

Biology controlling stereochemistry, and Stereochemistry controlling biology

A wide-angle landscape photograph showing rolling hills and mountains under a sky filled with large, white, fluffy clouds. The foreground is dark, suggesting trees or a shadowed area.

www.asn.leeds.ac.uk

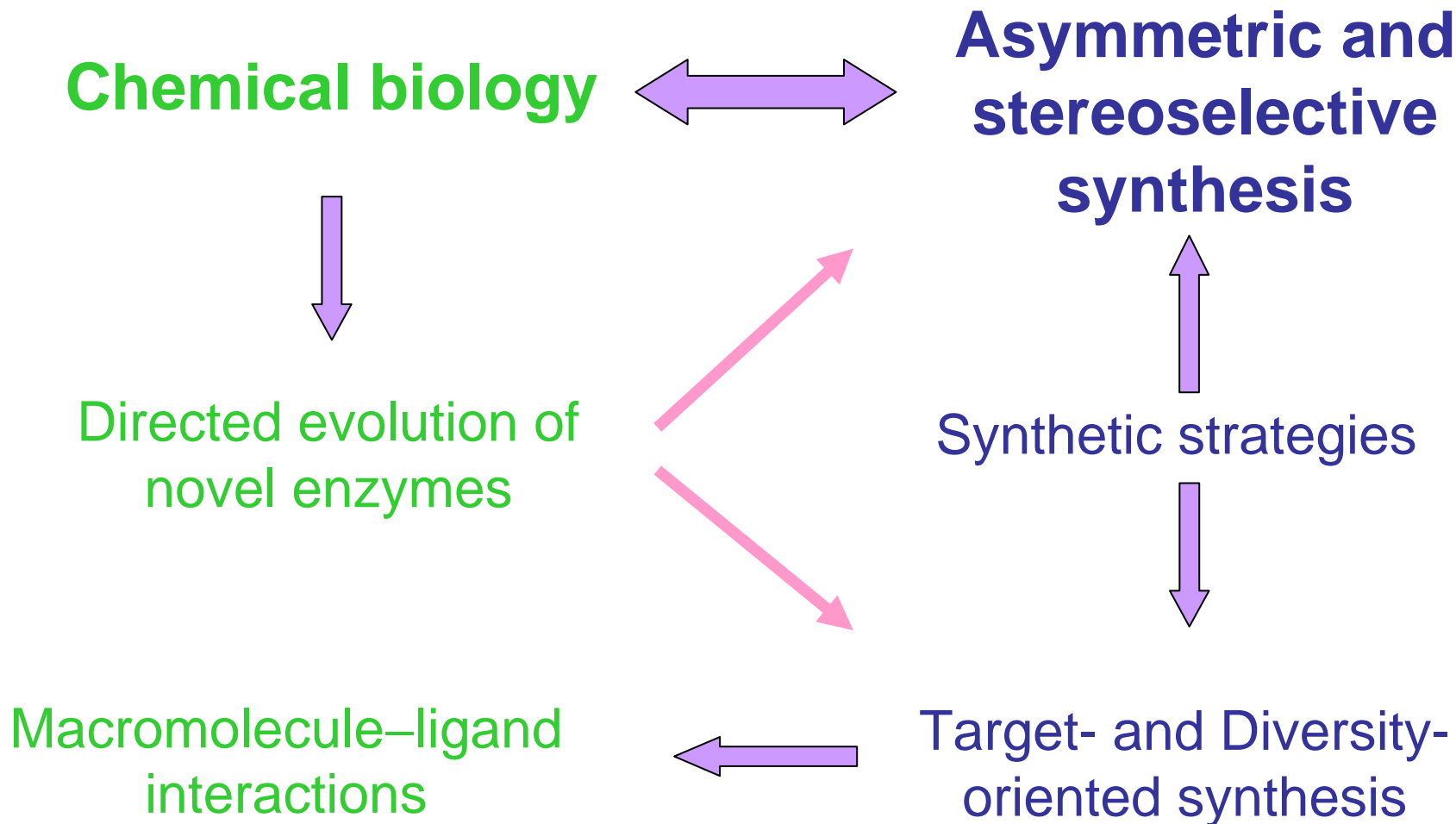
Prof. Adam Nelson

EPSRC Advanced Research Fellow

School of Chemistry, University of Leeds, UK

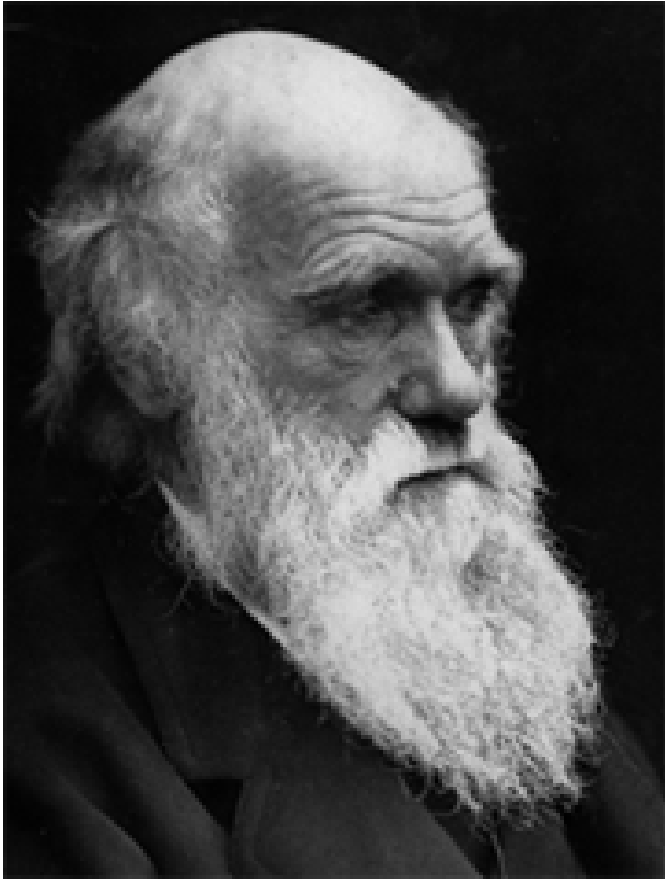
a.s.nelson@leeds.ac.uk

Current research themes



Directed evolution of enzymes with synthetically useful activity

Evolution



“ I have called this principle, by which each slight variation, if useful, is preserved, by the term of Natural Selection”

Darwin, Origin of Species, 1859

Directed Evolution

- Large libraries of random mutant proteins are generated
- Mutants possessing a desired property or activity are identified and their DNAs used in next generation
- The process of natural evolution is mimicked

Interplay between Chemistry and Biology

Biology

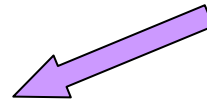


Chemistry

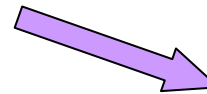
Identification of directed evolution targets



