ALMAC

Partnering to Advance Human Health

“Shortening the Path - Pharmaceutical Materials from Enzymatic Reactions”

Dr. Stefan Mix / ALMAC Group

Organic Process Research & Development
Prague, 17-19 October 2016

www.almacgroup.com

ALMAC Group Overview

- Founded in 1968
- Global Headquarters in Craigavon, Northern Ireland
- Turnover >$500m
- ~ 4000 Personnel Globally
- Unique ownership – charitable foundation
- All Profit Re-invested
- Arran Chemical Company - purchased 2015

European Business Awards
RSM
Ruban d'Honneur 2014/15

Ulster Bank Business Achievers Awards 2014
Visit businessachieversaward.com

Aer Lingus Viscount Awards

The Irish Laboratory Awards

Belfast Telegraph Business Awards 2014
In association with British Airways

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History of ALMAC

1968
GALEN
Galen founded by Allen McClay

Galen floated on the stock market
GALEN
1997

Galen merged with Warner Chilcott
GALEN
2000

Sir Allen McClay retires
2001
GALEN

Sir Allen McClay comes out of retirement and acquires Galen service divisions
2002
CSS

Galen service divisions are integrated
2004
CSS

Almac successfully rebrand all business units as part of the Almac Group
2006
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Almac Group achieves foundation status
McClay FOUNDATION
2009

Passing of Sir Allen McClay.

Opening of regional headquarters in North American (Souderton)
2010

Opening of regional headquarters in Asia (Singapore)
2015

Almac Group acquire DP manufacturing facility in Loughborough, England
2015

Almac Group acquire Arran Chemicals (non-GMP) facility in Athlone, ROI
2015

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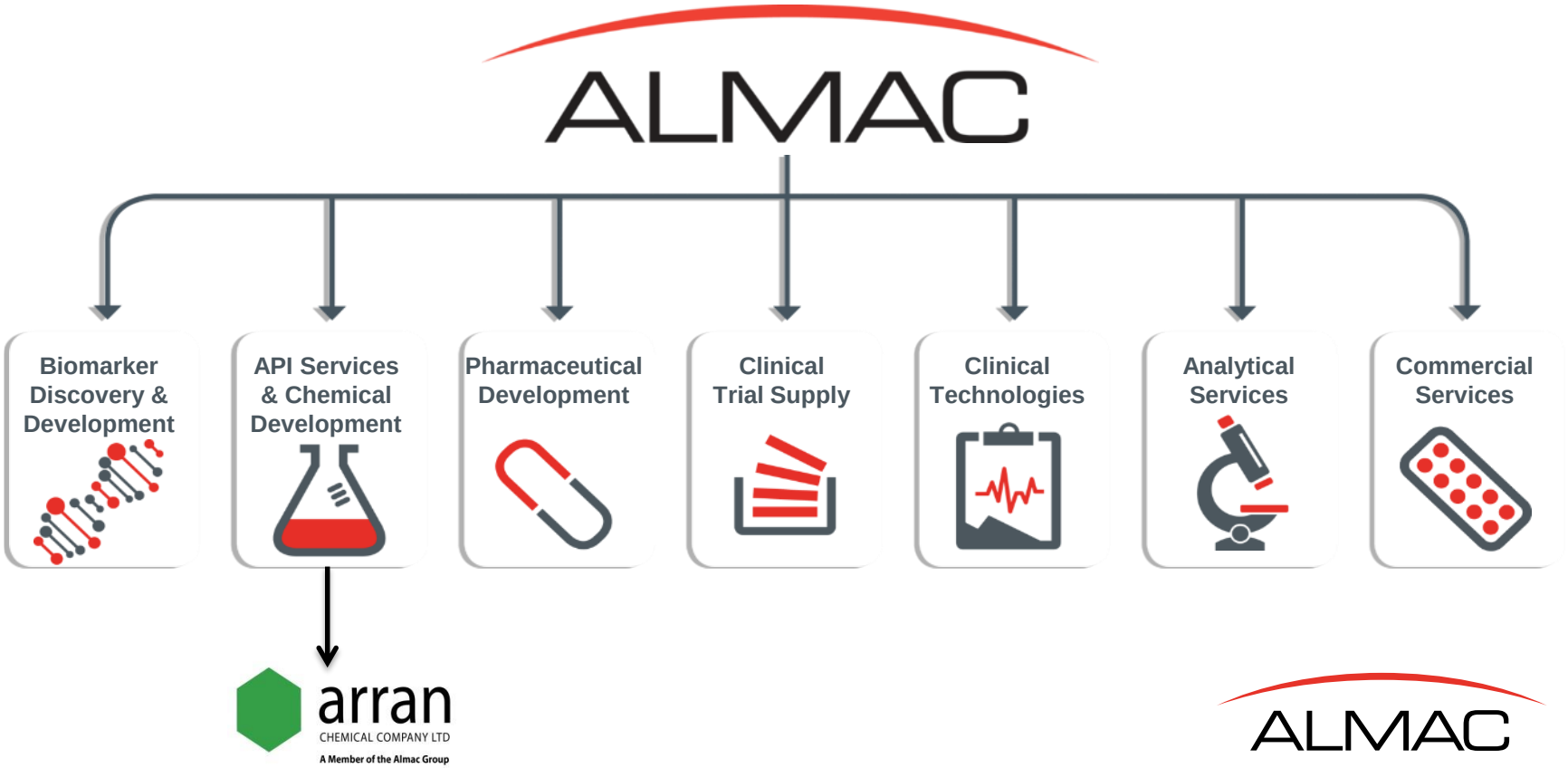
ALMAC Group: Who we are and what we do

Almac provides a range of integrated services to the Pharmaceutical and Biotechnology industries.

Services range across the full spectrum of drug development, from discovery through development to final delivery of commercial drug product.

Almac employs over 4000 people and has evolved into a world-class solution provider of integrated development services and product supply.



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Arran Chemical Company Overview

- Based in Athlone, Co. Roscommon, Ireland
- >25 Years experience as a fine chemical provider (building blocks and intermediates)
- ~60 Staff (chemists, analysts, production)
- Current output ~300 ton per annum
- Business split of 50% pharma and 50% speciality chemicals provisions

  e.g. flavour and fragrance, monomers, etc.

arran
CHEMICAL COMPANY LTD
A Member of the Almac Group

Advantages from using enzymes

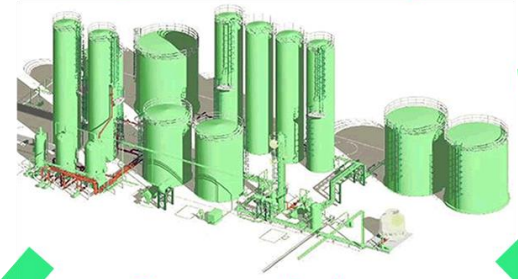
High selectivity

Avoidance of toxic waste

Conversion of waste to chemicals

Why biocatalysis?

Tandem biocatalysis



Application from mg to ton

Our vision

Low solvent usage

Renewable feedstocks

Rapid access to novel biocatalysts

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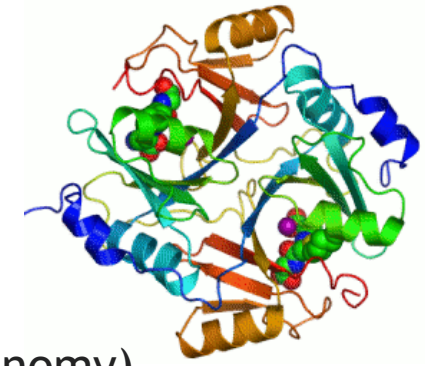
Biocatalysis at ALMAC

An integrated group of biologists and chemists who:



Discover. New enzyme discovery platforms

Screen. Screen for and utilize enzymes in processes

Evolve. Improve properties of enzymes (process efficiency, economy)

Supply. Manufacture (immobilised) enzymes and enzyme-derived products

Key Expertise:

- Enzyme discovery (genome mining, metagenomics)
- Building panels of enzymes
- Active site modelling and enzyme design
- Evolution tools (saturation/random mutagenesis)
- Fermentation development & scale-up
- Enzyme supply & immobilisation
- Bioprocess development and manufacture of products
- Metabolite synthesis

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What makes a biocatalytic process?

Natural diversity
- Characterised organisms
- Sequenced genomes
- Metagenomes

Protein engineering
- Directed evolution
- Rational design

- Recombinant enzymes
- Wild type organisms
- Pathway-engineered organisms

Biocatalysts

Key features:
- Selectivity
- Specificity
- Stability
- TON + TOF

- Process design
- Substrate engineering

Synthetic application

Key features:
- Cost
- Productivity
- Quality
- Sustainability

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Bioprocess challenges

Development effort and time → off-the-shelf *vs* bespoke biocatalyst

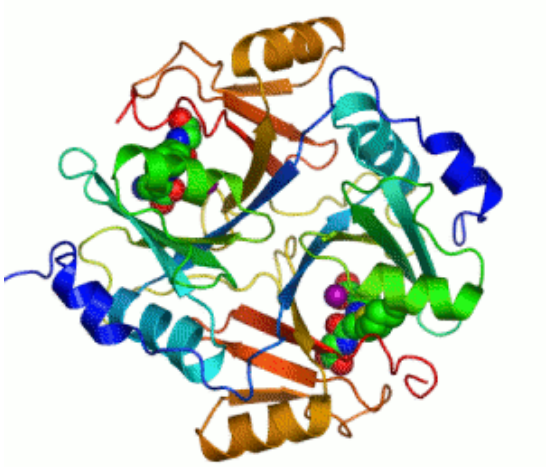
Catalyst cost contribution → choice of enzyme form

Space-time-yield and CAPEX / manufacturing cost → process design

Competing technologies → watch the market!

