Enabling Robot Scientists to screen for antiparasitic drugs

Workshop on Synthetic Biology and Robotics

February 24th, 2011

Bessie Bilsland
• Malaria
  – caused by *Plasmodium*
  – kills 3 million people/year
  – treated by chloroquine, artemisin and antifolates such as pyrimethamine; however, *Plasmodium* spp. have become resistant to many of these

• Schistosomiasis
  – caused by the blood fluke *Schistosoma*
  – affects 207 million people and causing the death 300,000 individuals/year
  – treated with praziquantel, however drug-resistant parasites may evolving

• Sleeping sickness (or African trypanosomiasis)
  – caused by *Trypanosoma brucei*
  – infects about 300,000 people each year leading to about 40,000 deaths

• Chagas disease
  – caused by *T. cruzi*
  – 17 million people are infected, leading to about 21,000 deaths/year
  – very few drugs are available for the treatment of patients infected with *Trypanosoma* spp. and their low efficacy, high cost, or unacceptable side effects suggest a pressing need for new treatments.
• Establishing reproducible culturing conditions that mimic *in vivo* host environments is difficult to achieve using parasites.

• When a potential new compound is identified and verified, the absence of information regarding its target and mechanism of action frequently makes the approval of the compound for clinical use problematic.

• Novel methodologies and tools enabling the identification of antiparasitics are urgently needed.
Overview of our experimental design

• We have designed a system to rapidly identify new antiparasitic drugs using a Robot Scientist.

• *Saccharomyces cerevisiae* was engineered to express target proteins from either parasites or humans.

• Dihydrofolate reductase (DHFR), N-myristoyl transferase (NMT) and phosphoglycerate kinase (PGK) were used as a model targets.

• We expressed the enzymes from *Plasmodium falciparum, P.vivax, Homo sapiens, Schistosoma mansoni, Leishmania major, Trypanosoma brucei*, or *T.cruzi*.

• We have fluorescently tagged strains expressing target enzymes from different species to allow drug screens with up to 4 strains/well.

• The system’s practicality was demonstrated by robotically screening a library of ~14,400 compounds and identifying novel agents selectively active against parasite targets.
The targets: dihydrofolate reductase (DHFR)

- DHFR is present in organisms ranging from bacteria to humans and is the target of pyrimethamine.
- \( dhfr \Delta \) yeast strains can be rescued by the expression of heterologous enzymes from human, *Plasmodium*, *Cryptosporidium* and *Toxoplasma*.
- These strains were used successfully for drug sensitivity assays on agar plates (Sibley lab).
The targets: N-myristoyl transferase (NMT)

- Enzymes responsible for co- and post-translational modifying various proteins.
- Transfer myristate groups to N-terminal gycine residues.
- Allow their targeting to various membranes.
- Essential enzymes conserved from kinetoplastid parasites to humans.
- Have been successfully demonstrated as drug targets.
The targets: phosphoglycerate kinase (PGK)

- Central enzymes in glycolysis and gluconeogenesis.

- Catalyze the high-energy transfer of phosphoryl groups from the acyl phosphate of 1,3-bisphosphoglycerate to ADP to produce ATP.

- Essential for the blood stages of parasites but are not expressed in erythrocytes.
DHFR expression plasmids

- pDHFR
- SsDHFR
- pDHFR
- TbDHFR
- TcDHFR
- RpDHFR
- HsDHFR
- URA3
- pCM188
- CEN
- tetO2-prom
Maximum growth rate of yeast strains expressing heterologous drug targets

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>Hs</th>
<th>Lm</th>
<th>Pf</th>
<th>PfR</th>
<th>Pv</th>
<th>Sm</th>
<th>Tb</th>
<th>Tc</th>
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<tbody>
<tr>
<td>DHFRs</td>
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<tr>
<td>NMTs</td>
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<tr>
<td>PGKs</td>
<td>0.6</td>
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</table>

max growth rate (relative to WT)
Similarity between DHFRs, NMTs, and PGKs
Pyrimethamine sensitivity of yeast strains expressing heterologous DHFRs

