

Why is bio-catalysis becoming a leading synthetic methodology?

Future challenges and thoughts from a synthetic organic process chemist

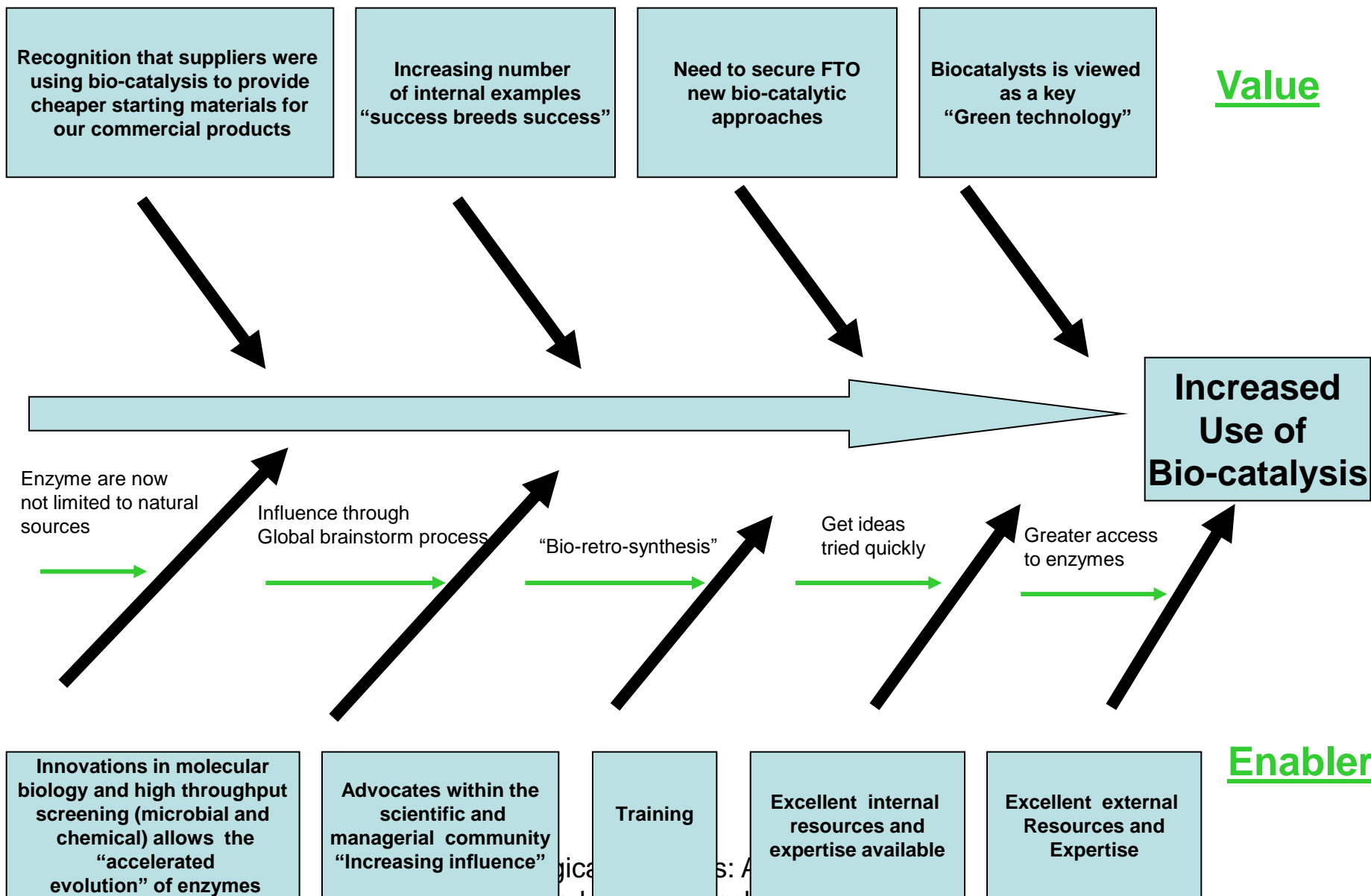
Alan Pettman

Pfizer Global Research and Development

Presentation Objectives

- Outline why Pharmaceutical and Fine chemical industries are using more bio-transformation's and some of the challenges to further application
- Provide “food for thought” from a somewhat “bio-naïve” process chemist on areas for greater exploitation of bio-catalysis and further academic collaborations?