Productivity limitations from

- Mass transfer in heterogeneous systems
- Enzyme stability and deactivation
- Substrate/product inhibition
- Thermodynamics



Choice of starting material, route and enzyme class

Product isolation, purification, reduction of bioburden

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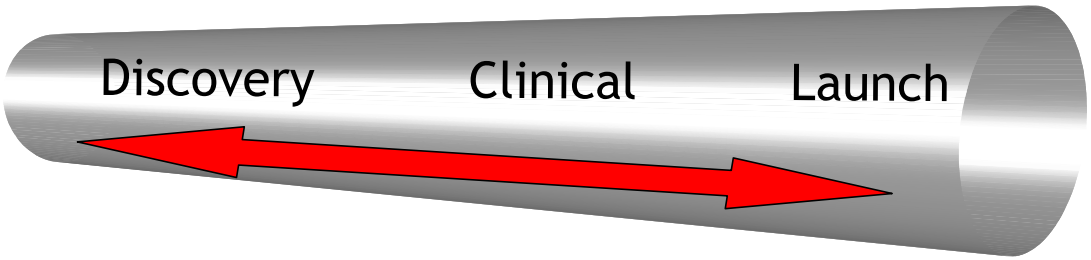
Biocatalyst libraries off-the-shelf: Speed matters!



mg-10g 100g-1Kg 1Kg-100Kg >100Kg

"Off-the-shelf" biocatalysts

Process Dev

Speed

COST

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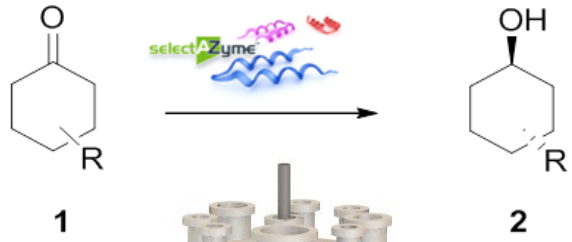
From screening to bioprocess scale up



Fermentation Development

Fermentation Scale-up

Bulk Enzyme supply

Scale-up
- Raw material sourcing
- Quality

Process Development
- Reaction design
- Reaction safety
- Enzyme form
- Product isolation

Successful POC
- Lab prod. sample

Enzyme Screening

Enzyme origin

Isolation

Product isolation+ purification *via*
- Solvent extraction
- Distillation
- Crystallisation
- Re-slurry
- Biopolish
- Ion exchange
- Charcoal treatment
- Ultrafiltration

Biocatalyst libraries off-the-shelf at ALMAC

Enzyme Platforms	Product Classes	Enzymes
Aldolases	Alcohols, Diols, Amino alcohols	96
Proteases	Peptides, Amines, Carboxyesters	20
Lipases and Esterases	Alcohols, Esters, Carboxylic acids	190
Ammonia lyases	Amino acids	40
Hydantoinases, Carbamoylases, Racemases	Amino acids	24
Amidases, Acylases	Amino acids, Amides	12
Imine reductases	Amines	100
Hydroxynitrile lyases	Cyanohydrins	24
Omega-Transaminases	Amines	400
Carbonyl Reductases	Alcohols	300
AA Dehydrogenases	Amino acids	96
Ene reductases	Ketones, Nitriles, Esters, Amides	180
Nitrilases and Nitrile Hydratases	Carboxylic acids, Amides	270
Amine oxidases	Amines	96
Monooxygenases (P450, BVMO)	Alcohols, Sulfoxides	300
Epoxide hydrolases	Epoxides, Diols	28
Halohydrin dehalogenases	Epoxides, Diols	18

Extensive portfolio of off-the-shelf enzymes:

- Made in-house
- From partners

On-going enzyme discovery programs:

- In-house
- With industry partners
- With academic partners

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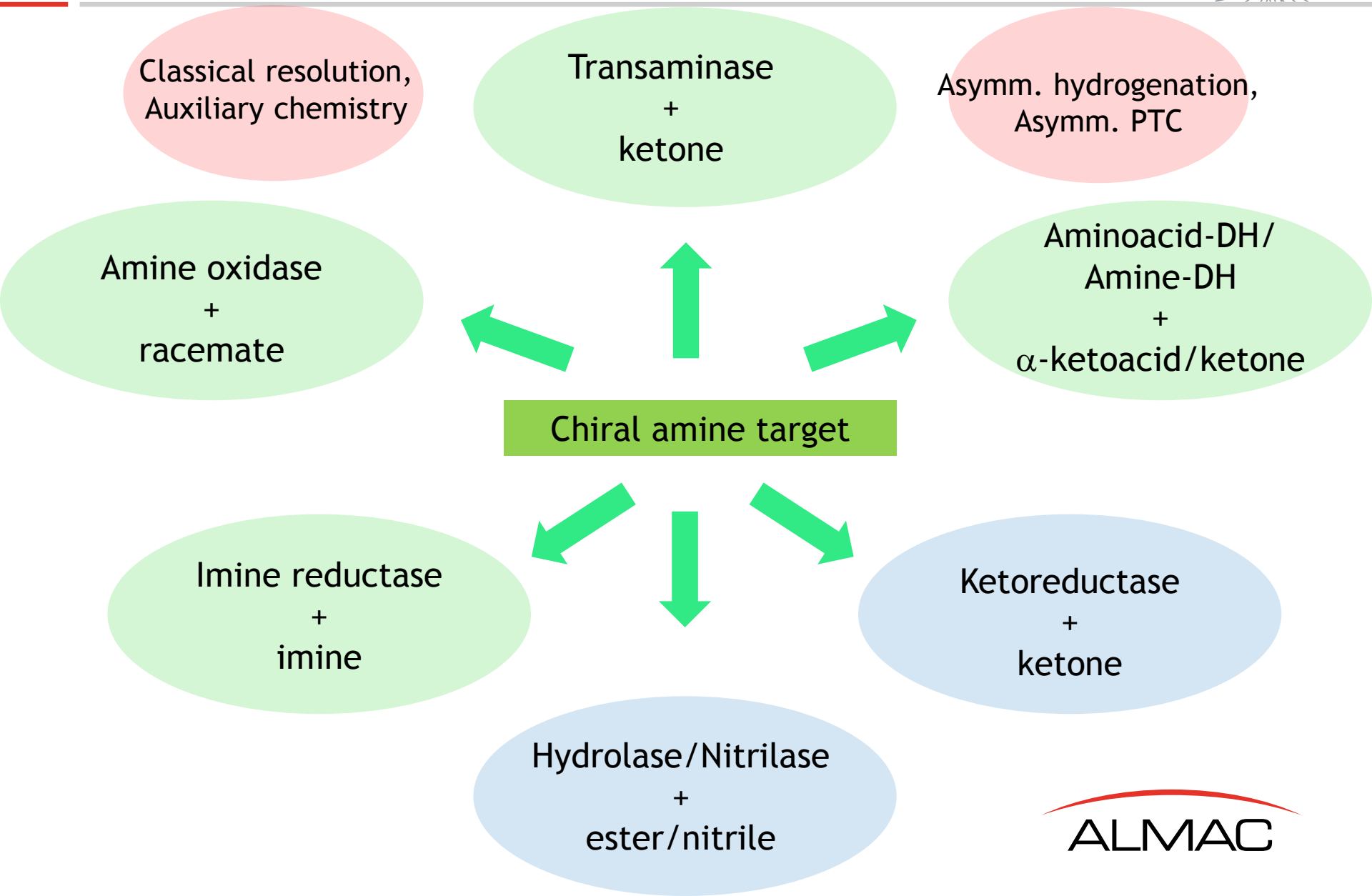
How to make chiral amines – spoilt for choice?



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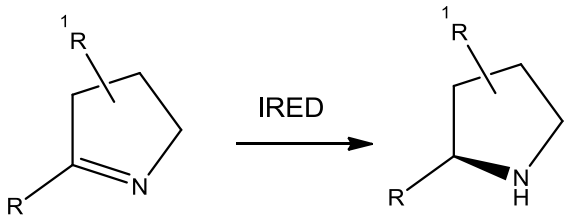
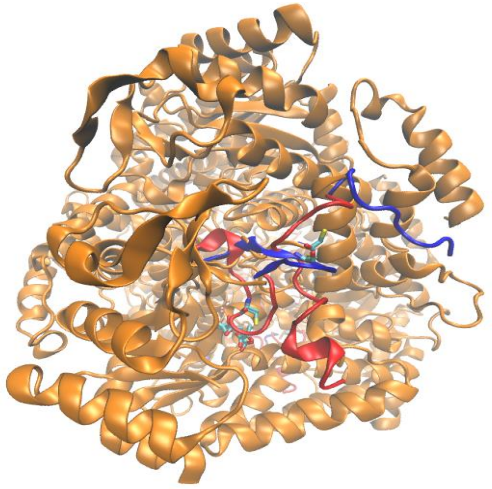
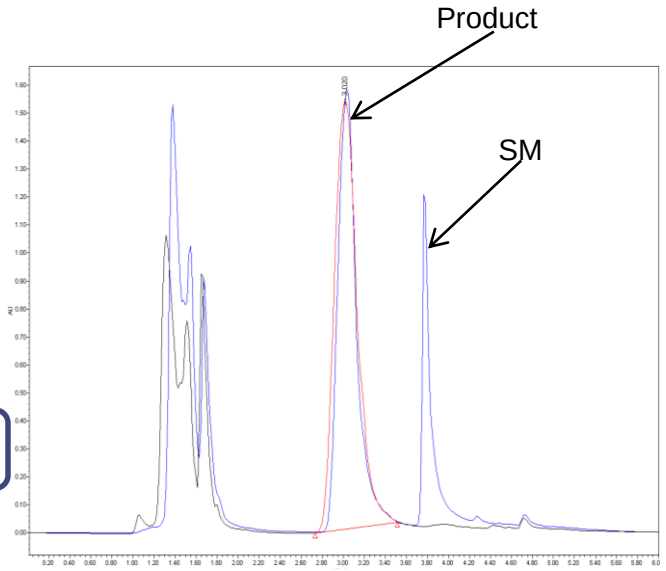
IRED found by *in-silico* screening