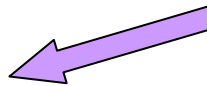
Synthesis of screening substrates



Directed evolution of enzymes with synthetically useful activities

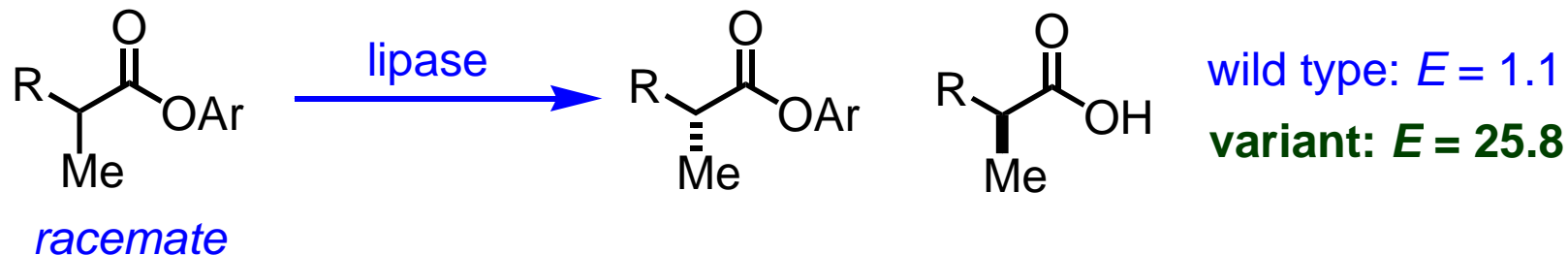


Application of products in chemical genetic studies



Application of evolved enzymes in synthetic chemistry

Directed evolution has been used to improve kinetic resolutions



Reetz et al., Chem. Biol. 2000, 7, 709

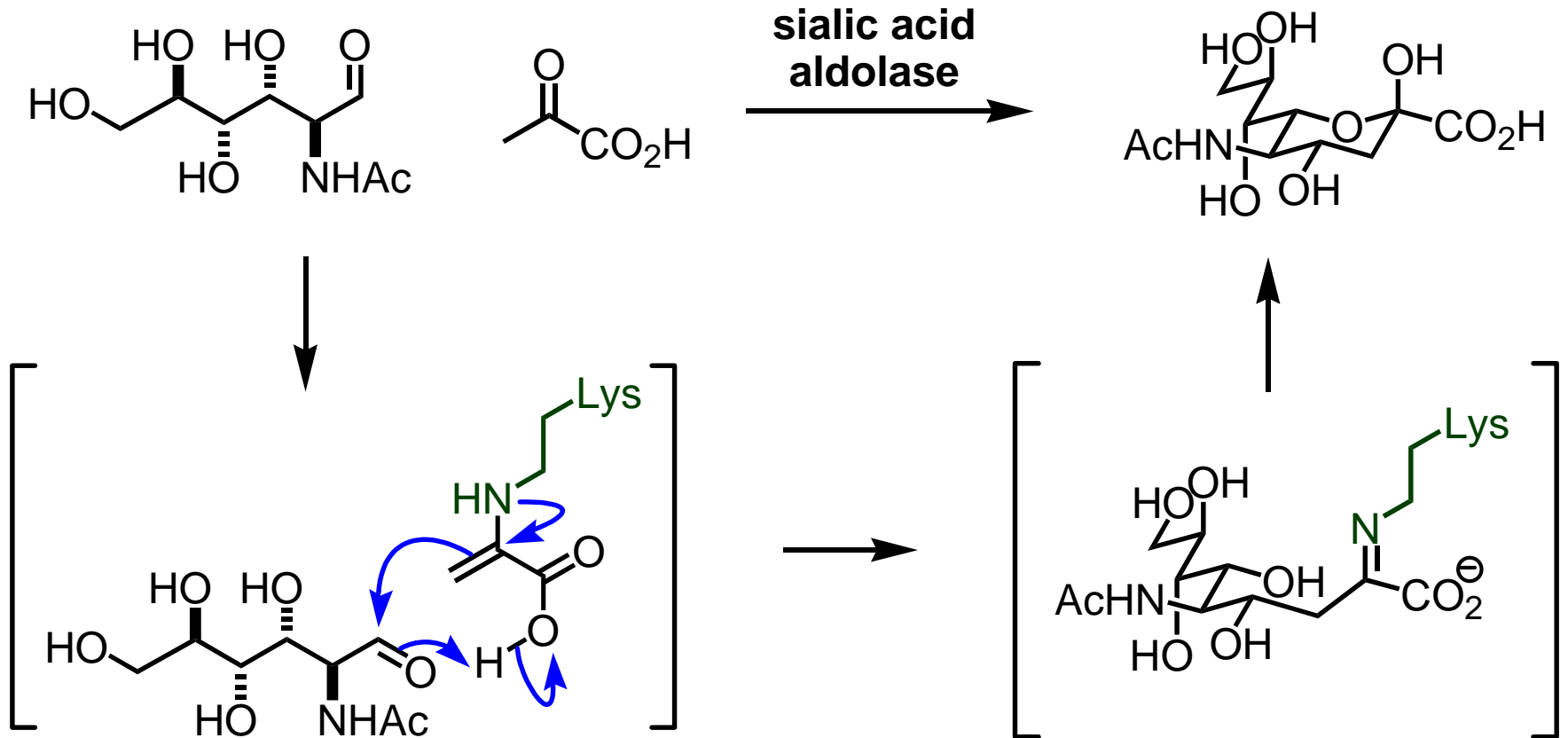
For (dynamic) kinetic resolution of hydantoins, see:

Arnold et al., Nature Biotech. 2000, 18, 317.

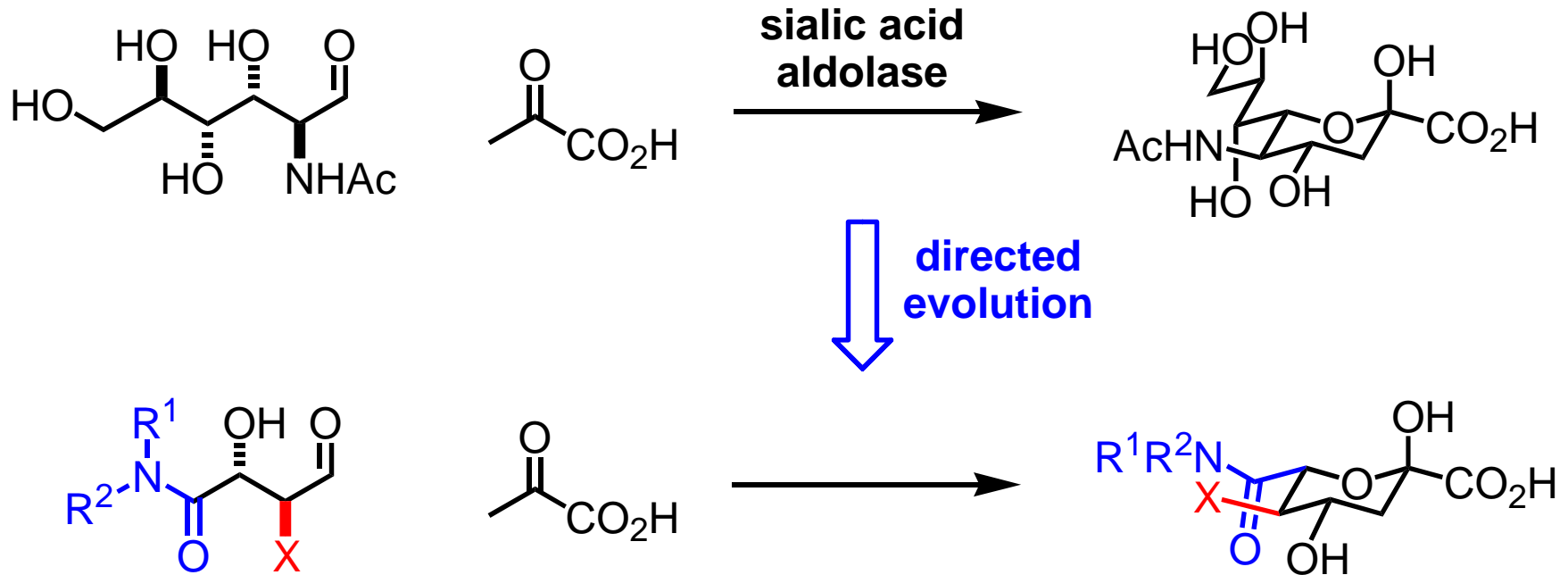
For deracemisation of amines, see:

Turner et al., Angew. Chem., Int. Ed. 2002, 41, 3309.

Sialic acid aldolase



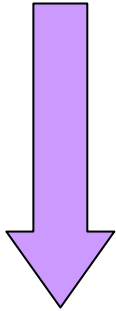
Directed evolution of enzymes for application in parallel synthesis



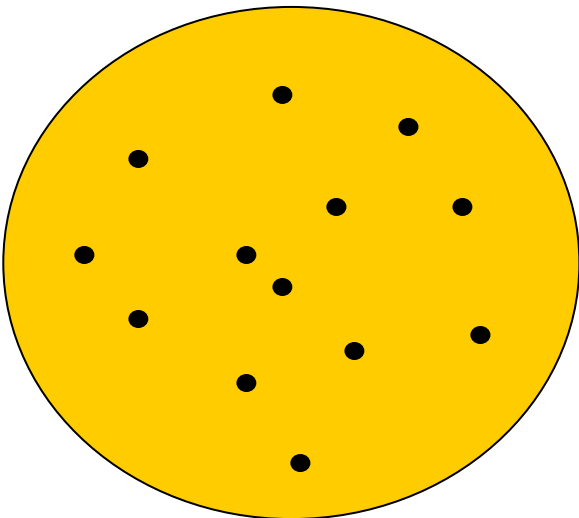
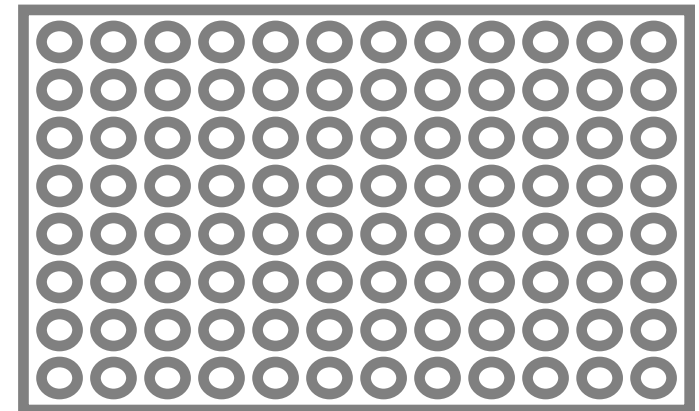
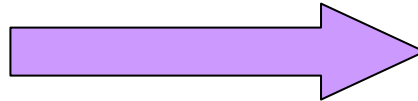
with Alan Berry, Gavin Williams, Tom Woodhall

How can variant enzymes be identified?

Mutant genes cloned into
and expressed in *E.coli*

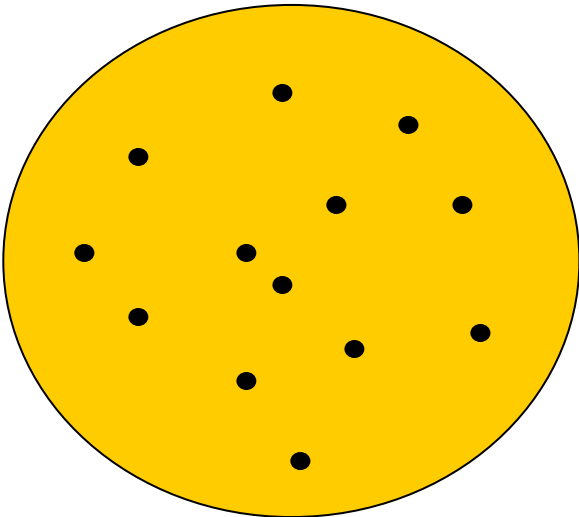
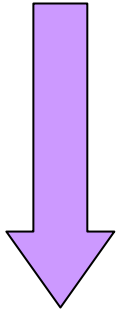


Pick colonies into
microtitreplates

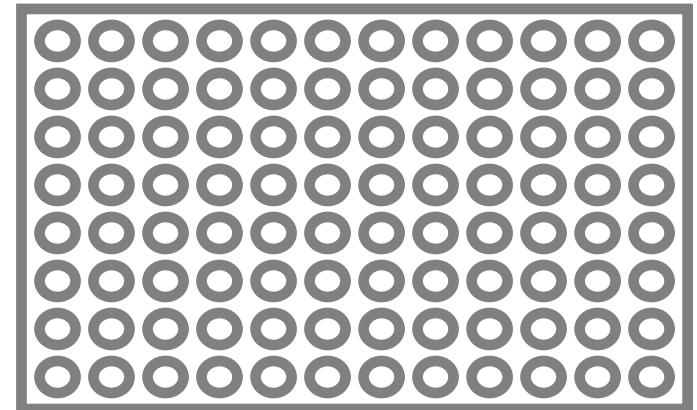
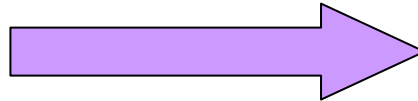


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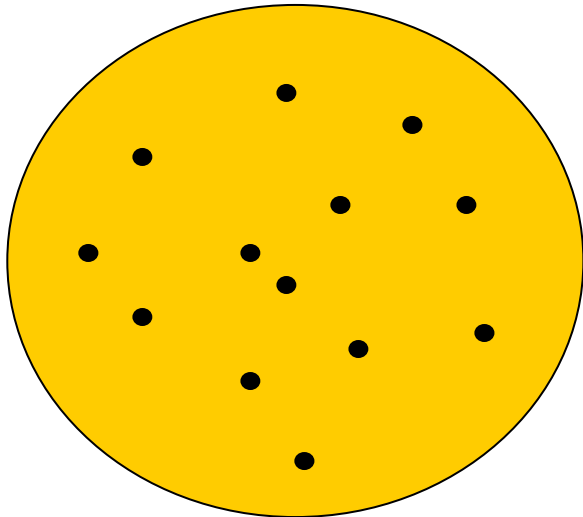
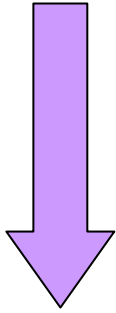


Pick colonies into
microtitreplates

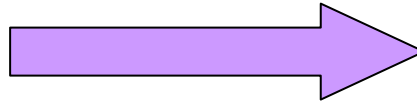


How can variant enzymes be identified?

