

Alteration of Enzyme Specificity by Computational Loop Remodeling and Design

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Baker Lab

RosettaCON

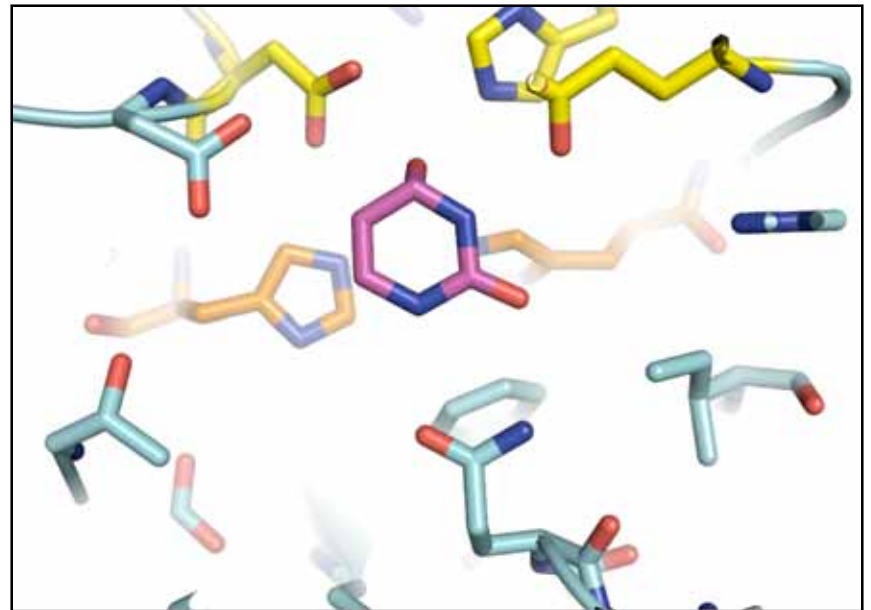
7/12/08

Enzyme Specificity

- Problem:
 - Assume catalytic machinery of an active site has been assembled
 - *de novo* design
 - reuse of biological active sites
 - How can activity/binding towards various substrates be optimized?

Enzyme Specificity & Backbone Flexibility

- Motif-based design
 - single residue
 - large contribution to ΔG_b
 - highly constrained
- Backbone flexibility greatly expands conformation space
 - better solutions
 - larger search space
 - How to search?



Crystal structure of bCD + TS analog

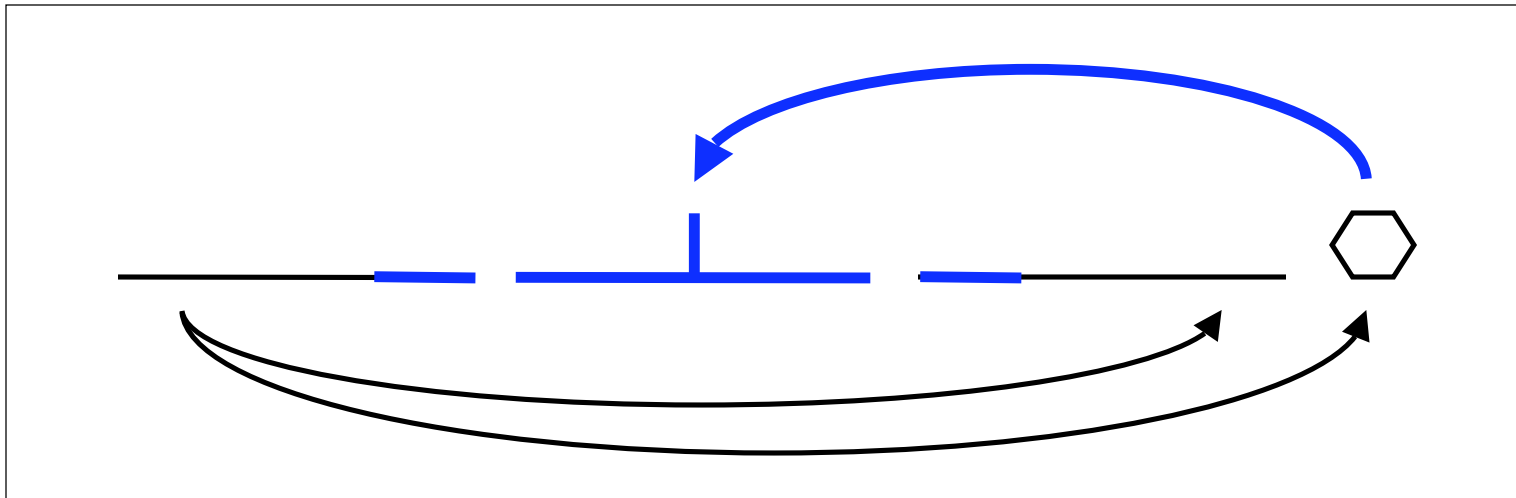
Enzyme Specificity & Backbone Flexibility

- How to search?
- Target-rich environment (eg. DNA interface)
 - Model many loops
 - Filter for motif-satisfaction
- Target-poor environment (eg. This problem?)
 - Model desired interaction
 - Work backwards to find loops that host the given interaction

Implementation in Mini

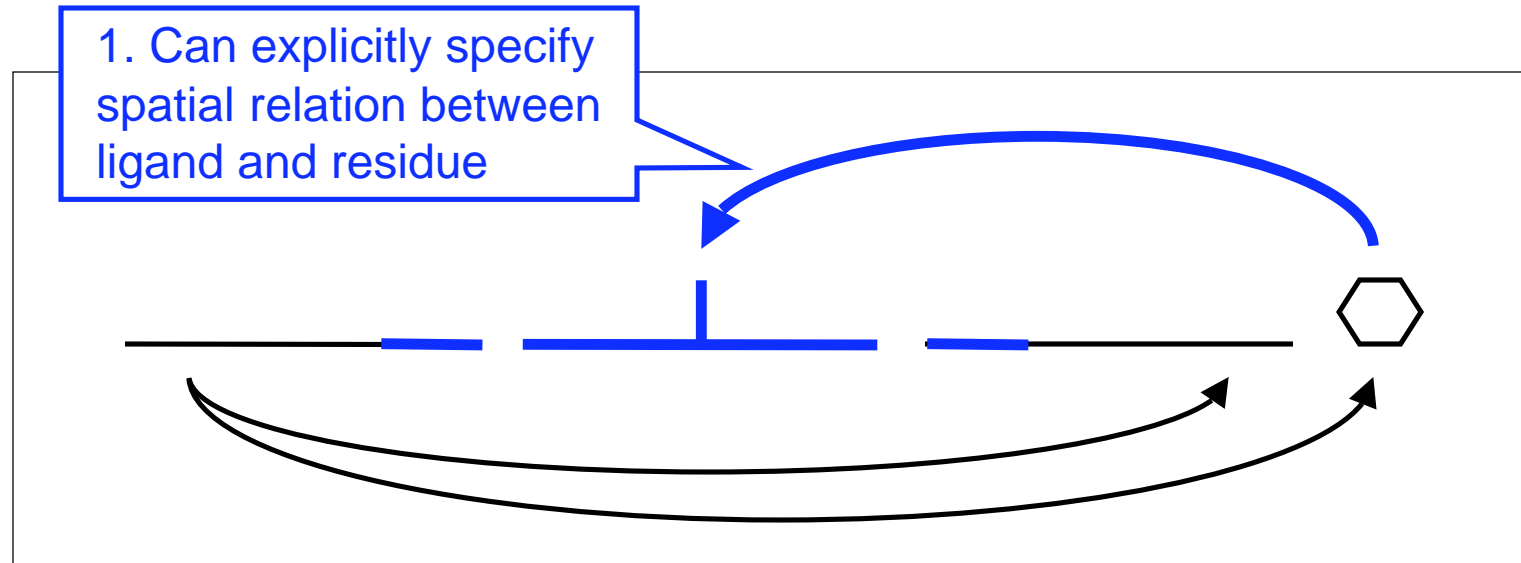
- How to code this type of search?
 - fold/atom-tree
 - multiple poses
 - loop closure algorithms

