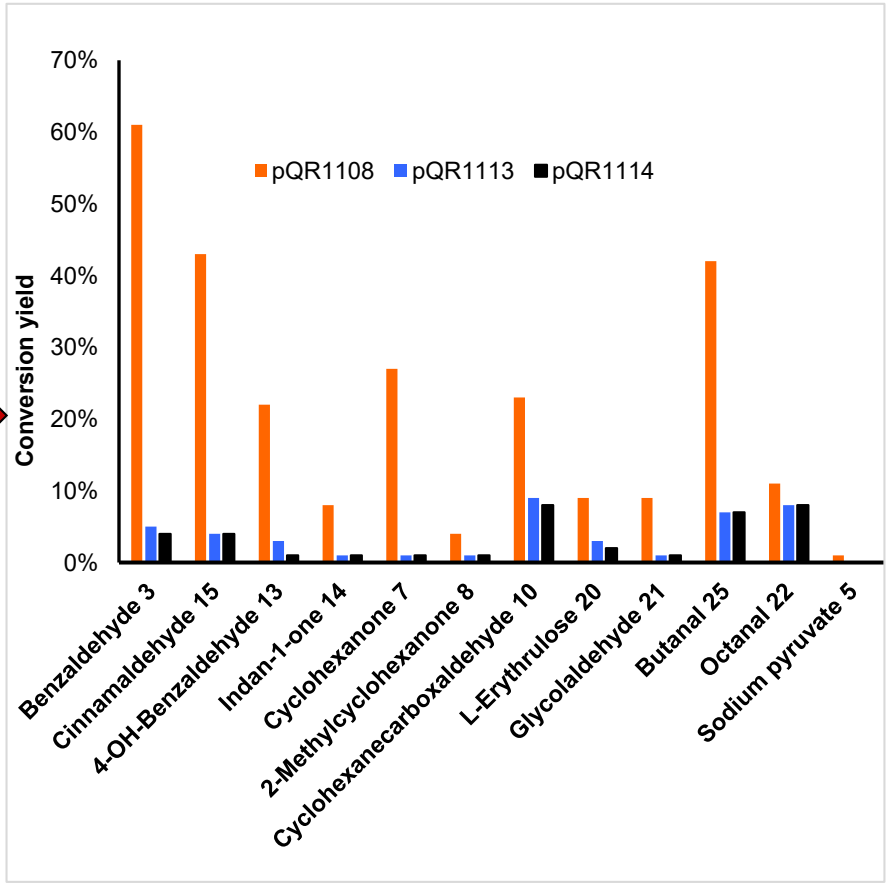


J. W. E. Jeffries, N. Dawson, C. Orengo, T. S. Moody, D. J. Quinn, H. C. Hailes, J. M. Ward, *ChemistrySelect*, **2016**, *1*, 2217

TAms from tongue metagenome

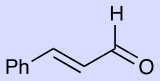
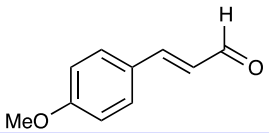
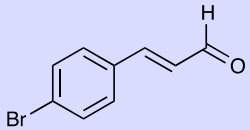
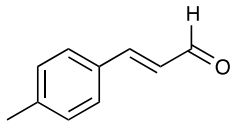
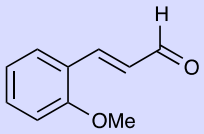
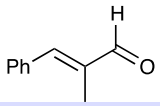
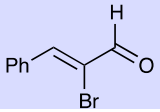
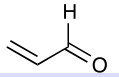
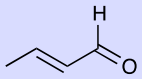
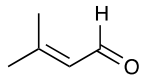
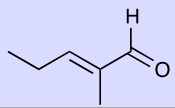
- Roche sequencing – 15 putative class III transaminases, 11 successfully retrieved and cloned.
- A range of different substrates and amine donors screened which highlighted 3 for further study.



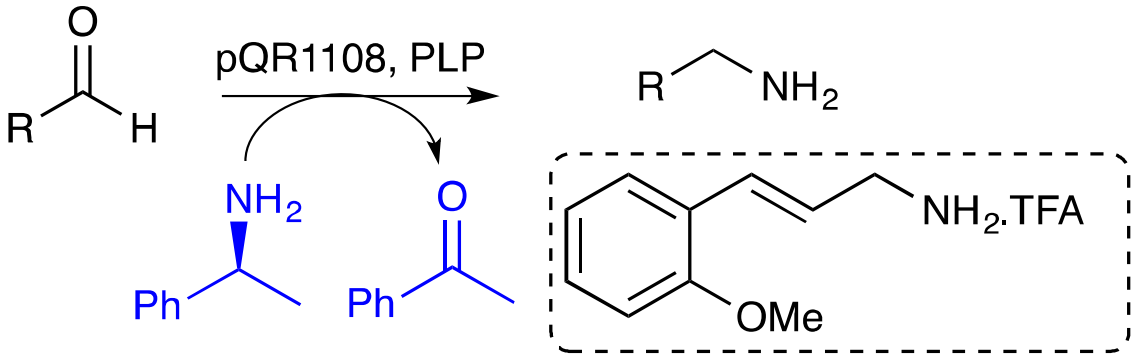


D. Baud, J. W. E. Jeffries, T. S. Moody, J. M. Ward, H. C. Hailes, *Green Chem.*, **2017**, *19*, 1134

TAms from tongue metagenome

Aldehyde	Yield
	75%
	39%
	48%
	49%
	58% 84% scale-up
	57%
	54%
	35%
	49%
	45%
	37%

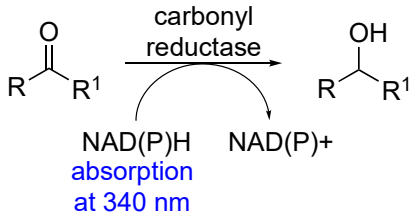
- pQR1108 was particularly active towards conjugated aldehydes

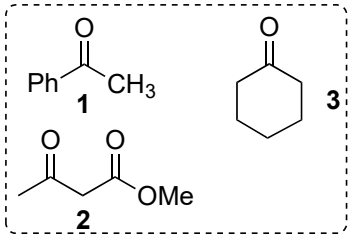


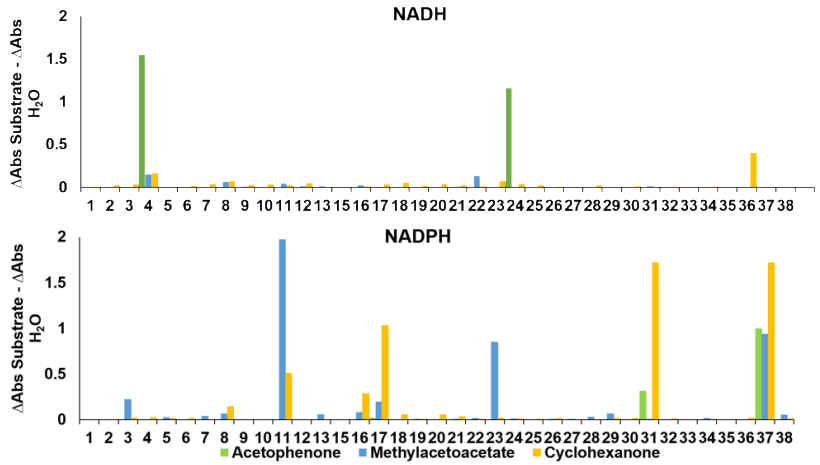
D. Baud, J. W. E. Jeffries, T. S. Moody, J. M. Ward, H. C. Hailes, *Green Chem.*, **2017**, *19*, 1134

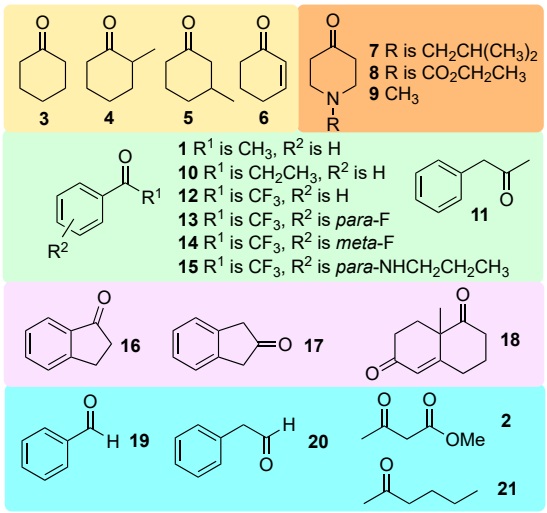
Tongue metagenome: Carbonyl reductases (SDRs)