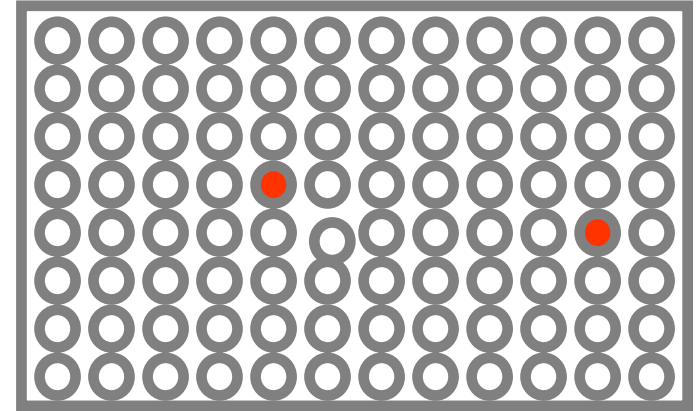
Mutant genes cloned into
and expressed in *E.coli*



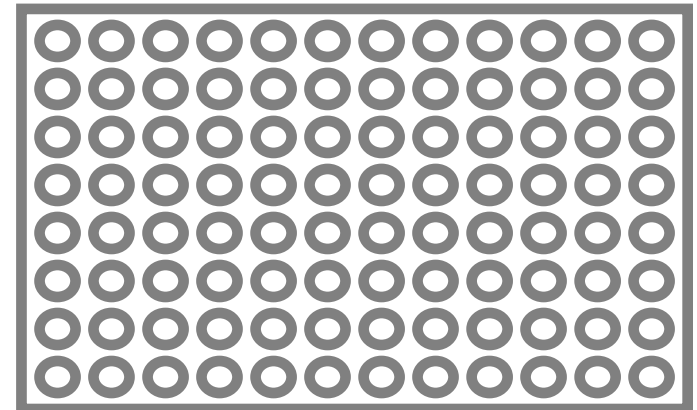
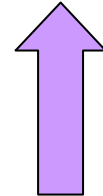
Pick colonies into
microtitreplates



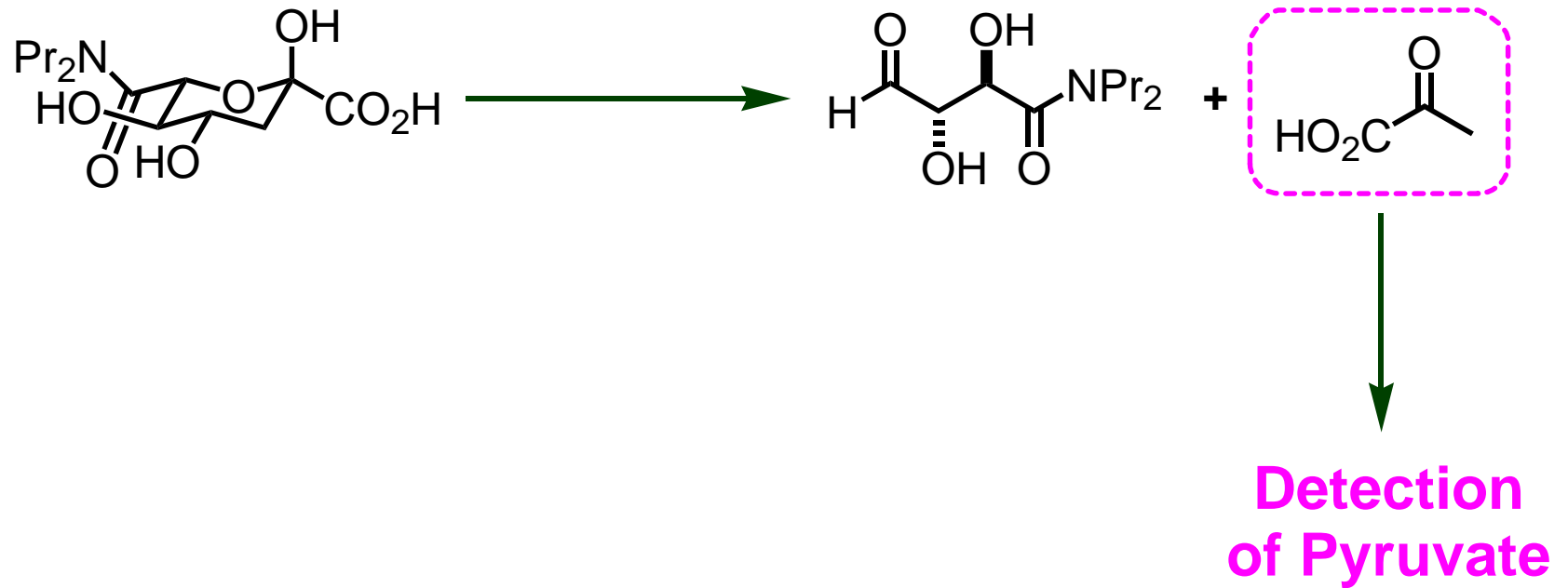
Assay for desired activity;
select positives



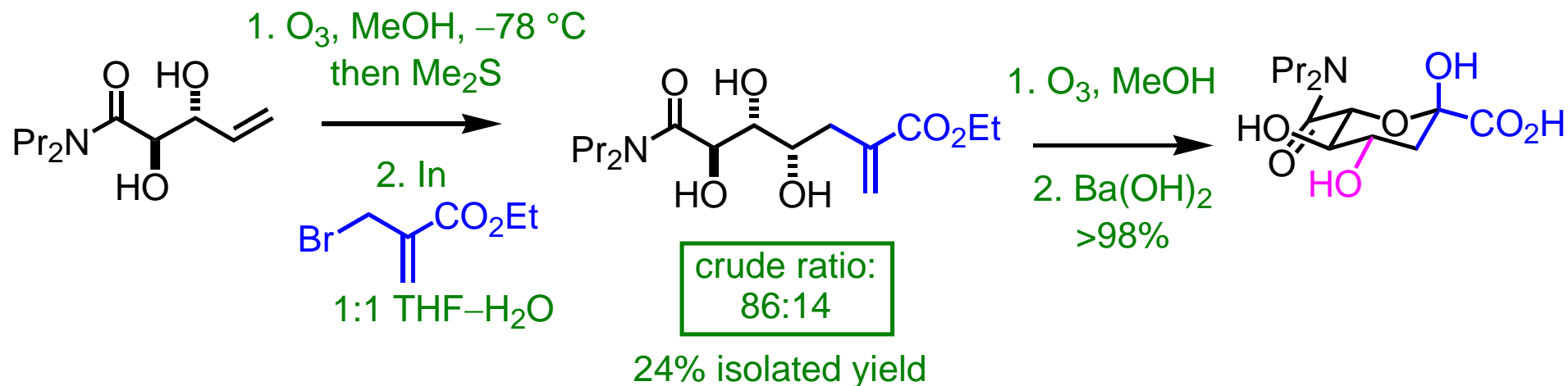
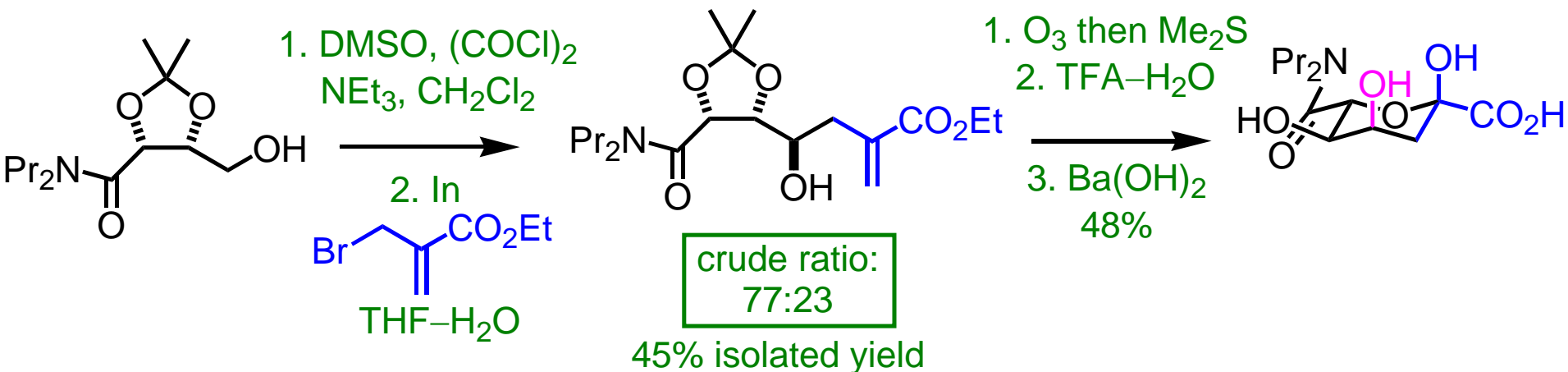
Lyse cells
Remove debris