Impl. in Mini: *Fold/Atom-Tree*



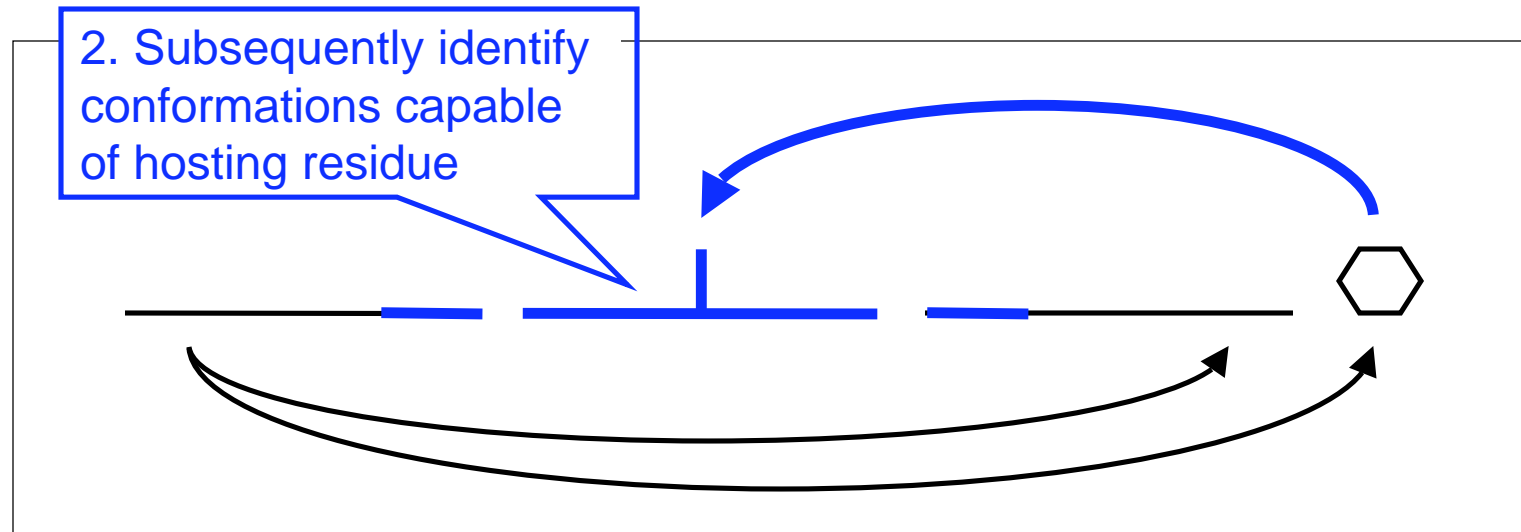
- Atom tree structure lets us search subspace with specific interaction
 - important to validate solutions outside of this subspace

Impl. in Mini: *Fold/Atom-Tree*



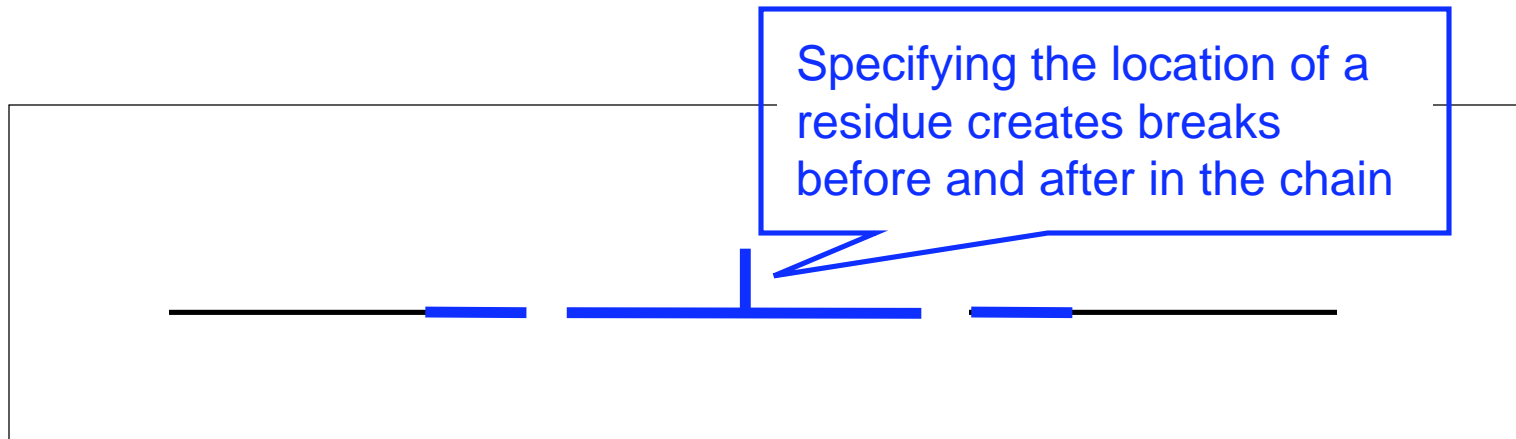
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Impl. in Mini: *Fold/Atom-Tree*



- Atom tree structure lets us search subspace with specific interaction
 - important to validate solutions outside of this subspace

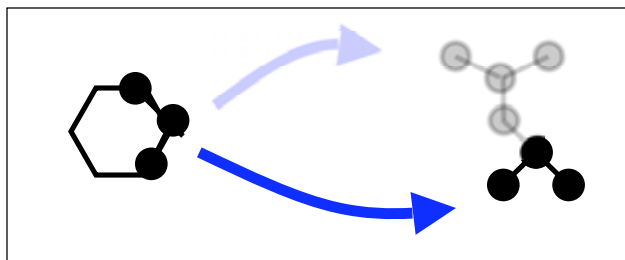
Impl. in Mini: *Loop Closure*



- Many available algorithms solve generic problem of closing chainbreaks
 - this search protocol is reasonable relative to other ways of imposing constraints

Impl. in Mini: *Multiple Poses*

- Several poses are used during search
 - Derive ligand:residue jump
 - Kinematics from a full-atom pose
 - Trimmed pose speeds scoring *
 - Score from a centroid pose *
 - needed for lo-res search phase
 - kinematically synced w/ full atom pose

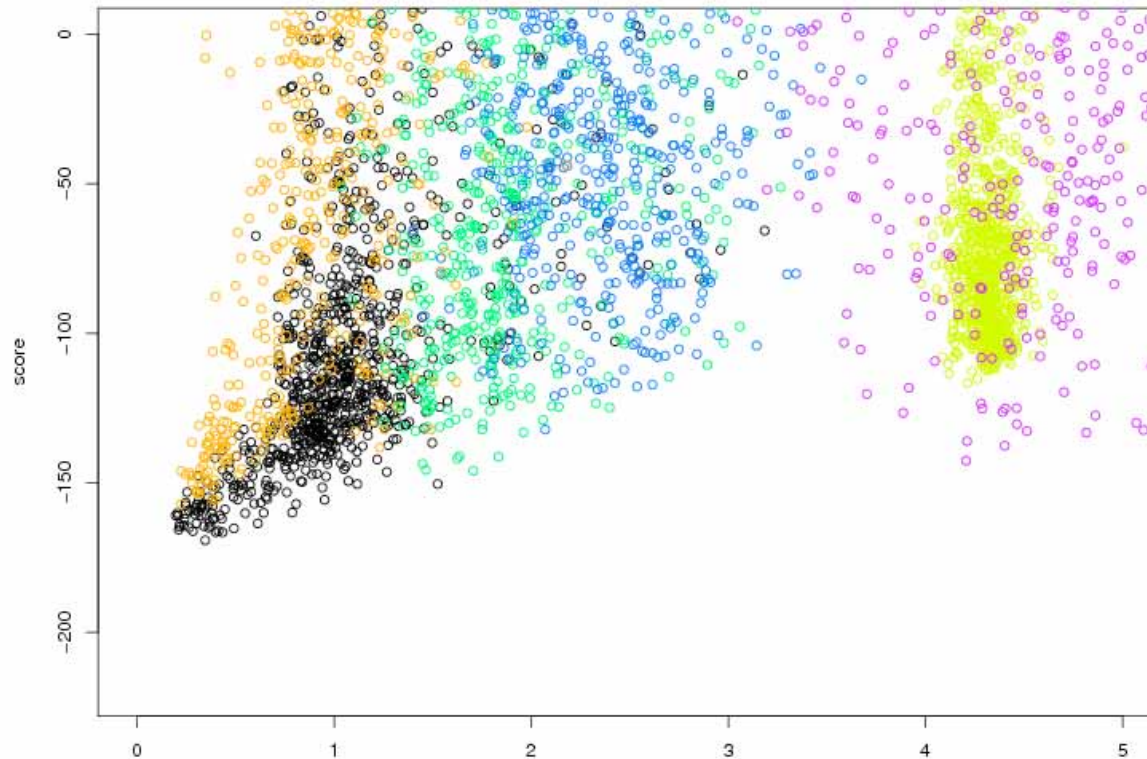


Prediction Performance

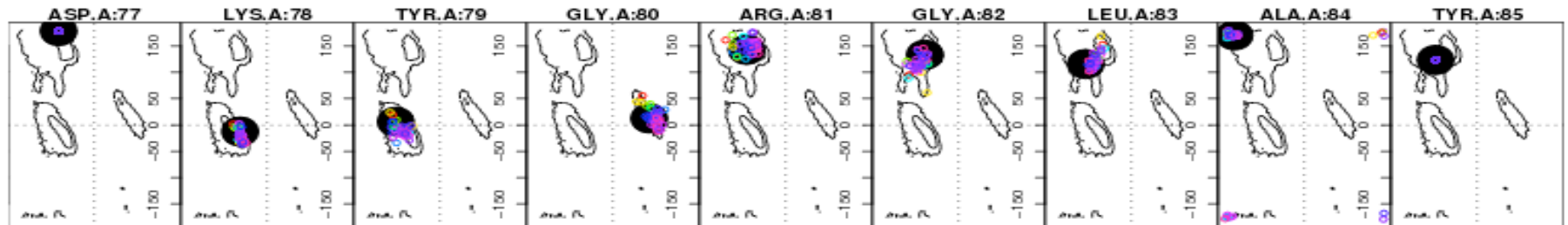
- Can we recover
 - Native structure?
 - Native sequence?
 - Native loop length?
- Benchmark
 - Set of enzyme:ligand complexes from PDB
 - Low calculated hbond energy between single residue and ligand
 - stronger argument about precision of designs