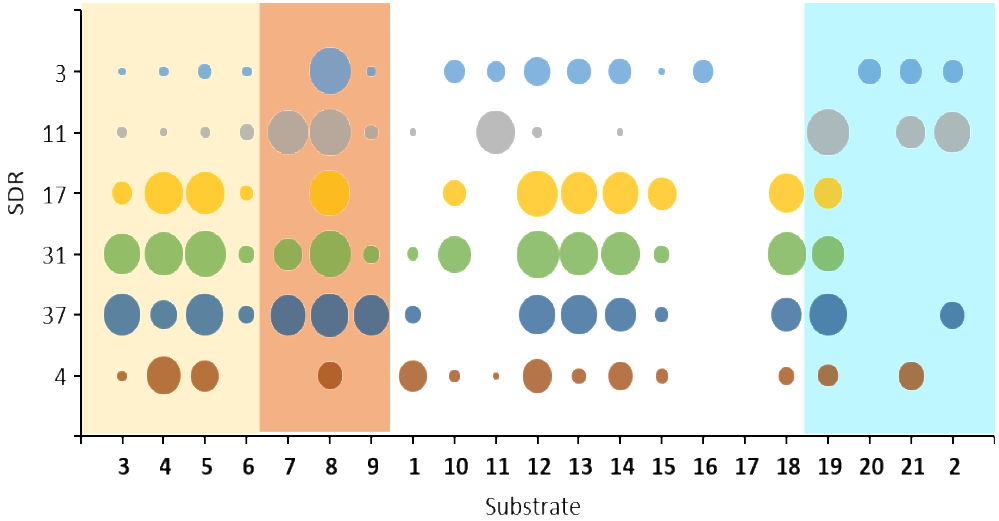
- Tongue metagenome *in silico* search: 38 short chain dehydrogenases (SDRs) selected
- 37 successfully cloned and expressed
- 6 SDRs showed good activity against a range of substrates





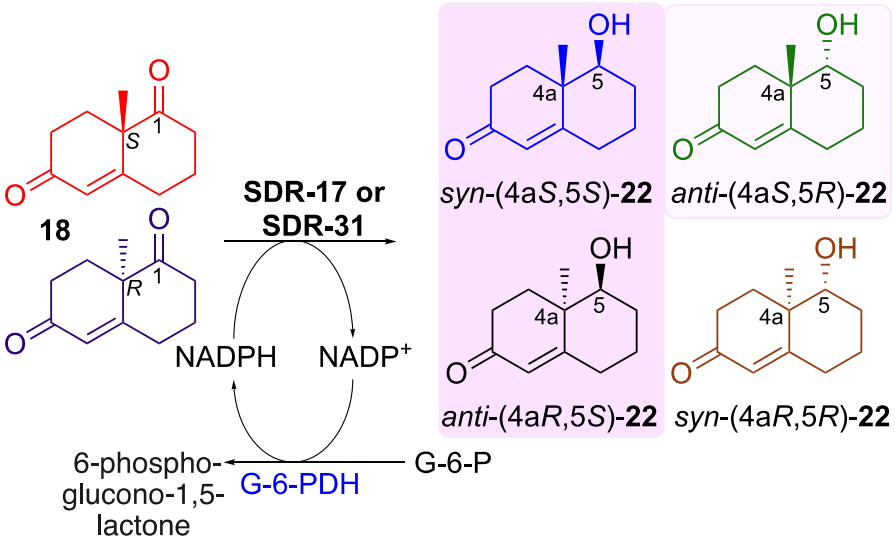


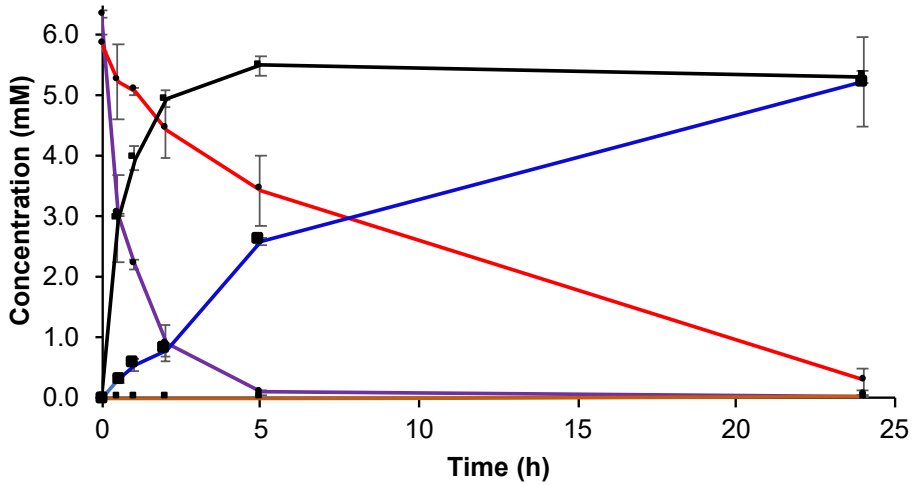




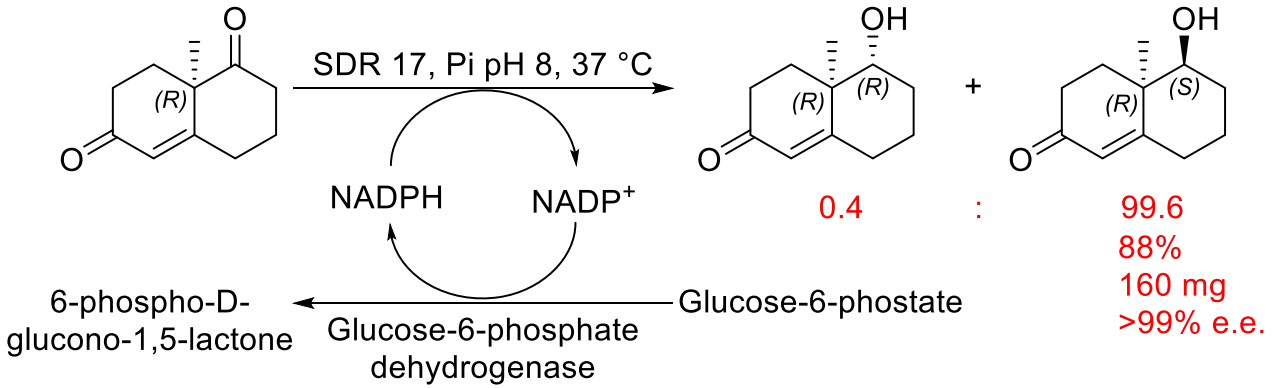
SDR-17

- SDR-17 had the best stereoselectivity towards the Wieland-Miesher ketone





- Preparative scale 50 mL volume

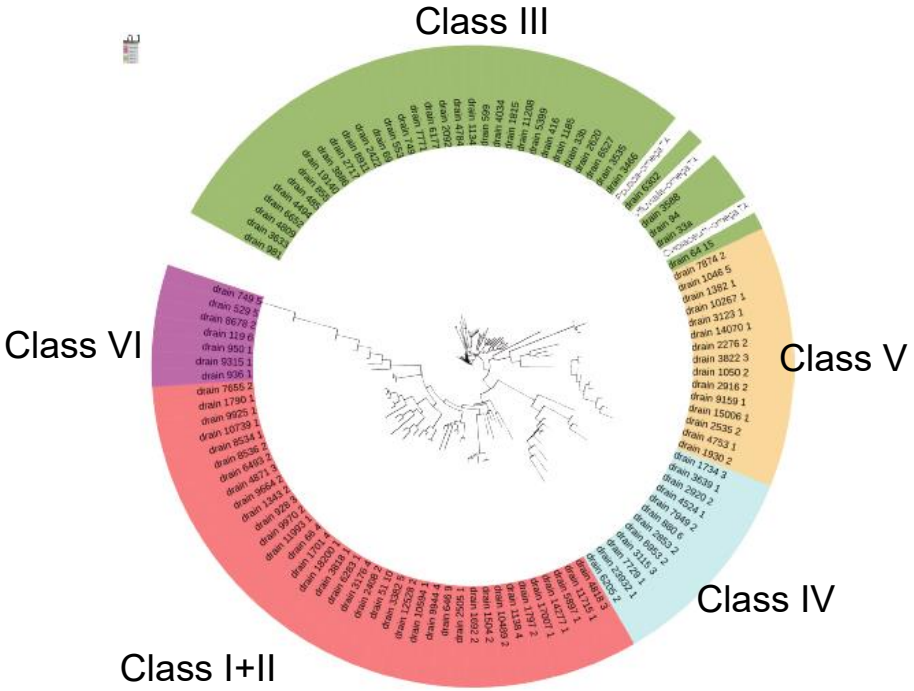


S. A. Newgas, J. W. E. Jeffries, T. S. Moody, J. M. Ward, H. C. Hailes, *Adv. Syn. Catal.*, **2021**, *363*, 3044

The drain metagenome

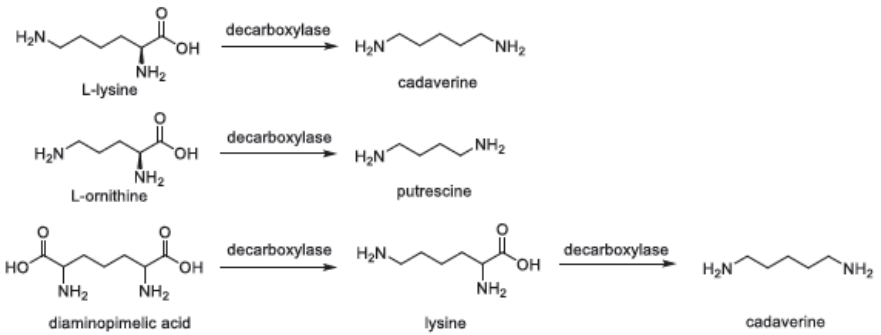
- Blocked domestic drain – source of microbial mass and a lot of biomass.
- Hair/biomass removed and metagenomic DNA extracted
- DNA sequenced using Illumina MiSeq and contigs built *in silico* - 219,776 contigs containing 307,101 genes

