Info all on web page

Links and papers.

Feel free to look for more info!

1

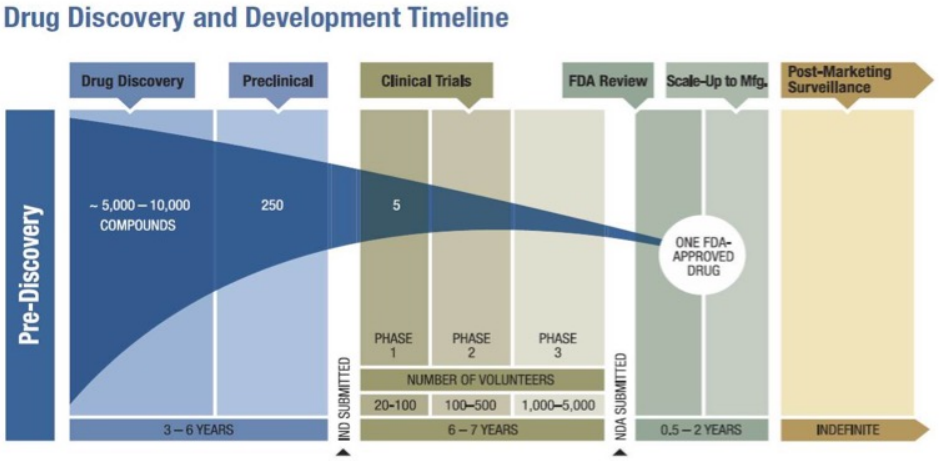
What is Drug Discovery?

- The Process by which new medicines are identified.
- Involves interdisciplinary approaches of
  - Biochemistry, molecular biology, organic chemistry, physical biochemistry, pharmacology, cell biology and physiology

2

Drug Discovery Process

- Workflow for novel drug therapy
- Time to market and costs have skyrocketed
  - Discovery is expensive and high-risk >10 years!



3

Investigational New Drug (IND) Application

A request for authorization from FDA to administer an IND or biologic product to humans

  - This is literally seeking permission to perform a clinical trial.
- The trial can proceed if the FDA does not file a hold with 30 days of receiving the application
- The application must contain information on:
  - Animal pharmacology and toxicology studies
  - Manufacturing processes and procedures for the drug or biologic
  - Previous clinical trials as well as clinical protocols for the investigational study
  - The investigator brochure

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New Drug Application (NDA)

When the sponsor of a new drug has enough evidence of the drug's safety and effectiveness has been obtained to meet FDA's marketing approval an NDA can be submitted with the required PDUFA fee.

- If the NDA is approved by the FDA the product can be marketed in the U.S.
- The application must contain data from specific technical viewpoints including:
  - nonclinical pharmacology and toxicology
  - chemistry, manufacturing and controls
  - clinical pharmacology
  - Medical
  - Statistics

5

Pharmaceutical Development

- The question becomes, what is the real number?
- During the Super Bowl, in 2012, a representative of the pharmaceutical company Eli Lilly posted on the company's corporate blog that the average cost of bringing a new drug to market is $1.3 billion.
- 2020 estimated drug development costs range $314Million to $2.8 Billion

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Pharmaceutical Development

Company	Number of drugs approved	R&D Spending Per Drug ($Mil)	Total R&D Spending 1997-2011 ($Mil)
AstraZeneca	5	11,790.93	58,955
GlaxoSmithKline	10	8,170.81	81,708
Sanofi	8	7,909.26	63,274
Roche Holding AG	11	7,803.77	85,841
Pfizer Inc.	14	7,727.03	108,178
Johnson & Johnson	15	5,885.65	88,285
Eli Lilly & Co.	11	4,577.04	50,347
Abbott Laboratories	8	4,496.21	35,970
Merck & Co Inc	16	4,209.99	67,360
Bristol-Myers Squibb Co.	11	4,152.26	45,675
Novartis AG	21	3,983.13	83,646

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Pharmaceutical Development

- U.S. Pharmaceutical firms invest as much as five times more in research and development relative to their sales as an average manufacturing firm
  - $65 Billion annually in R&D
  - 3,000 new products being developed

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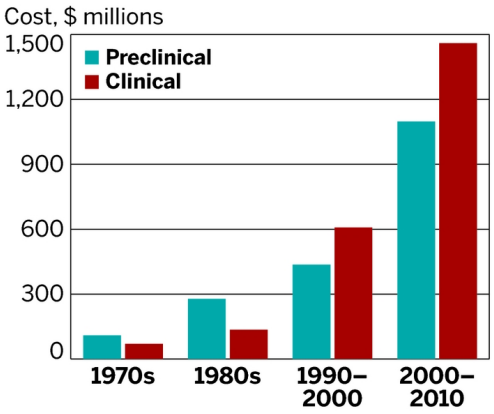
Skyrocketing Costs in Drug Development

