The role of Binding Kinetics in Drug Action

David C Swinney

Mastering Medicinal Chemistry

Boston MA

June 15, 2016

Institute for Rare and Neglected Diseases Drug Discovery

Mountain View, CA

www.irnd3.org

iRND3

Take home message

- Binding kinetics influence
  - Molecular mechanism of action (MMOA)
    - efficacy
    - safety
    - therapeutic index.

Outline

Part 1. How do medicines work?

Part 2. The impact of MMOA on PK/PD relationships.

Part 3. Practical application at iRND3.

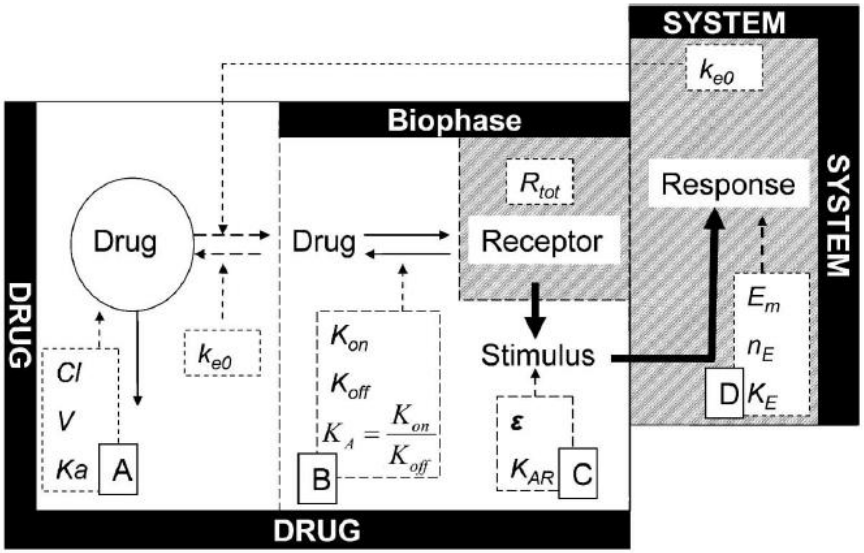
Part 1: How do medicines work?