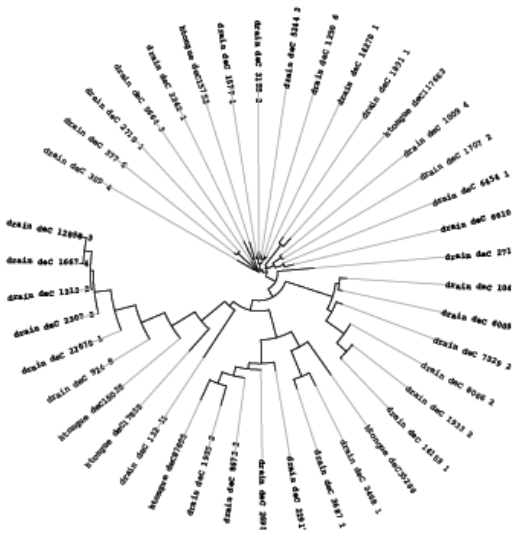
Transaminases



115 new transaminases

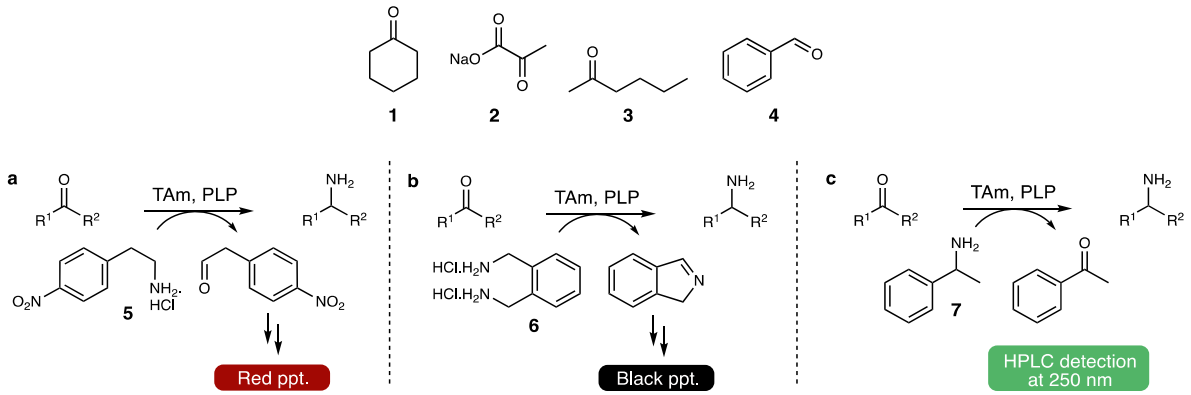
Decarboxylases

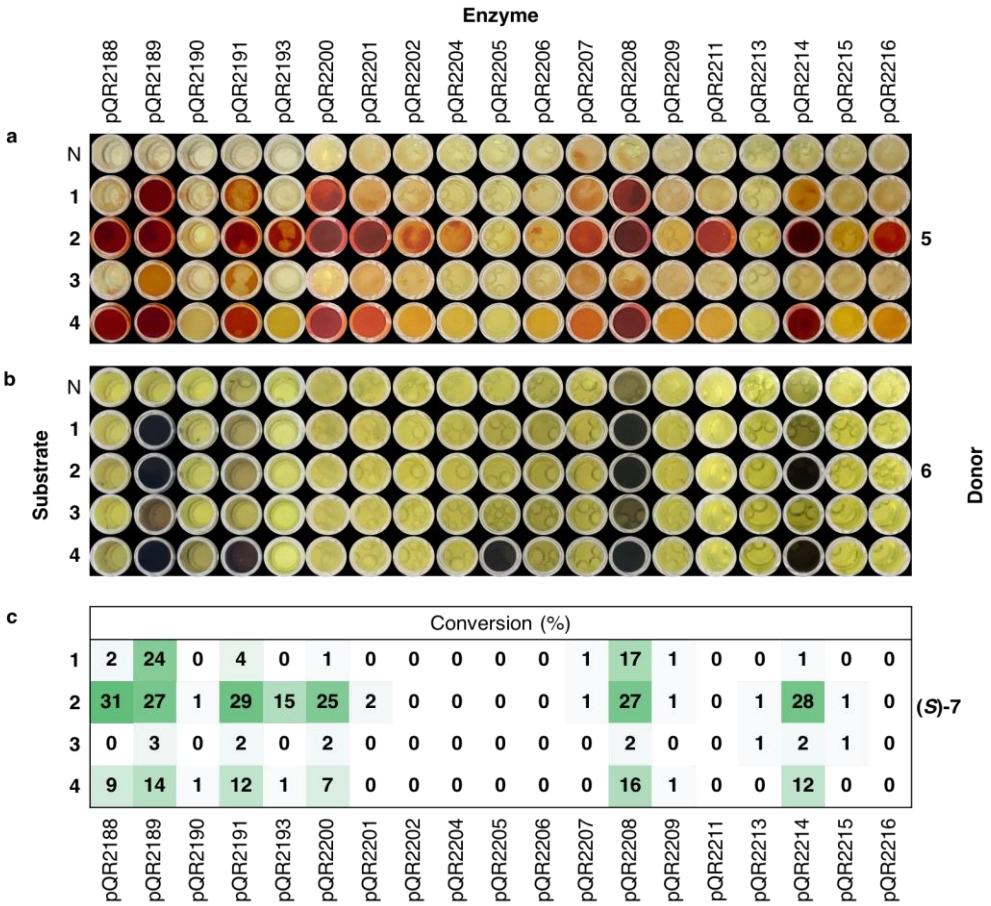




36 new decarboxylases

Robust TAms from a drain metagenome

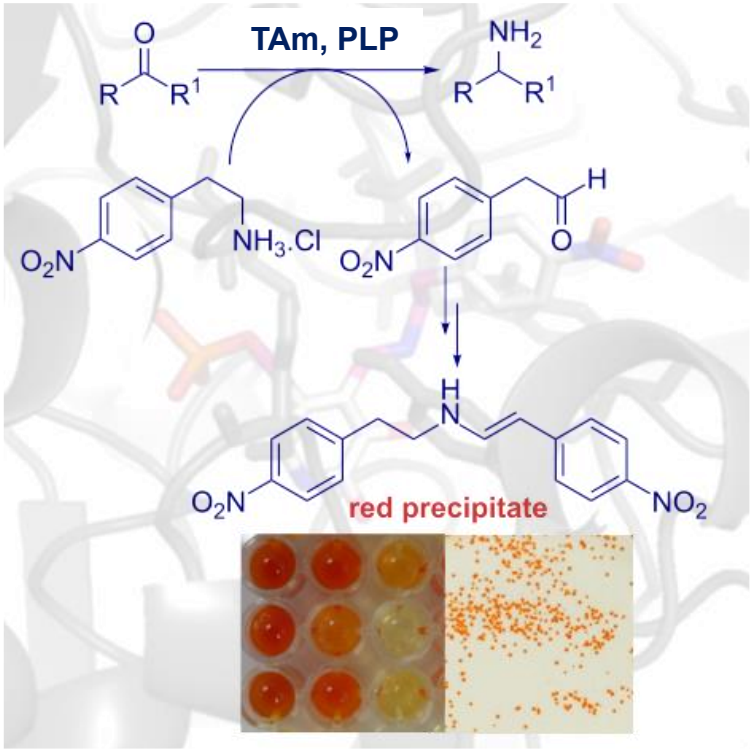


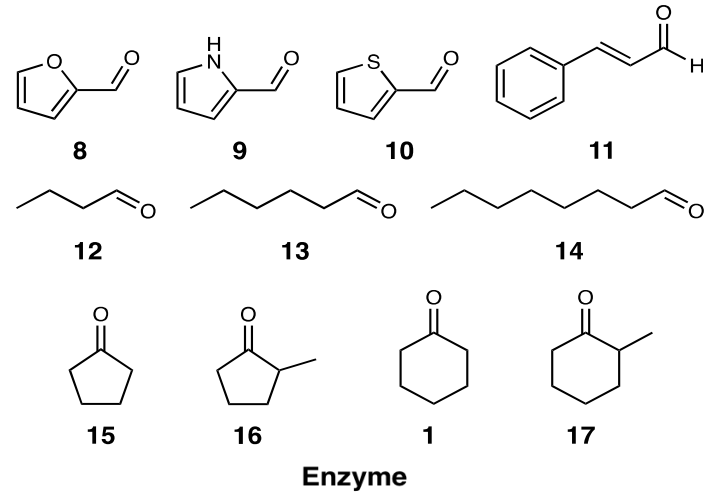


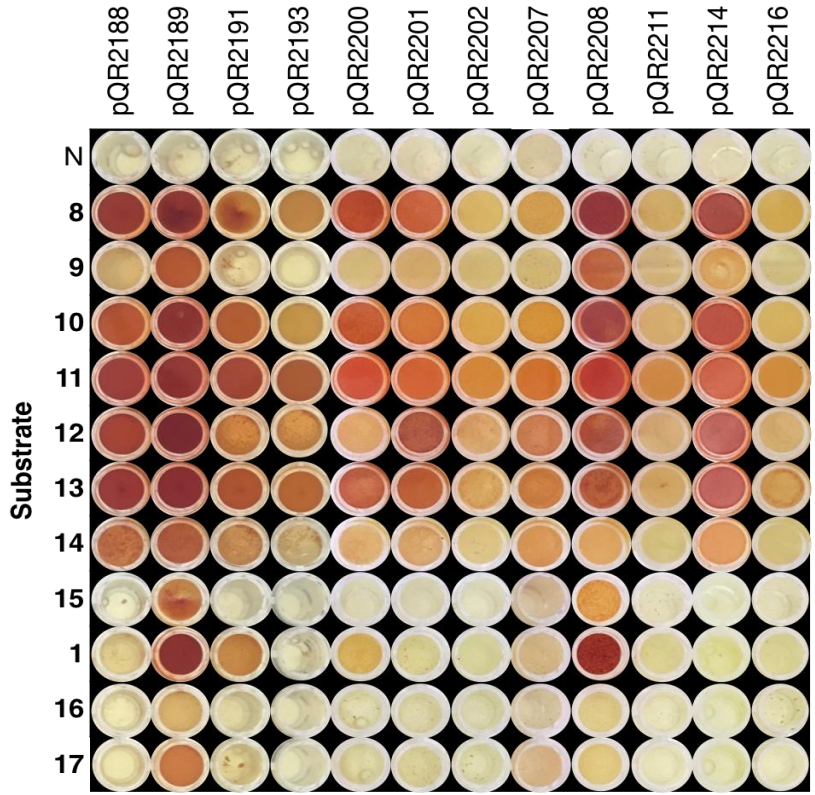
- **36 Class III TAms** identified
- **29 successfully expressed** in *E. coli*
- **19 displayed activities** against substrates

L. Leipold *et al*, *Green Chem.*, **2019**, *21*, 75

Robust TAms from a drain metagenome



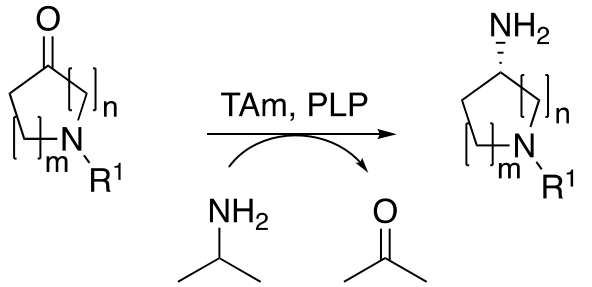


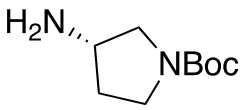


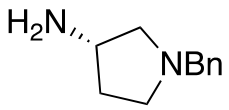
- **12** of the most versatile TAms were explored in more detail against a range of substrates
- **3** selected for further studies