- Cost of bringing a drug to market now $2.6 Billion!
- 145% increase from 2003



C&EN Nov 20, 2014

- $0.4 Billion in '80s
- Increase in costs are due to complexity of clinical trials, a greater focus on chronic and degenerative diseases and tests on comparator drugs to accommodate payer demands

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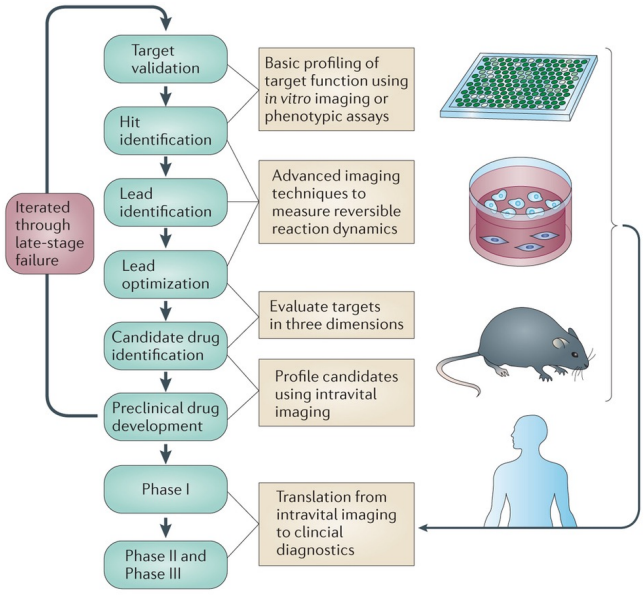
Focus on early phases of DD



Nature Reviews | Cancer

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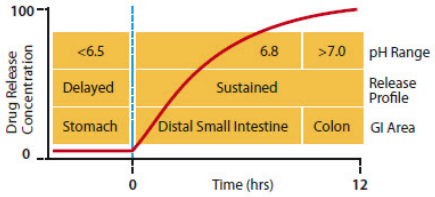
Drug Development Terminology

- Pharmacokinetic (PK) Studies
  - Are done to monitor mechanisms of absorption, distribution, metabolism and excretion (ADME) of the test drug in animals.

Figure 1. Colpermin® Pharmacokinetic Profile



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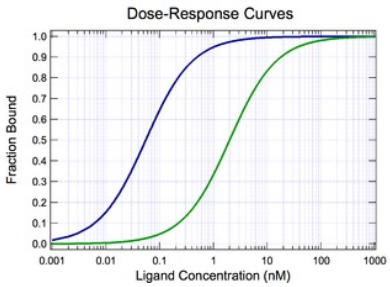
Drug Development Terminology

- Pharmacodynamic (PD) Studies
  - Evaluate the biochemical and physiological effects of the drug, and the relationship between drug concentration and effect in test animals.



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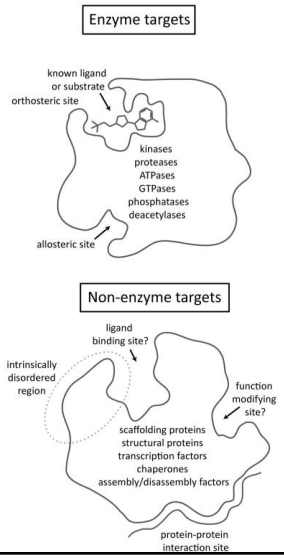
Terminology

- Target (drug target)
  - A biological target of a drug. Proteins and nucleic acids are the most common targets
    - Receptors, ion channels, nuclear receptors, kinases, enzymes, some structural proteins
  - Small molecule () vs Biological
  - Druggablility is the ability of a compound to bind and effect with high affinity
  - Non druggable targets – protein-protein interactions, sometimes but not always "non-enzyme" targets



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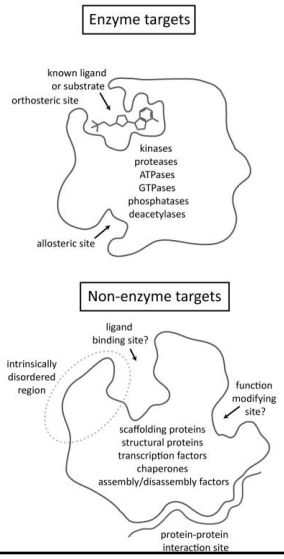
Terminology

- Hit
  - Some positive result, usually with low specificity and low efficacy (mM range) of a drug screen, often times via high throughput screen
- Lead (compound)
  - Suboptimal drug that needs modification to better fit the target for higher efficacy and less off-target effect