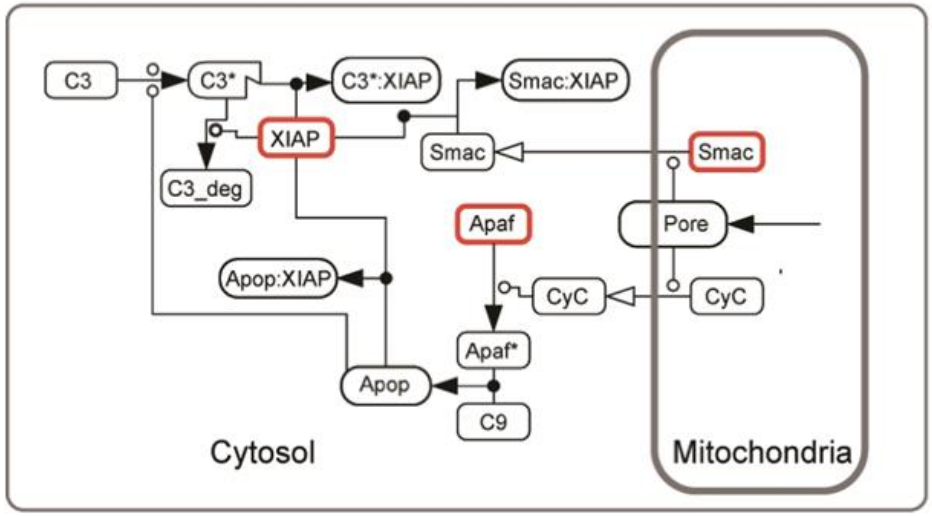
Drug action begins with binding

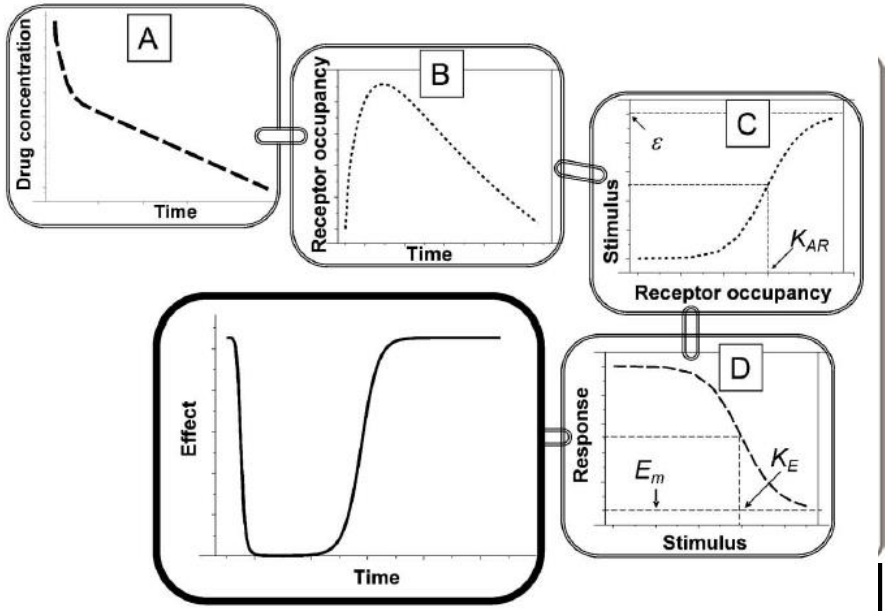
‘Corpora non agunte nisi fixata’

A substance will not work unless it is bound

-Paul Ehrlich, 1913







Ploeger BA, van der Graaf PH, Danhof M. Drug Metab Pharmacokinet. 2009;24:3-15.

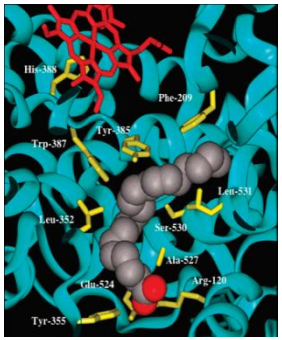
Molecular Mechanism of Action (MMOA)

- MMOA: mechanism through which specific molecular interactions between the drug and its target result in a effective and **safe** pharmacological response.
  - Includes **binding kinetic** and **conformational** changes that specifically provide a therapeutically useful response.

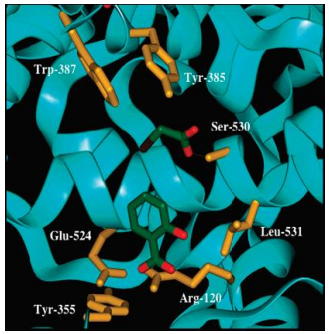
MMOA-pharmacological hot spot

Aspirin and Ibuprofen: Two Medicines, One Target, Different Molecular Mechanisms, Different Uses

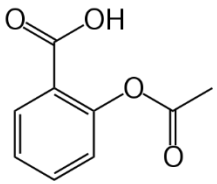
- **Aspirin has anti-platelet activity whereas NSAIDs do not**
  - Effective for prevention of atherothrombotic disease
- **Both bind to the active site of cyclooxygenase 1 and 2**
  - Aspirin irreversible inactivation via acetylation of Ser530
  - Ibuprofen and other NSAIDS are reversible
- **Irreversible action of aspirin in platelets leads to long lasting anti-thrombotic effects**
  - Platelets do not have the capacity to resynthesize new protein

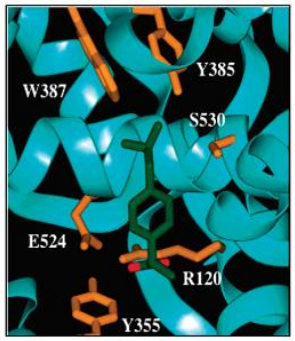


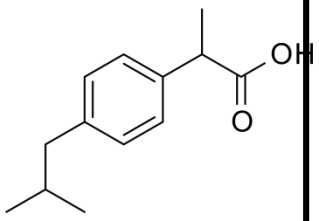
Substrate - arachidonic acid



Aspirin







Ibuprofen






- **Communication of information** as an analogy of MMOA
  - Proximity is rarely sufficient for effective sharing of specific information
  - **MMOA is a language to communicate specific information.**

'Pharmacological hot spots'

Why is molecular mechanism important?

- Physiology
  - Uses molecular mechanism to provide selective and safe responses
  - spatial and kinetic control of physiologic responses
- Drug Discovery
  - flood the system with drug.
  - maintain drug concentrations above IC_{50} per dosing interval.