Prediction Performance - 2of1

2of1_THP_A:501_ARG_A:87.rtable

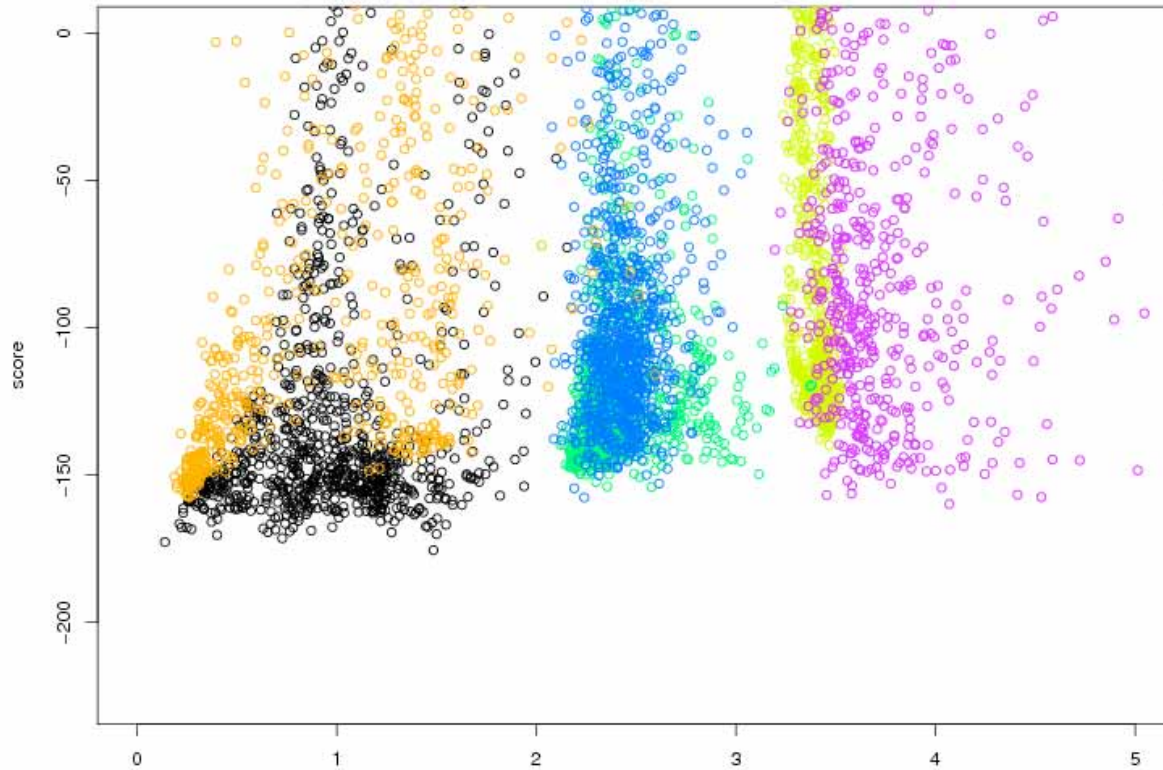


DKYGRGLAY
DAAARAAAY
DAARRAAY
DAARAAAAY
DAAAARAAY
DAAAARAAAAY



Prediction Performance - *1gua*

1gua_GNP_A:170_ASP_A:119.rtable



GSKCDLEDE

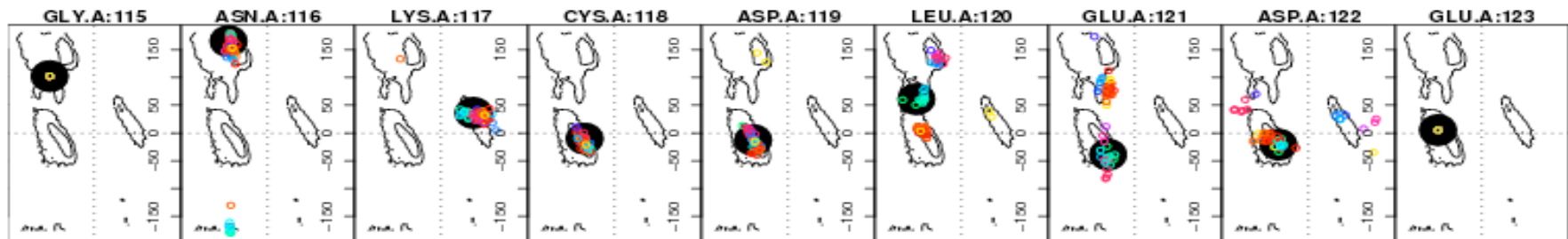
GAAADAAAE

GAADAAE

GAADAAAEE

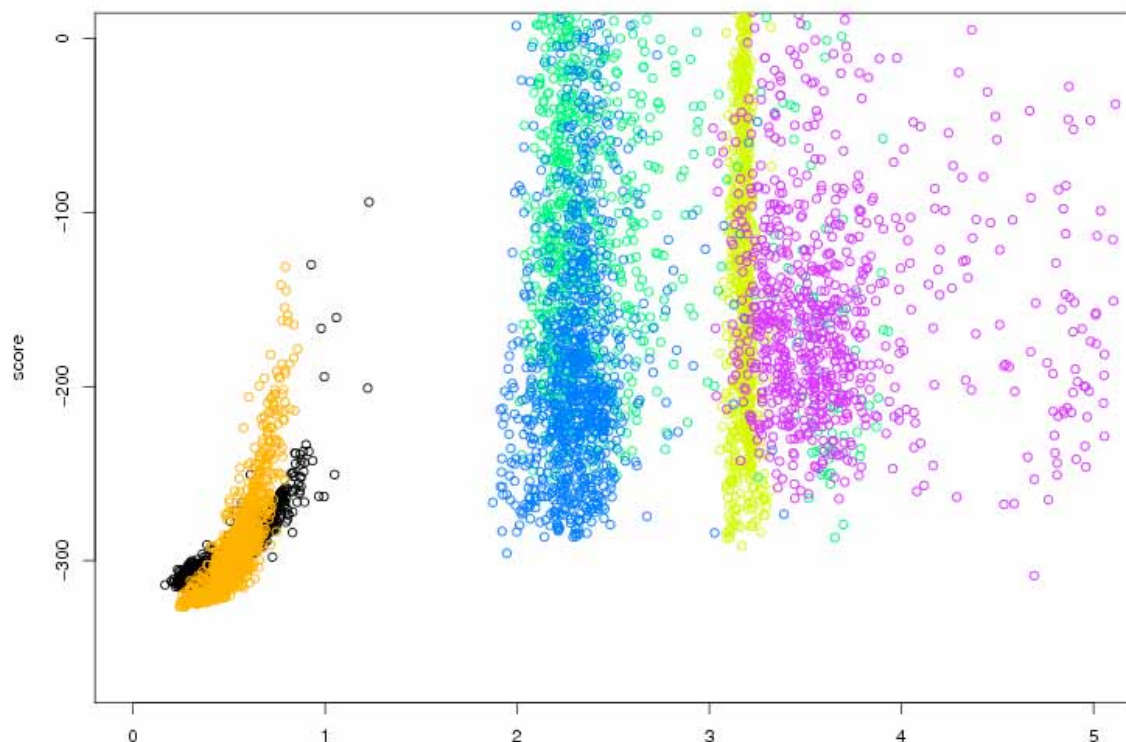
GAAAADAAE

GAAAADAAAEE

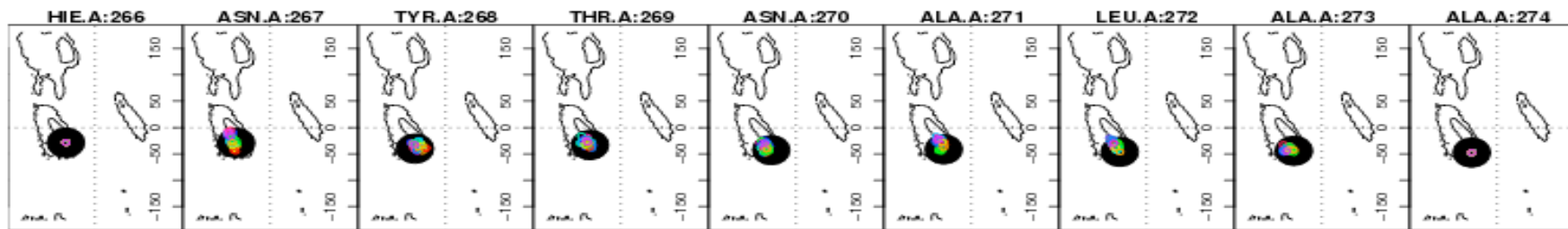


Prediction Performance - 2jfg

2jfg_ADP_A:1441_ASN_A:271.rtable



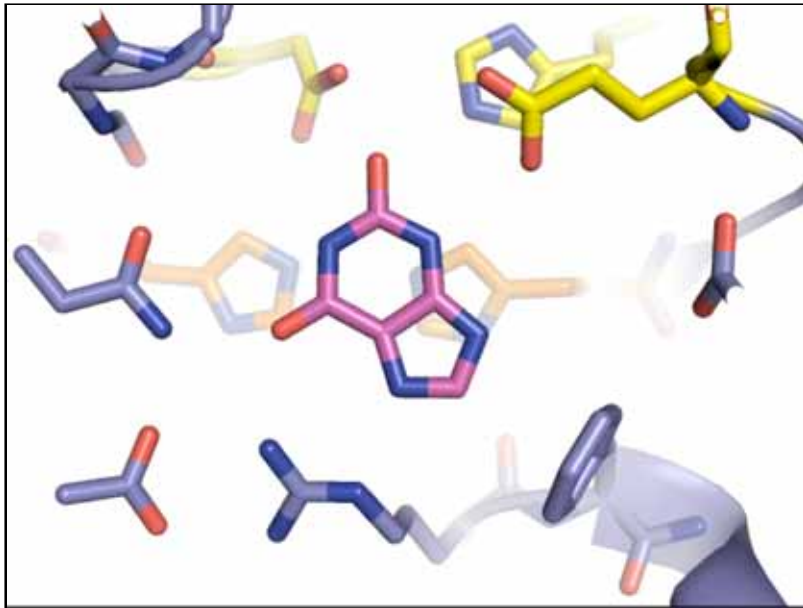
HNYTNALAA
HAAANAAAA
HAANAAA
HAANAAAAA
HAAAANAAA
HAAAANAAAAA



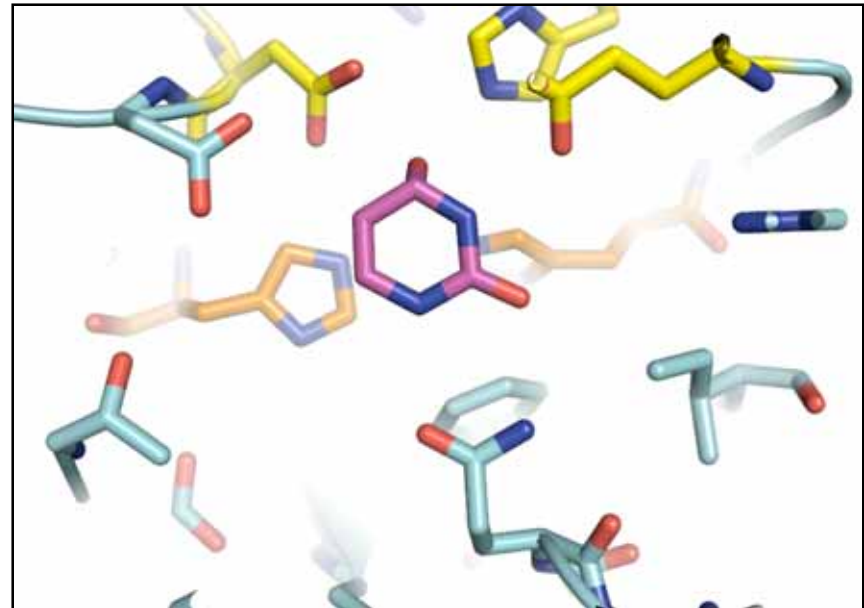
Design Performance

- Can we transplant sc:ligand interactions into a new scaffold by altering bb conformation?

hGDA=>hCD



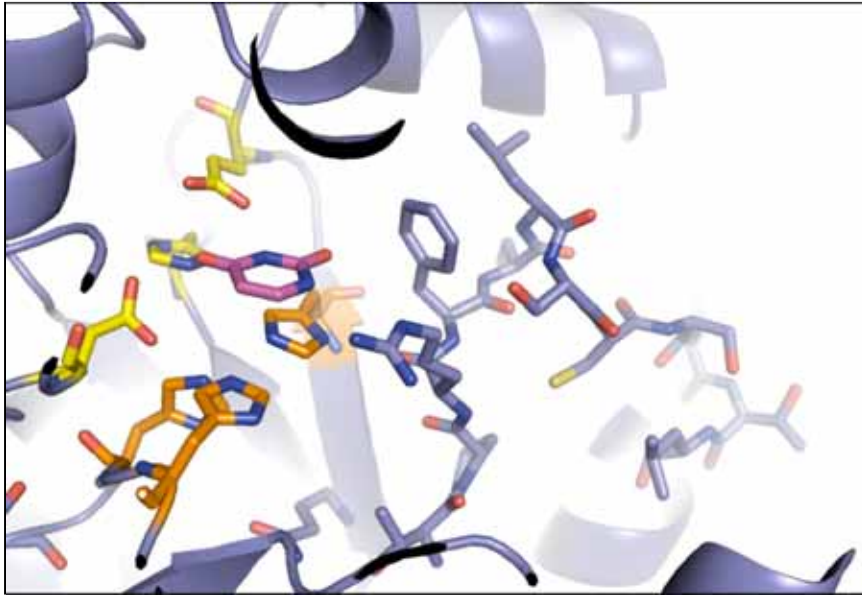
Crystal structure of hGDA + xanthine



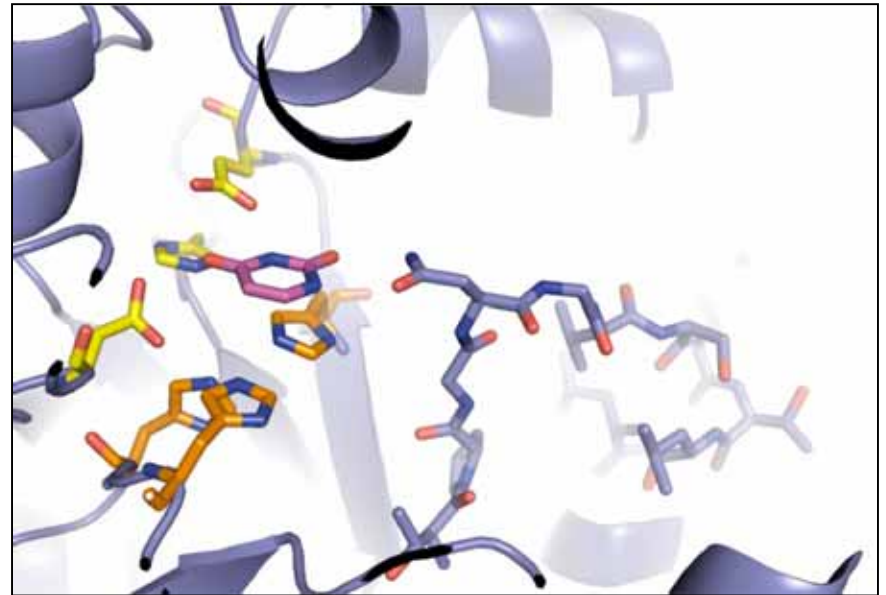
Crystal structure of bCD + uracil

- Towards a human cytosine deaminase (hCD)
 - Alter the specificity of human guanine deaminase (hGDA)