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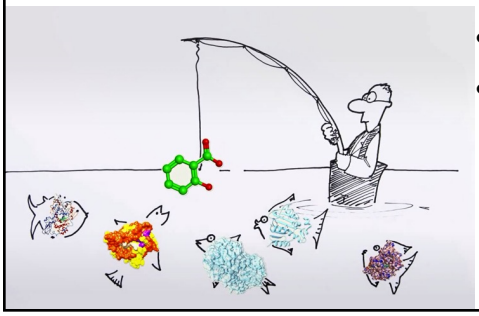
Drug Discovery Process

Target Identification

- Must be efficacious, safe, meet clinical needs and be druggable.
- GPCRs Kinases are some of the most common



- Structure based
- Computational/informatics
- Start with known target or more broadly ask what will impact disease of interest – less effective!

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Drug Discovery Process

Screening Strategies

• High Throughput	Large # of compounds
• Focused Screen	Use identified classes of small molecules
• Fragment Screen	Portions of compounds
• Virtual Screen	Docking and molecule dynamic approaches
• Physiological Screen	Tissue or animal impact
• NMR Screen	Often part of fragment screen or library

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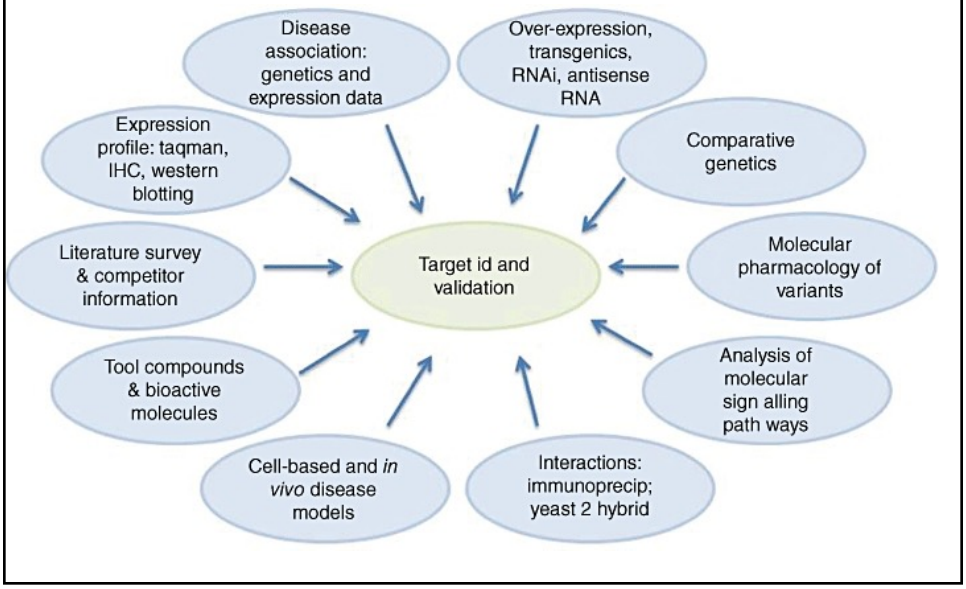
Drug Discovery Process



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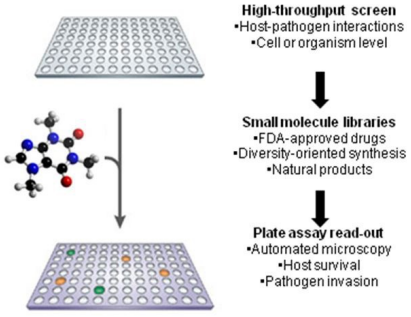
Drug Discovery Process

High Throughput Screening (HTS)

- Hundreds of small molecule libraries exists and are created
- Need way to evaluate each quickly and inexpensively.
- 96 well (multiples of to 1,152!) wells holding compounds, plasmid DNA, shRHA, RNAi and other reagents.
- Purified proteins (impact of genome) and cell based assays allow for screening of "activity" to discover a "hit"



Look for a small molecule library in google
- what kinds of libraries are there?

