Discovery and development of kinase inhibitors for trypanosome diseases.

September, 30 2015

47th Brazilian Congress of Pharmacology and Experimental Therapeutics

Aquas de Lindoia, Brazil

David C Swinney

Institute for Rare and Neglected Diseases Drug Discovery

Mountain View, CA USA

iRND3.org

iRND3

Take home message

- Combinations of hypothesis directed and empirical strategies can be successful
- Analysis of MMOA for binding kinetics and substrate competition can create new opportunities
- To progress a compound from the lab to the clinic requires many different skill sets

Goal

- GOAL: Identify new therapies for human African trypanosamiasis (HAT)…African sleeping sickness

- STRATEGY: Combine molecular hypothesis driven and empirical approaches

- Considerations
  - Start with genetically validated proteins

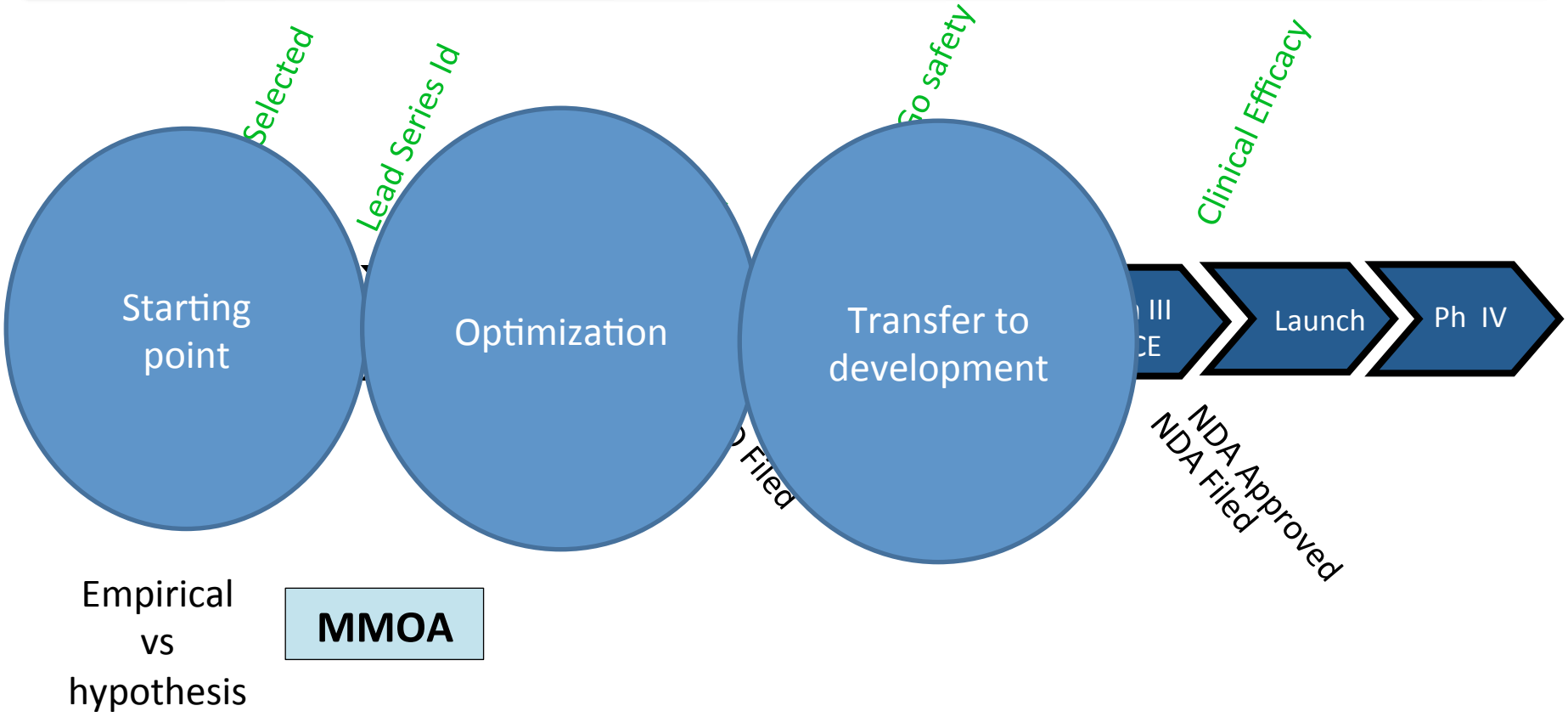
African Sleeping sickness- unmet medicial need

- Progressive and fatal disease caused by parasites *Trypanosoma brucei*
- Transmitted to human by bite of Tse fly
- 20,000 new cases/ year in sub-Sarahan Africa and 65 million at risk
- Current therapies either can not be given orally or are very toxic
- Closely related to *Trypanosoma Cruzi* which causes Chagas
- Chagas is transmitted to humans by the feces of the kissing bug.