Pyrimethamine  control  10 μM  25 μM  100 μM  250 μM  500 μM
Pyrimethamine sensitivity of yeast strains expressing heterologous DHFRs

<table>
<thead>
<tr>
<th>Yeast DHFR</th>
<th>Control</th>
<th>10 µM</th>
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<td>y^{Tb}DHFR</td>
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DHFR expression plasmids

- dhfr
- ura3
- tetO2-prom
- pCM188
- doxycycline
Effect of doxycycline on pyrimethamine sensitivity

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<tr>
<th>Pyrimethamine</th>
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<td>y^{Tc}DHFR</td>
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5 mg/l doxycycline

no doxycycline
The drug efflux pump Pdr5

- Yeast cells are inherently resistant to drugs:
  - protective cell wall
  - multiple drug efflux pumps

- Pdr5p is the most abundant drug export pump in *S. cerevisiae*

- *pdr5Δ* strains are sensitive to multiple drugs
Quantification of the effect of the efflux pump Pdr5 in drug sensitivity

**A**

<table>
<thead>
<tr>
<th>PDR5</th>
<th>pdr5Δ</th>
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<tbody>
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<td>y^{Hs} DHFR</td>
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**B**

![Image B]

**C**

![Image C]

**D**

![Image D]
**In vivo labelling of yeast cells**

- We have constructed a series of plasmids expressing high levels of different fluorescent proteins to allow the *in vivo* labelling of yeast cells expressing different heterologous drug targets.

- The labelling of the different strains allowed us to perform high-throughput drug screens with up to 4 strains, each expressing a different DHFR, growing in competition.
Fluorescent plasmids

- CFP
- Sapphire
- Venus
- mCherry
- LEU2
- TDH3prom
- 2 micron
- yEp
- URA3
- HIS3
Fluorescent-labeled yeast cultures

<table>
<thead>
<tr>
<th></th>
<th>Venus</th>
<th>Sapphire</th>
</tr>
</thead>
<tbody>
<tr>
<td>mCherry</td>
<td>CFP</td>
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</table>

BF

UV

Fluorescent-labeled yeast cultures
Experimental design
Fluorescent-labeled yeast cells grown ON in the absence or presence of pyrimethamine

No drug

Pyrimethamine

- $y^{Hs}_{DHFR} + y_{EpmCherry}$
- $y^{pf}_{DHFR} + y_{EpSapphire}$
- $y^{Rpf}_{dhfr} + y_{EpVenus}$
- $y^{Sm}_{DHFR} + y_{EpCFP}$
The Robot Scientist Eve

• High-throughput (HTS) automated laboratory system

• Aims:
  – execute a standard blind HTS against thousands of compounds
  – switch over to a intelligent mode that uses machine learning and QSARs (quantitative structure-activity relationships)
  – perform cycles of intelligent selective HTS and home in on the optimal chemical structure to inhibit the action of the target enzyme
  – Select lead compounds in less time and using fewer resources than traditional screens.
Negative controls in 10µM screen:

Compounds in 10µM screen:

Confirmation screening growth:

**key:**  
- Red: HsDHFR  
- Blue: PvDHFR  
- Yellow: PfRDHFR

**Pyrimethamine**  
**P. vivax hit**  
**Pf-R/Pv hit**
Network of hits from Eve’s initial high-throughput screens
Network of hits from Eve’s initial high-throughput screens
Network of hits from Eve’s initial high-throughput screens
Conclusions

• Method suitable for automation (less labor intensive than agar streaks)

• Low volumes of compound required (70μl/4 strains; 10μM)

• Internal controls:
  – Toxicity
  – Specificity
  – Positioning effect

• Sensitivity due to competition

• High-throughput
Future work

• Develop Eve’s machine learning software

• Perform screens with compounds selected based on the similarities between hit compounds

• Validate our hits using whole parasites
Thanks!

• Andrew Sparks (RDK lab): high-throughput drug screens (Eve)

• Kevin Williams (RDK lab): analysis of Eve results

• Pınar Pir (SGO lab): quantification of \textit{pdr5}\textsubscript{Δ} effects in pyrimethamine sensitivity

• All members of SGO and RDK groups