- *In silico* selection of 50 IRED enzyme library
- Gene synthesised, cloned and expressed
- Screened against client substrate – 20% hit rate

IRED no.	% HPLC peak area @ 260 nm		% HPLC peak area of product enantiomers		ee
	Product	SM	(S)	(R)	
3	9	91	54	46	8
5	2	98	0	100	100
8	77	23	89	11	78
9	2	98	13	87	74
12	3	97	100	0	100
16	3	97	77	23	54
20	100	0	100	0	100
26	1	99	89	11	78
27	31	69	92	8	84
28	8	92	0	100	100



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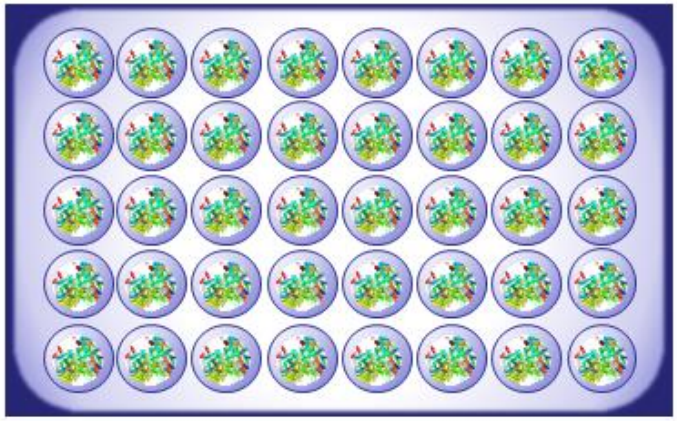
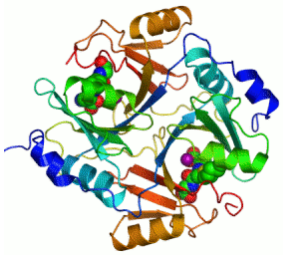
Enzyme panel building

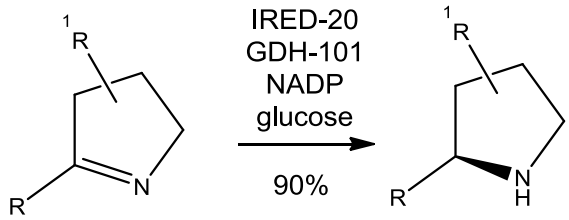
Bespoke enzyme panel





- Bespoke enzyme panels can be developed within weeks using Almac bioinformatics GIDS platform
- GIDS – gene informed database searching
- Tailored panel of enzymes expressed in 96 well plate format
- 1 enzyme only possible; typical is 25, 50 or 96
- Panels can contain mutants, homologues, or enzymes selected by in-silico screening

IRED process design challenges + solution



- IRED-20 selected for development
  (100% ee and best activity in screening)
- Enzyme form defined: Spray-dried cells suitable
- Challenges encountered during PRD:
  Inhibition of enzyme limits product titre
  Product highly water soluble → difficult recovery by extraction
- Solution implemented: Ion exchange column in recirculation mode
  → Solves inhibition problem in aqueous reaction medium
  → Enables efficient product recovery with ammonia in methanol

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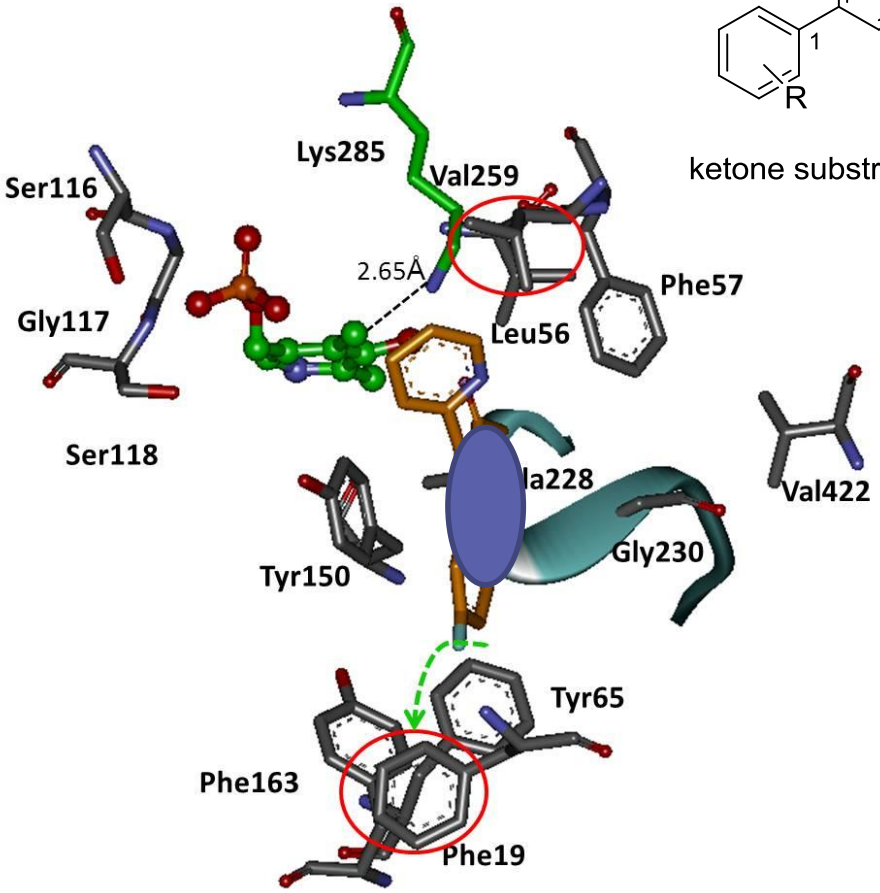
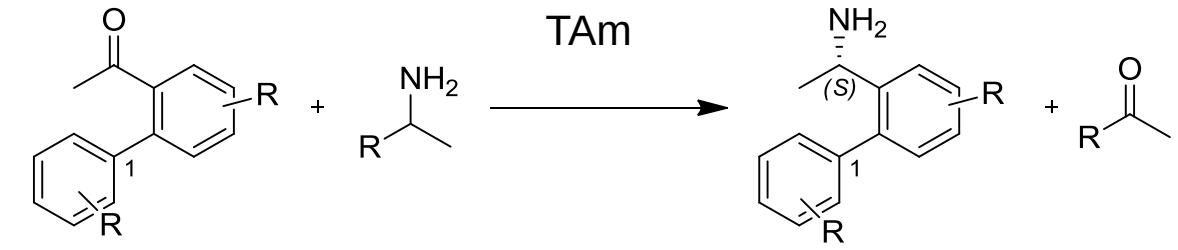
Engineered transaminase for arylethylamine (1)

Rational variant design by protein-substrate interaction modelling





ketone substrate | amine donor | S-amine product | side product

- Bulky substrate – no good for wildtype TAm enzymes
- Smart library approach chosen for enzyme engineering
- Only rationally selected residues were mutated
- Increased chance of success from short engineering program

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Rational protein engineering