Target Product Profile (TPP) for HAT

- Effective for early and late stage disease
- Orally administered
- Short time course (7 days)
- Safe for all persons including children and pregnant women
- Cost less than $30 Euros/course

Drug discovery and development interfaces



Drug discovery strategy
Bridging TDD and PDD

Knowledge-based rationale approach
Best with complete knowledge

TDD
-Choose specific target
-Validation e.g. genetic (RNAi)
-Identify compound that interacts with protein

Test in phenotypic assay

Empirical approach
Effective with incomplete knowledge

PDD
-physiologically relevant assay
-identify compound that works in assay

Identify target

Pharmacological MMOA

Tactics

- Target hypothesis
  - Potential targets identified with RNAi.
- However it is not known which are pharmacologically tractable
  - Empirically evaluate essential targets to determine pharmacologically tractable ones
- Use previous knowledge of compound class…kinases
- Look for molecules with MMOAs that limit ATP competition….via slow kinetics.

Program Flow chart

Express and purify enzymes

Establish assays

Run focus HTS

Verify HTS, chemical expansion

Evaluate MMOA

Phenotypic assay

Run in parasite assays

PK & efficacy in animals

iRND3

Binding: First principles

$A + B \rightarrow AB$

Binding/association

- k_a, k_{on}, k_1 represent association rates
  - Second order
  - Units are M^{-1}, s^{-1}
  - Association rates are concentration dependent
  - Diffusion control association
    ~10^7 M^{-1},s^{-1}

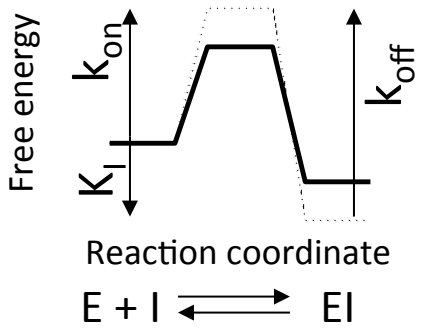
$K_I = k_{off}/k_{on}$
Equilibrium dissociation constant