Screening of Variants



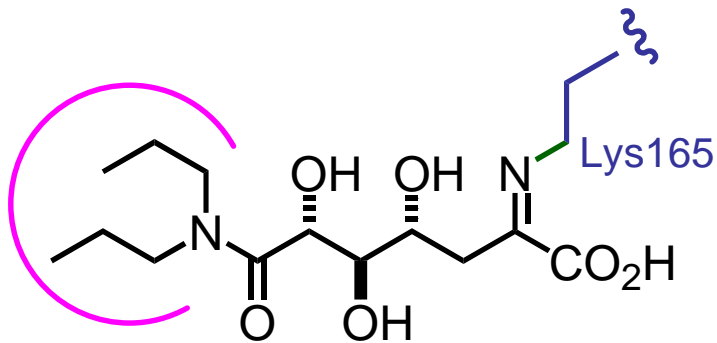
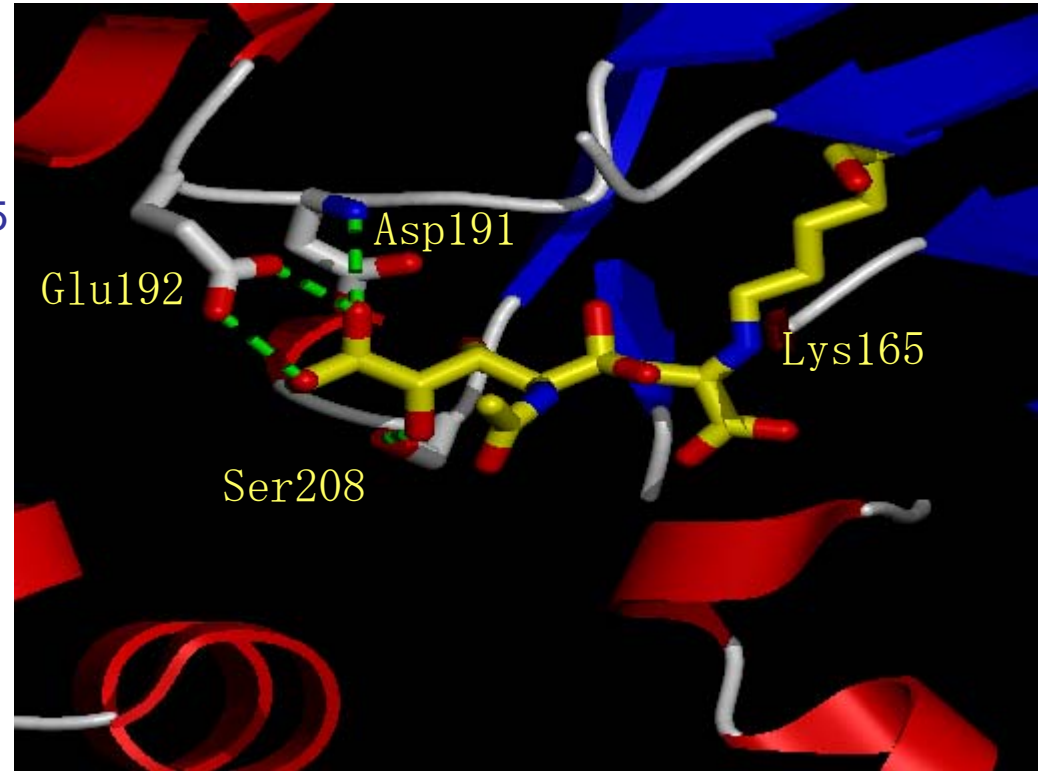
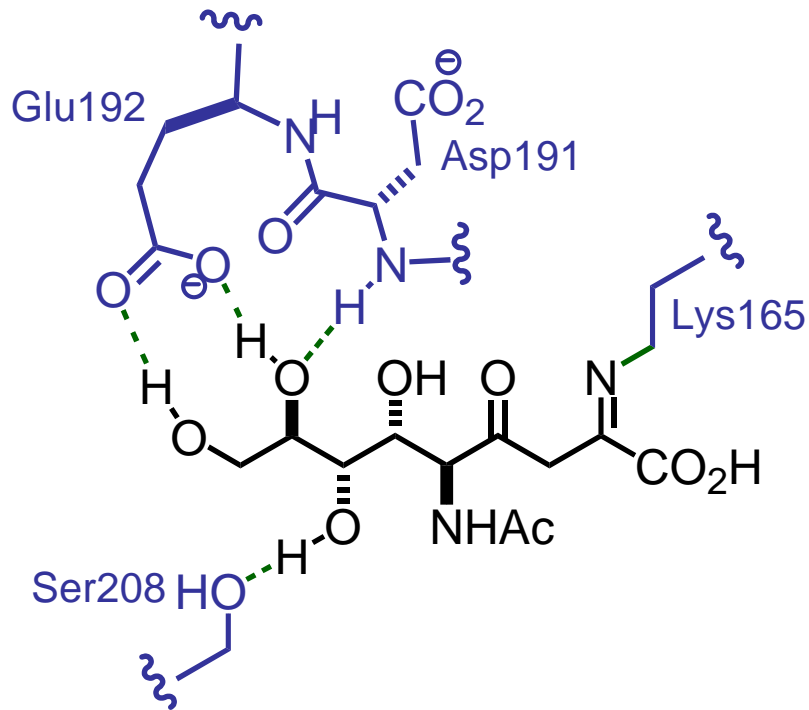
Synthesis of Screening Substrates



Org. Biomol. Chem. 2005, **3**, 1795

with Alan Berry, Gavin Williams, Tom Woodhall

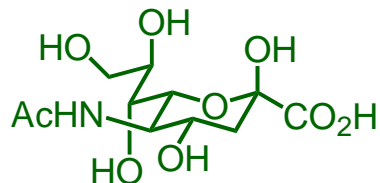
Evolution of Activity



Prot. Eng. Des. Sel. 2005, **18**, 239

with Alan Berry, Gavin Williams, Tom Woodhall

Initial Studies on Crude Lysates

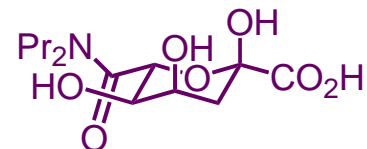
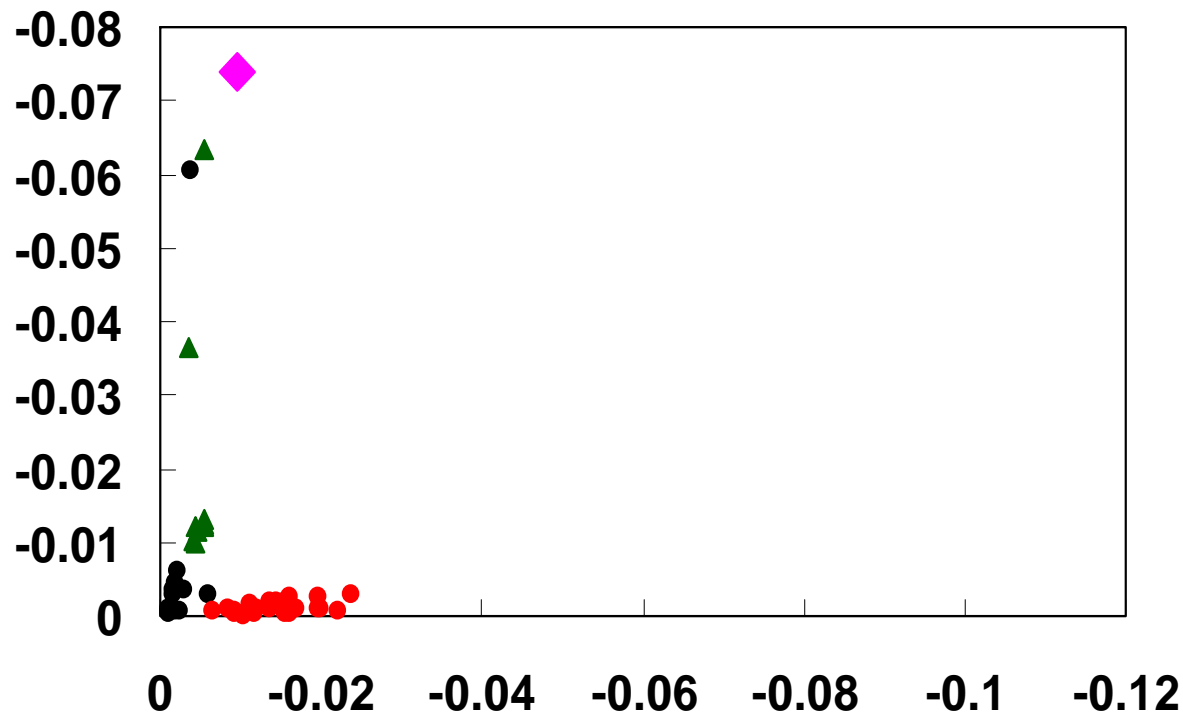