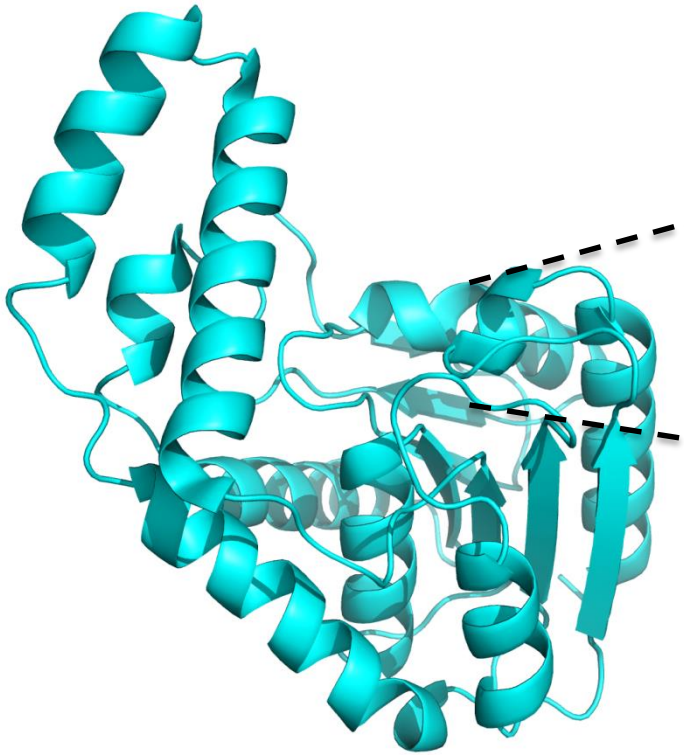
Initial Structural analysis

Docking/ Mechanism determination

Point mutations

In silico prediction of mutant Enzymes

Wet lab validation



Typically 20-50 rationally designed mutant enzymes per round

Wet lab validation

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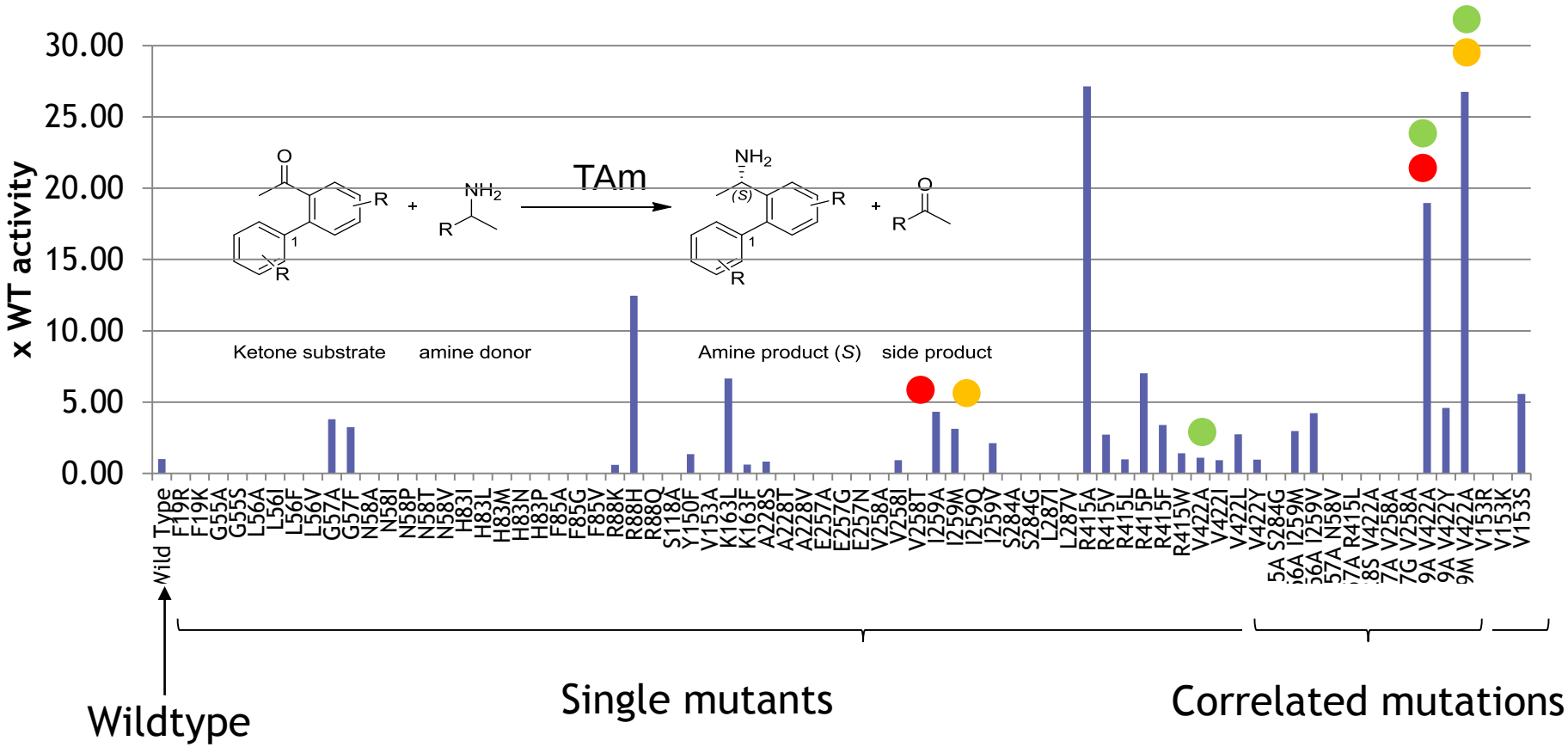
Engineered transaminase for arylethylamine (1)



⇨ 72 Mutants screened => first round improvement: 25 x wildtype activity

Huang *et.al.* ACS Catalysis **2016**, DOI: 10.1021/acscatal.6b02380

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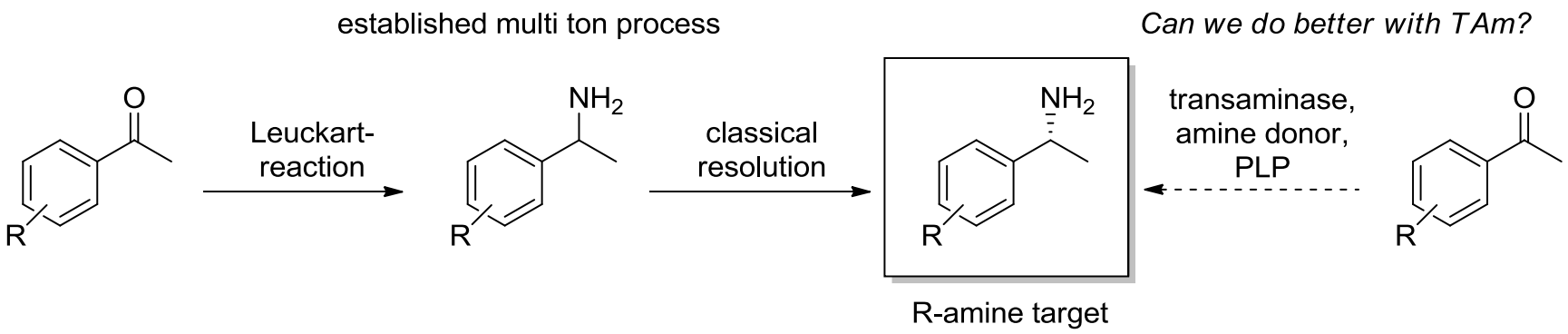
Engineered transaminase for arylethylamine (2)



- Large volume product made by Arran *via* classical resolution
- Cost improvement wanted via implementation of transaminase process using cheap amine donor isopropylamine
- Screening of off-the-shelf R-transaminases gave hit with excellent ee
- However: can't get to high enough titre AND low enough enzyme loading with screening-hit enzyme
- Challenge by interplay of thermodynamics and enzyme stability
- Enzyme engineering program initiated

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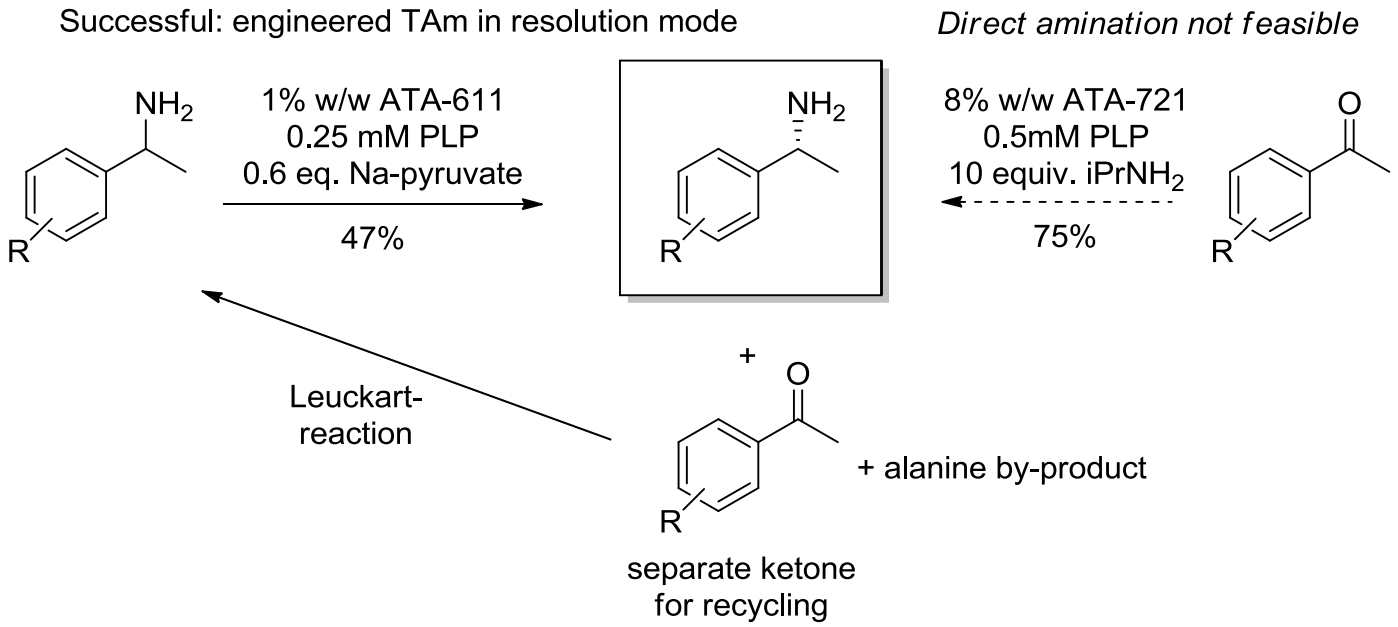
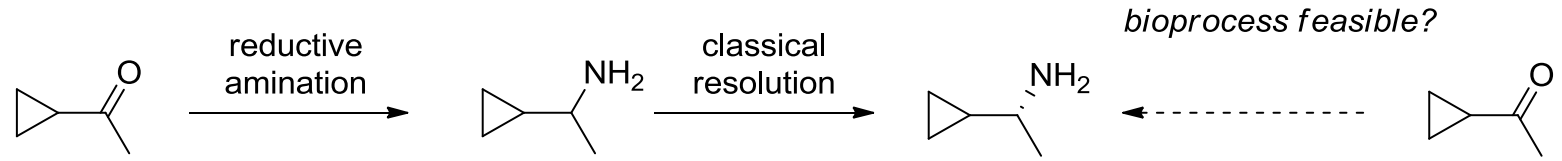
Engineered transaminase for arylethylamine (2)



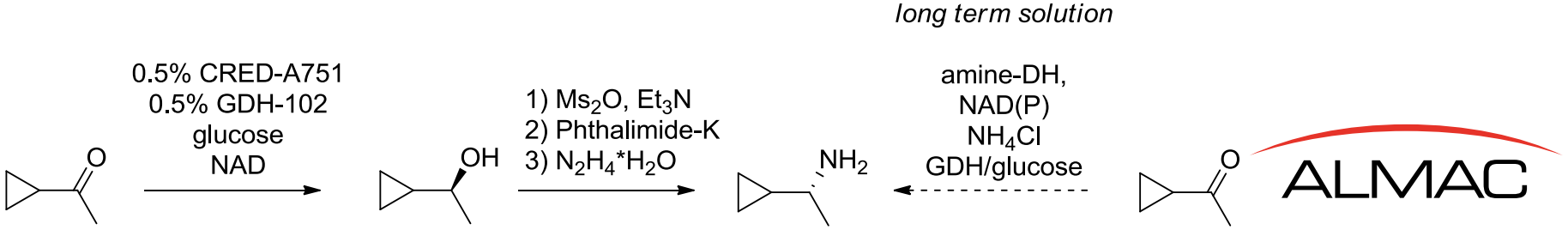
- Limited TAm engineering program conducted
- Best direct amination process with iPr-amine donor gave insufficient improvement of yield, enzyme loading and product titre
- Using ATA-611 in resolution mode resulted in much better overall process (ketone recycling required, but easily accommodated in existing synthetic route)

Are further improvements possible? Sure!