Rate constants are lower case k
Equilibrium constants are capital K

$A + B \leftarrow AB$

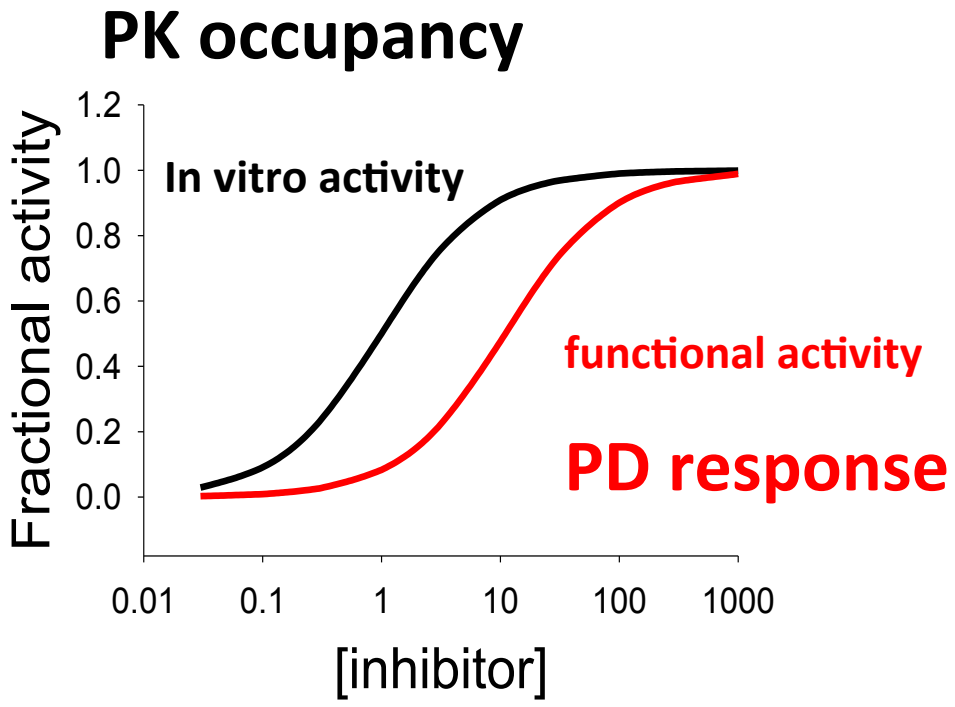
Debinding/dissociation

- k_d, k_{off}, k_{-1}, k_2 represent dissociation rates
  - First order
  - Units are in reciprocal time
  - Dissociation rates are concentration independent
- Half life = ln2/k
- Residence time (T) = 1/k



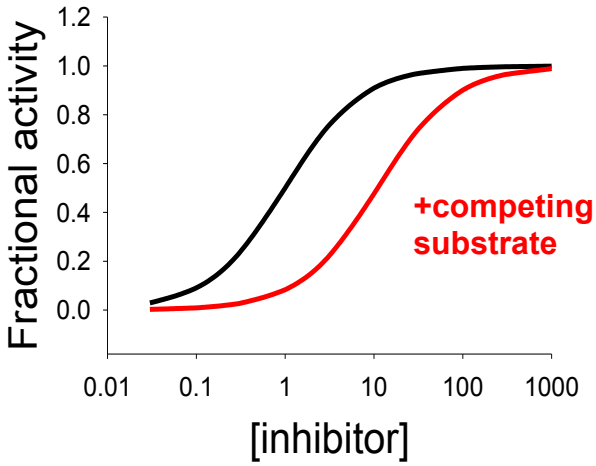
Dose response curves (IC_{50}) can shift in physiological systems vs isolated targets

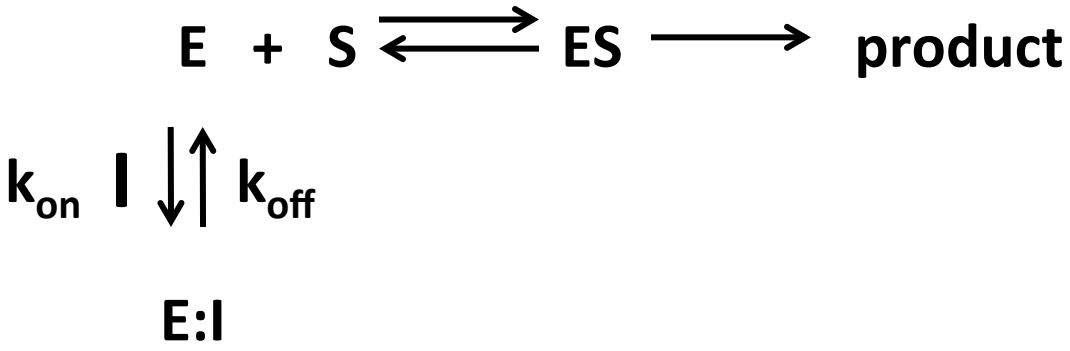
Fractional occupancy = Drug/(Drug + K_I)



Competition causes a shift in dose response curves under equilibrium conditions

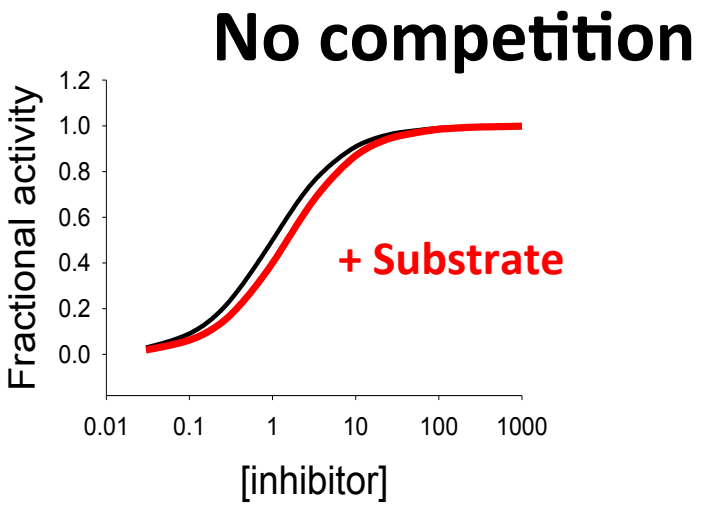
- IC_{50} relationship to affinity (K_I) depends on the binding mechanism
  - IC_{50} is an operational term
  - Competition shifts dose response curve
  - $IC_{50} = K_I (1 + S/Km)$