Why is bio-catalysis becoming a leading chiral synthetic methodology?



Summary comments

- Advances in molecular biology, high throughput screening (microbial and chemical) and “bio-retrosynthetic” training have resulted in greater use of bio-catalysts in for commercial route identification
- The time and cost to evolve a “designer enzyme” can now be justified for a commercial application within development
 - Weak hits can now be evolved in less than 1 year to produce highly cost effective systems
- Bio-transformations compete well with expensive metal catalysts in other asymmetric methodologies although:
 - Expensive metals can be recovered
 - Metal loadings can be very low
 - There is a significant focus on catalysts immobilisation and re-use
 - Growing academic focus on asymmetric chemistry with cheap Fe, Ni, etc

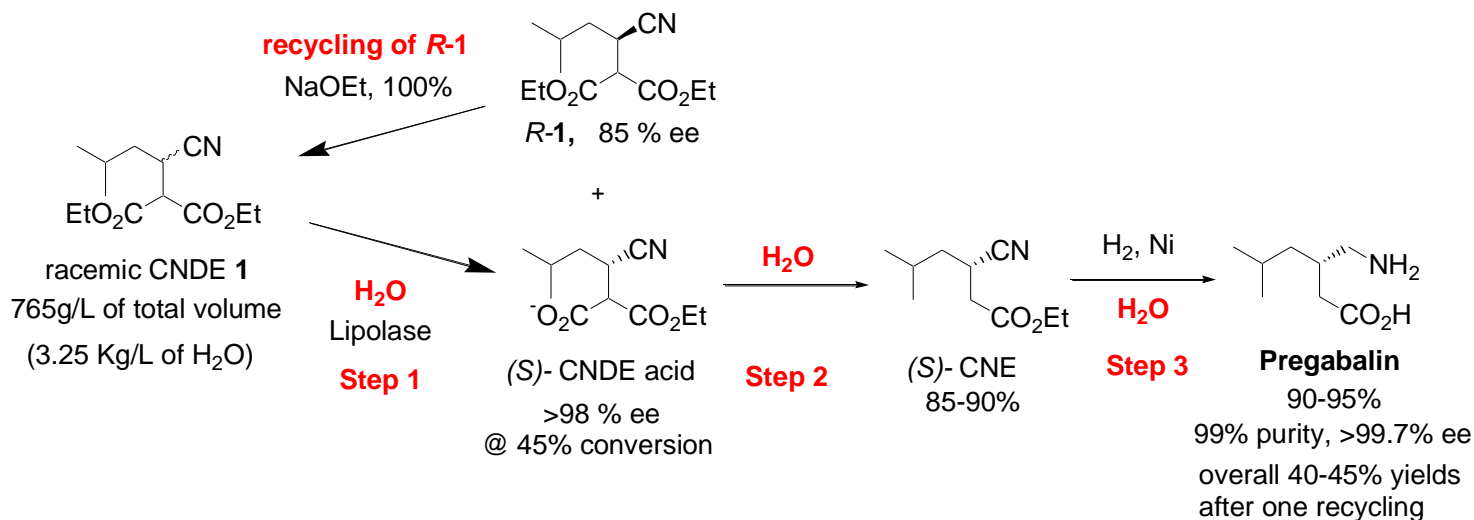
Challenges

- Biocatalysis is still not used frequently in medicinal chemistry and early development unless an enzyme can be bought off the shelf
 - Enzyme engineering is generally considered to be slow and the cost to evolve a “designer enzyme” is prohibitive
 - Modest ee’s and yields with “metal catalysis” is not an issue and there are a large number of ligands and catalysts to try with availability for rapid kilogram scale-up
 - Medicinal Chemistry generally requires technology that works on a range of related structural analogues and not specifically designed for one substrate
- Dogma and a lack of bio-catalysis knowledge and capability in the synthetic chemistry community
- Even in parts of the process chemistry community, development of enzymatic process is still viewed by many as only cost effective for making small chiral molecules and for products with large commercial volumes where investment to find the “designer enzyme” can be justified

However we have moved on past using only hydrolytic systems and hydroxylations to make metabolites - so what's next?

What enzyme processes have become routine over the past few years!