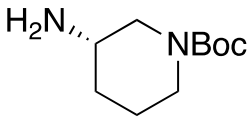
L. Leipold *et al*, *Green Chem.*, **2019**, *21*, 75

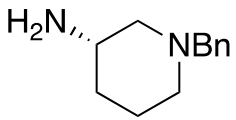
Robust TAms from a drain metagenome



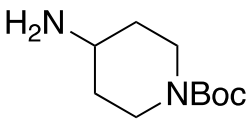


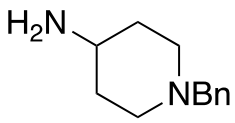


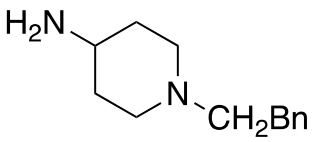




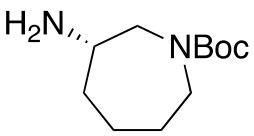
pQR2189	72%	20%	60%	2%
pQR2191	25%	4%	19%	1%
pQR2208	67%	10%	55%	1%







pQR2189	100%	75%	100%
pQR2191	100%	46%	100%
pQR2208	51%	30%	70%

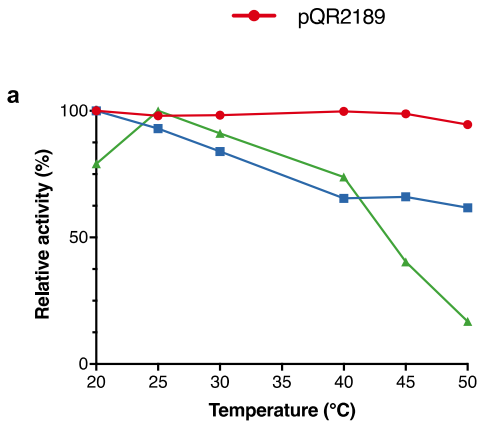


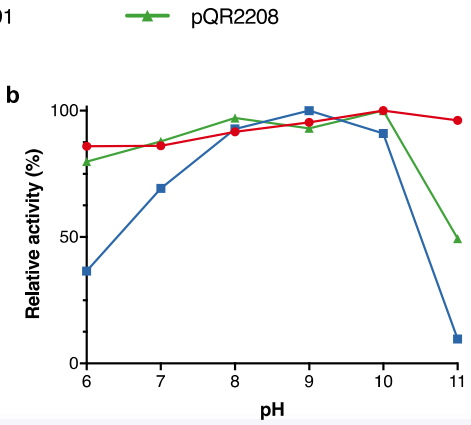
pQR2189	75%
pQR2191	13%
pQR2208	32%

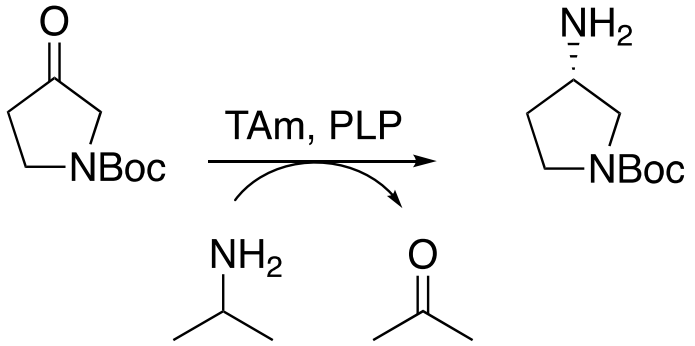
- 3 most productive TAms explored with further substrates using IPA as the amine donor

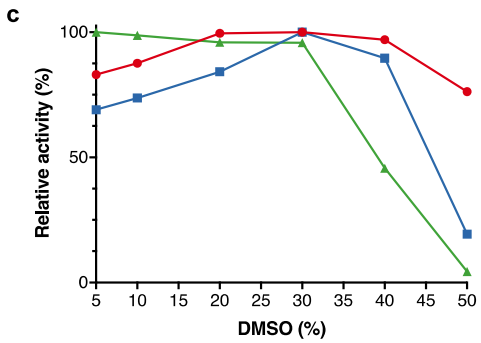
L. Leipold *et al*, *Green Chem.*, **2019**, *21*, 75