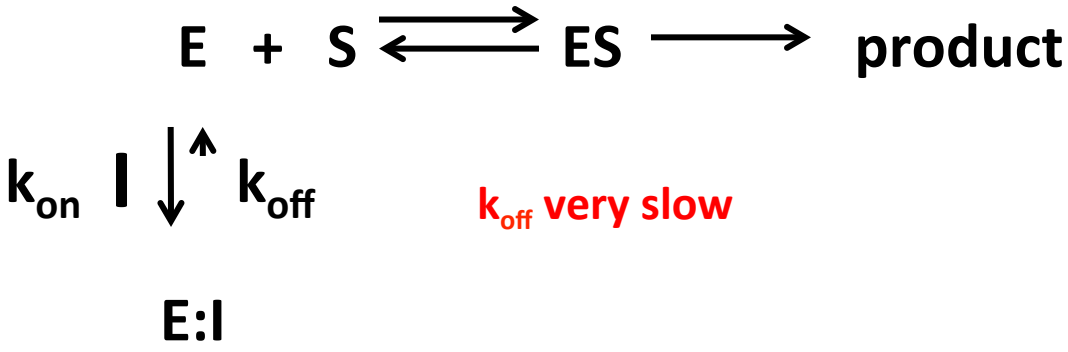




Slow binding kinetics can limit competition in non-equilibrium systems

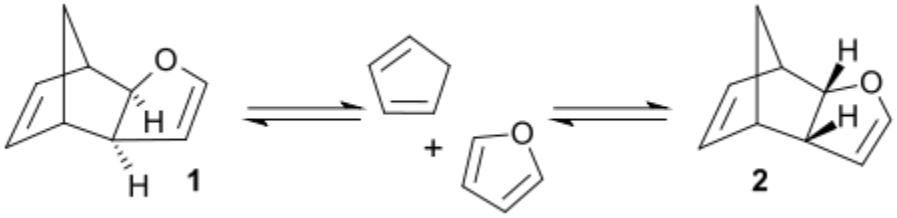
- Irreversible
- Slow dissociation kinetics in non-equilibrium system
- Functionally insurmountable





Kinetic vs thermodynamic control in chemistry

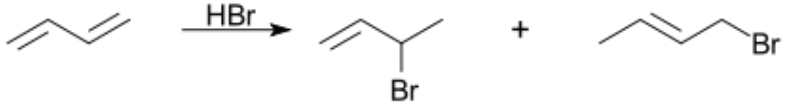
Diels alder



thermodynamic product

Kinetic product

Electrophilic addition



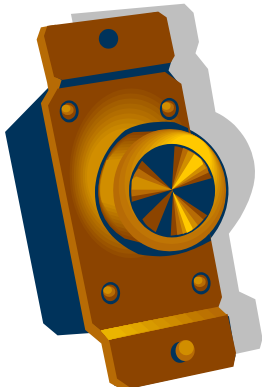
T = 0 °C	71%	29%
T = 40 °C	15%	85%

Kinetic product

thermodynamic product

Kinetic vs thermodynamic control

	Kinetic	Thermodynamic
	Non-equilibrium	equilibrium
competition	Rate-dependent	Concentration-dependent
behavior	Switch-like	adjustable



Four point MMOA assay

Determines time-dependence with and without preincubation of inhibitor with enzyme prior to starting the enzyme reaction
Determines competition of inhibitor with substrate at two substrate concentrations