Approved molecular medicines have evolved with specific, selective MMOAs

The optimal MMOA depends on the potential for mechanism-based toxicity

No Mechanism based toxicity

- hit target as hard as possible
- results in lower concentrations of drug
- increase therapeutic index
  - full agonist
  - irreversible inhibitors
  - noncompetitive inhibitors
  - insurmountable antagonists
  - slow dissociation
  - PAMs(positive allosteric modulators)

Mechanism-based toxicity

- Molecular mechanism can provide opportunity to differentiate efficacy from mechanism-based toxicity.
  - partial agonists
  - rapidly reversible inhibitors
  - uncompetitive
  - functionally selective receptor modulators
  - use dependent channel blockers

Swinney, *NRDD*, **3**, 801, 2004
Swinney ARMC, 46, 301, 2011

Many drug class evolved to have optimal MMOA

No mechanism based toxicity evolve to slow off rates

Drug class	Fast-off, surmountable	Slow-off insurmountable
ARBs	losartan	Candesartan/valisartan
Antihistamines	Diphenhydramine	desloratadine
Antimuscarinincs	Ipratropium	tiotropium

With mechanism based toxicity evolve to fast off rates

Cyclooxygenase inhibitors	kinetics
aspirin	irreversible
indomethacin	Slow reversible
ibuprofen	Fast reversible
COX2 selective	COX2 irreversible
COX2 selective	COX2 reversible??

Two types of kinetic responses

Responses:

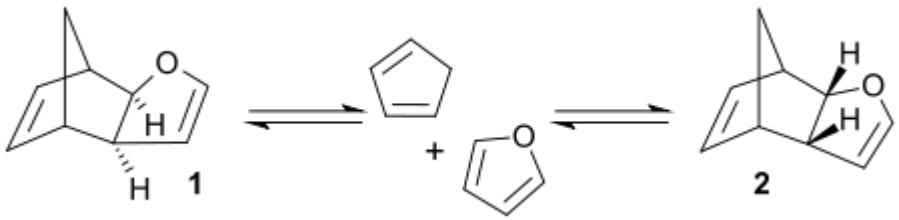
1) equilibrium

2) non-equilibrium

Equilibrium vs non-equilibrium in drug action

Kinetic vs thermodynamic control in chemistry

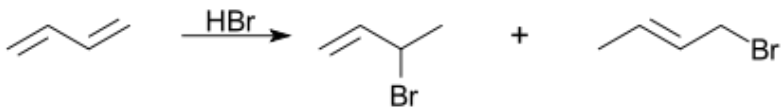
Diels alder



thermodynamic product

Kinetic product

Electrophilic addition



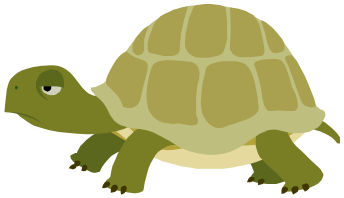
T = 0 °C	71%	29%
T = 40 °C	15%	85%

Kinetic product

thermodynamic product

Outcome controlled by competing rates and relationship to equilibrium

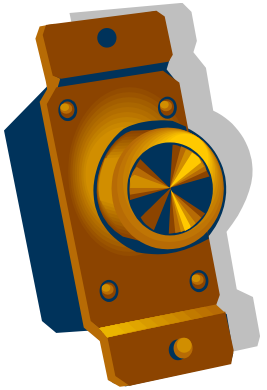
- Definition of ‘fast’ and ‘slow’ with regard to binding kinetics is only relative to a competing rate
  - A turtle could be fast in comparison to a snail



- For example: a 10-min dissociation half-life is…
  - …very fast when the competing rate is elimination of medicine from the body with a half-life measured in hours
  - …very slow when the competing rate is opening and closing of a channel with a half-life measured in milliseconds

Kinetic vs thermodynamic control

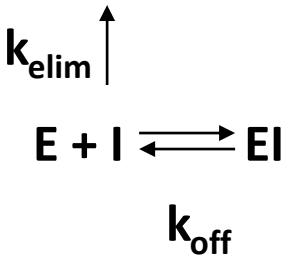
	Kinetic	Thermodynamic
	Non-equilibrium	equilibrium
competition	Rate-dependent	Concentration-dependent
behavior	Switch-like	adjustable