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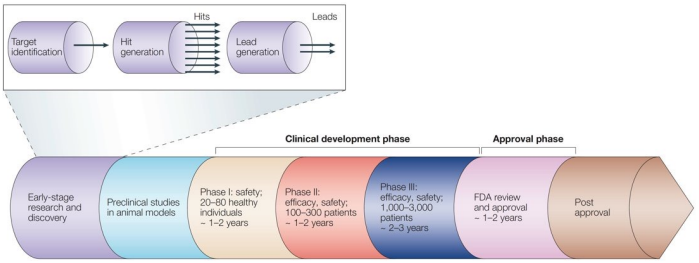
High Throughput Screening

Early Stage Research and Discovery



The overall success rate of HTS by measuring progression to lead optimization is 45-55%

- Estimated size of drug-like compound library is 10^{60} compounds, while corporate chemical library is only 10^{6} compounds
- Increasing the size of the screening library does not proportionally yield more hits
- Twice as likely to fail for newer targets

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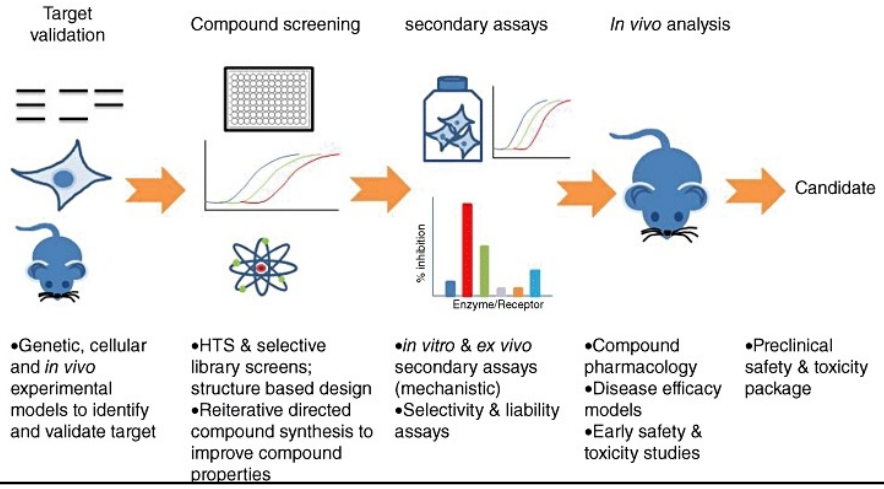
High Throughput Screening

- Typical HTS workflow.
- Need cell proliferation and cytotoxicity data and 10 point dose response curve for IC_{50}



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High Throughput Screening

- HTS targets
  - Recombinant purified proteins – Kinases
  - Other "plate assays"
    - Need to have some measurable change in activity
    - Fluorescence over absorbance for greater sensitivity
  - Cell based assays
    - Cell growth, death, GPCR response…
      - Often rely on cameras and automated readouts – to difficult to achieve by hand
- Z' factor – plate assay quality factor which factors in signal to noise intensity AND the variance around both high and low signals – allows for large data to be collected and automatically analyzed with confidence

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High Throughput Screening

Defining a hit series

- Libraries are often organized by known function or targets
- Some are chosen based on prior approval for human use
- Can also be created based on chemical definition/behavior
- One school of thought is to start simple hits to generate a lead that can be optimized in the next step.
- Select for active and inactive (algorithm), solubility and ADME

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ADME: Lipinski's rules of five

ADME: Absorption, distribution, metabolism and excretion. Method to determine potential biological activity in an oral drug.

- Also known as Pfizer's rule of five

- Number of H-Bond donors no more than 5
- Number of H-bond acceptors, 10 or less
- Molecular Weight <500 da
- ClogP (partition coefficent between octanol/water) is a measure of lipophilicity. <5 ClogP

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Developing Hits to Leads

- Additional screens using several biological outcomes – usually cell based
- Screen hits using structure function / docking programs if possible
- Dose response curves of select drugs identify efficacy
- Structure-activity relationship (SAR) looks at 3D structure of drug to predict bioactivity and impact of potential changes
- Determine cell permeability, metabolism by p450 and changes in activation

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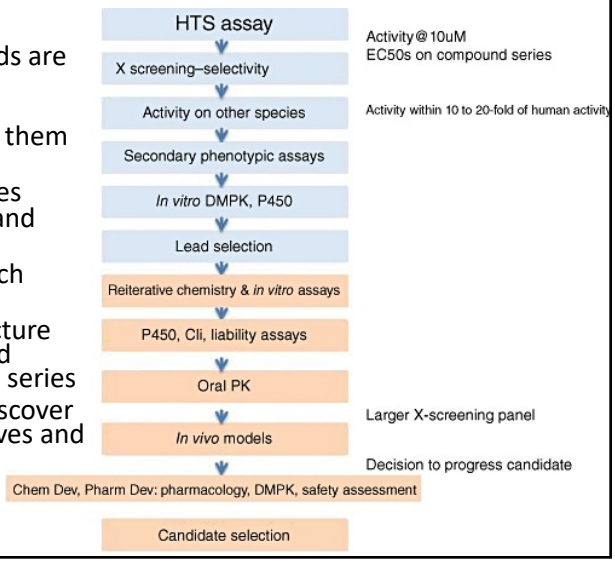
Strategy for HTS Triage