- Enzymatic hydrolytic resolutions with esterase, lipases, nitrilases and particularly desymmetrisation approaches are a popular approach



- Resolution at first step (wrong enantiomer can be recycled)
- High throughput (**3.25 Kg/litre** of H₂O)

- However recently we have seen greater use of and number of other systems

Keto-reductases for the preparation of chiral alcohols

- Popular as a result of less waste and less safety concerns with “chiral hydride” reductions
- Also work well with non aryl ketones which is a limitation of asymmetric hydrogenation and transfer hydrogenation in addition to high pressures and temperatures used



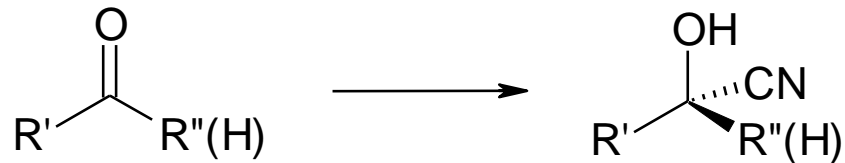
- *Z. bailii* gave 78% conversion with 99% ee at 20 g/L substrate

Transaminases to synthesis chiral primary amines from ketones

- A very difficult transformation for asymmetric hydrogenation or transfer hydrogenation
- However we need to develop “R” selective transaminases and the development of IP-free cofactor recycling systems

Oxy-nitrilases

- However they are difficult to evolve because some systems have been almost completely closed off by patents filings



Where are the new opportunities?

- What would medicinal chemists like?
 - New chemical space not accessible through traditional chemistry
 - Novel heterocycles?
 - Novel CH activations in un-activated positions of aryl and aliphatic carbon frame works
 - What else?
 - Rapid divergent synthesis
 - Hydroxylation, halogenation (fluorinase!)
 - What other hetero atoms could be introduced into carbon frame with enzymes?

Where should we focus efforts in chiral methodology?

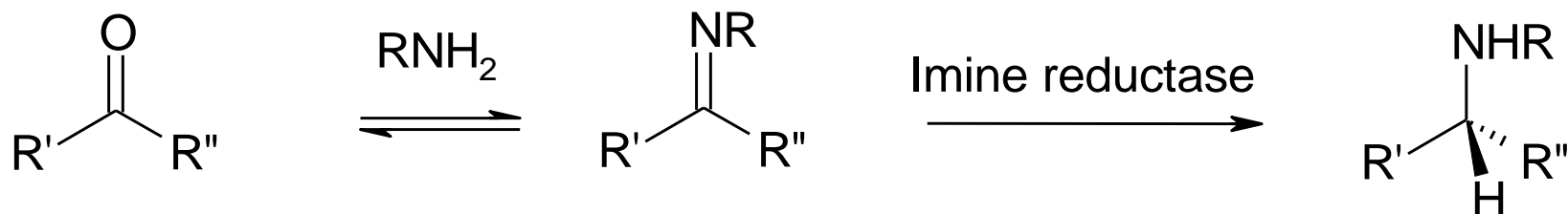
- What chiral molecules do we tend to make in Pharma and are generally a challenge in asymmetric chemistry?
 - Chiral amines, chiral alcohols and chiral acids/ esters/ aldehydes/ nitriles are common functionalities with no universal methodology to make them

Where could bio-catalysis add added value over other asymmetric approaches?