Swinney *Let Drug Design Disc*, 2006, 3, 569-574

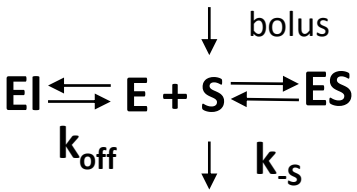
Competing rates define a response

PD outlast PK



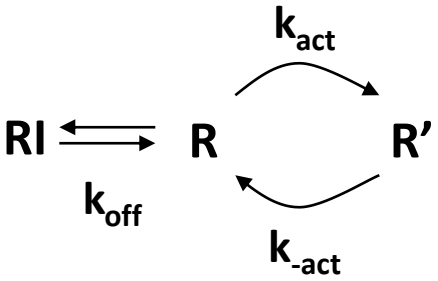
k_{off} slower the k_{elim}

insurmountable



when $k_{off} < k_{-s}$ inhibition will be functionally irreversible

use-dependence



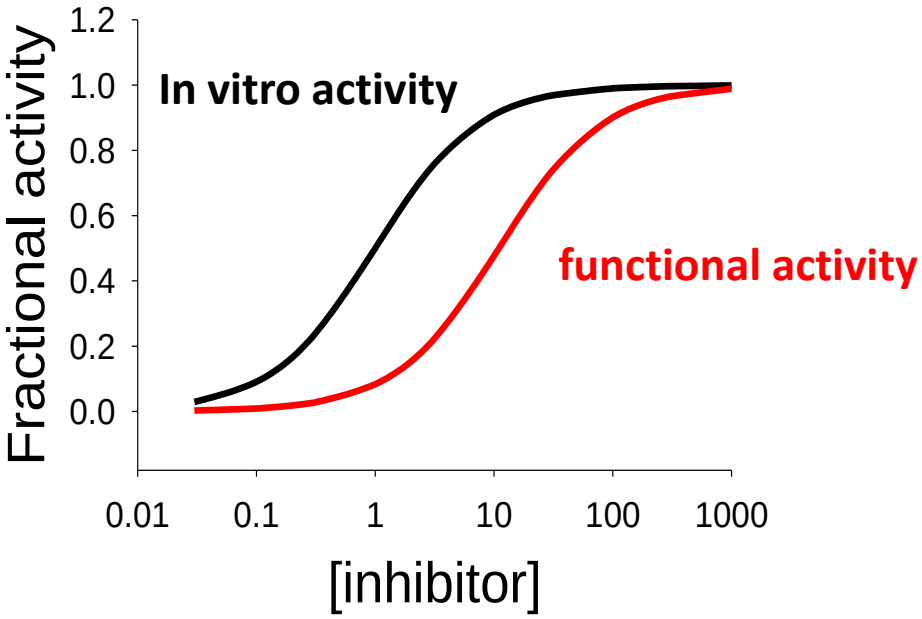
when $k_{off} < k_{act}$ the block will accumulate

PART 2

MMOA effects dose-response (PK/PD) relationships.

Dose response curves (IC_{50}) can shift between in vitro and phenotypic assays

Fractional occupancy = Drug/(Drug + K_I)