- Run HTS
- Decided which compounds are "active" and which are "inactive"
- Cluster the actives to put them into series
- Visualize clusters of actives (showing 2D structures) and pick series of interest
- Identify "scaffold" for each series
- Use similarity or substructure search on inactives to find inactives related to these series
- Use SAR techniques to discover differences between actives and inactive in a series



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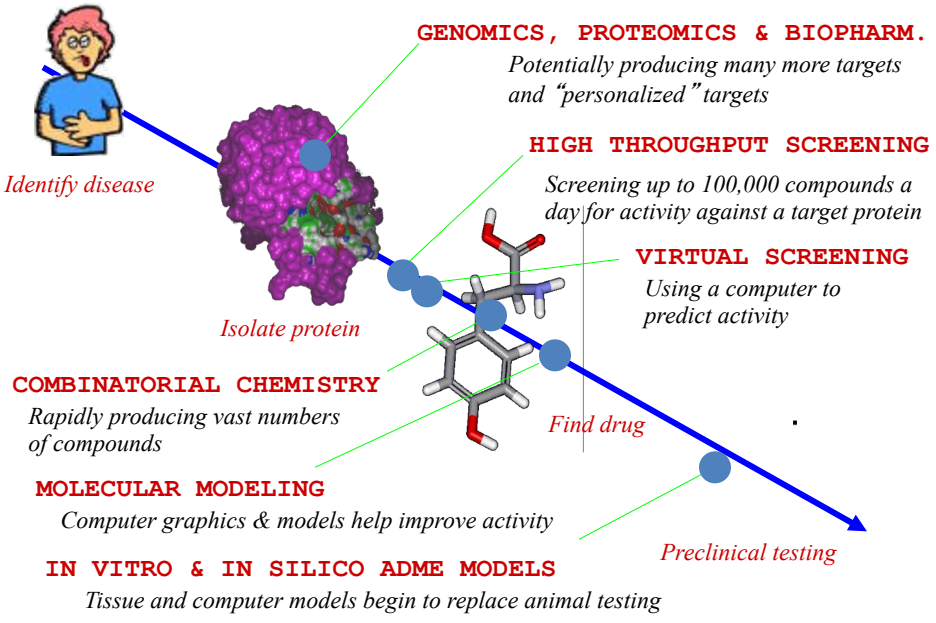

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Drugs from FBDD to Reach Clinical Trials

Using variety of techniques, a handful of drugs developed by FBDD have entered the clinic

Drug	Company	Target	Phase
PLX-4032 (Vemurafenib)	**Plexxikon**	**B-Raf V600E**	**FDA Approved**
ABT 263	**Abbott**	**Bcl-2/Bcl-x_L**	**Phase 2**
ABT869	Abbott	VEGF and PDGFR	Phase 2
AT9283	Astex	Aurora	Phase 2
AT5719	Astex	CDKs 1,2,4,5	Phase 2
LY-517717	Lilly/Protherics	Fxa	Phase 2
Indeglitazar	Plexxikon	PPAR agonist	Phase 2
VER-52296	Vernalis/Novartis	Hsp90	Phase 2
ABT-518	Abbott	MMP-2 and MMp-9	Phase 1
ABT-737	Abbott	Bcl-2/Bcl-x_L	Phase 1
AT13387	Astex	Hsp90	Phase 1
LP-261	Locus	Tubulin	Phase 1
PLX-5568	Plexxikon	Kinase	Phase 1

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Erlanson, D.A. *Top. Curr. Chem.* **2012**, *317*, 1.

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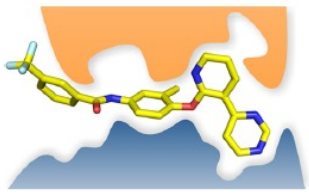
New Approach FBDD

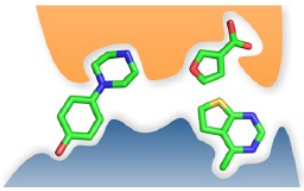
Fragment Based Drug Design – novel approach using smaller compounds…



Typical compound hit from HTS screen

- Large molecule (MW between 250 – 600)
- Broad surface contact with no high quality interactions in key pockets
- May contain functional groups that contribute poorly to protein binding
- Emphasis on potency (30 µM – nM hit activity)

The idea that large molecules can be considered combinations of two or more individual fragments is a fundamental principle of fragment-based drug discovery



Typical compound hits from FBDD

- Smaller molecule (MW between 150 – 300)
- High proportion of the functional groups involved in binding
- Clearly interacts with pockets
- Potency in the range of mM to 30 µM
- Emphasis on efficiency and design

Rees, D.C.; Congreve, M.; Murray, C.W.; Carr, R. *Nature* **2004**, *3*, 660.
Scott, D.E.; Coyne, A.G.; Hudson, S.A.; Abell, C. *Biochemistry* **2012,** *51,* 4990.