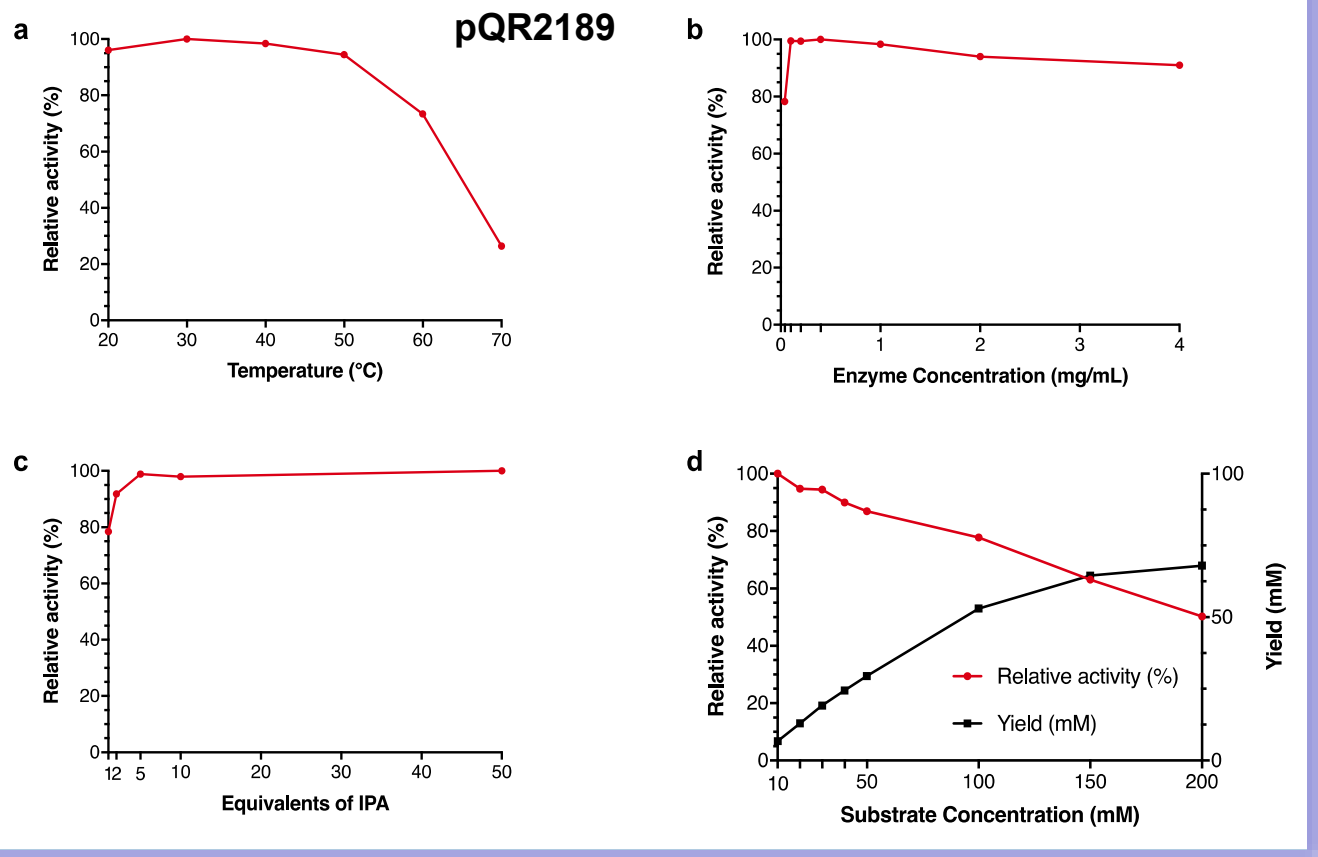
Robust TAms from a drain metagenome





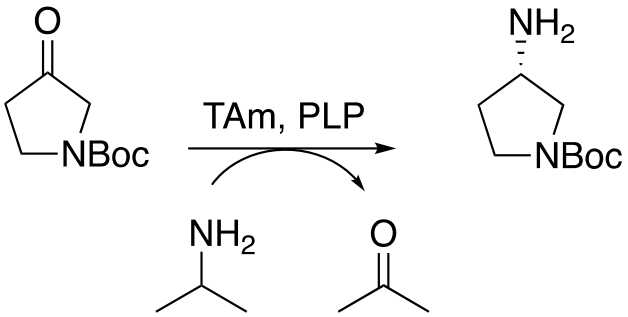


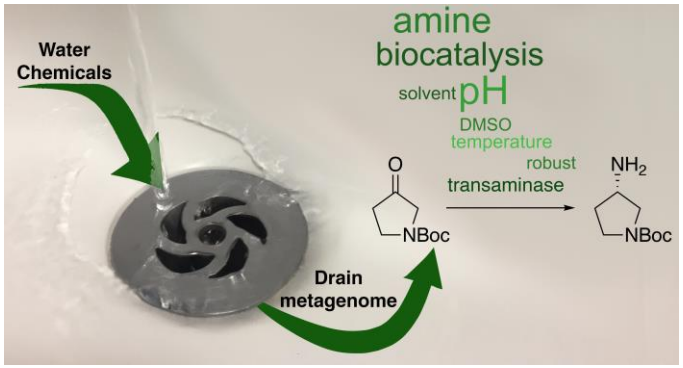


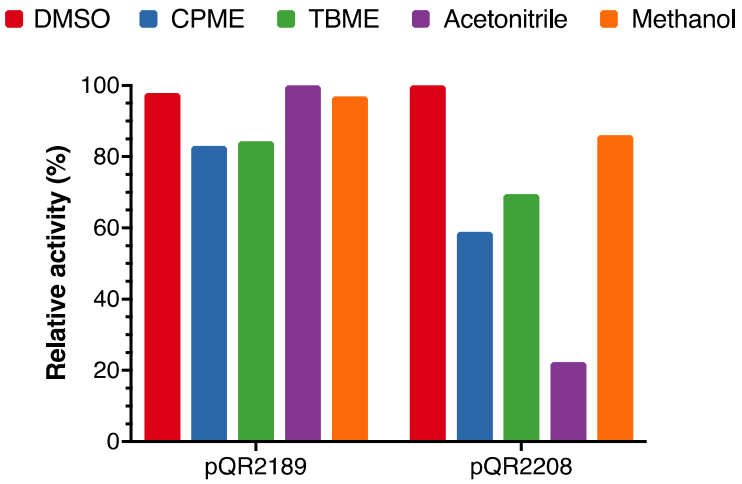


L. Leipold *et al*, *Green Chem.*, **2019**, *21*, 75

Robust TAms from a drain metagenome







- Highlights the value of using metagenomics for biocatalyst discovery from niche environments
- **One of the most robust native transaminases described to date**
- Enzme-preparative scale reactions performed (50 mL scale). Yields up to 100% (82% isolated yield, >99% ee)

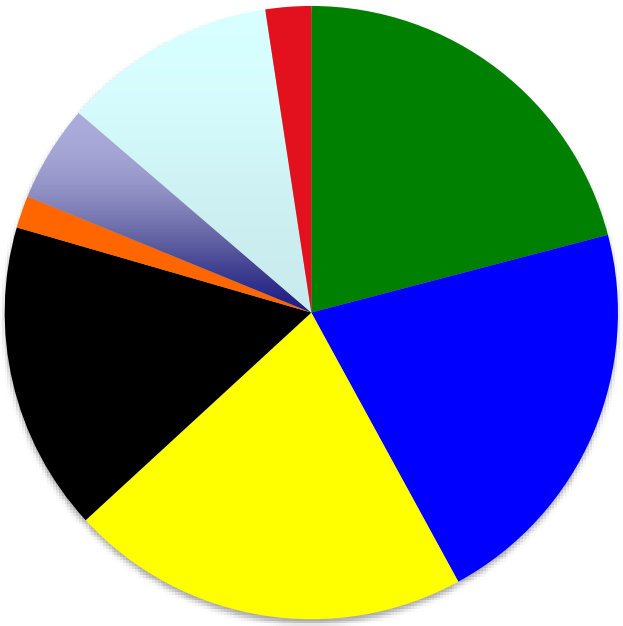
L. Leipold, D. Dobrijevic, J. W. E. Jeffries, M. Bawn, T. S. Moody, J. M. Ward, H. C. Hailes, *Green Chem.*, **2019**, *21*, 75

Bio-derived Feedstocks: Sugar Beet Pulp

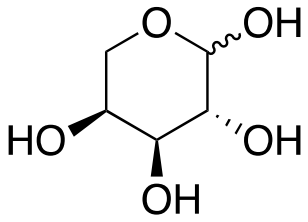
Cellulose → **Enriched cellulose fraction Feedstock for bioethanol**

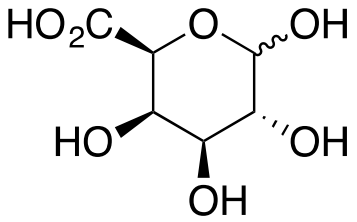
Pectin → **Selective release of pectin fraction, source of galacturonic acid and arabinose**