Chiral amines

- **From ketones**

- Transaminases or amine dehydrogenases are excellent for chiral primary amine synthesis
- But should there be more focus on *iminases* for chiral secondary amine synthesis
- If successful can we evolve systems to asymmetric reductive amination and addressing imine vs ketone chemo-selectivity



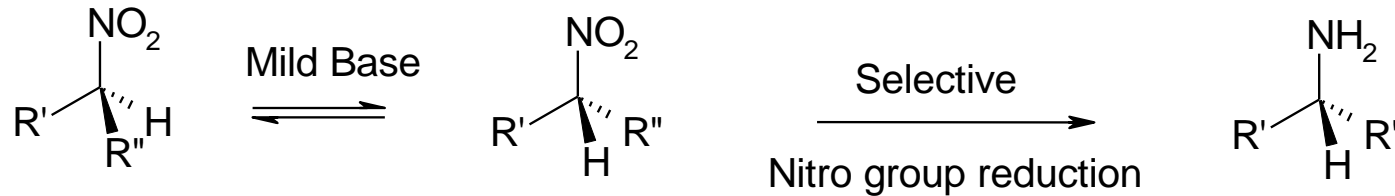
- Active area in the asymmetric catalysis community

- **Asymmetric addition of ammonia or amines and across double bonds**

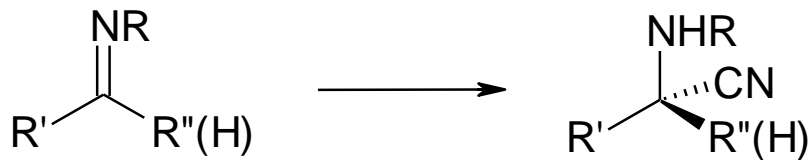
- Activated and deactivated e.g. asparatase and phenylalanine aminomutase leads
- Asymmetric hydroamination with metal systems is difficult and limited to anilines, amides, sulphonamides and simple double bonds
- Organo-catalysis is best in unsaturated aldehydes

Chiral amines

- Aliphatic nitro reduction including dynamic resolution
 - Using the labiality of the highly acidic alpha proton at slightly basic pH



- Strecker synthesis by modifying oxy-nitrilases and the addition of cyanide to imines rather than aldehydes or ketone?



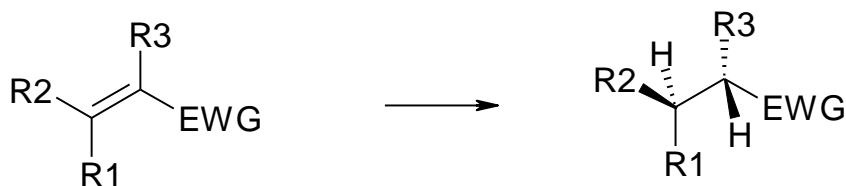
Chiral alcohols

- Need to build further on the use of keto reductases, asymmetric epoxidations
 - What are the limitations?
- More applications of Baeyer Villigerases and in particularly ketone de-symmetrisation's
- Can enzymes carry out asymmetric addition of water “asymmetric hydration” across double bonds?
 - Not easy in asymmetric chemistry c.f hydroboration/ oxidation
- Organic chemists naively and frequently suggest asymmetric CH hydroxylation of cheap starting materials e.g. in aliphatic chains and particularly saturated heterocycles for chiral piperidine and pyrrolidine building blocks
 - Microbiologist frequently reply “P-450 enzymes are complex and need to be made more robust through “structural engineering” before they can be contemplated for general commercial application”
- Significant gap in all methodology for asymmetric tertiary alcohol synthesis and a increasingly frequent need