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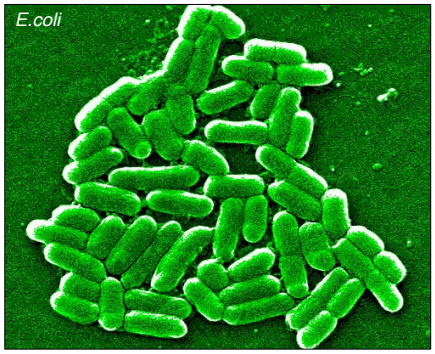
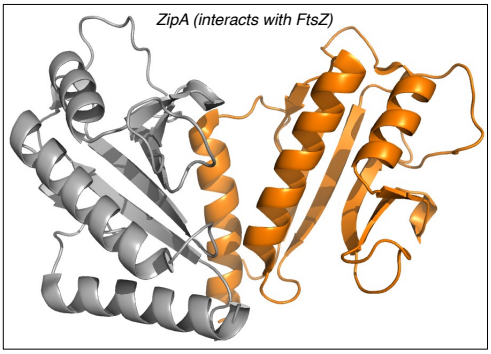
Fragment Based Drug Discovery - Why is there the need for new methodology?

While HTS generally works for most enzyme classes in some cases this does not work

The limitations of HTS was highlighted by researchers from GSK who examined success rates in antibiotic drug discovery over a five year period. Of the 70 campaigns (67 target based, 3 whole screening) only 5 leads were found





The reason for this failure was that the physicochemical properties of compounds that bind to anti-bacterials are different (higher MW and lower logP) than other drug targets so HTS libraries are not suitable

This trend is also showing up with some protein-protein interaction targets (AZ – 15 targets and no hits)

FBDD the way forward with these targets?

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Fragment Library Design

Rule of Three for fragments

- Molecular weight < 300 Da, ofteen ~25 "heavy atoms"
- ClogP $\leq$ 3
- Number of hydrogen bond donors $\leq$ 3
- Number of hydrogen bond acceptors $\leq$ 3
- Number of rotatable bonds $\leq$ 3
- Polar surface area $\leq$ 60 Å^2

Privilege is given to fragments known to bind to proteins

"Bad actors" are removed – those fragments known to react or bind non-specifically or aggregate and chelate

- *Higher water-soluble/H bonds contribute to enthalpy driven binding/higher affinity binding*
- *Faster to prepare*
- *Multiple fragments can combine (from larger mixture) to forman optimized ligand.*
- *Form fewer interactions than lead library compounds but higher quality target interactions*
- *Fragments are less likely to block other groups*
- *Increased flexibility in binding to unpredictable active, allosteric, or regulatory sites*
- *Can find "undruggable" targets*

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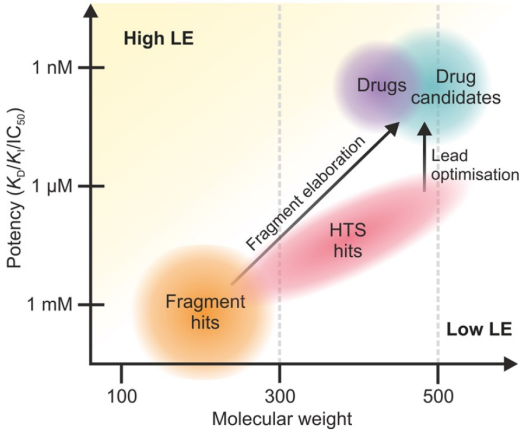
Comparison

- HTS yield low µM – nM
- Fragments lower potent but can be "built"



Scott, D.E.; Coyne, A.G.; Hudson, S.A.; Abell, C. *Biochemistry* **2012,** *51,* 4990.