Other



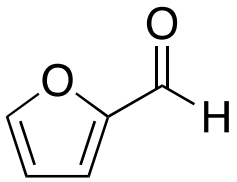
L-Arabinose

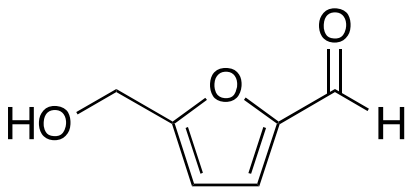




D-Galacturonic Acid

Furfurals





M. Cardenas-Fernandez *et al, Faraday Diss.*, **2017**, *202*, 41525

Upgrading biomass to aminopolyols

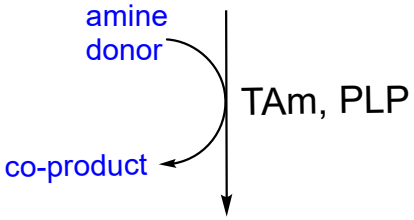
sugars

D-xylose

L-arabinose

D-fructose

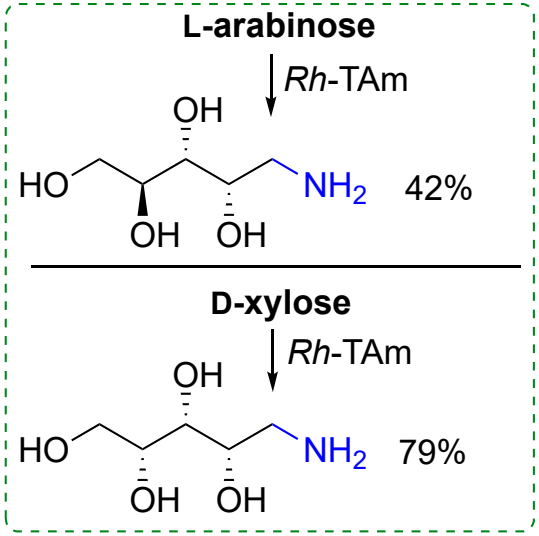
+ 7 more examples

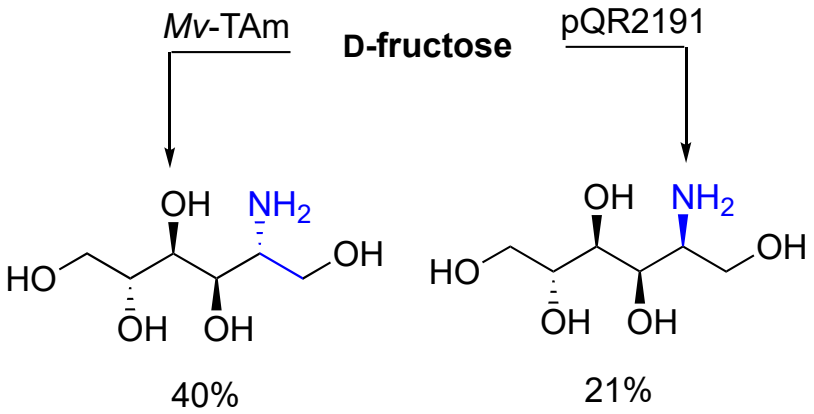


acyclic aminopolyols, e.g.

	Cv-TAm		*Rh*-TAm[a]		*Mv*-TAm		pQR2189[b]		pQR2191[b]		pQR2208[b]	
	5	(S)-MBA	5	(S)-MBA	5	(R)-MBA	5	(S)-MBA	5	(S)-MBA	5	(S)-MBA
D-GalAc		7%		n.a.		n.a		8%		29%		2%
L-arabinose		7%		16%		n.a.		6%		12%		1%
D-ribose		15%		14%		n.a.		12%		54%		3%
D-xylose		7%		28%		n.a.		4%		9%		n.d.
L-rhamnose		6%		6%		n.a.		n.d.		19%		n.d.
D-ribulose		9%		10%		45%		n.d.		12%		4%
D-fructose		1%		n.a.		40%		1%		31%		n.a.
L-sorbose		n.d.		n.a.		16%		1%		29%		n.a.
D-tagatose		n.d.		n.a.		22%		n.d.		24%		n.a.
L-glucohept		n.d.		n.a.		n.d.		n.d.		23%		n.a.
(+)		44%		75%		95%		74%		82%		62%
(-)		-		-		-		-		-		-

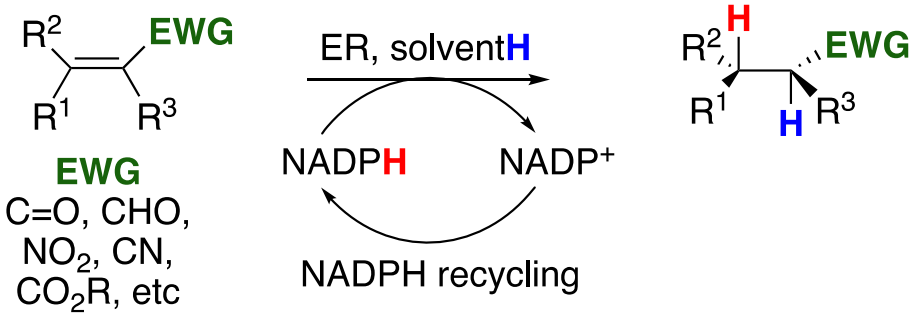
[a]Reaction at 30 °C rather than 45 °C; [b]Reaction in the presence of 25% of DMSO



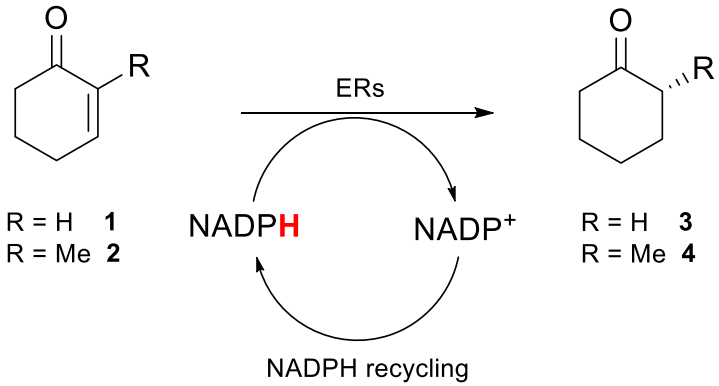


F. Subrizi, L. Benhamou *et al*, *Angew. Chem. Int. Ed.*, **2019**, *58,* 3854

Ene-reductases (ERs) : Drain metagenome



- **10 putative ERs** identified from the drain metagenome
- 9 were successfully amplified from the drain metagenomic DNA
- 7 were classical ERs and 2-thermophilic-like and they were expressed in *E. coli*

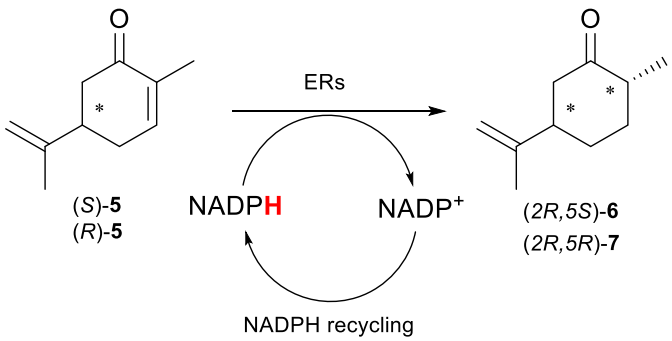


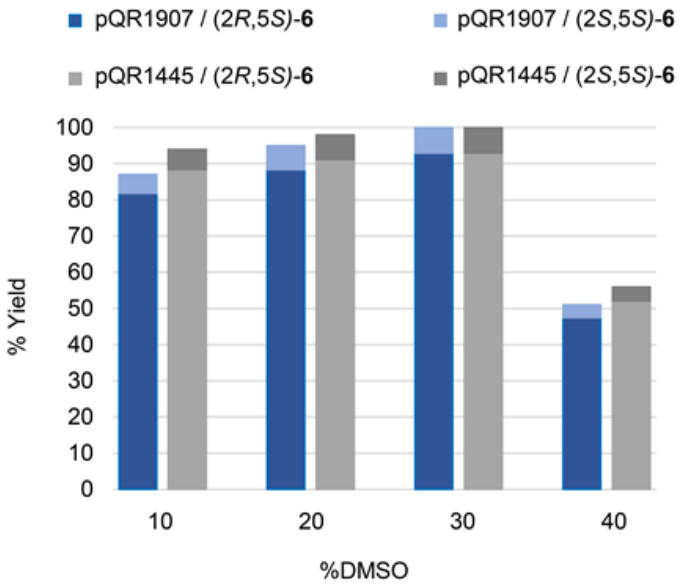
ERs	3	4	
	Yield [%]	Yield [%]	ee [%]
pQR1907	83	> 99	90 (*R*)
pQR1908	76	90	99 (*R*)
pQR1909	80	> 99	92 (*R*)
pQR1439	0	0	-
pQR1440	70	87	92 (*R*)
pQR1442	72	86	90 (*R*)
pQR1443	29	39	83 (*R*)
pQR1445	84	> 99	99 (*R*)
pQR1446	82	98	83 (*R*)
NCR	65	69	99 (*R*)

D. Dobrijevic, L. Benhamou, *et al., RSC Adv.,* **2019**, *9,* 36608

Ene-reductases (ERs) : Drain metagenome

- Co-solvent tolerance was investigated with dehydrocarvones
- Enzymes co-expressed with co-factor recycling enzymes G6PDH