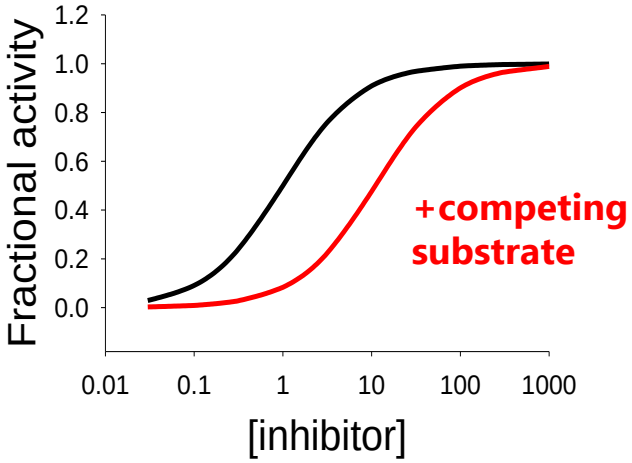


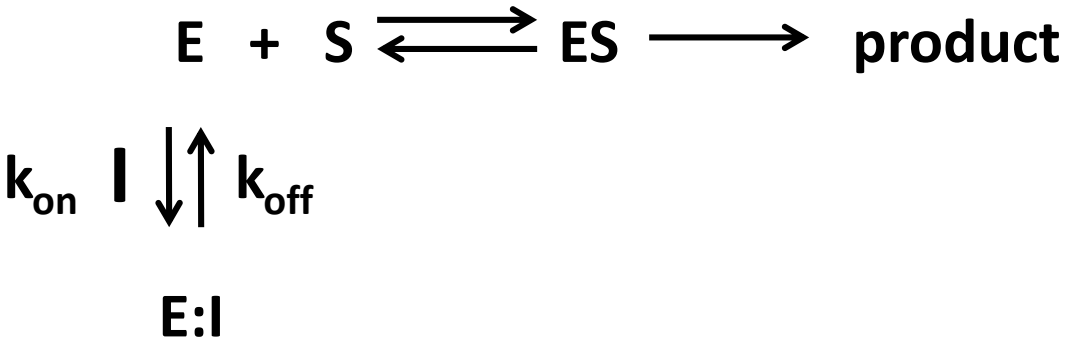
In vitro- purified protein- **target**
Functional- cells, tissues, animals- **phenotypic**

iRND3

Competition may cause a shift in dose response curves under equilibrium conditions

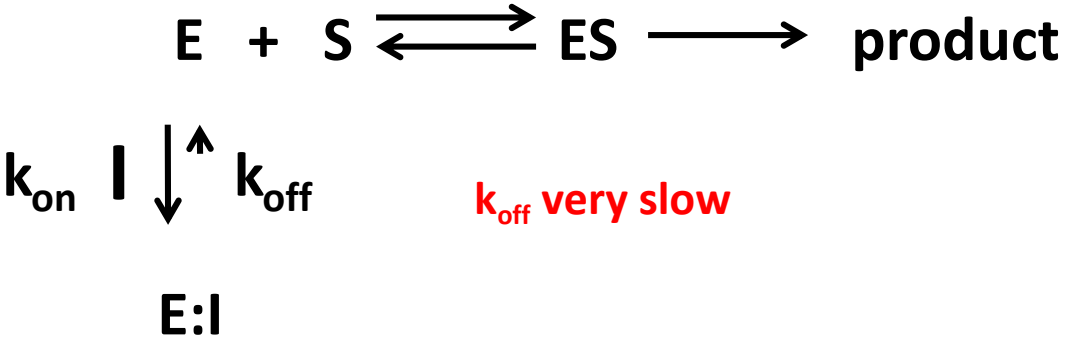
- IC_{50} relationship to affinity (K_I) depends on the binding mechanism
  - IC_{50} is an operational term
  - Competition shifts dose response curve
  - $IC_{50} = K_I (1 + S/Km)$

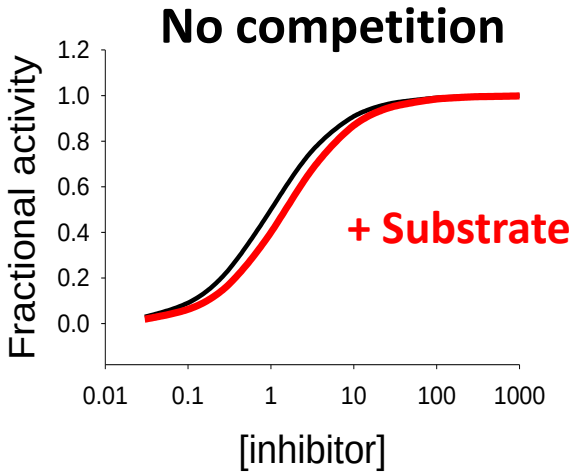




Slow binding kinetics can limit competition in non-equilibrium systems

- Irreversible
- Slow dissociation kinetics in non-equilibrium system
- Functionally irreversible (insurmountable)