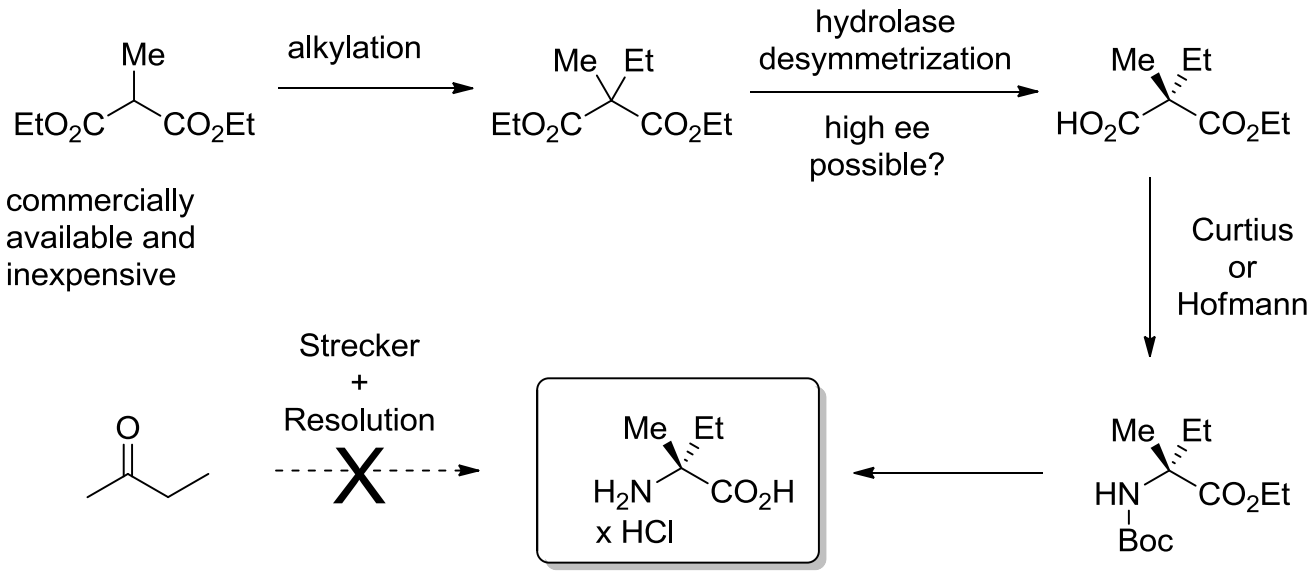
Wanted: asymmetric bioprocess for (R)-CPEA



- R-CPEA is building block of great interest and limited, costly supply
- Very small molecule = challenge for any technology
- Existing process suffers from high cost of resolving agent, manufacturing, and low yield
- Transaminase process investigated, but cost is too high
- CRED + displacement works better than resolution route despite 1 more step
- Long term outlook: Amine dehydrogenase process (still needs some work)



Desymmetrization route design: D-isovaline



Existing D-isovaline supply: too expensive, too low ee, under IP.

Initial attempts to establish quick Strecker+Resolution process failed.

Can we establish a desymmetrization process meeting requirements?

Can the strategy be applied to L-isomer and other amino acids?

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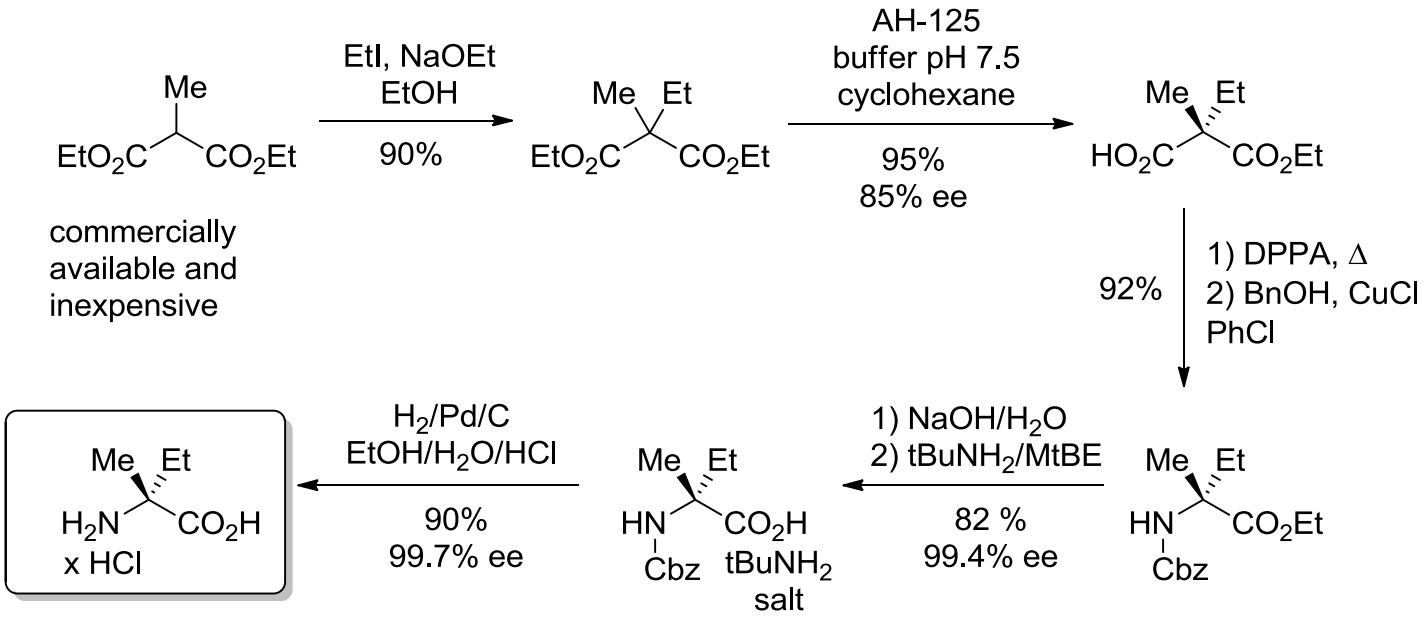
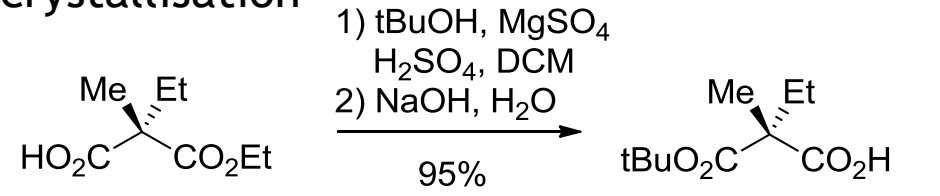
Desymmetrization route design: D-isovaline



Desymmetrization strategy worked – multi kg process implemented
High ee spec of client met via ee upgrade crystallisation

L-isomer accessible via ester switch:



key intermediate

to L-isomer from here

The strategy has been applied to 5 other “exotic” aminoacids.

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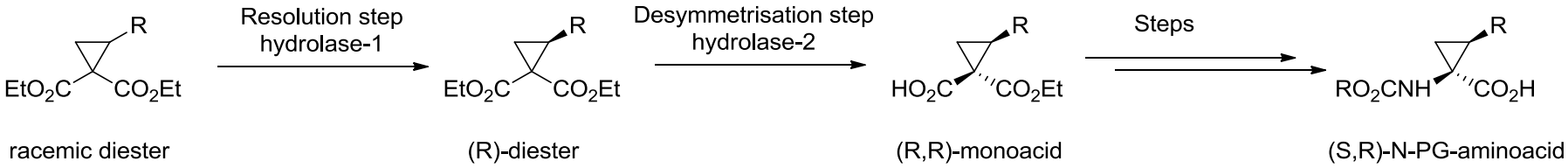
Desymmetrization for 2nd generation process



Background:

- Client wanted enzymatic process to replace chiral SMB process for late phase and commercial API

Problems:

- Expensive racemate – yield matters a lot
- Hydrolase-1 had low E-value of 13 → (R)-diester yield < 30%
- Hydrolase-2 had low stability → high enzyme loading required with resulting isolation problems and yield impact

Solutions sought:

- Improve E-value of resolution step via substrate engineering
- Improve stability of desymmetrization enzyme via homologues panel

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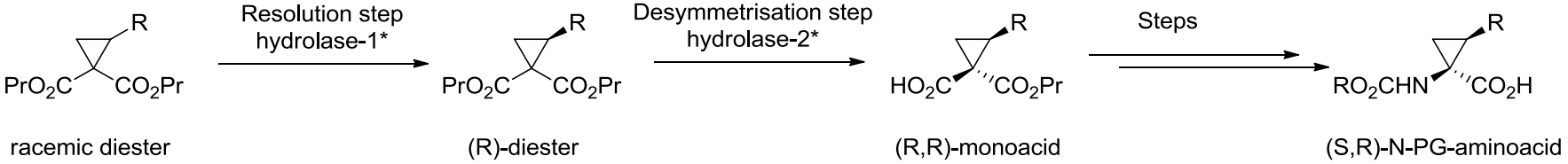
Desymmetrization for 2nd generation process



Problems solved:

- Hydrolase-1* has E-value of 60 with dipropyl ester → (R)-diester yield is now 45+%.
- Hydrolase-2 accepts (R)-propyl diester with unchanged selectivity.
- Hydrolase-2* found via homologues search. It was reported to be thermo-stable, and has a published crystal structure. At maintained selectivity the process now runs at 50 deg C with low enzyme loading.