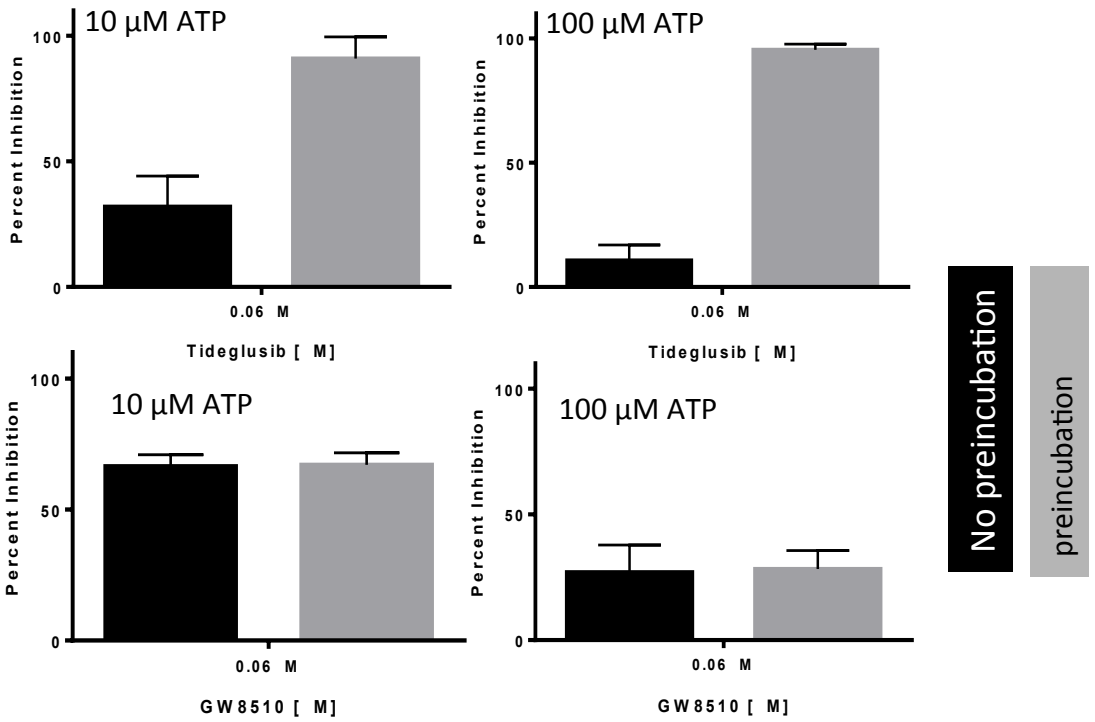
Enzyme-TbGSK3β kinase
Substrate- ATP
Inhibitors-