hGDA=>hCD - *Design*



Model of wild-type hGDA + uracil

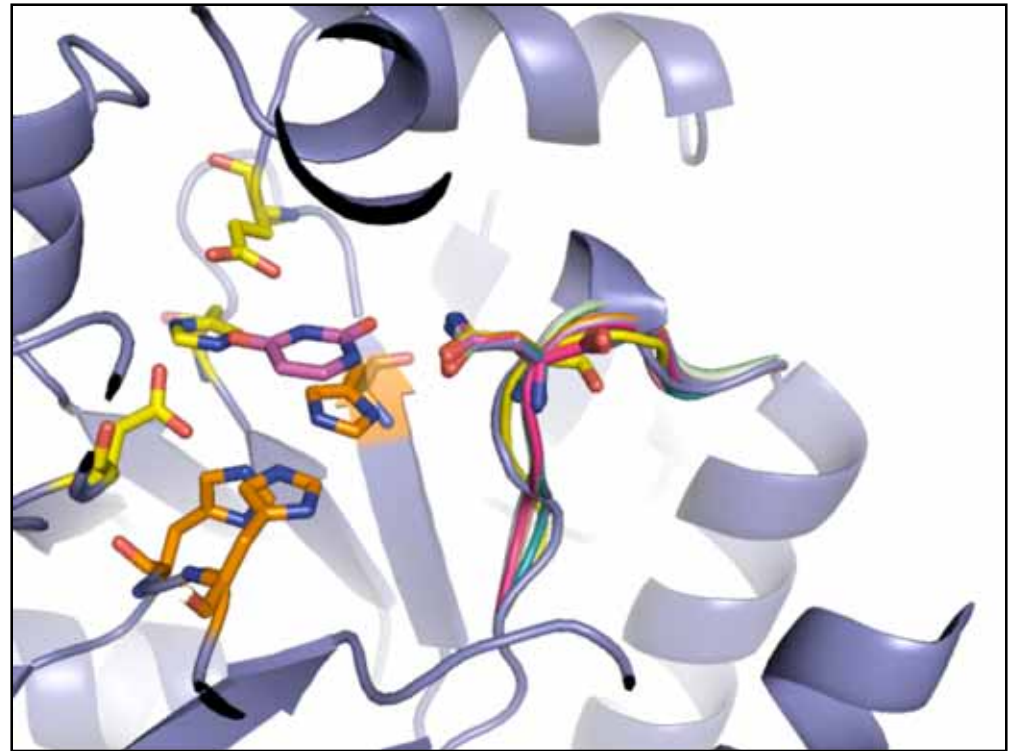


Designs of hGDA + uracil using interaction from bCD

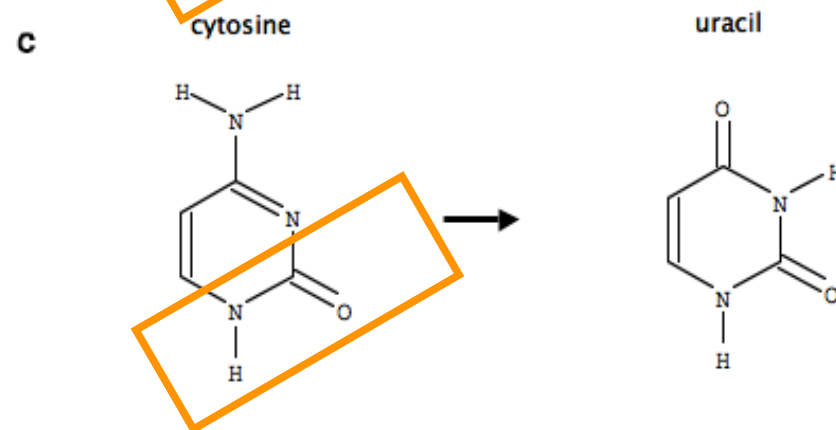
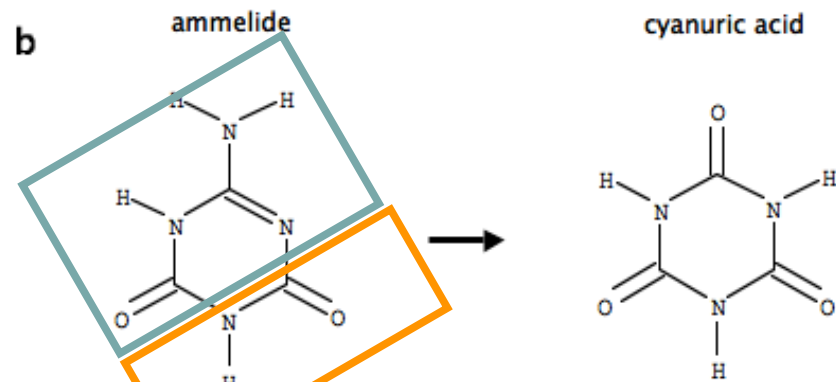
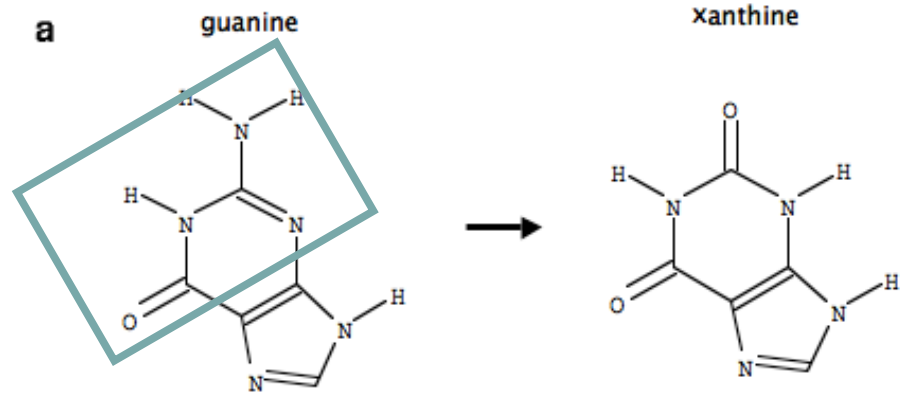
- Protocol
 - Superimpose new TS structure
 - Use sc:ligand interaction from bCD
 - Design loop as described