WT

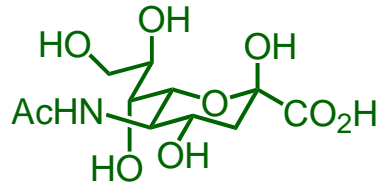
Library A

Library B

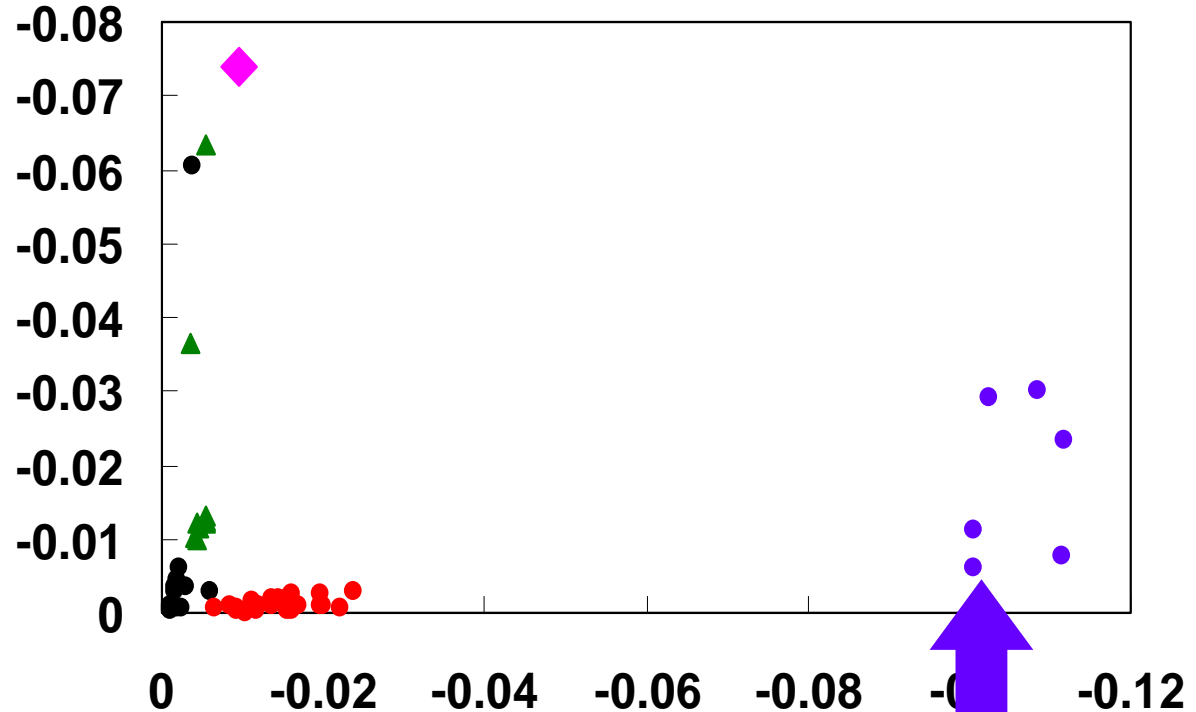
Library C



Initial Studies on Crude Lysates

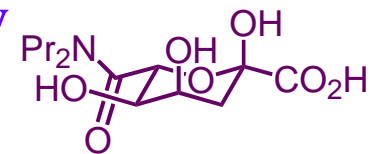


WT
Library A
Library B
Library C
Glu192X

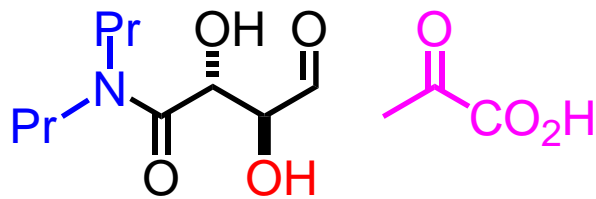


Mutants with:

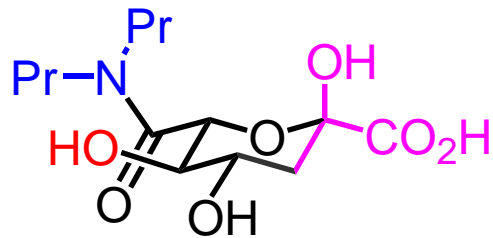
- 4-fold increase in activity
- up to 3000-fold substrate specificity switch



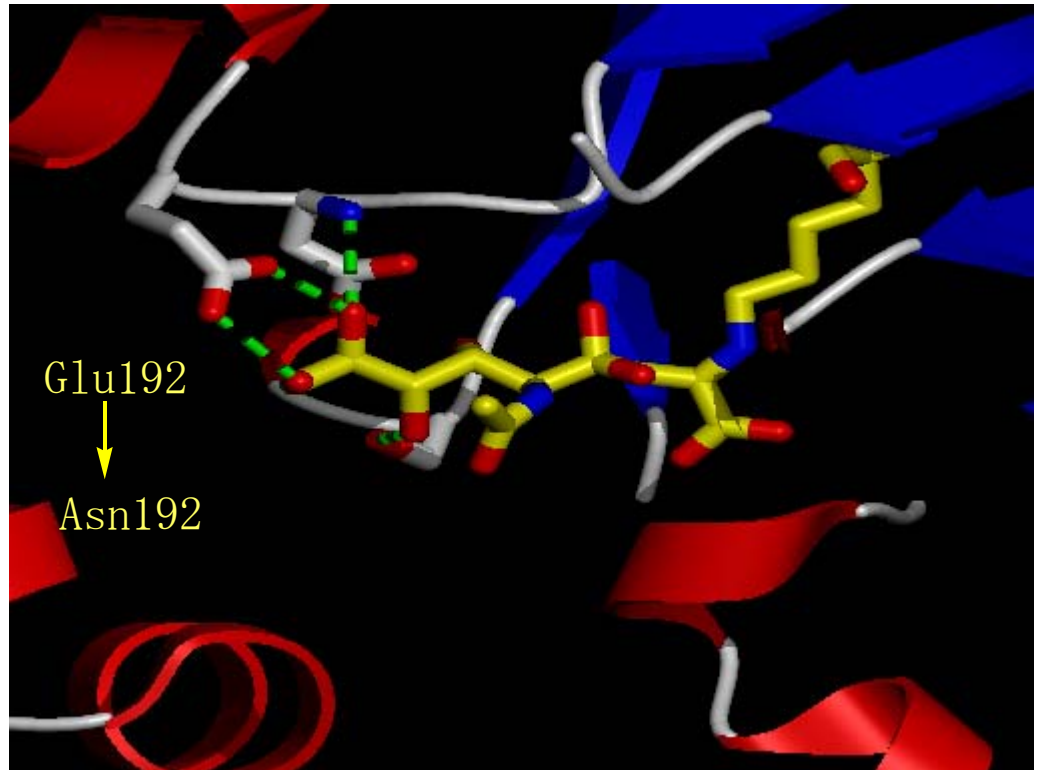
Directed evolution of enzymes for application in parallel synthesis



E192N
pH 7.4



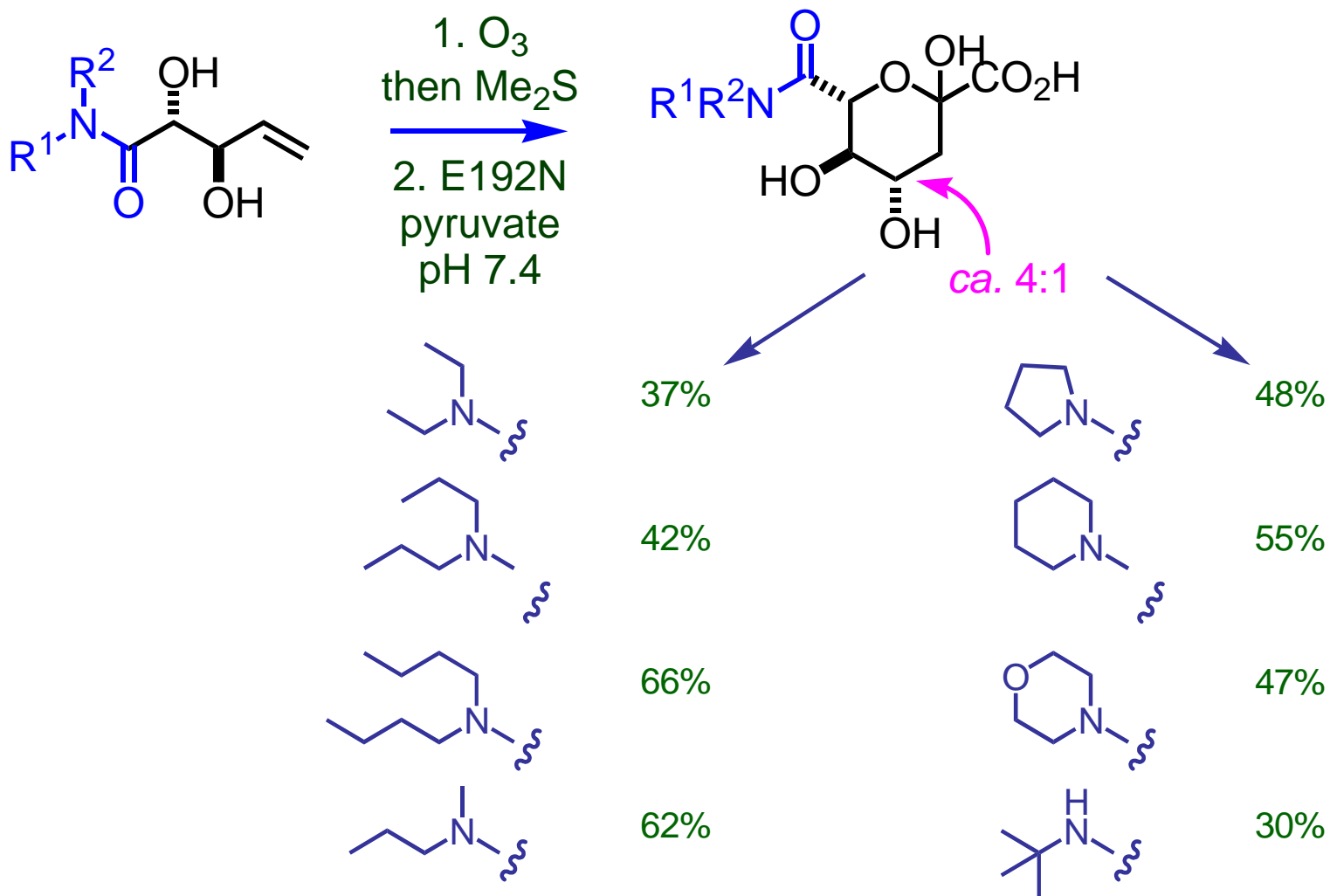
>600-fold switch
in substrate selectivity