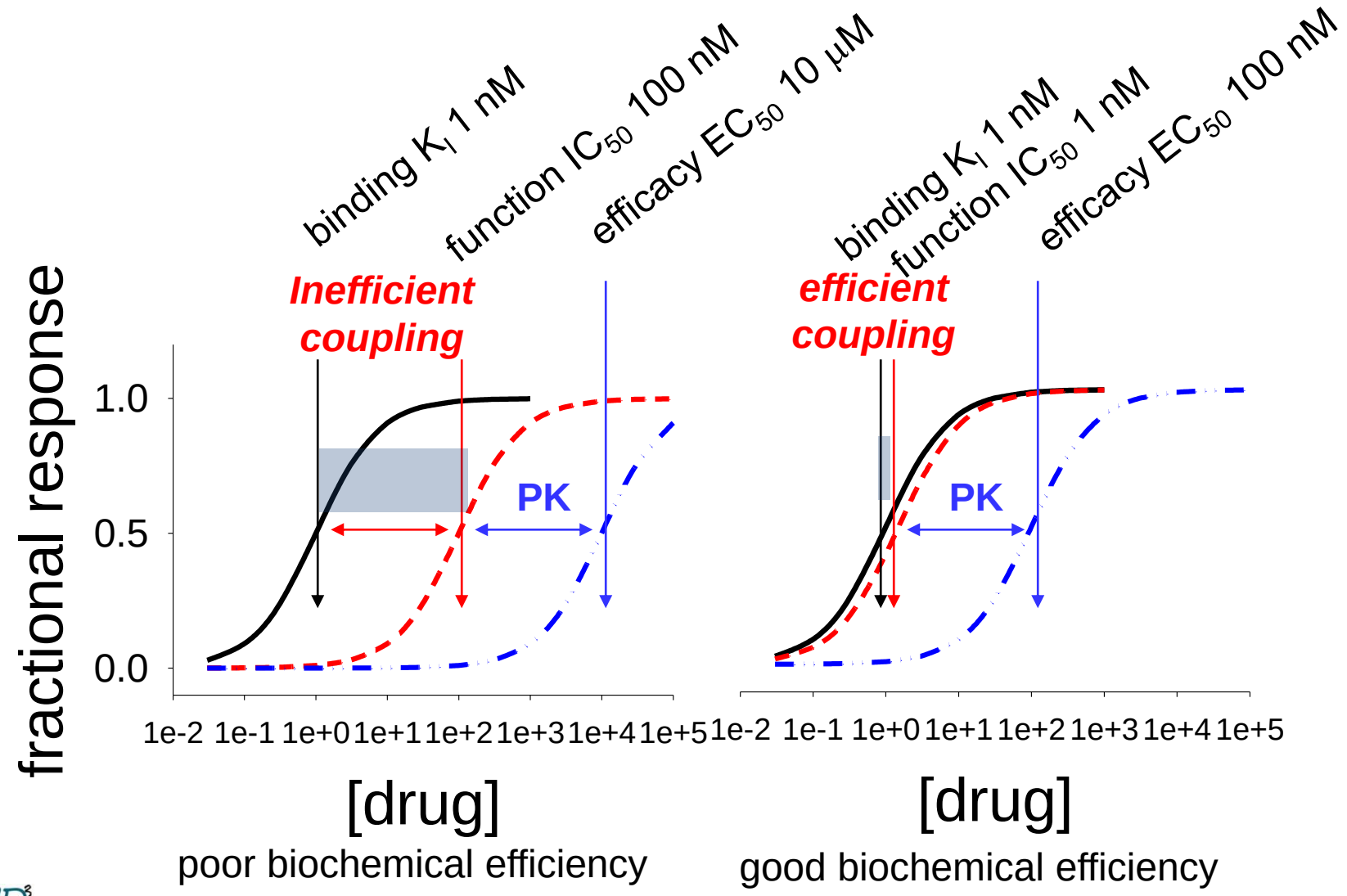
Mutations to EGFR kinase increase affinity for ATP

- Decrease effectiveness of inhibitors because of equilibrium competition
- Next generation inhibitors limit competition with irreversible or slowly reversible binding kinetics

iRND3

Yun et al *PNAS* 105, 2070 (2008)