hGDA=>hCD - *Design*

- Results
 - Asn, 2 res del
 - RFSLSC=>GNGV



Designs of hGDA + uracil using interaction from bCD

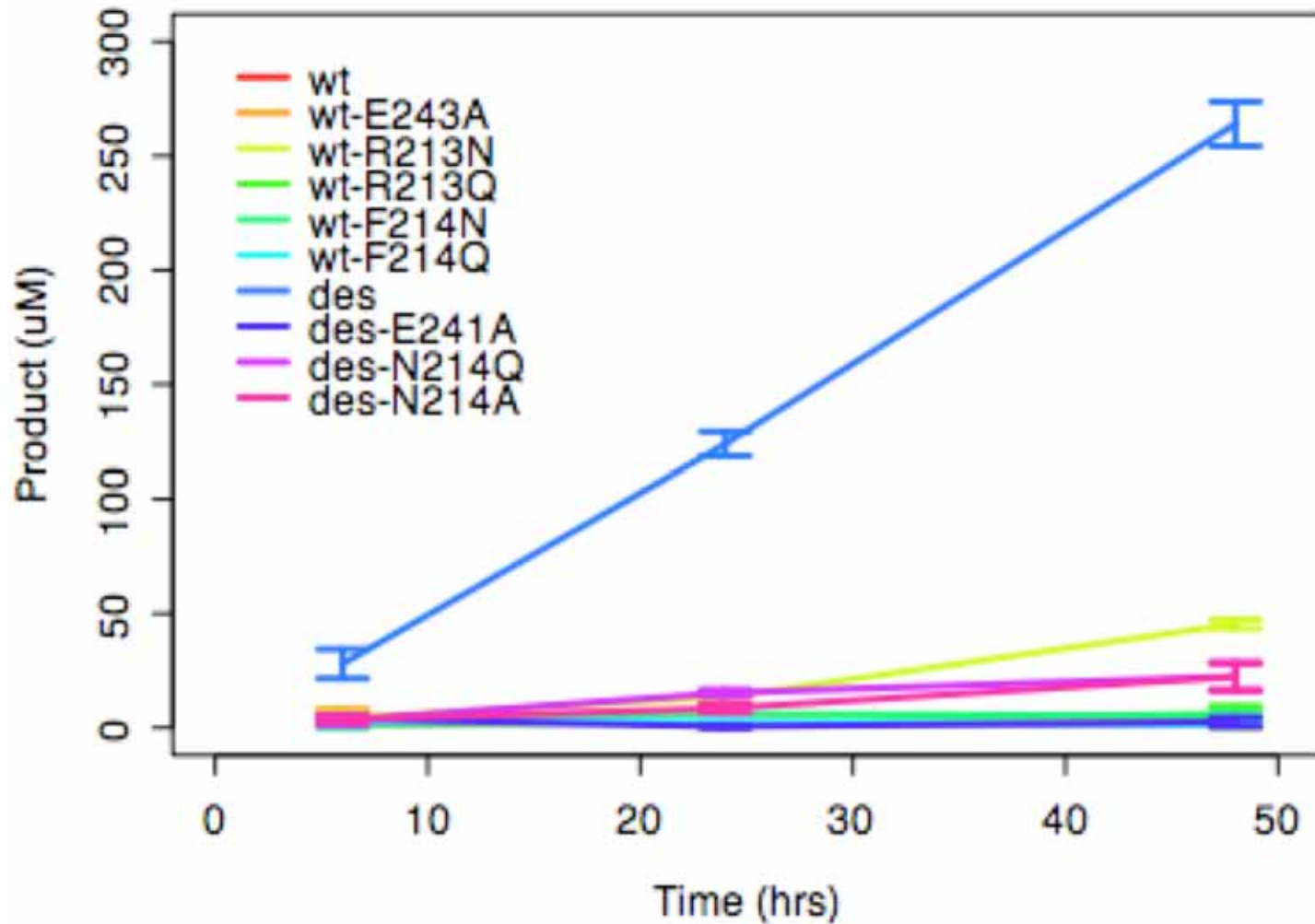


Ammelide

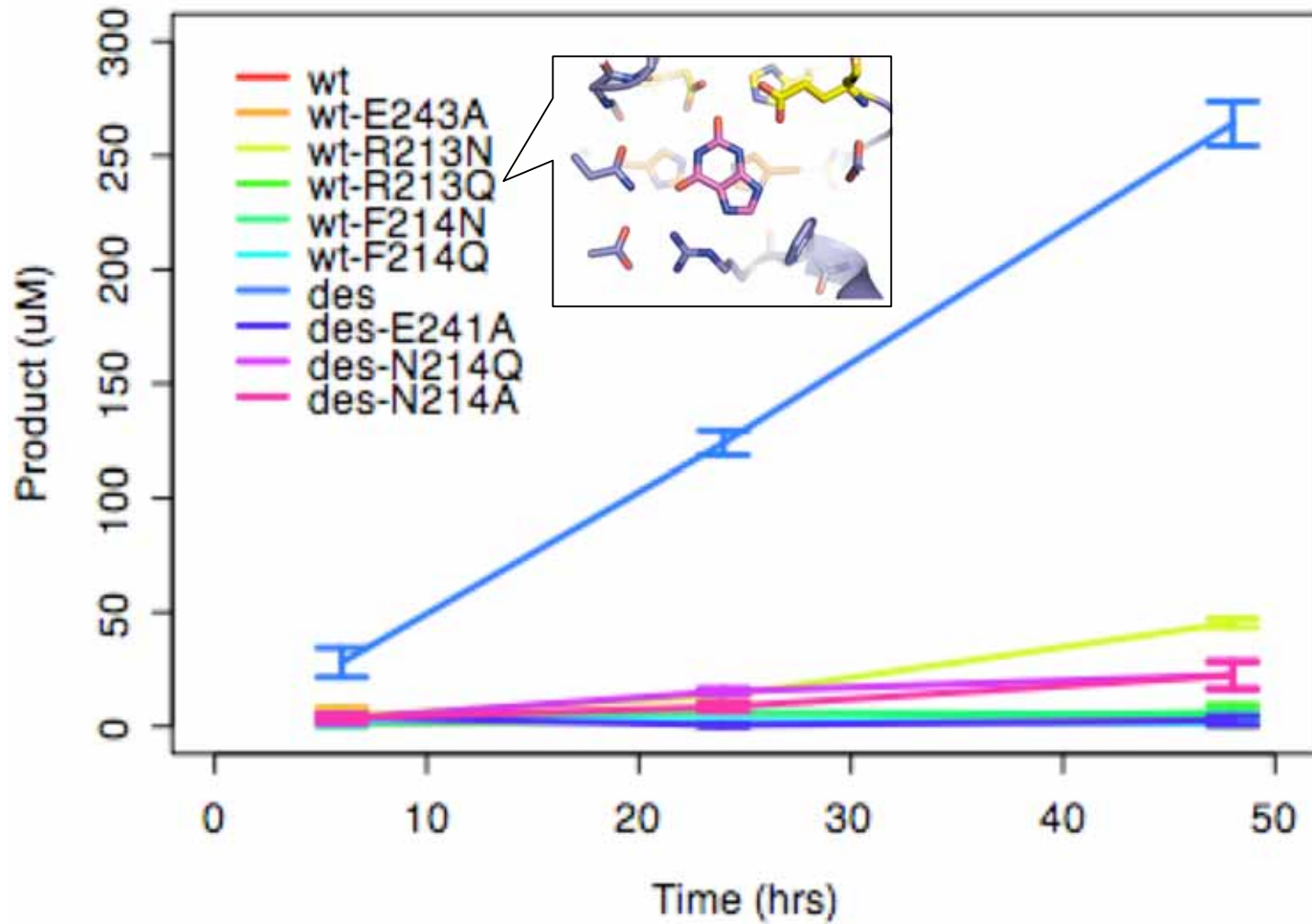
- stepping stone from G to C
- no bAD *

(cf 2-sided design)

