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 $\Delta G_b$
  – 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
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Atom tree structure lets us search subspace with specific interaction
  - important to validate solutions outside of this subspace
• 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

DKYGRGLAY
DAARAAAAAY
DAARAAAY
DAAAARAAY
DAAAARAAAAAY
Prediction Performance - 1gua

GSKCDLEDE
GAAADAAAE
GAADAAAE
GAAAADAAAE
GAAAAADAAAAAE
Prediction Performance - 2jfg

HNYTNALAA
HAAANAAAA
HAANAAAAA
HAAAANAAAAA
HAAAANAAAAAA
Design Performance

• Can we transplant sc:ligand interactions into a new scaffold by altering bb conformation?
hGDA => hCD

- Towards a human cytosine deaminase (hCD)
  - Alter the specificity of human guanine deaminase (hGDA)
hGDA=>hCD - *Design*

- **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
hGDA$\Rightarrow$hCD - *AD Activity*
hGDA=>hCD - *AD Activity*

\[ \text{kcat/Km} = 0.15 \text{ s}^{-1} \text{ M}^{-1} \]
hGDA=>hCD - AD Activity

• Biochemical results consistent with structural model, except…
• Why such low kcat/Km?
  – 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 A
  - Loop=0.93 A
  - Lid=2.7 A
- Apo structure
  - No e ≡ for
    - Ligand
    - Asn214.sc
- Active site
  - same conformation
  - zinc present

\[mesh=e \; \text{≡ from MR, cyan}=\text{final structure}\]
hGDA=>hCD - Structure

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

*mesh* e density from MR, *cyan* = final structure, *yellow* = model of design,
hGDA=>hCD - **Structure**

- **Ca-RMSD**
  - Overall=0.82 Å
  - Loop=0.93 Å
  - Lid=2.7 Å

- **Apo structure**
  - No e ś for
    - Ligand
    - Asn214.sc

- **Active site**
  - same conformation
  - zinc present

*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 Å
  - Functionally incomplete?
    - sequence => structure => function
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