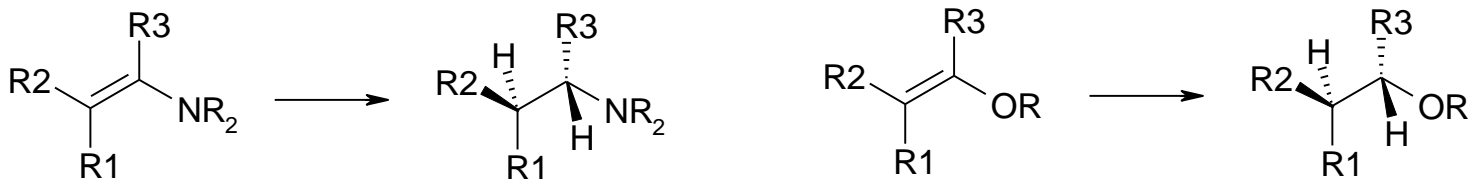
Chiral acids/ esters/ aldehydes/ nitriles

- **Asymmetric alkene reduction**

- Exciting new area for Pfizer are enoate reductase for electron deficient double bonds in general



- Strong interest in scoping out the functionality requirements and are coordinating groups needed e.g. asymmetric hydrogenation?
- What about electron rich double bonds for reductions of enamines/ enamides to amines and enols to ethers?



- **Generally more asymmetric CC bond forming reactions**

- Not just aldehyde electrophiles and what about ketones?

Achiral opportunities

- Although an increasing number of chiral centres are being introduced into synthetic sequences through bio-catalysis, these steps are a low proportion of the total number of steps
- So should bio-catalysis only be used to make chiral molecules?

What are the opportunities in achiral chemistry?

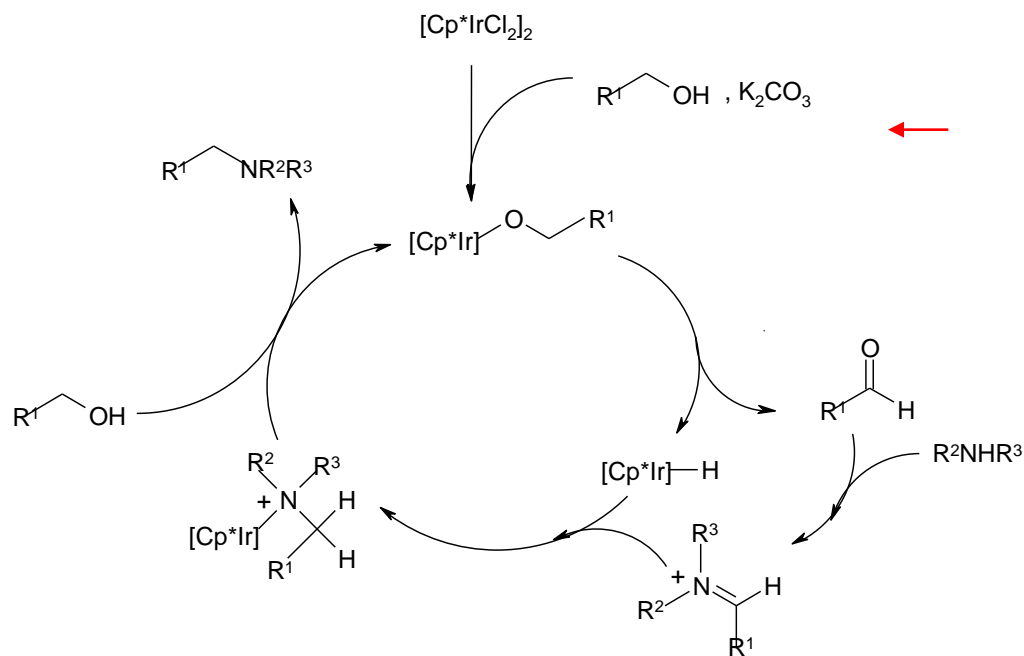
Achiral transformation challenges

(ACS Roundtable and UK Pharma process groups)