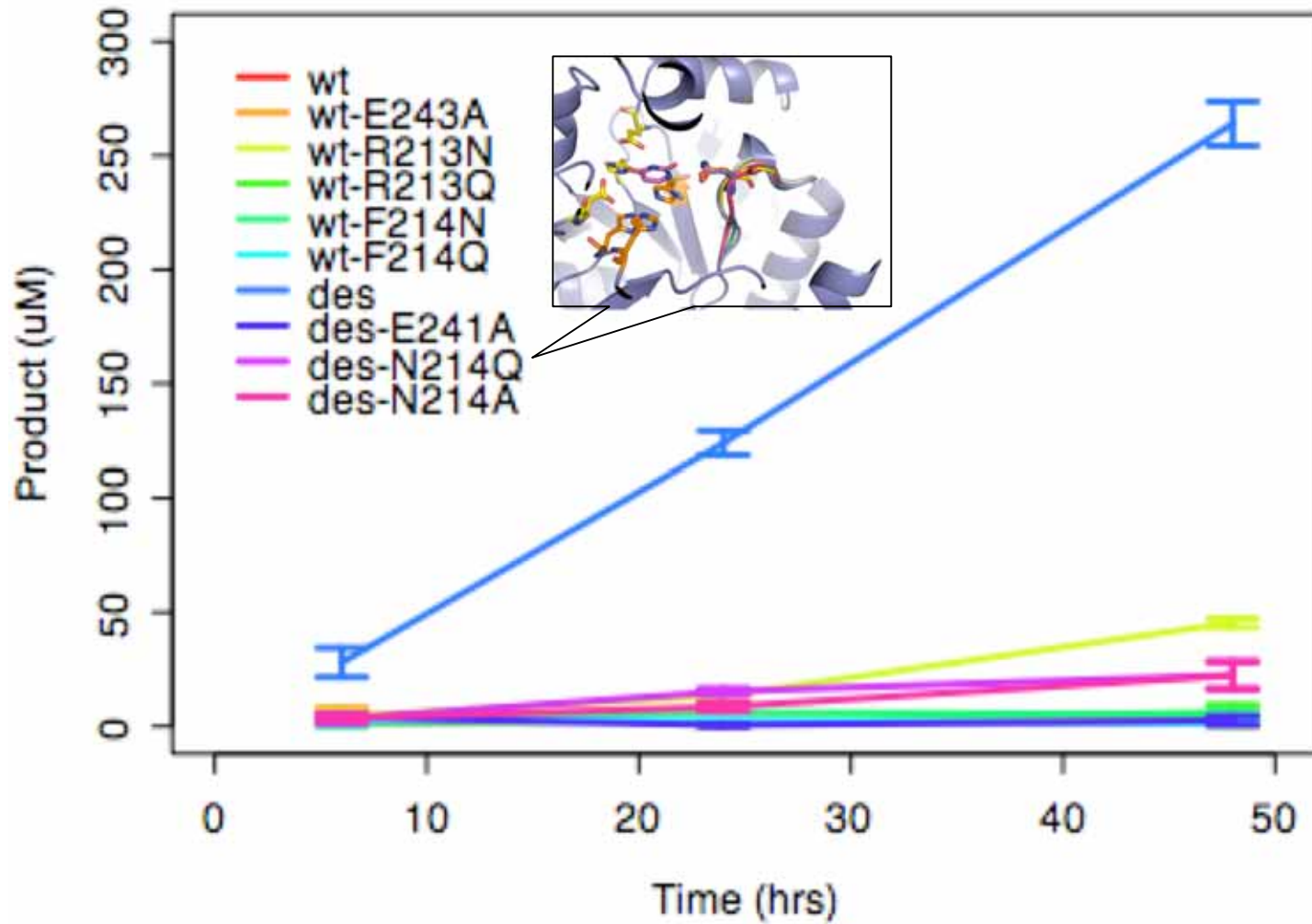
hGDA=>hCD - *AD Activity*



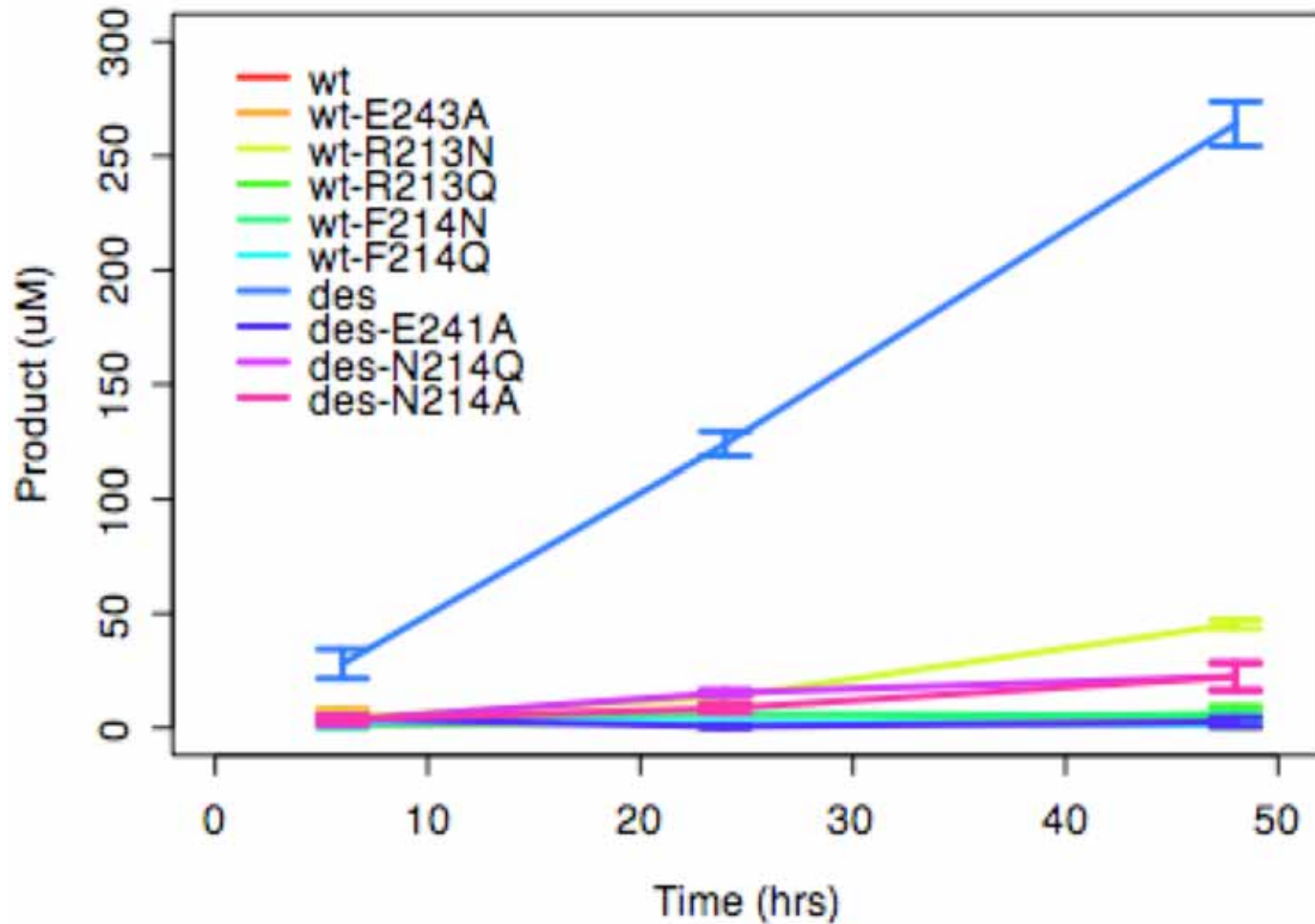
hGDA=>hCD - *AD Activity*



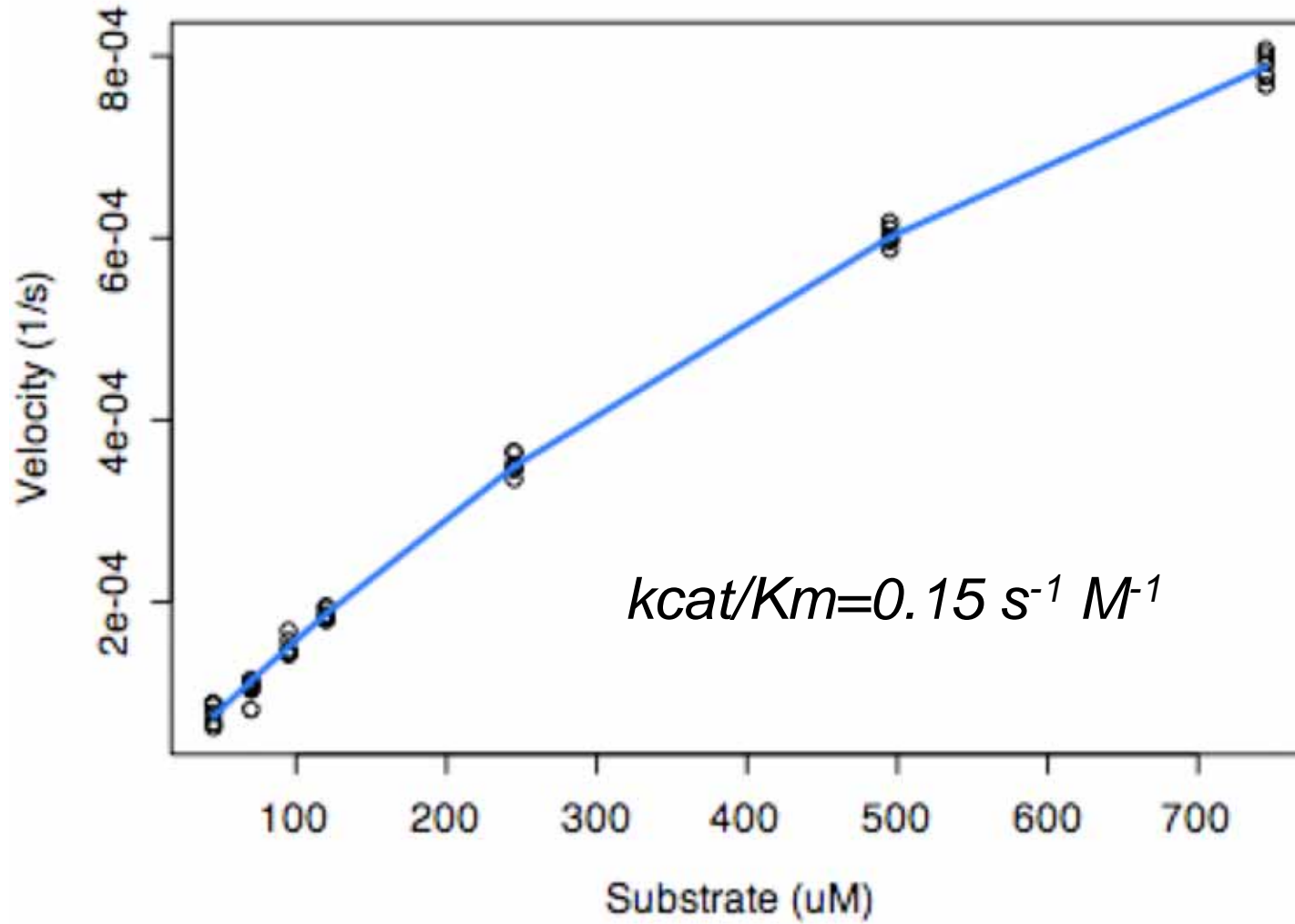
hGDA=>hCD - *AD Activity*



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hGDA=>hCD - *AD Activity*

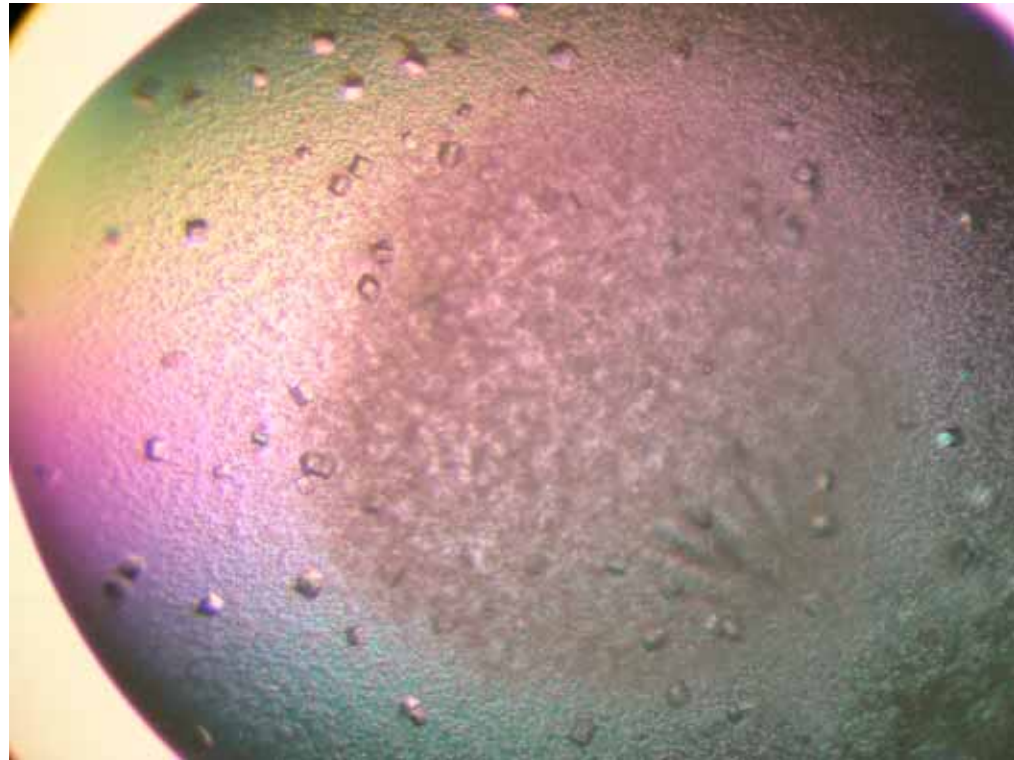


hGDA=>hCD - *AD Activity*