Thinking forward:

- Solutions so far enable maintaining registration schedule but are not all we could wish for.
- Protein engineering of hydrolase-2* to open the way to 3rd generation single enzyme process.

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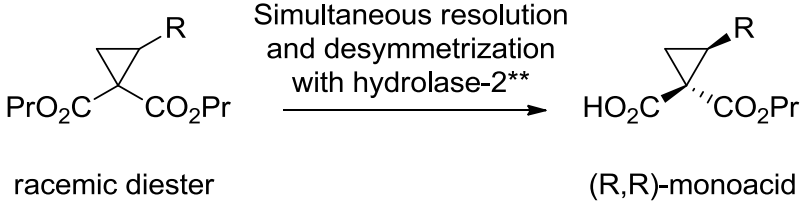
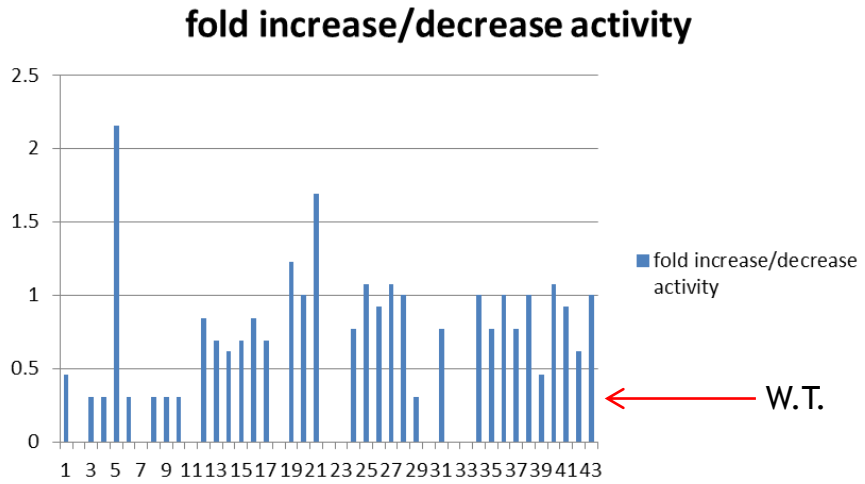
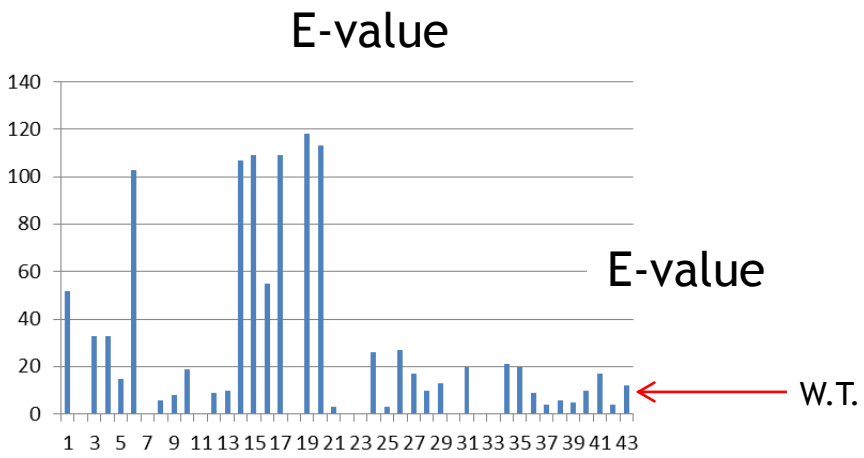
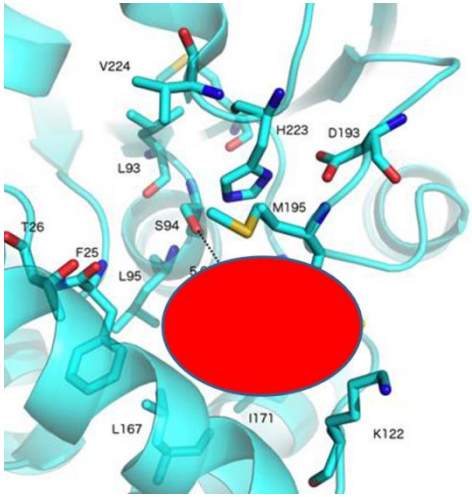
Hydrolase engineering for 3rd generation process



- Want single enzyme approach
- Substrate docking and rational mutagenesis: 2 rounds of 20 completed
- Greatly increased selectivity and catalytic rate
- Mutations combined to hit target E value of >100







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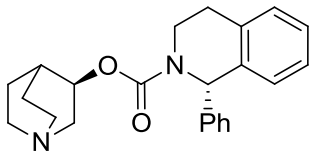
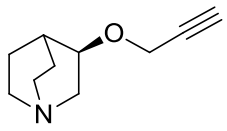
CRED application to (R)-quinuclidinol

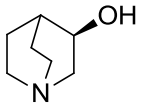


Solifenacin

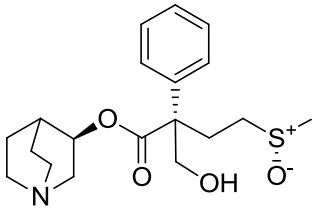
Key building block



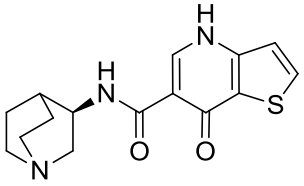
Talsacladine



(R)-Quinuclidinol



Revatropate



Pumosetrag

(R)-Quinuclidinol is key building block for commercial APIs.

Some APIs are becoming generic, increasing need for low cost BB supply.

Historically made by lipase resolution, but racemate is expensive.

ALMAC had investigated Noyori CTH approach, but ee was low, and so was yield after ee upgrade crystallization.

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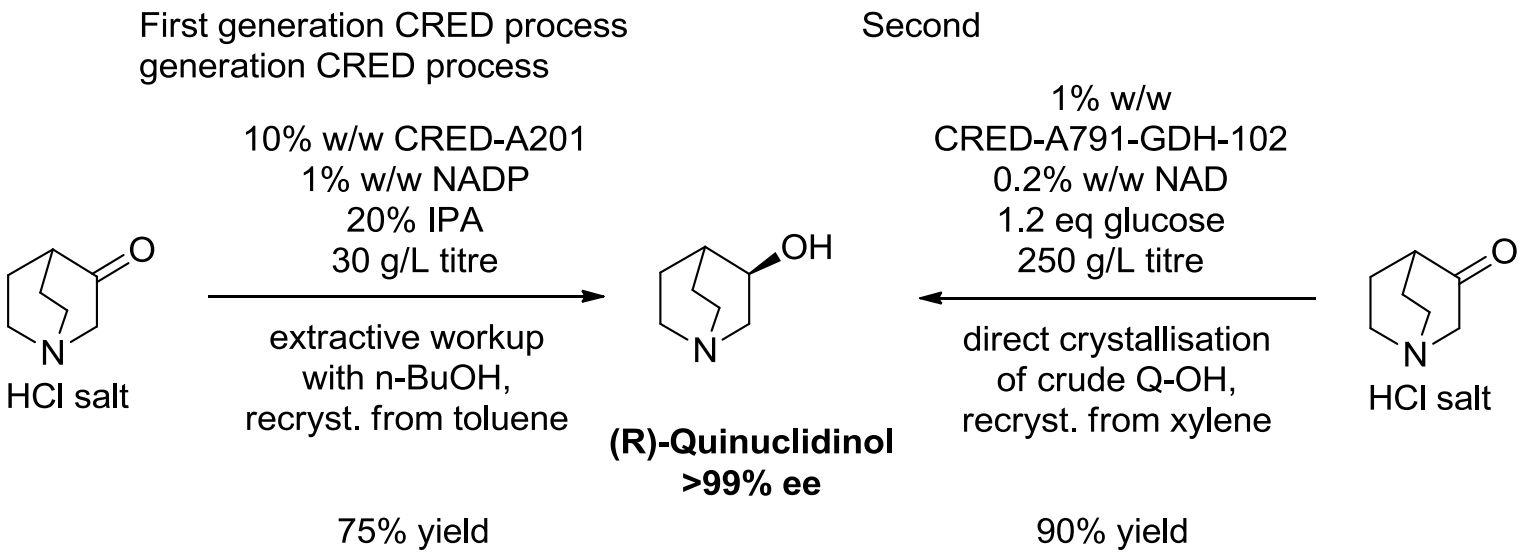
CRED application to (R)-quinuclidinol



Our first generation CRED process had drawbacks: higher enzyme loading, low titre, tedious workup, high volume recrystallization, limited yield.

Also, our enzyme manufacturing cost was much higher back then.

Our second generation CRED process can now meet <<$1000/kg price target. Cost is dominated by ketone price.

This was enabled by co-expression of improved CRED and GDH, ability run high titre and now uses direct crystallisation.