- **Reductions of esters, acid, amides (nitriles)**
 - Generally produce a lot of waste and hazardous
 - However some good progress being made with catalytic hydrogenation
- **Oxidations of alcohols**
 - Generally produce a lot of waste and hazardous reagents
 - Although clean methods are evolving e.g. tempo/ bleach, air/ cat, acetone redox neutral systems
- **Making amide bonds and in hindered systems and addressing waste issues**
 - A broadly applicable amide forming catalyst would be great
 - System that is able to accommodate “large acids/esters and large amines”
- ***(Could acylations of non amine nucleophiles possible through active esters?)***
- **Mild deprotection strategies e.g. mild Boc-deprotections/ N acetyl**

Additional areas of current interest in the process chemistry community

- Methodology to removing the need for using “intermediates of genotoxic potential” c.f “Borrowing hydrogen/ redox neutral” methodology



- Can a single enzyme or mixed system be both an alcohol oxidant and an imine reductant?

Additional areas of current interest in the process chemistry community

- Enzymes in flow chemistry?
 - What are the opportunities for biocatalysis?
 - Can flow help bio cascade processes
 - Separating incompatible enzymes
 - Helping reaction sequencing?
- Tandem processes and synthesis of API's through multi-enzymatic processes “synthetic enzymatic cascades” (e.g. Steroid pathways)
 - However the majority of chemical transformations we currently use in the synthesis of API's are not natural transformations for enzymes so clearly we need to expand chemical diversity that enzyme can perform to build cooperative pathways
 - How do we engineer or identify bio-catalytic systems to do unnatural functional group transformations?

Solvents and building blocks for the future as carbon sources deplete

- Most bio derived solvents and building blocks are based on oxygen containing molecules; ethers, alcohols, esters and phenolic compounds etc
- How to we get to non polar bio derived solvents as substitutes for hexanes and aromatics?
- Where will we get our aromatic building blocks from; particularly non oxygenated ones
- Do we need more reducing enzymes?