GW8510

Not time-dependent
ATP competitive

Tideglusib

Time dependent
ATP competitive no preincubation
Non-competitive preincubation

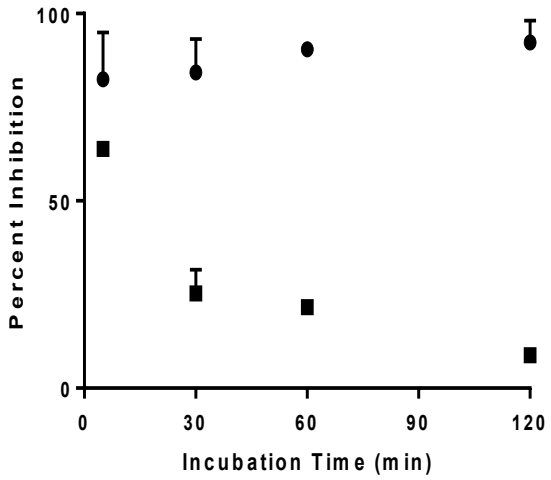


Identify compounds with different MMOAs

Z Swinney

iRND3

Tideglusib irreversible inhibitor TbGSK3β



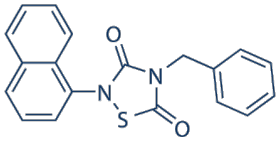
Evidence for irreversibility

Preincubation of enzyme and tideglusib followed by a 1 to 100 dilution

Tideglusibs inhibits TbGSK3b

GW8510 does not

Z Swinney



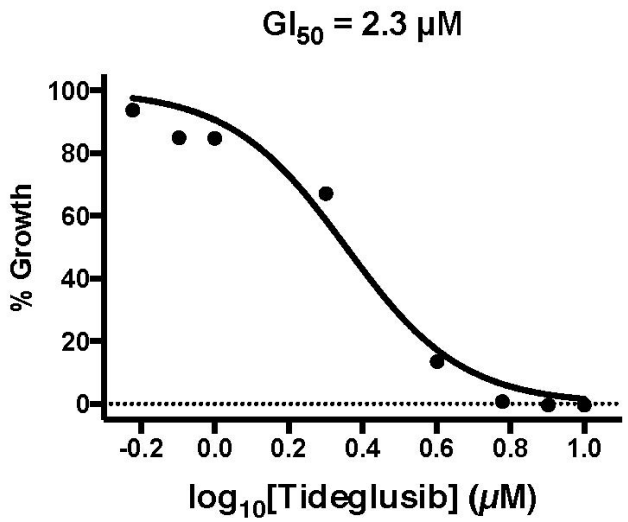
Tideglusib

- Irreversible inhibitor of human GSK3b
- Developed for Alzheimer's
- One year of dosing in phase II trials
- Well tolerated
- Did not meet efficacy end points
- Program terminated
- IP sold to ASD therapeutics

Tideglusib inhibits trypanosoma parasite growth

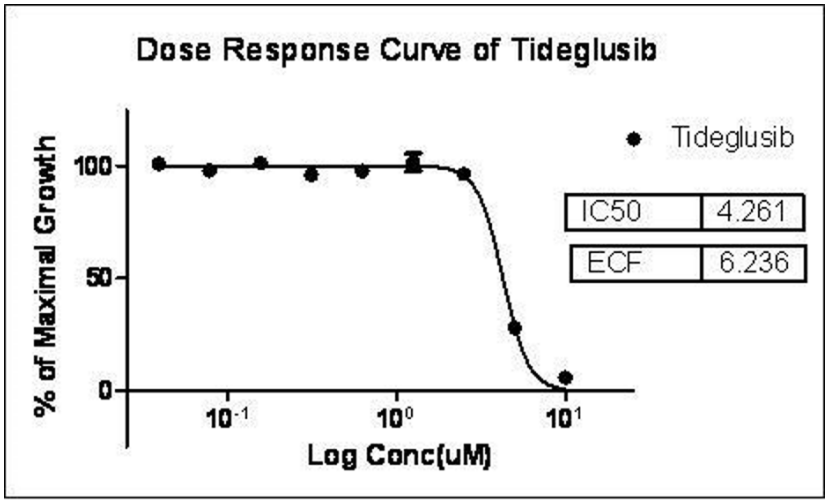
Blood levels in mice and man reported to be around 6 uM;

T. brucie



Paul Guyett
Kojo Mensa-Wilmot UGA

T. cruzi



Rick Tarleton, UGA

Steps to optimize for POC studies

- Production of drug substance
- Pharmaceutics-formulation/stability
- ADME
- POC in animal
- Safety in animals

Steps towards repurposing

- Target Product profile
- Partners (do they want a program or company)
  - Pharma
  - Biotech
  - VCs
  - Foundations
    - Welcome Trust
    - DNDi
    - Gates

Take home message

- Combinations of hypothesis and empirical strategies can be successful
- Analysis of MMOA for binding kinetics and competition can create new opportunities
- To progress a compound from the lab to the clinic require many different skill sets

Acknowledgements

- iRND3 team
  - Brad Haubrich
  - Zach Swinney
  - Steve Gomez
  - David Chereau protein production
  - Chakk Ramesha HTS screen
  - Dave Rotstein- Med chem

- University of Georgia
- Kojo Mensa-Wilmont, Paul Guyett T brucei parasitology
- Rick Tartleton T Cruzi parasitology

- Funding from NIAID R01 AI103476

Institute for Rare and Neglected Disease Drug Discovery, aka iRND3

Non-profit 501c3 drug discovery organization
Well equipped laboratory Mountain View, CA
Experience drug discovery team with many years of Pharma experience
www.irnd3.org

Mission

iRND3's mission is to discover new medicines for rare and neglected diseases

Vision

-to utilize our understanding of how successful new medicines are discovered to implement and execute drug discovery strategies that supply our growing pipeline of new candidate medicines for rare and neglected diseases.

-to evolve an innovative, socially responsible operational model for successful collaborations between the non-profit, private and public to translate scientific discoveries to a continuous source of new medicines.