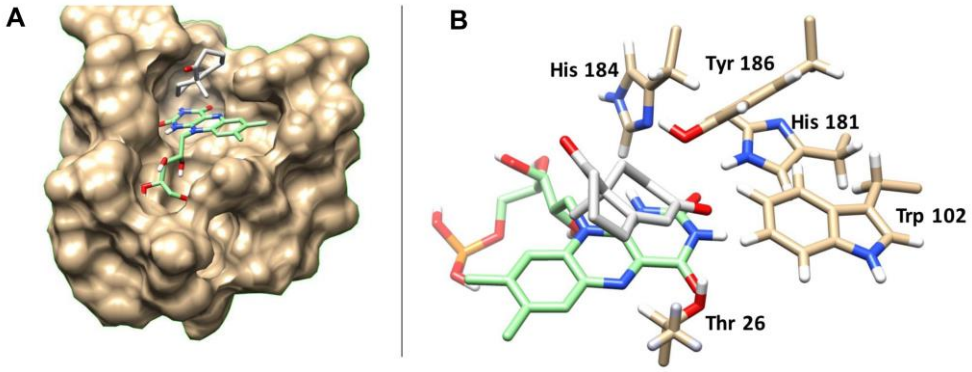




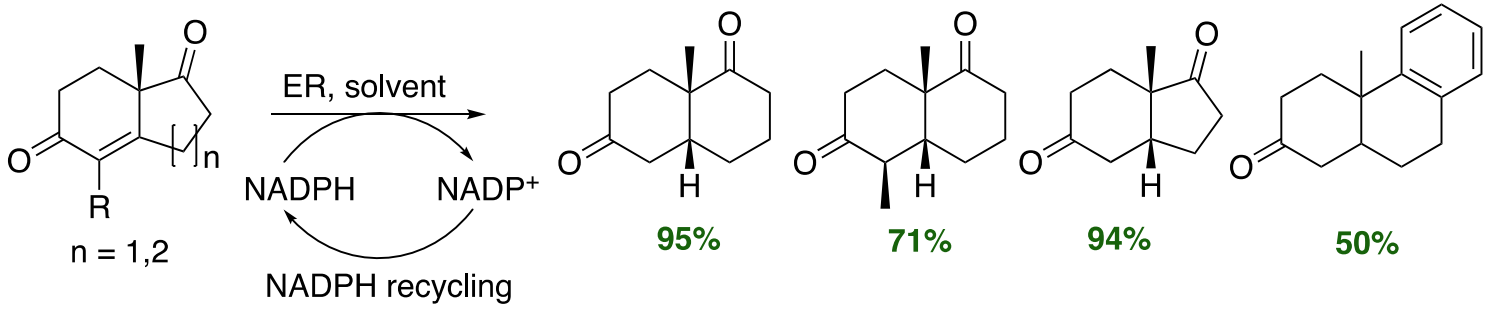
D. Dobrijevic, L. Benhamou, *et al., RSC Adv.,* **2019**, *9,* 36608

Ene-reductases: Drain metagenome

- ERs used with sterically challenging substrates and on a preparative scale

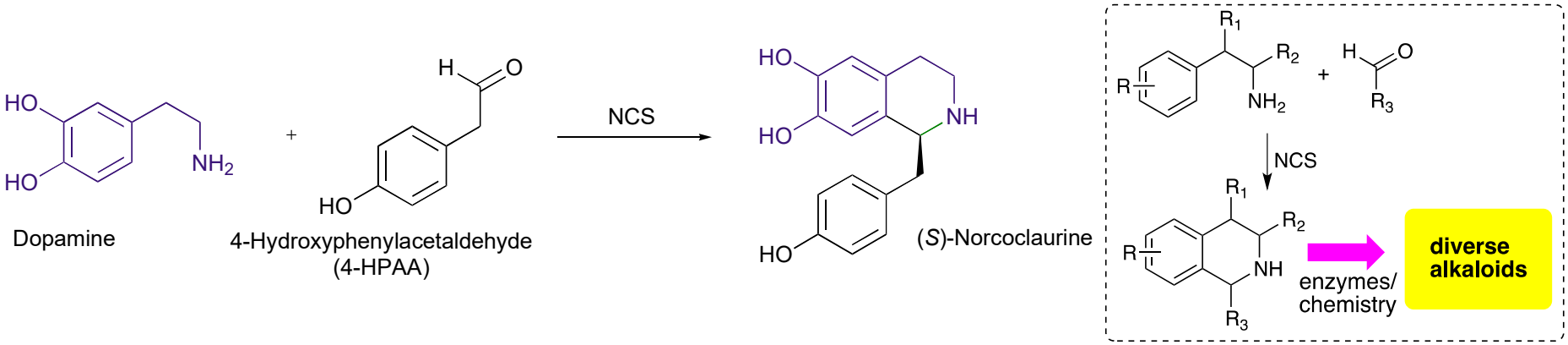


ERs from the drain metagenome → **pQR1445/pQR1907 Selective reduction of sterically challenging bicyclic ketones** → **Excellent organic solvent tolerance: E.g. 30% DMSO, MeOH**

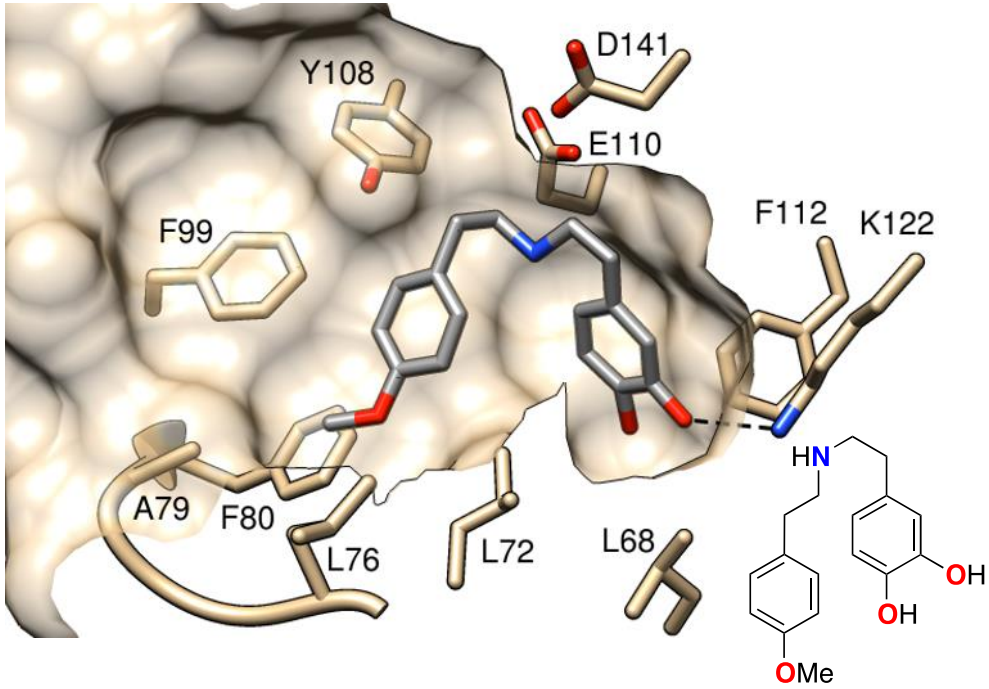


D. Dobrijevic, L. Benhamou, T. S. Moody, C. A. Orengo, *et al., RSC Adv.,* **2019**, *9,* 36608

Norcoclaurine Synthase (NCS)



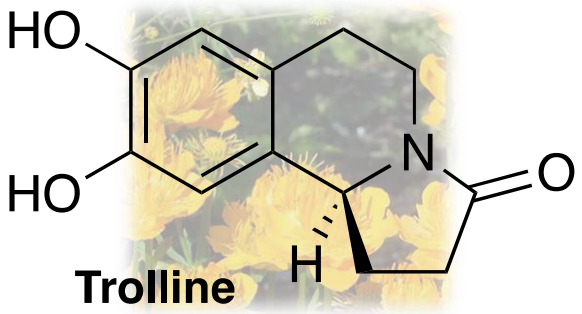
- **Several NCSs** known (e.g. ***Tf*NCS, *Cj*NCS2** and *Ps*NCS)
  - Small enzyme (22 kDa for monomer)
  - Crystal structures solved for *Tf*NCS (Δ33C196*Tf*NCS)
- 'dopamine first' mechanism

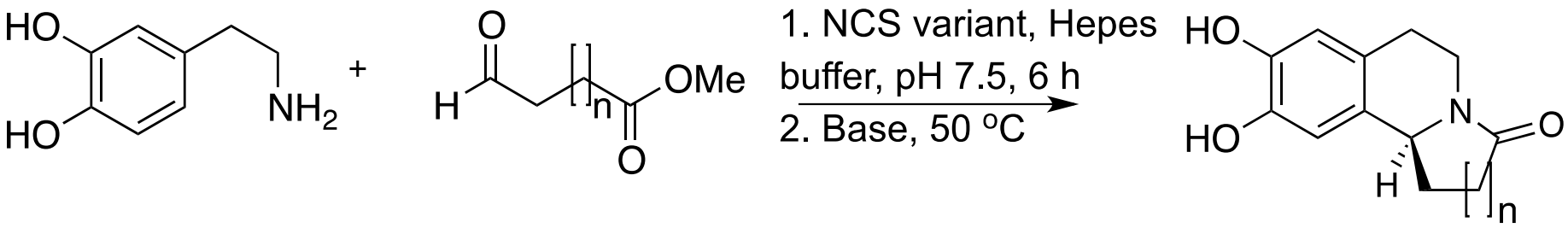


A. Ilari *et al, J. Biol. Chem.*, **2009**, *284*, 897; H. Minami, F. Sato *et al, J. Biol. Chem.* **2007**, *282*, 6274; T. Pesnot *et al*, *Adv. Synth. Catal.*, **2012**, *354,* 2997; B. M. Ruff *et al Tetrahedron Lett.*, **2012**, 53, 1071; T. Pesnot *et al*, *Adv. Synth. Catal.*, **2012**, *354,* 2997; B. R. Lichman *et al FEBS J.*, **2015**, 282, 1137; B. R. Lichman *et al Biochemistry*, **2017**, *40*, 5269

Cascades using NCS: (*S*)-Trolline

- Extracted from flowers of *Trollus chinensis:* Anti-viral activity influenza virus A and B
- Anti-bacterial against respiratory bacteria e.g. *Staphylococcus aureus*, *Streptococcus pneumoniae*