Both biochemical efficiency and PK effect PK/PD relationships



iRND3

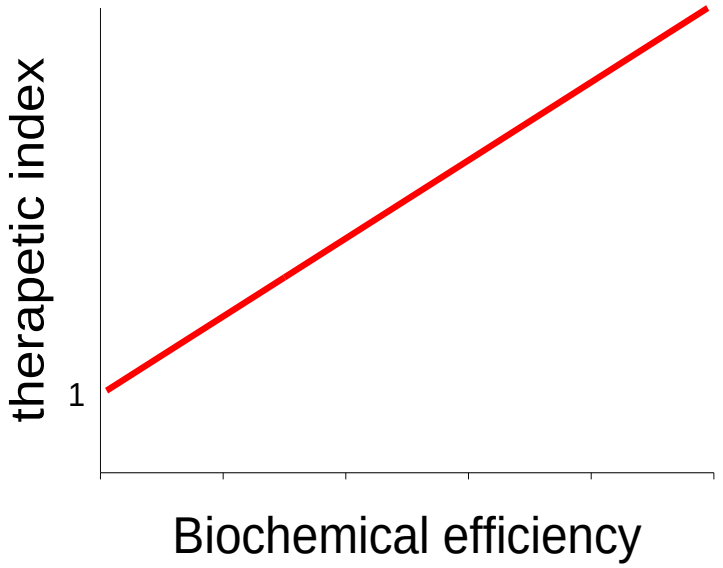
Many medicines do not have a shift in activity. They have good biochemical efficiency (K_I/EC_{50}).

The shift in activity between binding and function (K_I/EC_{50}) is called Biochemical Efficiency-(BE)

-Good BE is a property of many best in class medicines (>90% of drugs in study with BE > 0.4)

-Molecules that couple more efficiently to the desired response will have a greater therapeutic index.

-Enables efficacy at lower drug concentrations



Swinney NRDD, 3, 801 (2004)

Swinney CTMC 6, 461 (2006)

Part 3: Practical application at iRND3

- We look to identify compounds with time-dependent activity.
- Difficult to predict
- Establish simple screen
- Shift in activity with preincubation

Four point MMOA assay

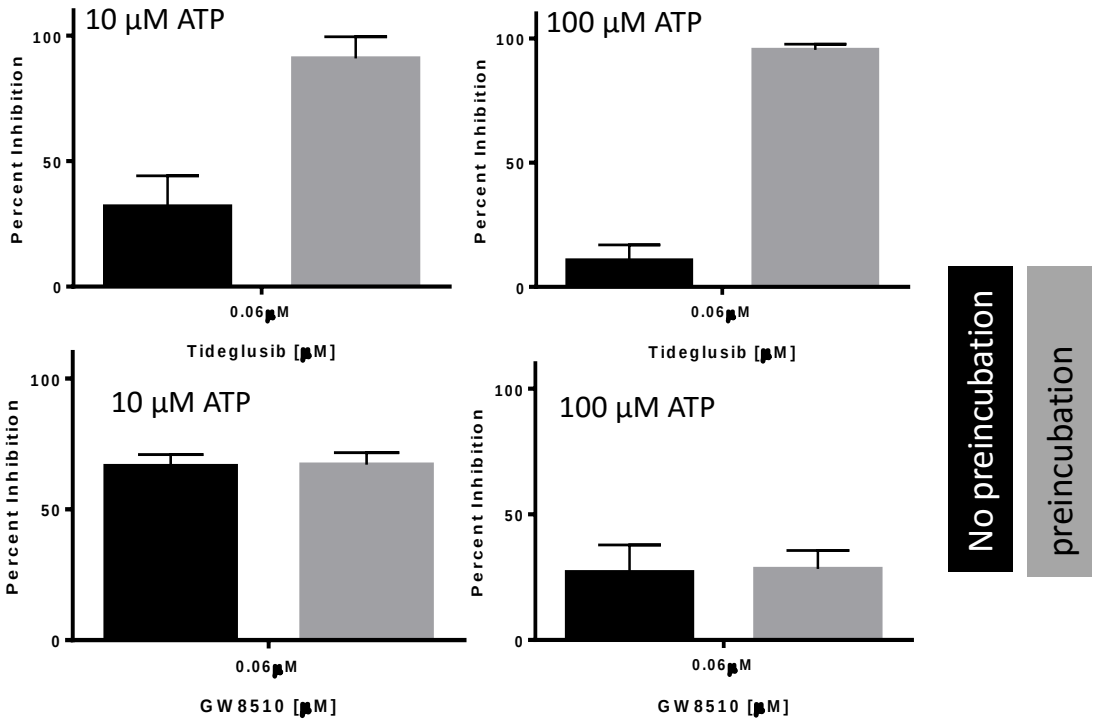
Determines **time-dependence** with and without preincubation of inhibitor with enzyme prior to starting the enzyme reaction
Determines **competition** of inhibitor with substrate at two substrate concentrations