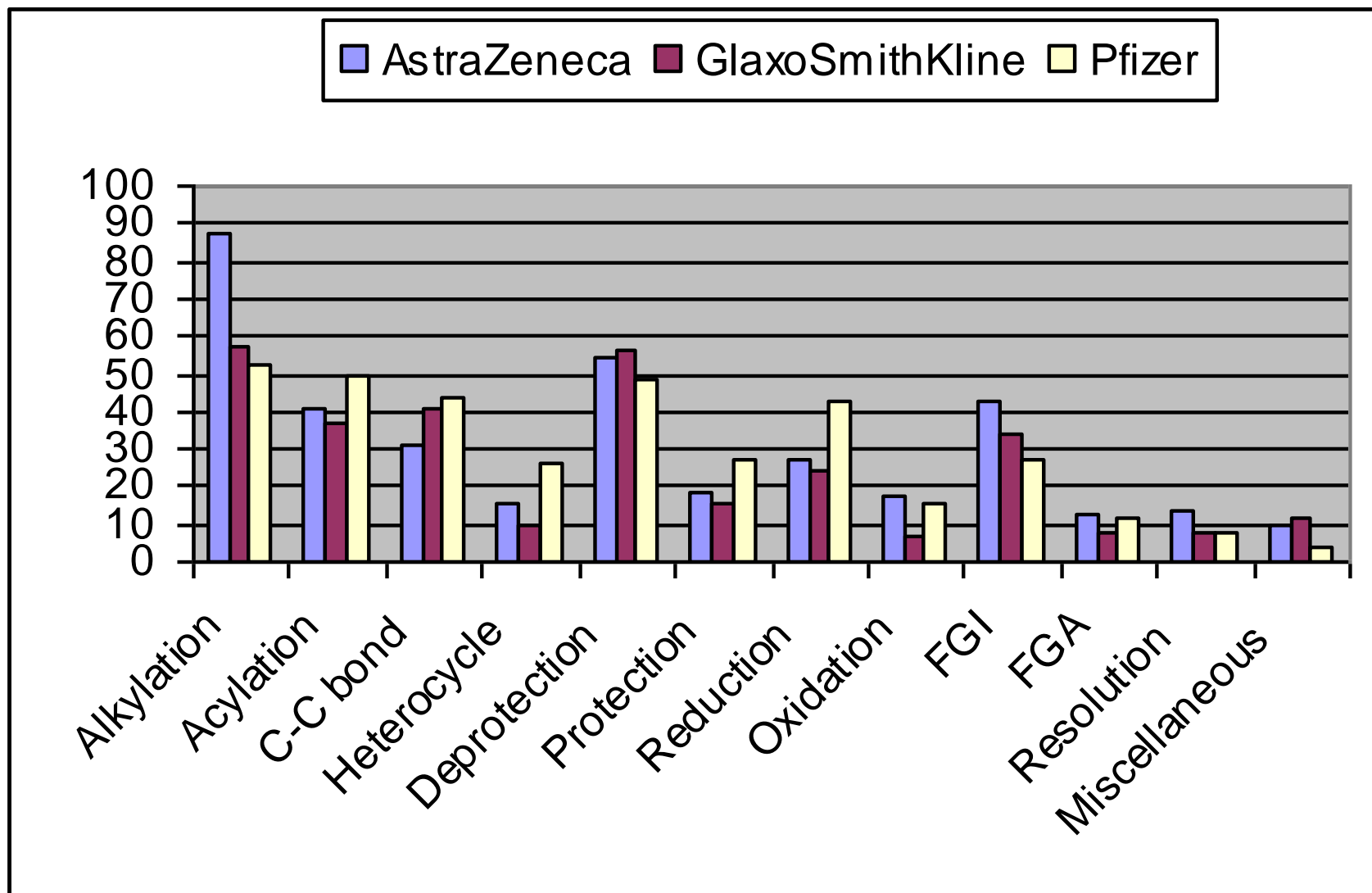
Summary

- I hope I have given an in-sight into why biotransformations are being used to a greater extent in Pharma and fine chemical manufacture
 - Value to our business and application of new tools and expertise to get there
- Hopefully stimulated and supported some current thinking and focus in applications of bio-catalysis and where the academic community may be able to help expand the “organic chemists tool box”

Acknowledgements

- I would like to acknowledge the Pfizer experts!
 - Members of Pfizer “Biotransformation Steering Group” but in particular Van Martin, John Wong, Roger Howard and Hamish McArthur

Reaction Categories



More “blue sky” thoughts from the biotrans experts

- Synthesis of API's through multi-enzymatic processes “synthetic enzymatic cascades”
 - *In vivo* or *in-vitro* isolated enzyme sequences
 - Recombinant microbes containing enzymes from multiple sources
 - Use of 2 recombinant microbes in “one pot”, one to produce an intermediate from the first part of the pathway, the second to convert the intermediate to the desired product
- Clearly we need to expand chemical diversity that enzyme can perform to build cooperative pathways
- How do we engineer biocatalytic systems to do novel transformations?
- Artificial enzymes and applying understanding from natural systems to expand the chemical diversity that enzymes can perform
 - Advances in DNA sequencing have uncovered many new potential enzymatic activities which can be searched for “in silico”.
 - Enzymes could be theoretically designed to carry out a desired reaction by stitching together active sites from several different enzymes

Enabling Technologies

- Still a need to further increase the Speed of Enzyme Engineering
 - Operational Technologies help with the use of automation, building and access to mutant libraries and the use of computational chemistry and crystal structure determination
 - But computational chemistry and assay development are still significant road blocks