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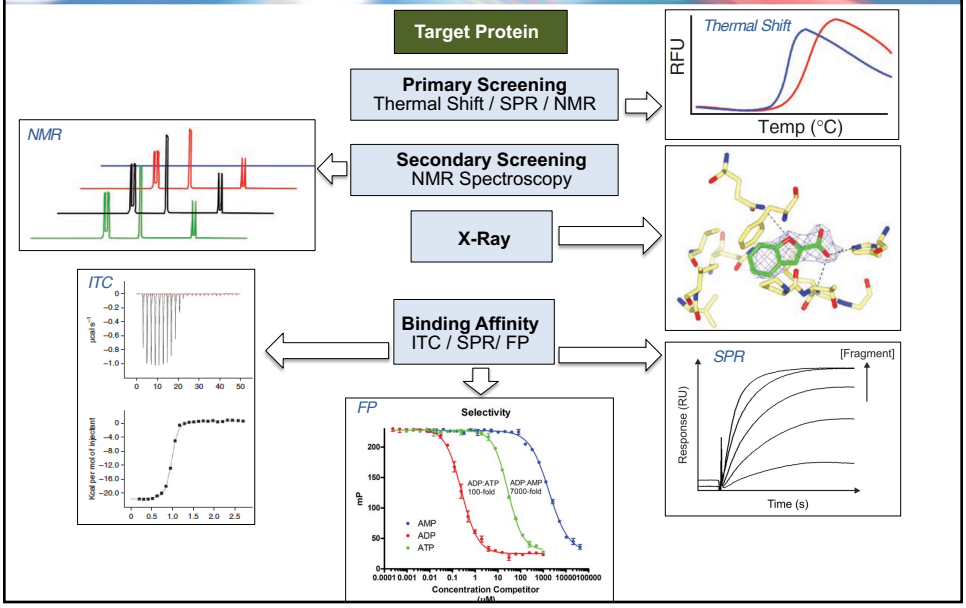
Fragment Binding – Biophysical



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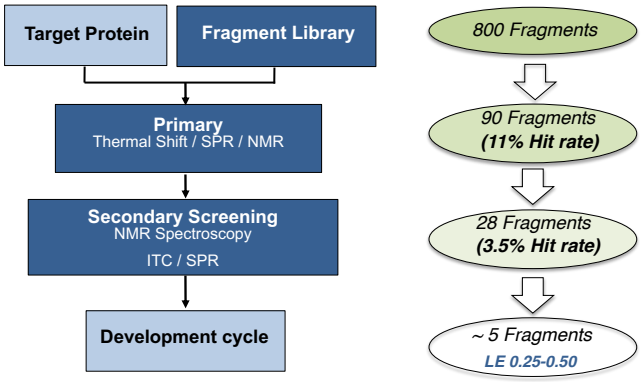
Hit Rate and Ligand Efficiency

Typical fragment screen – the numbers



The hit rate is dependent on the library size and composition. It also depends upon the type of target where the more ''challenging' the target the fewer hits that arise from a fragment screening

*Ligand Efficiency is one of a number of metrics used to look at fragment development (**Lecture 1**)*
(Binding energy per atom in a ligand)
LE = ΔG/NHA
NHA = number of heavy atoms
ΔG = Gibbs free energy of binding (from K_D)

Typically no more than 5 fragments are taken forward for development

Murray, C.A.. *et al, ACS Med. Chem. Lett.*, **2014**, *asap article*

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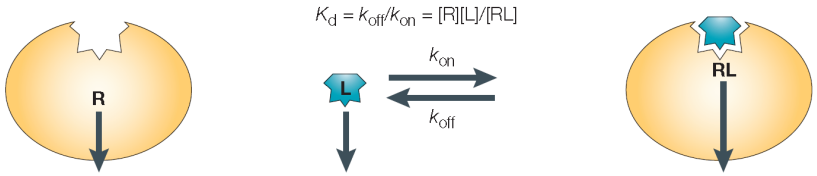
Determination by NMR

NMR methods for detecting ligand binding are divided into two categories

1) Monitor NMR signals from the protein in the presence of ligand
   - **Chemical-shift mapping and "SAR by NMR"**
2) Monitor the ligand bound to target relative to the free ligand
   - **T_2 and $T_{1\rho}$ relaxation**
   - Transferred NOEs
   - Saturation transfer difference (STD)
   - Water-ligand Observed via Gradient Spectroscopy (Water-LOGSY)
   - Diffusion editing



Slow Brownian motion: fast relaxation; negative NOE; slow diffusion

Fast Brownian motion: slow relaxation; positive NOE; fast diffusion

Motional properties of L similar to those of R

Pellecchia, M.; Sem, D.S.; Wuthrich, K. *Nature* **2002**, *1*, 211.
Meyers, B.; Peters, B. *Angew. Chem. Int. Ed.* **2003**, *42*, 864.

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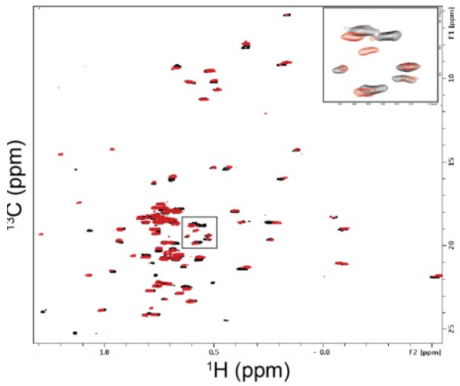
NMR Chemical Shift Mapping

Label the target with ^{15}N and/or ^{13}C and observe changes in the chemical environment with the addition of a ligand or mixture of ligands



^{1}H-^{13}C-HSQC of a 30 kDa protein selectively ^{13}C-labeled on Ile, Leu, and Val methyl groups in the presence (red) and absence (black) of a twelve-fragment mixture (75 μM protein, 600 MHz spectrometer, 12 minute collection time). The expansion in the top right corner is of the boxed portion of the spectrum.