Prot. Eng. Des. Sel. 2005, **18**, 239

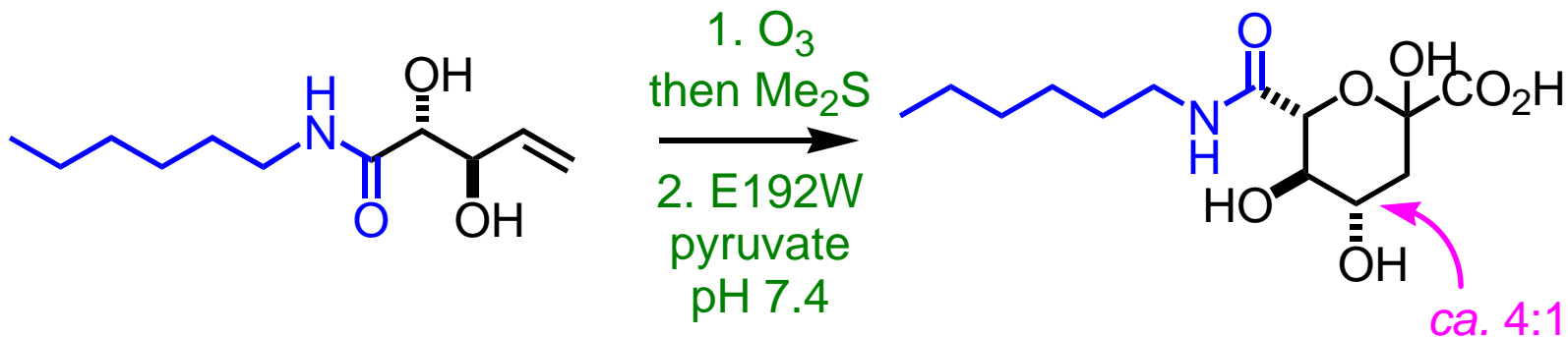
with Alan Berry, Gavin Williams, Tom Woodhall

Enzymatic parallel synthesis of sialic acid mimetics

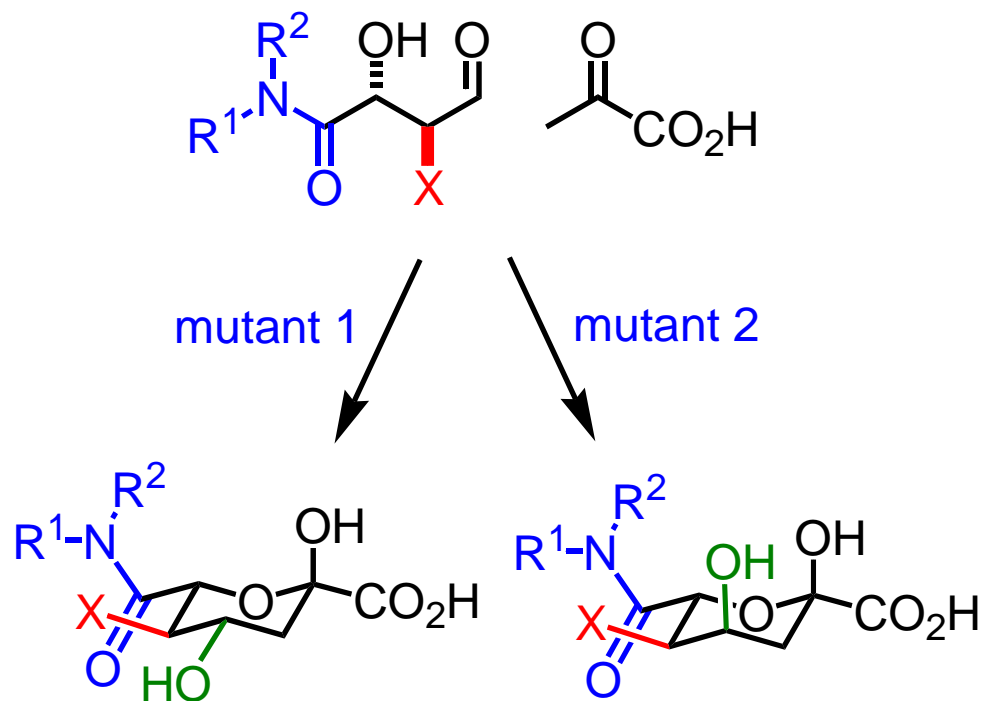


Angew. Chem., Int. Ed. 2005, **44**, 2109

E192W is the enzyme of choice for secondary amides



Creation of complementary enzymes for diastereoselective C–C bond formation

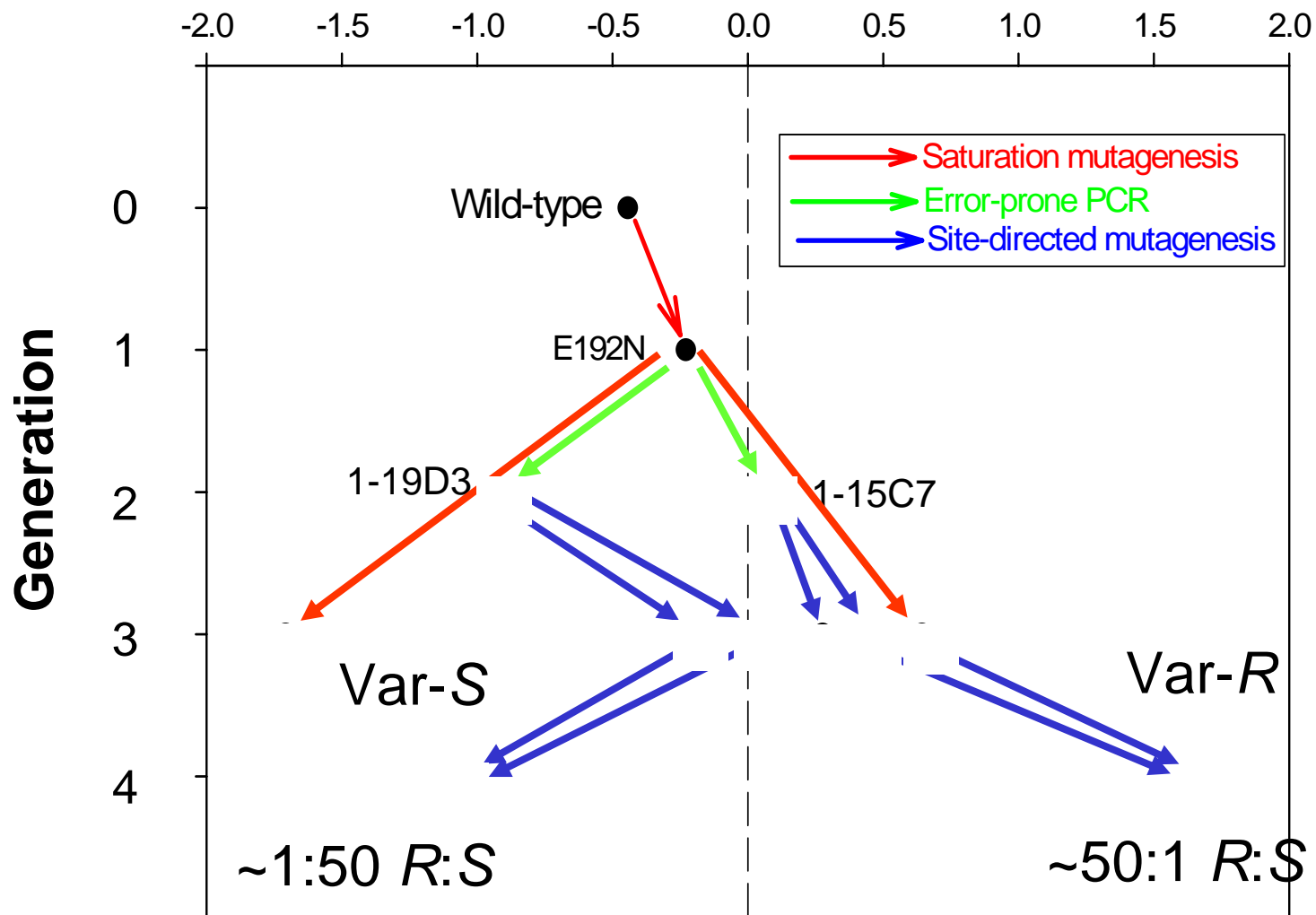


for related work: *PNAS* 2003, **100**, 3143

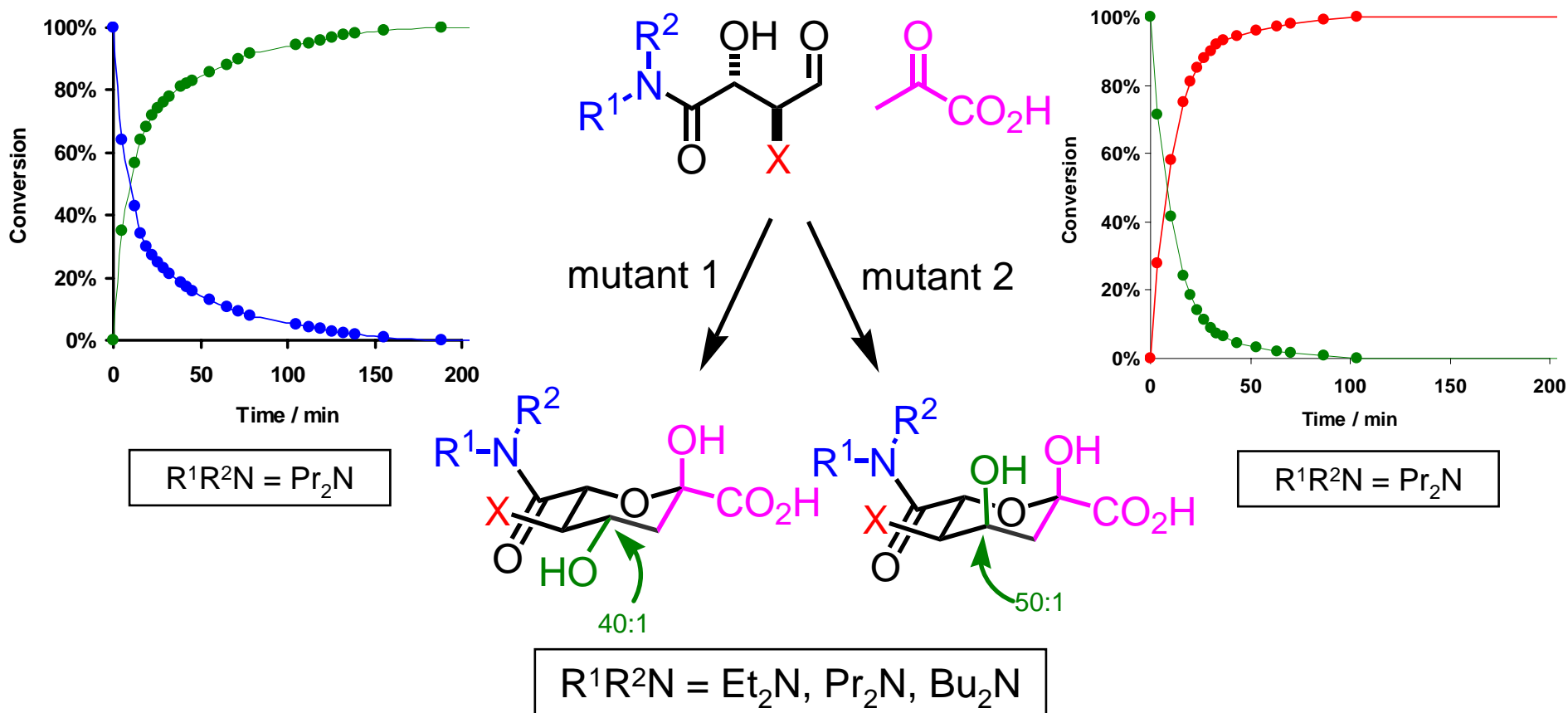
with Alan Berry, Gavin Williams, Tom Woodhall