- Biochemical results consistent with structural model, except...
- Why such low k_{cat}/K_m ?
 - incorrect modeling in mini?
 - need xtal structure to determine this

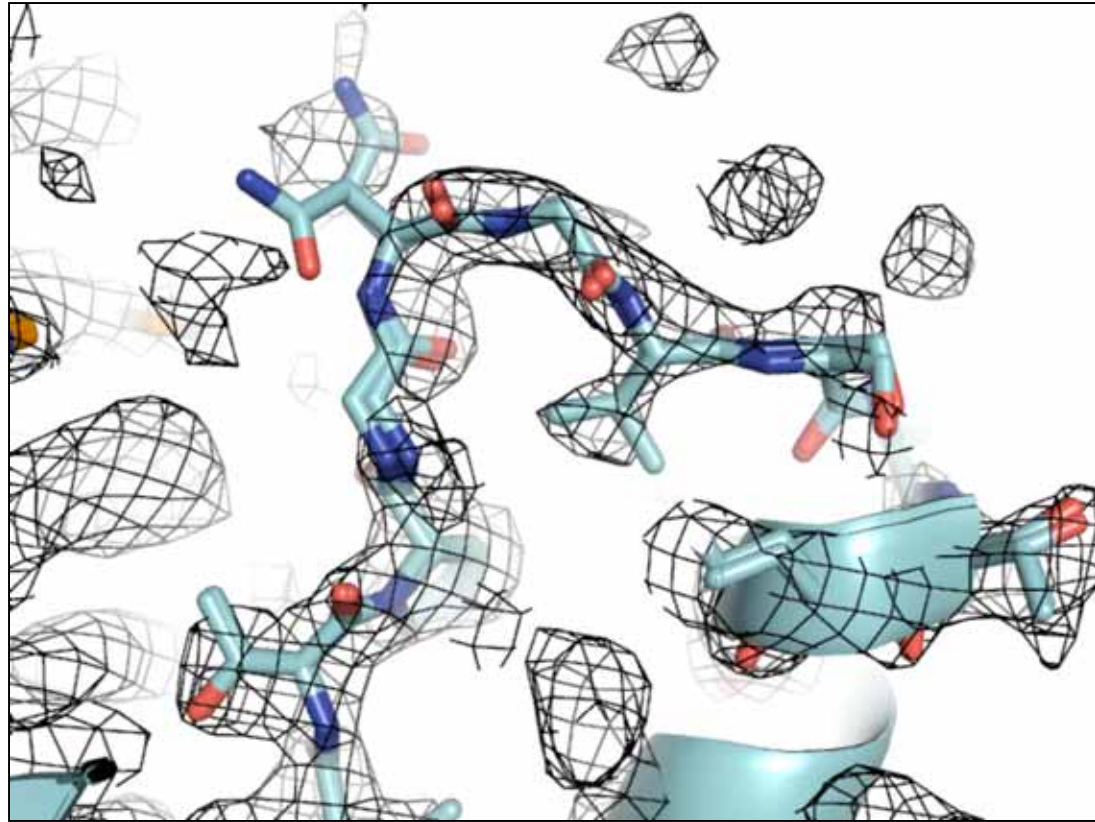
hGDA=>hCD - *Structure*

- Crystallography
 - Jill Bolduc
 - Barry Stoddard
 - Lei Zhou
- Resolution=2.4 Å
- Phaser_MR
 - search template:
2uz9 w/ loops
omitted
- $R_{\text{work}}=0.22$
- $R_{\text{free}}=0.26$