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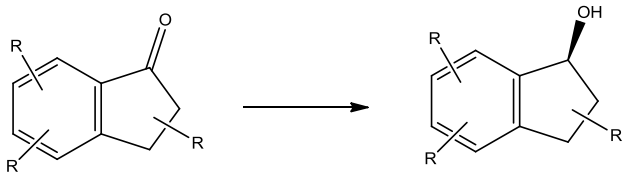
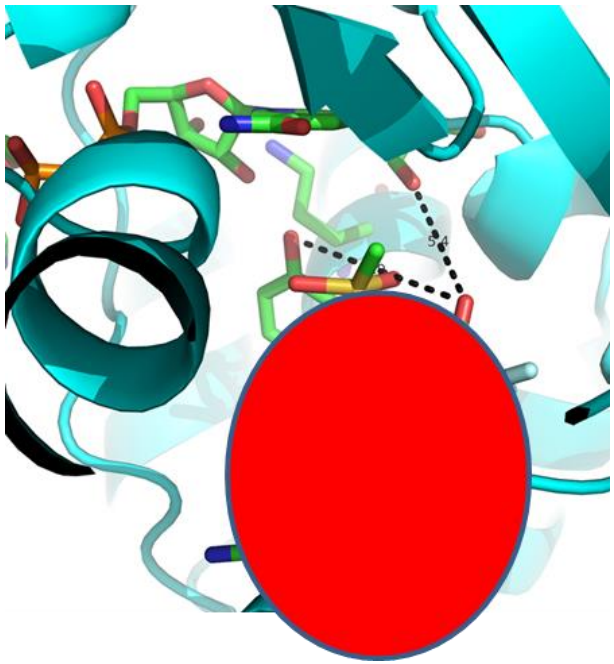
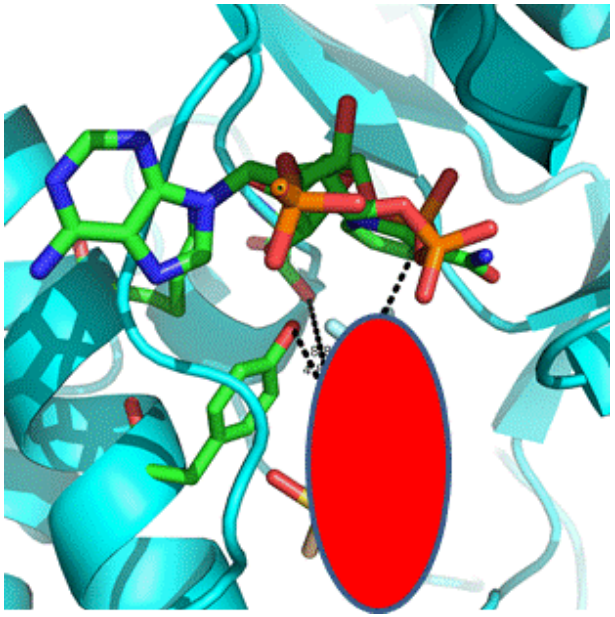
Addressing inhibition *via* CRED engineering

- Substrate inhibition: nM affinity
- Molecular modelling, substrate docking, rational mutant selection, gene synthesis, wet screening
- Affinity reduced to mM range with 20 enzyme panel
- Inhibition eased, selectivity of enzyme retained.







CRED-A231_F97A_ L241A_W226A + NADPH docked

Wild type: 88.9 nM

Variant: 12.7 mM

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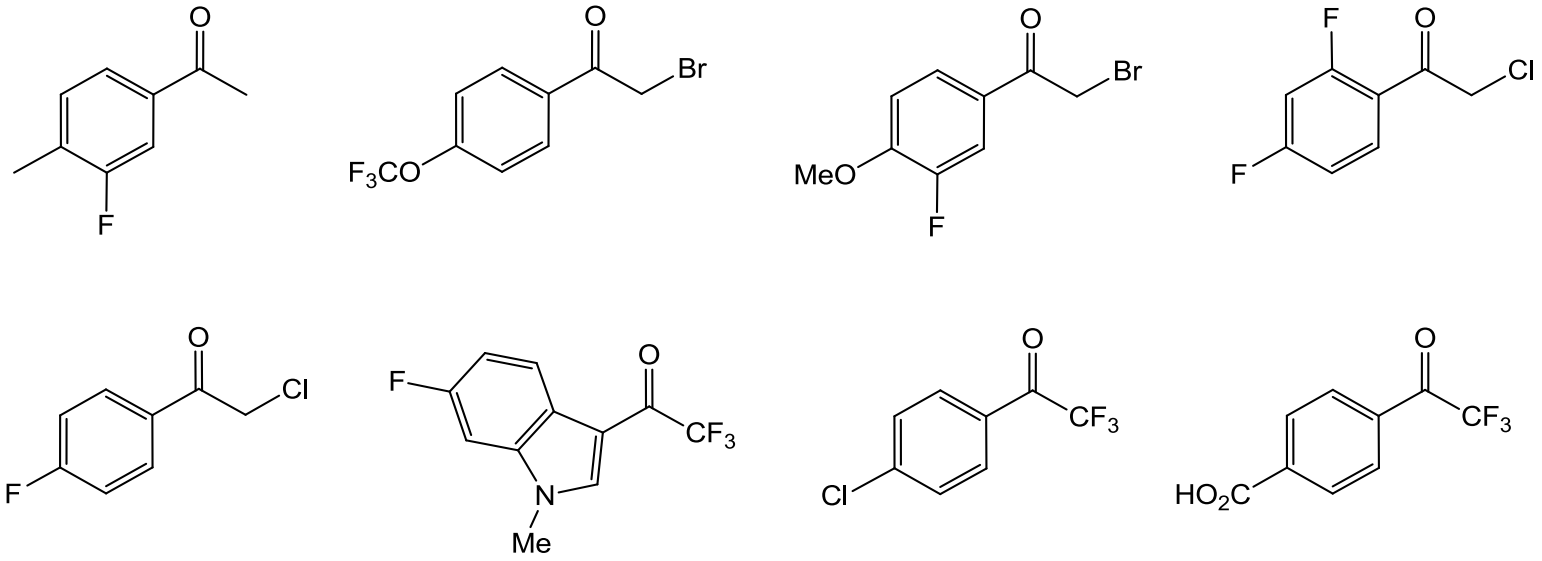
Fluorinated building blocks accessed with CRED technology



All ketones were reduced to both R- and S-alcohols with Almac's CRED kit at >98% ee.

CREDs are particularly powerful for asymmetric reduction of trifluoromethyl-ketones, when Noyori-catalysis struggles to accept hydrated substrates.

Rowan *et.al*. Tetrahedron: Asymmetry **2013**, *24*, 1369-1381

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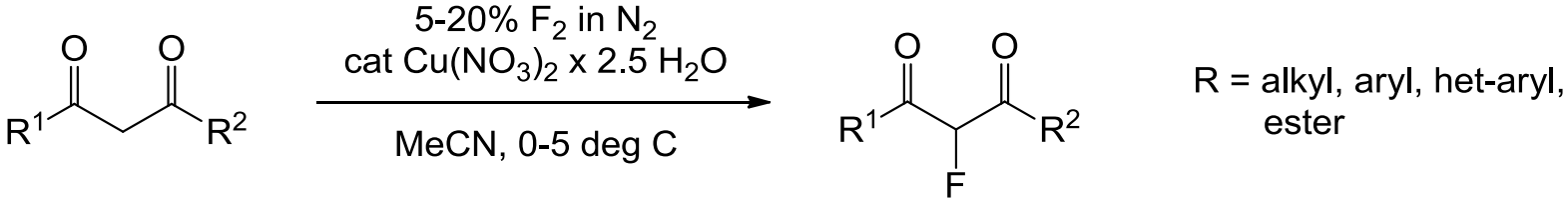
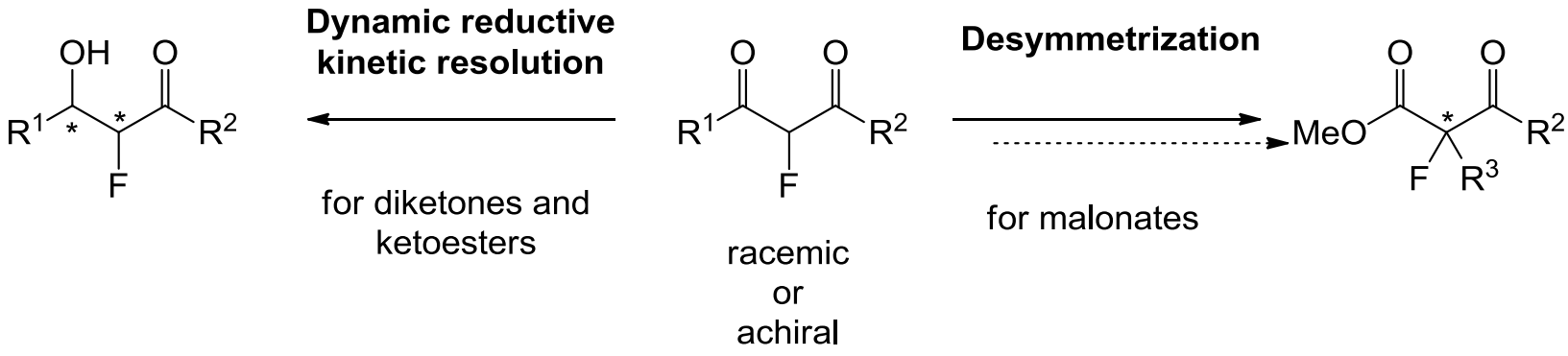
Novel access to C-F chirality

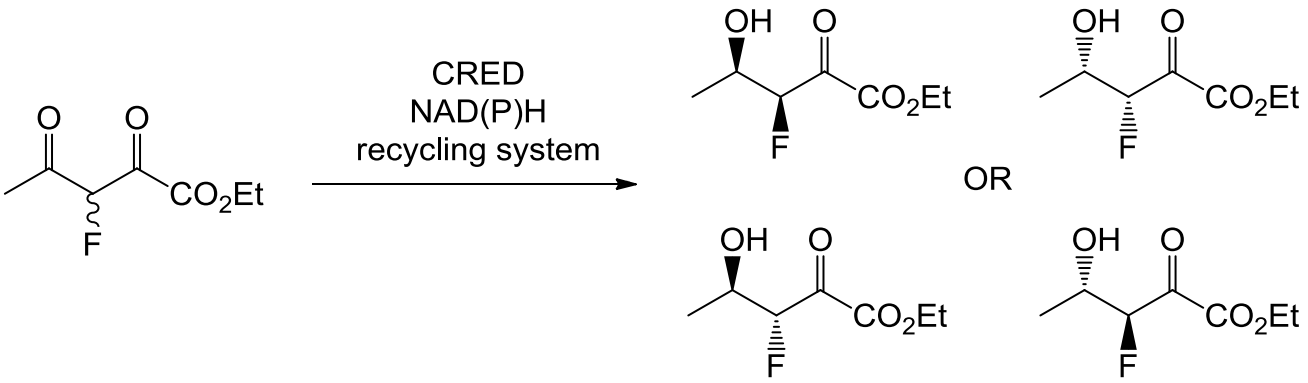


Selective monofluorination of 1,3-dicarbonyl compounds is versatile, efficient and inexpensive.