Evolution of complementary stereocontrolled catalysts

$$\mathbf{S} \leftarrow \boxed{\log\left(\frac{(k_{\text{cat}}/K_{\text{m}})_{\text{R}}}{(k_{\text{cat}}/K_{\text{m}})_{\text{S}}}\right)} \rightarrow \mathbf{R}$$



Creation of complementary enzymes for diastereoselective C-C bond formation



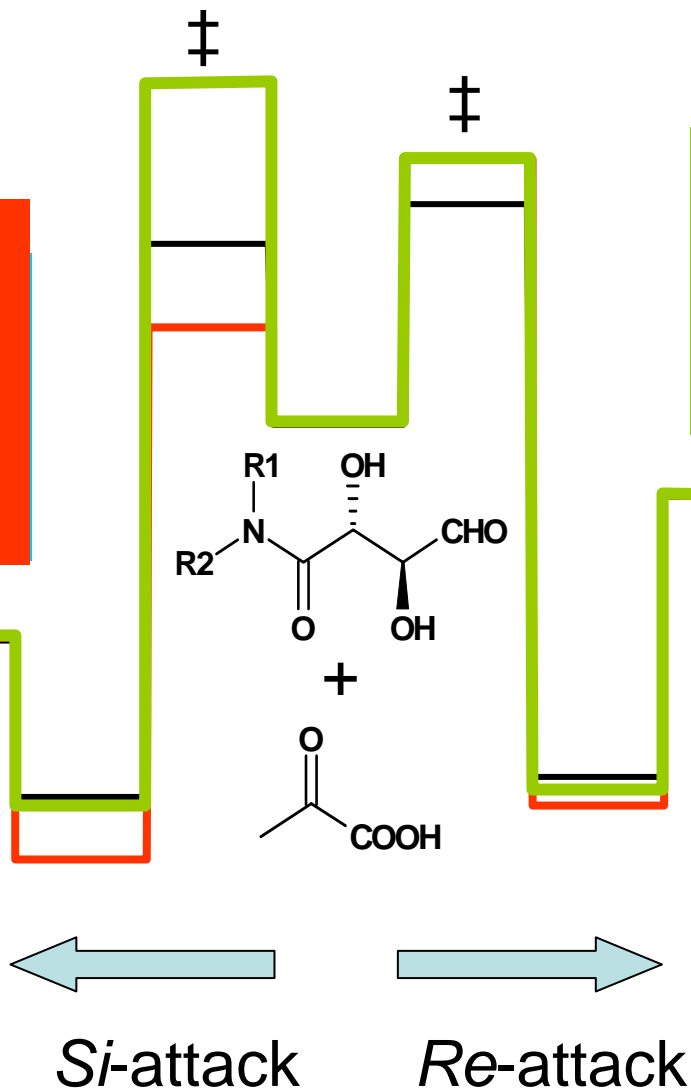
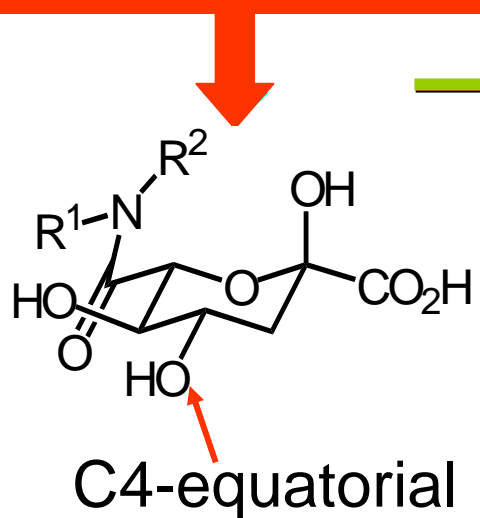
with Berry, for related work: *PNAS* 2003, **100**, 3143

with Alan Berry, Gavin Williams, Tom Woodhall, Lorna Farnsworth

Energy profile of variant aldolases

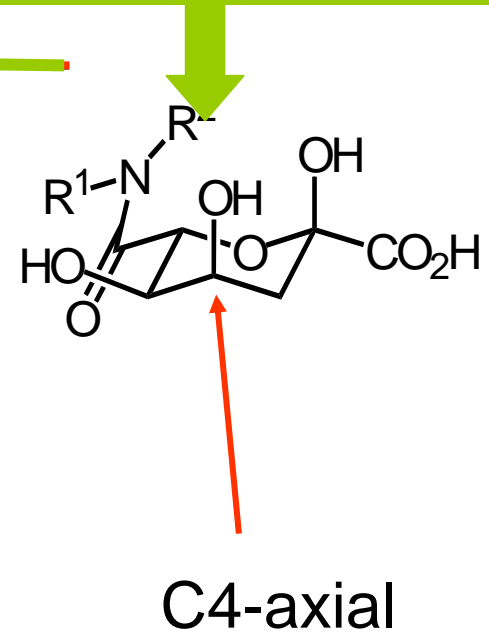
Variant S

C4-equatorial is more kinetically favoured so enzyme is more discriminatory in favour of S



Variant R

C4-axial is now kinetically favoured so R form now the kinetically controlled product

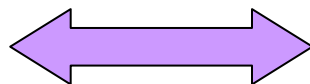


Using Ligand Conformation to Control Protein Kinase Inhibition

J. Am. Chem. Soc. 2005, **127**, 11699

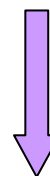
Interplay between Chemistry and Biology

Biology



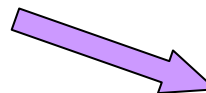
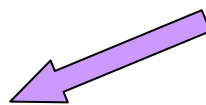
Chemistry