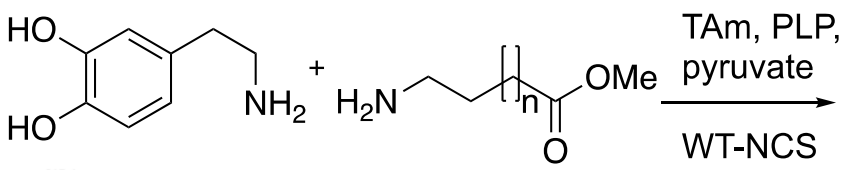


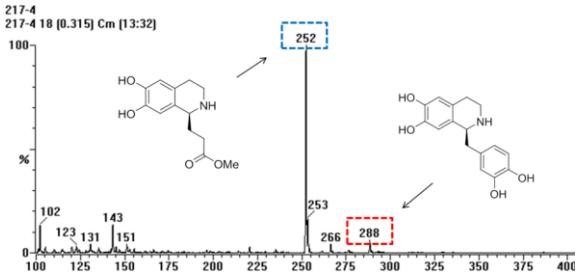


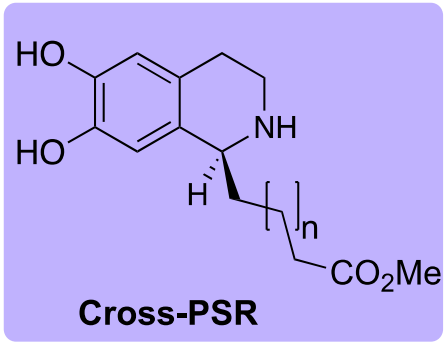
75-96% yield >95% *ee*

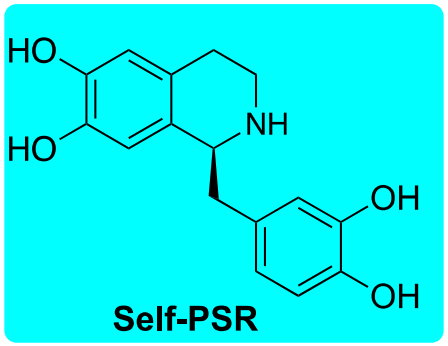
J. Zhao *et al, Chem Commun.*, **2018**, *54,* 1323

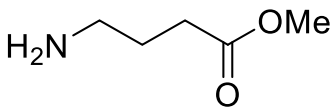
Transaminase-NCS Cascades

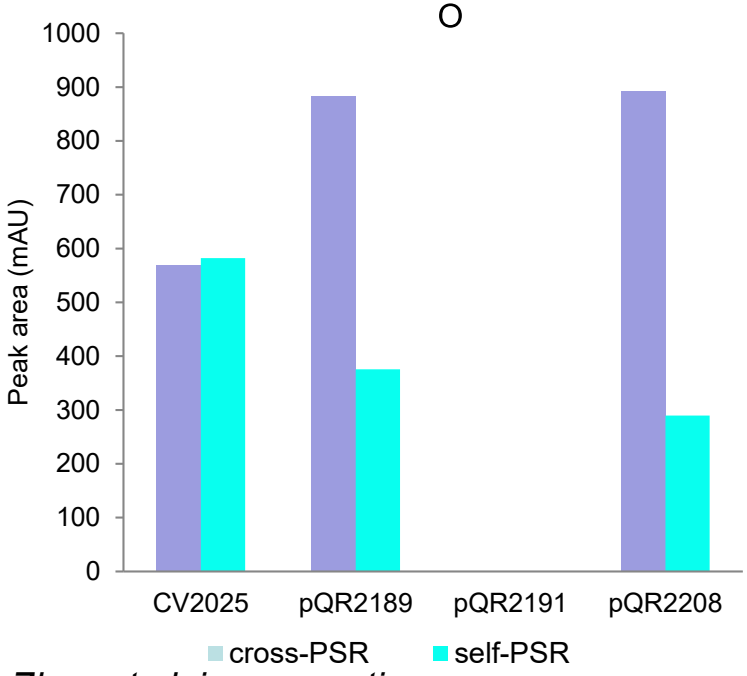


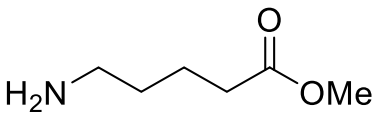


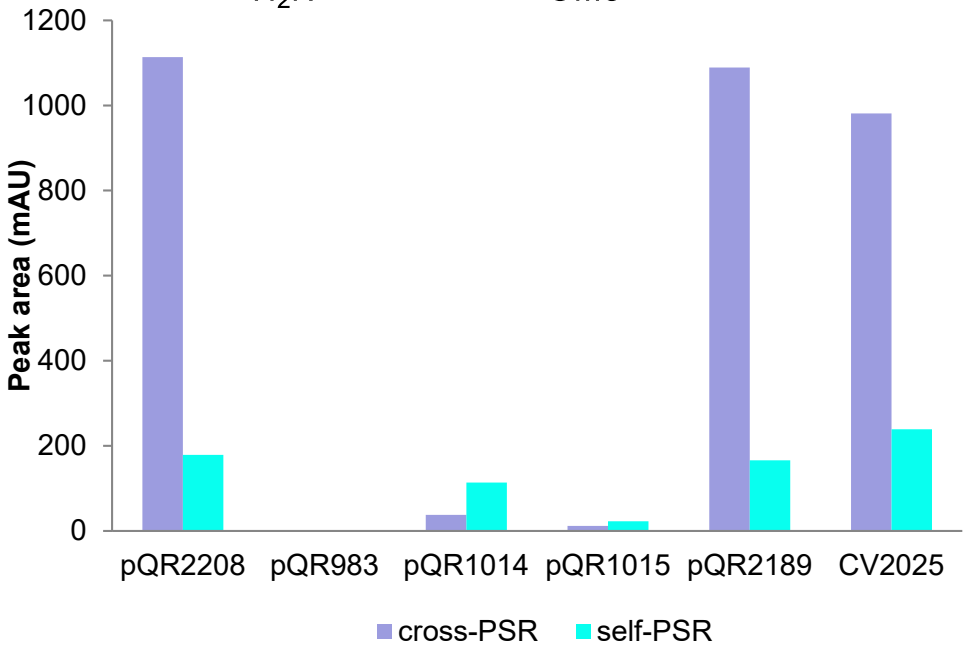








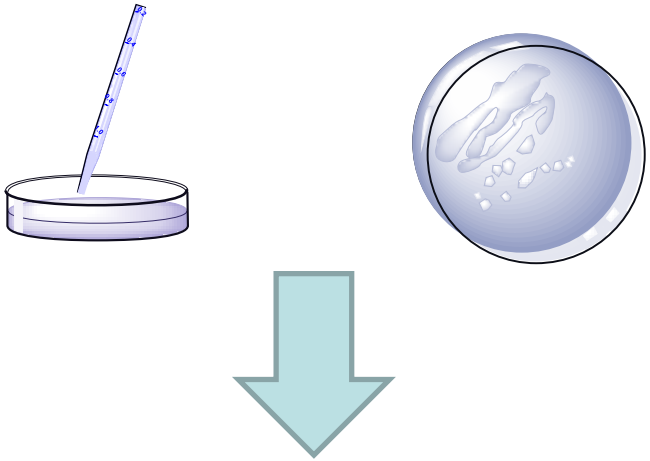




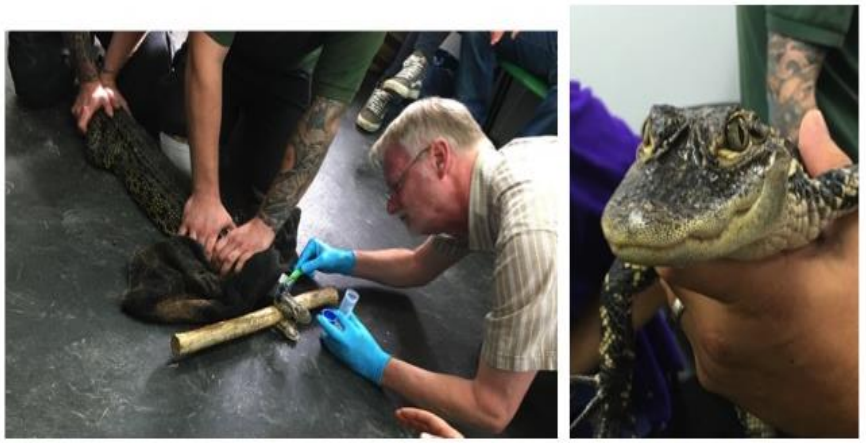
J. Zhao et al, in preparation

Crocodile oral metagenome

- Why do crocodile bites become infected? – oral bacteria!



16s Sequencing for Genus information



- Visited a crocodile sanctuary to collect microbiome samples from the mouths of several crocodiles.
- Genome mining for novel enzymes
- Continuing to sequence metagenomes from a range of environments

Acknowledgements

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Biotechnology and Biological Sciences Research Council

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