Enzyme-TbGSK3β kinase
Substrate- ATP
Inhibitors-

Tideglusib
Time dependent
ATP competitive no preincubation
Non-competitive preincubation

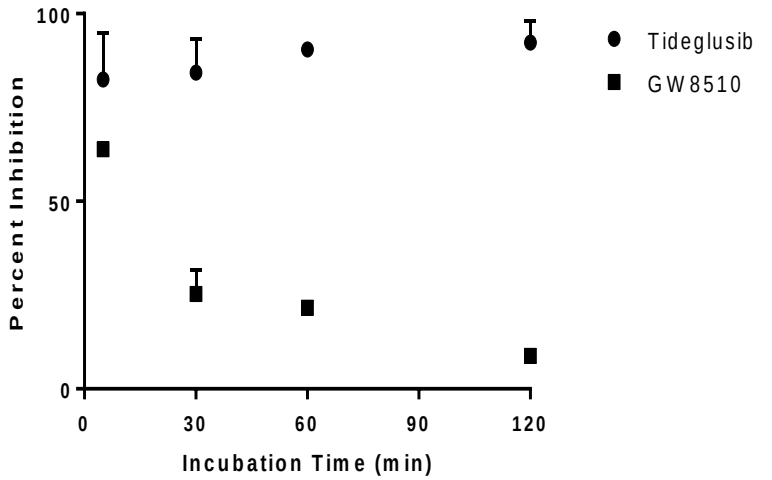
GW8510
Not time-dependent
ATP competitive

Identify compounds with different MMOAs



Swinney, ZT et al PLOS NTD, 2016

Tideglusib irreversible inhibitor TbGSK3β



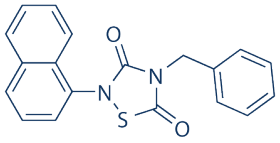
Evidence for irreversibility

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

The effect of equilibrium and non-equilibrium kinetics on drug action

equilibrium | **nonequilibrium**

biochemical

reversible | **irreversible**

Fast kinetics | **Slow kinetics**

occupancy dependent on substrate competition
$= [drug]/([drug] + K_I(1+S/Km))$

occupancy less dependent on substrate competition
$= [drug]/([drug] + K_I)$

functional

surmountable | **insurmountable**

Minimize mechanism-based toxicity

Good biochemical efficiency
Minimize off-target toxicity

clinical

improved therapeutic index

iRND3

Take home message

- Binding kinetics influence
  - Molecular mechanism of action (MMOA)
    - efficacy
    - safety
    - therapeutic index.

Institute for Rare and Neglected Disease Drug Discovery, aka iRND3

Non-profit 501c3 drug discovery organization
Well equipped laboratory Mountain View, CA, USA
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.

iRND3

Medicines with slow or irreversible binding kinetics.

They are discovered in many therapeutic areas

- Slow dissociation reversible ($t_{1/2}$)
  - Amlodipine (77 min) hypertension
  - Aprepitant (154 min) emesis
  - Buprenorphine (166 min) pain
  - Candesartan (11.5 h) hypertension
  - Darunavir (>240h) antiviral
  - Desloratadine (>6 h) antihistamine
  - Efavirnenz (4.1 h) antiviral
  - Lapatinib (300 min) anticancer
  - Maraviroc (10.5 h) antiviral
  - Olmesartan (72 min) hypertension
  - Oseltamivir (33-60 min) antiviral
  - Saxagliptin (5.1 h) diabetes
  - Telaprevir (2.9 h) HCV (phase III)
  - Tiotropium (34.7 h) COPD

- Irreversible
  - Aspirin; anti-platelet
  - Azacitidine; anticancer
  - Cefditoren; antibiotic
  - Clavulanic acid, Sulbactam, tazobactam; β-lactamase inhibitors
  - Finasteride; BPH
  - Formestan; anticancer
  - Omeprazole, Lansoprazole; GERD
  - Orlistat; obesity
  - Penicillin; antibiotic
  - Procarbazine; lymphoma
  - Selegiline,Tranylcypromine; depression
  - Ticlopidine, clopidogrel, prasugrel; anti-platelet
  - Vigabatrin; epilepsy