Ligand Synthesis



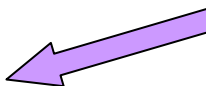
Conformational Analysis

Determine Protein Kinase
Inhibition Profiles



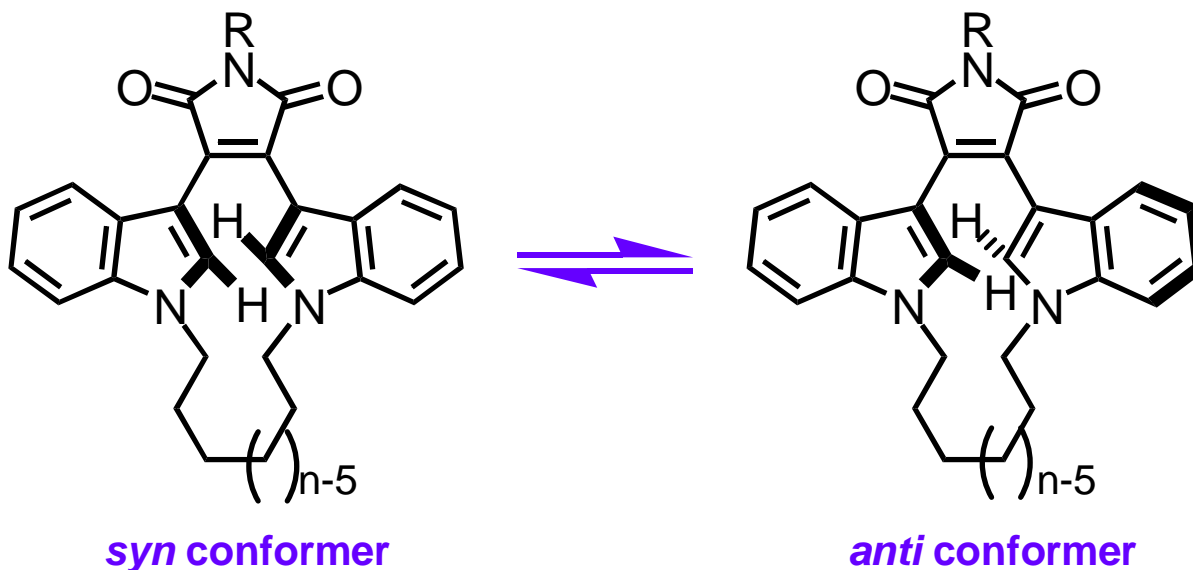
Infer bound conformation
of ligands

Application of products in
chemical genetic studies



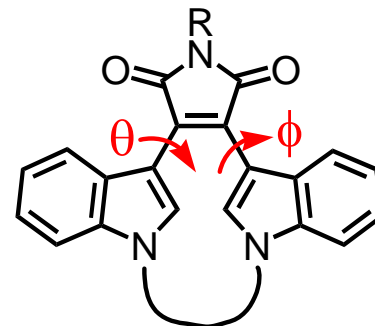
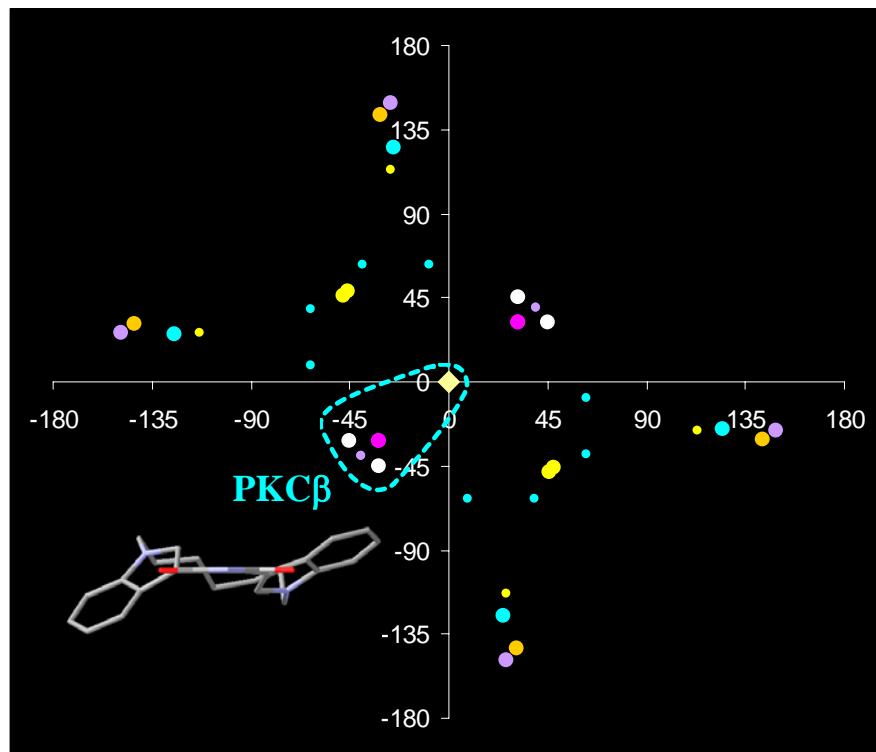
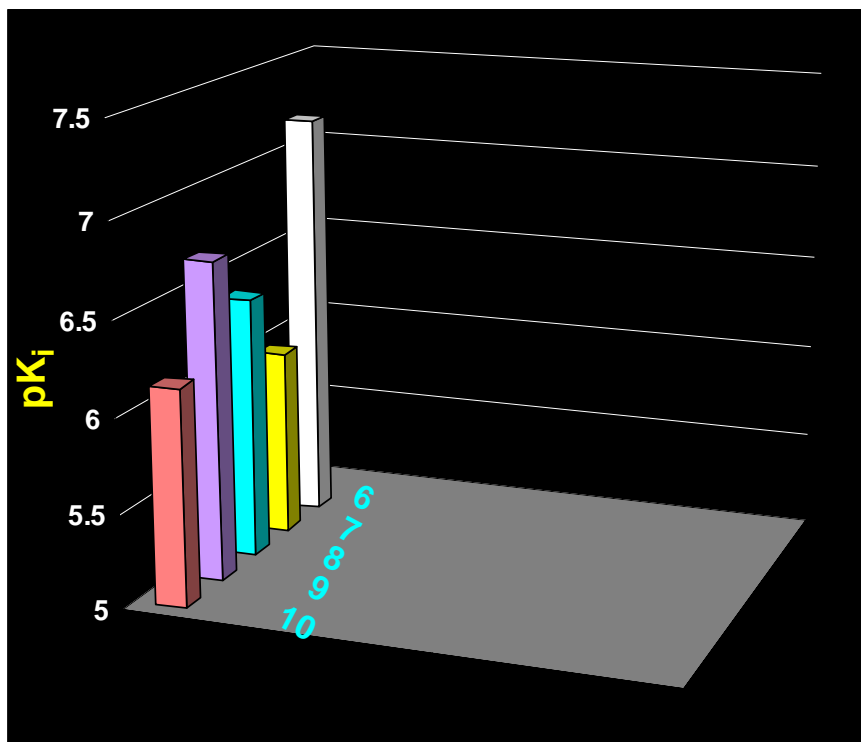
J. Am. Chem. Soc. 2005, **127**, 11699

Limiting conformations of the cyclophanes

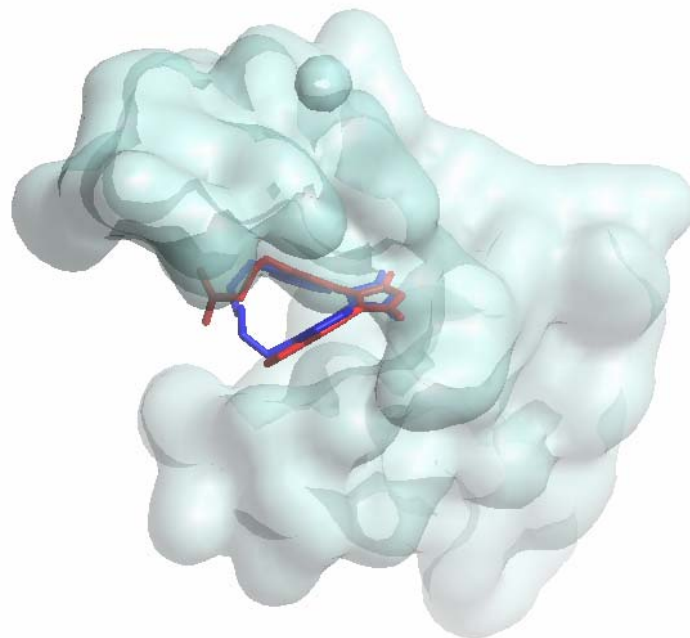


J. Am. Chem. Soc. 2005, **127**, 11699

Correlating conformation with inhibition of PKC β

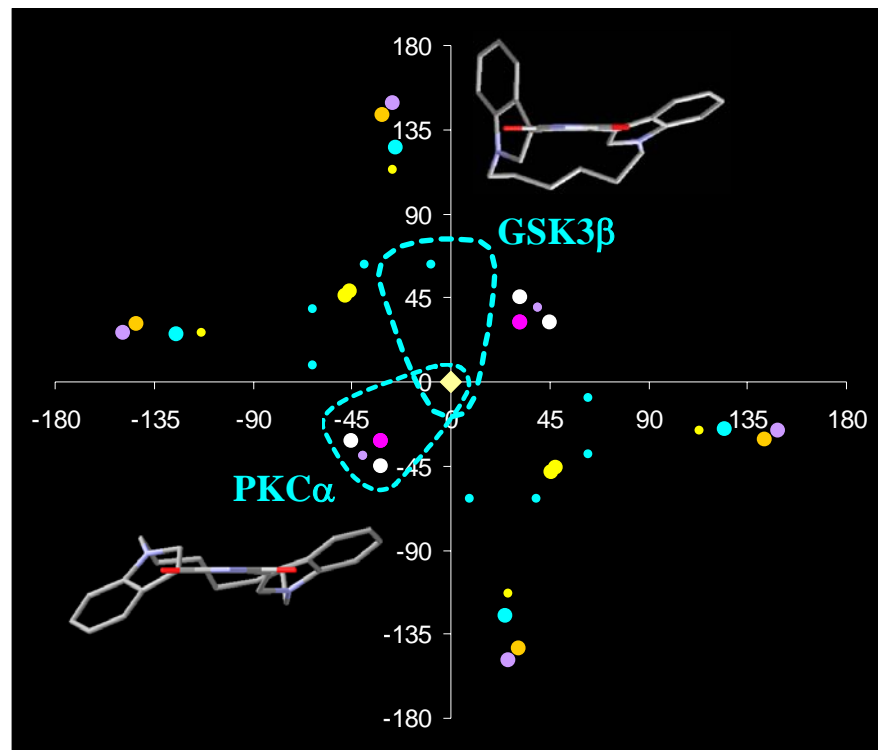
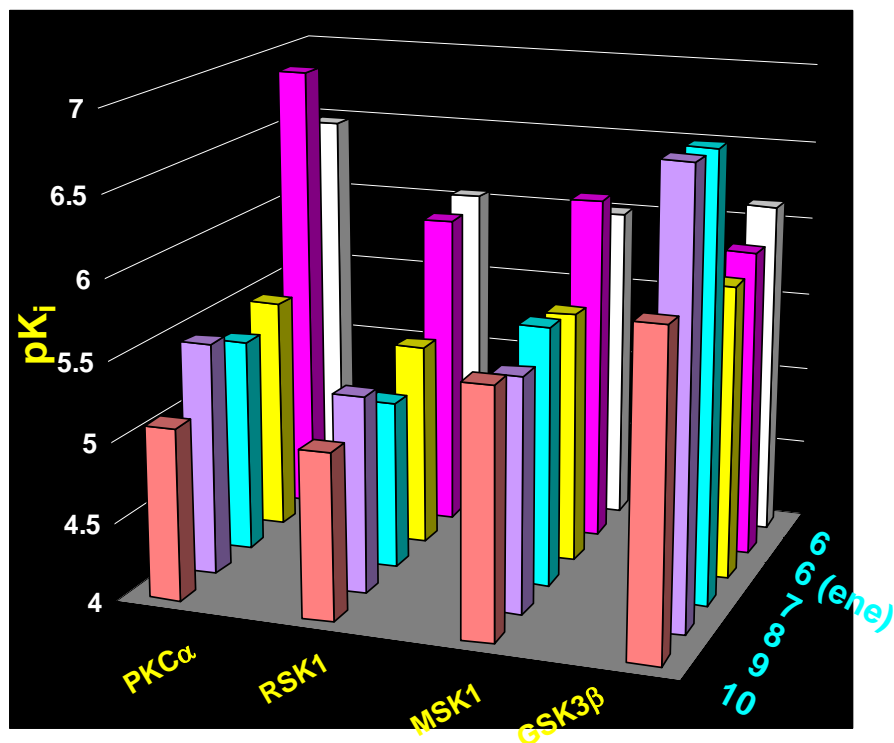


Proposed bound conformations of
ligands have now been observed
in complex with PDK-1



Structure 2004, **12**, 215

Correlating conformation with inhibition of other protein kinases



J. Am. Chem. Soc. 2005, **127**, 11699





Acknowledgments

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