Highways, biways, and detours: the IspD story

RAMC 2011 11-14 September

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RAPID: Rational Approaches to Pathogen Inhibitor Discovery
How to choose a target?

- Essentiality studies
- Homology
- Important biochemical pathways
How to choose a target?

• Essentiality studies
• Homology

  *M. smegmatis, M. tuberculosis*

• Important biochemical pathways
How to choose a target?

• Essentiality studies
• Homology
• Important biochemical pathways

MEP (a non-mevalonate pathway) for isoprenoid synthesis
Outline of Crystallization Strategy

- What initial screen to use?
- What to do if you get a hit
- What to do if you don’t get a hit

—with our real-life example IspD
## Our setup

<table>
<thead>
<tr>
<th>Cloning</th>
<th>His-His-His-His-His-His-His</th>
</tr>
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<tbody>
<tr>
<td>Expression</td>
<td>Arabinose 1-1.5 liter</td>
</tr>
<tr>
<td>Purification</td>
<td>Nickel IMAC Superdex</td>
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</tbody>
</table>
The initial search problem: how many conditions to try?
Is minimal screening efficient?

<table>
<thead>
<tr>
<th># conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>192</td>
<td>20</td>
</tr>
<tr>
<td>192 + 500 more</td>
<td>21</td>
</tr>
</tbody>
</table>

How similar/different are two screens?

A metric for:

- internal diversity
- diversity between screens
- search for a particular chemical

The C6 Web Tool at CSIRO C3
pH BUFFER SCR.
pH 3.0 - 10.0
1M buffers

JCS G+ (MD)
2009 06 25
3 (12)

Morpheus
2009 06 25
6 (12)

JCS G+ (MD)
2009 06 25
4 (12)

Morpheus
2009 06 25
7 (12)
bis-tris-propane
pH 6.5
## Examples of start buffers

<table>
<thead>
<tr>
<th>Location</th>
<th>Buffer Composition</th>
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<tbody>
<tr>
<td>RAPID, Uppsala</td>
<td>Tris pH 7.5, NaCl, 10% glycerol</td>
</tr>
<tr>
<td>JCSG, California</td>
<td>Tris pH 7.9, NaCl, 0.25 mM TCEP</td>
</tr>
<tr>
<td>SECSG, Georgia</td>
<td>Tris, pH 7.5, NaCl, 0.1 mM protease inhibitor mix</td>
</tr>
<tr>
<td>Toronto, Canada</td>
<td>Hepes, 7.5, NaCl</td>
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</tbody>
</table>
# Optimum Solubility Buffer Screen

<table>
<thead>
<tr>
<th>Problem</th>
<th>14 proteins with poor solubility</th>
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</thead>
<tbody>
<tr>
<td>Action</td>
<td>Screen for better buffer conditions---improved solubility for 11 of them</td>
</tr>
<tr>
<td>Result</td>
<td>9 of them now crystallized</td>
</tr>
</tbody>
</table>

Optimum solubility buffer screen

How to do it:

Google these words:
Optimum Solubility Screen
SOP

also: Kits available
Thermofluor

Jancarik et al. Acta Crys D60, 2004
After the buffer change from Hepes, 7.5 to btp, 6.5
Outline

• How many conditions to screen?
• What to do if you get a hit
• What to do if you don’t get a hit
Two different search problems

Initial hit

Optimization

- grid search
- seeding
- ligands/substrates
- additive screens
- change kinetics
Screening

Optimization with CTP
Dataset collected on MAR345
180 images x 15 min exposure = 45h collection time.
Resolution: 2.2Å
Spacegroup: $p2_1^2_1^2_1$
Outline

• How many conditions to screen?
• What to do if you get a hit
• What to do if you don’t get a hit
Run SDS gel

Minimal screens

Rescue strategies

Screen more

yes

optimize

no

yes

no

no
Check the protein concentration!

- Minimal screens
  - yes: optimize
  - no: Screen more
    - yes: Rescue strategies
    - no: no
3 mg/ml or 40 mg/ml?

Initial Screening

Optimized with ligand

0.3 mm
What rescue method to use?

• in situ proteolysis
• reductive methylation
• truncations
• remove disordered regions
• use an ortholog: cross-seed
M. smegmatis IspD + CTP
M. smegmatis IspD + CMP
M. tuberculosis IspD + CTP

Björkelid, Bergfors et al Acta D 2011
The IspD Story: Summary

- Protein concentration
- A truncation
- Change of buffer
- Add substrate
- Good teamwork
xray.bmc.uu.se/terese
Predicting crystallizability

- Dynamic light scattering (DLS)
- Bioinformatics
- Thermofluor
- Calorimetry
- Mass spec
- NMR
Predicting crystallizability vs. predicting disorder

- XtalPred
- PDPredictor
- MetaPPCP
- ParCrys
- OB-Score, etc.

www.disprot.org
TCA precipitation test
TCA precipitation test

- 0.5 mg/ml
- 2.5 mg/ml
- 5.0 mg/ml
Example of conformational heterogeneity

Celllobiohydrolase digesting a chain of cellulose
Why is it so hard to crystallize proteins?

- Evolution is against it!
- Crystallization conditions are unique for each protein.
- Proteins can be unstable, different conformations, floppy, etc.
DLS after freeze/thaw

Grey: before freezing
Black: after thawing
Stability Test at 4 and 20°C

Reduced

Non-reduced
Two analyses of disorder

Glob Plot

Fold Index
Not much consensus!

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<th>Pre-link</th>
<th>Fold Index</th>
<th>Glob Plot</th>
<th>RONN</th>
<th>Dis-prot</th>
<th>IU Pred</th>
<th>POND R</th>
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Overlap of methods

Vapor-diffusion

hanging-drop
Overlap of methods

Vapor-diffusion
hanging vs. sitting
Overlap of methods

Vapor-diffusion

Microbatch
Overlap of methods

Vapor-diffusion
Microbatch

Counterdiffusion