hGDA=>hCD - *Structure*

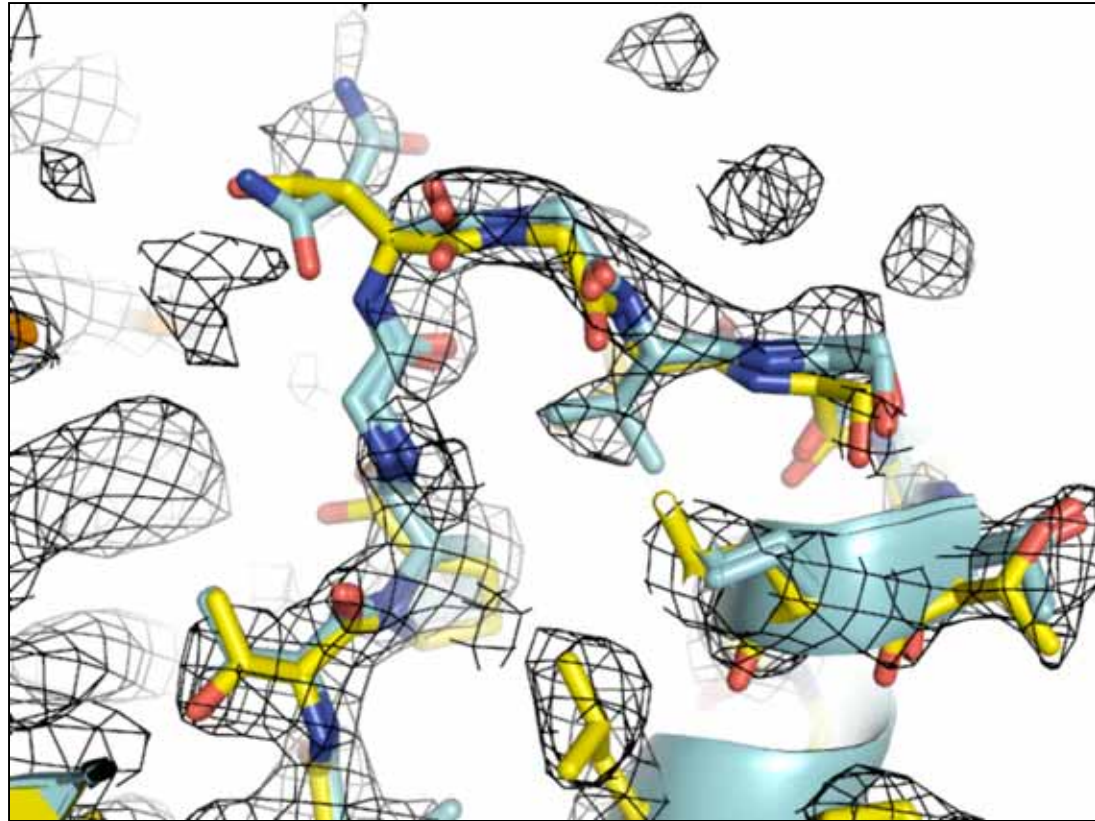
- Ca-RMSD
 - Overall=0.82 Å
 - Loop=0.93 Å
 - Lid=2.7 Å
- Apo structure
 - No e δ for
 - Ligand
 - Asn214.sc
- Active site
 - same conformation
 - zinc present



mesh=e δ from MR, *cyan*=final structure

hGDA=>hCD - *Structure*

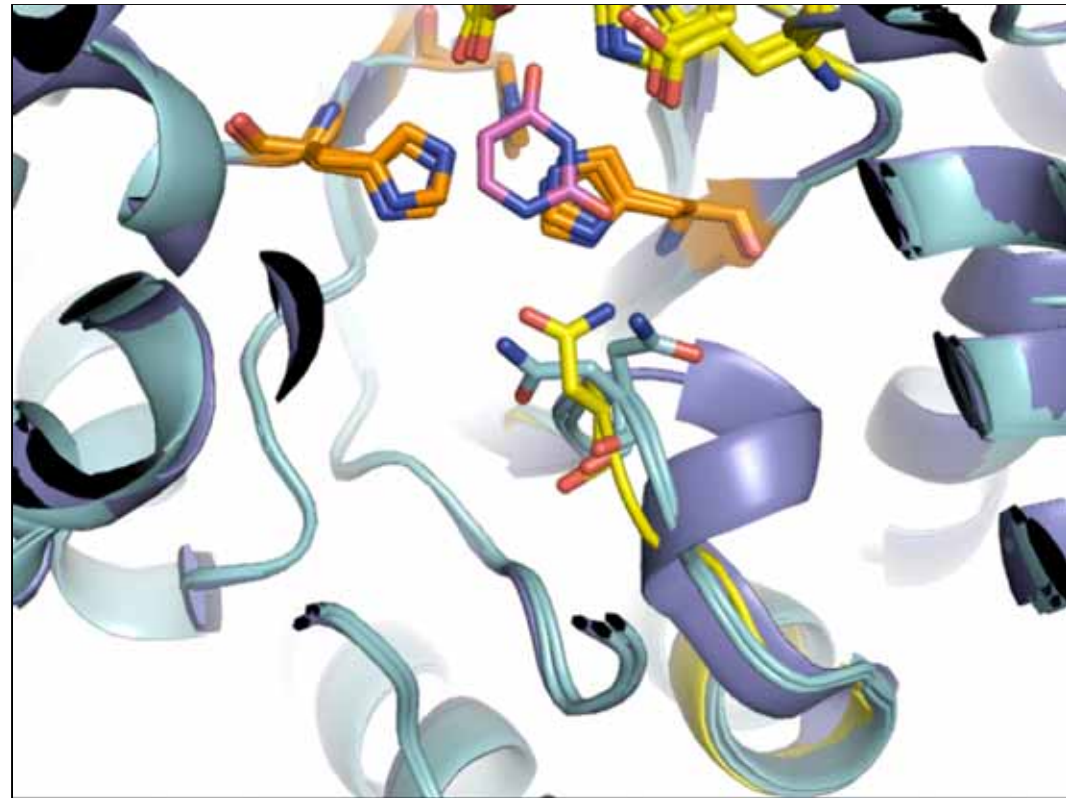
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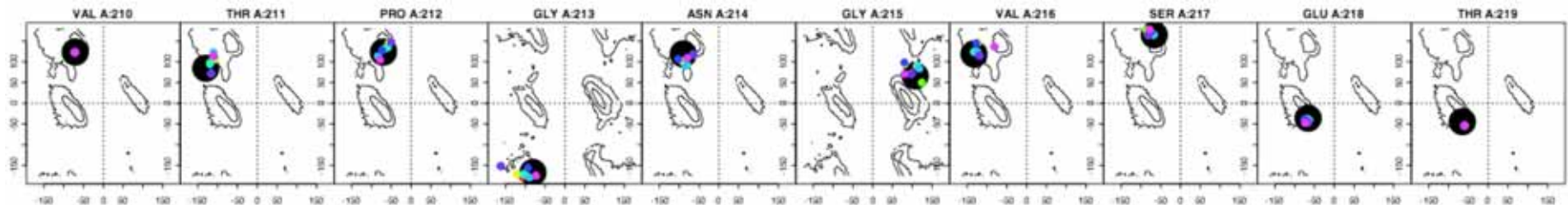
mesh=e δ from MR, *cyan*=final structure, *yellow*=model of design,

hGDA=>hCD - *Structure*

- Ca-RMSD
 - Overall=0.82 Å
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- Apo structure
 - No $e \delta$ for
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- Active site
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yellow=model of design, *cyan*=xtal of design, *slateblue*=xtal of wt



hGDA=>hCD - *past/future directions*

- Ammelide Deamination
 - fill hole left by deletion?
 - pre-order Asn214?
 - other loops?
 - 2nd/nth shell mutations?
- Cytosine Deamination
 - other face of the active site
 - random mutagenesis...

- Application to *de novo* active sites

hGDA=>hCD - *Conclusions*

- Modeling in mini
 - Structurally accurate to $< 1 \text{ \AA}$
 - Functionally incomplete?
 - sequence => structure => function

Acknowledgements

- David
- Phil, ALF, Ian, Bqian, mini
- Siegel, Jasmine
- Jill Bolduc, Lei Zhou, Barry Stoddard