See e.g. Hutchinson *et. al.*, J. Fluorine Chem. **1998**, *92*, 45-52;
Sandford *et. al.*, Green Chem. **2015**, *17*, 3000-3009



Accessing C-F chirality – DRKR with model substrate



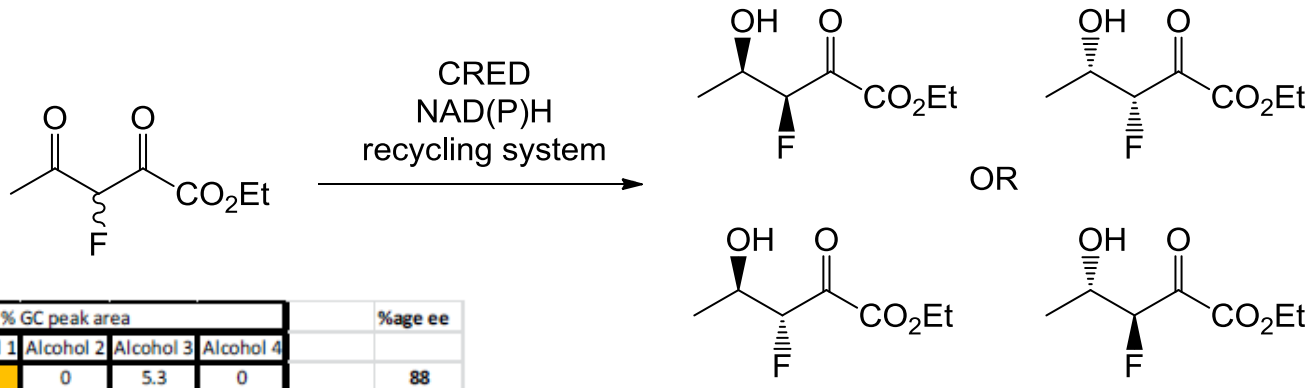
2-F-acetoacetate was investigated as model substrate.

Literature precedent is slim:

1x CRED published to produce 2R,3S-alcohol at 80% de/98% ee.

1x Pt-cat + quinine/quinidine published giving syn-alcohols at low de/ee and with by-products.

Accessing C-F chirality – DRKR with model substrate



	% GC peak area					%age ee
CRED	Ketone	Alcohol 1	Alcohol 2	Alcohol 3	Alcohol 4	
50	0.5	94.1	0	5.3	0	88
42	0.2	93.36	0	6.3	0	
32	0.3	91.5	0	8.3	0	
25	0.3	90.1	0	9.4	0	
43	0.3	87.3	0	12.1	0	
55	0.3	83.4	15.8	0	0	
27	0.3	82	0	17.5	0	
47	6.8	80.6	0	2.9	9.6	
59	0.3	4.1	94.5	0	0	92
29	5.3	0	82.9	0	11.1	
17	0.2	3	0.2	96.6	0	93
44	0.2	0.7	4.5	94.1	0	
5	0.3	2.2	5	92	0	
23	0.3	7.7	0.2	91.9	0	
12	0.1	0	8.2	91.3	0	
63	0.2	10.5	0.7	88.6	0	
14	0.1	11.6	0.7	87.7	0	
15	0.3	15.2	0.4	83.4	0	
1	9.2	4.4	5.9	80.5	0	
18	0.2	0.3	1.1	0.2	98.2	96
30	0.1	0.1	1.7	2	95.9	
6	0.6	0	6.9	7.6	85.6	
24	1.7	0	11.8	0	83.3	

200 off-the-shelf CREDs were screened.

All four stereoisomers can be accessed with high ee/de (not optimised).

Substrate scope investigation is in progress.

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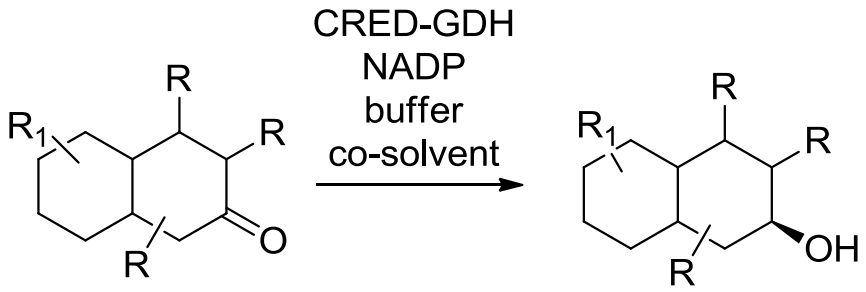
Highly competitive commercial CRED process



- Generic API = tight catalyst cost target
- High SM cost → high yield is critical for product cost
- CRED process offers superior selectivity over chemical reduction
- Co-solvent required for high space-time-yield
- Enzyme deactivation by co-solvent leads to reduced TON
- Enzyme loading reduced by extended co-solvent screen and introduction of improved GDH co-enzyme
- Co-expression of GDH and CRED has lowered enzyme cost per kg
- Low cost, long shelf life enzyme formulation: Spray-dried whole cells
- Going forward: continuous improvement of catalyst and process

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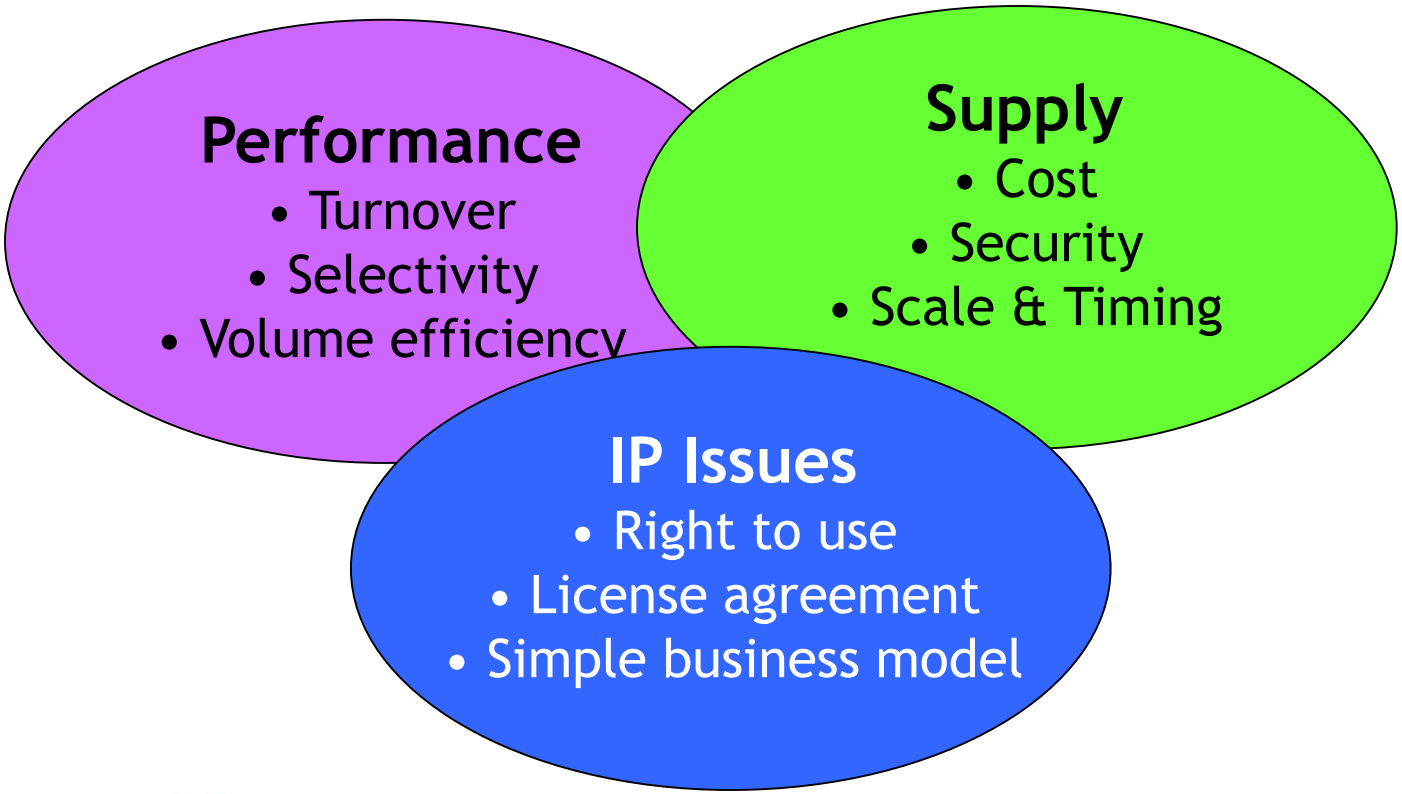
A commercially viable biocatalyst is defined by:



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