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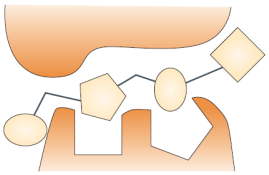
How is a Fragment Increased

Goal is to increase potency, to move from mM to ~10 nM

Elaboration of HTS hit



Modified compound with higher potency

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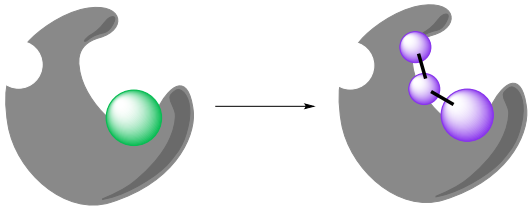
Fragment Elaboration

Fragment Growing



- *Most successful and frequent method of increasing potency*
- *Typically a single fragment in a binding pocket is 'grown' using chemical synthesis to pick up further interactions with the protein.*
- *This is the case that is the most likely to arise where a single fragment binds to protein or multiple fragments bind to a specific area of the binding pocket*

X-Ray information on how the ligand binds to the protein is key to guiding fragment development

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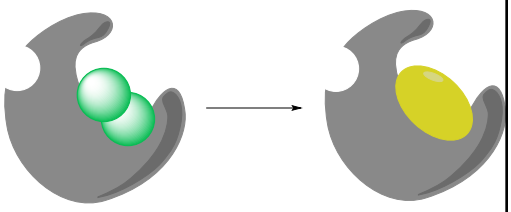
Fragment Elaboration

Fragment Merging



- *Find several fragments binding in close proximity*
- *Chemical synthesis uses overlap to design lead compound*

Xray information on how the ligand binds to the protein is key to guiding fragment development, steps of merging overlapping compounds is "tricky" sometimes loss of binding occurs

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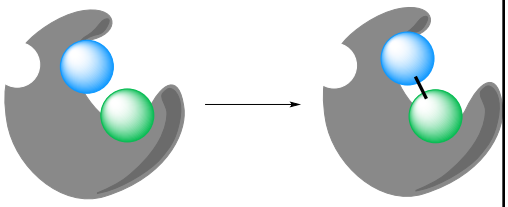
Fragment Elaboration

Fragment Linking



- *Create flexible linkers between different binding pocket/ligands*
- *Considered best way to increase potency can be created similar to HTS lead development*
- *Linked compound should have higher binding afinity with correct linker*
- *Considered the most difficult*

Few examples of this have been successfully produced – but often against protein-protein undruggable interactions

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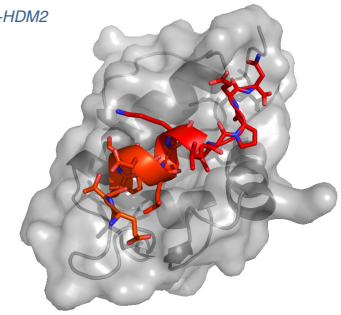
Fragment Linking – Protein-Protein Interactions

Why protein-protein interactions as targets?

p53-HDM2

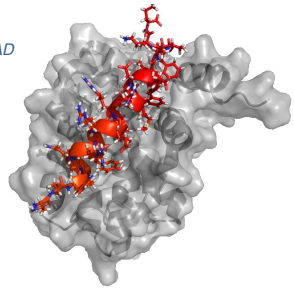


*Protein-Protein interactions (PPI's) are found throughout biological systems. Typically these are defined as **difficult targets** as success rates in targeting these has been low especially using HTS approaches.*

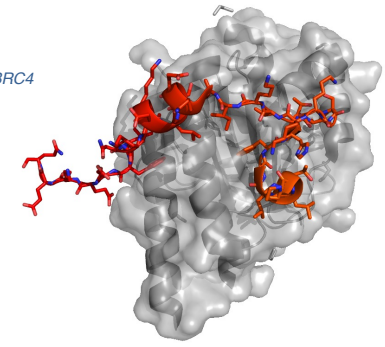
Unlike conventional targets they do not have distinct binding pockets however they have what is known as 'hot-spots' typically on the surface of the protein

FBDD has been used successfully against a number of these targets however none to date have been approved as drugs although in a number of cases there are compounds in Phase I/II development.

Bcl-BAD



RAD51-BRC4



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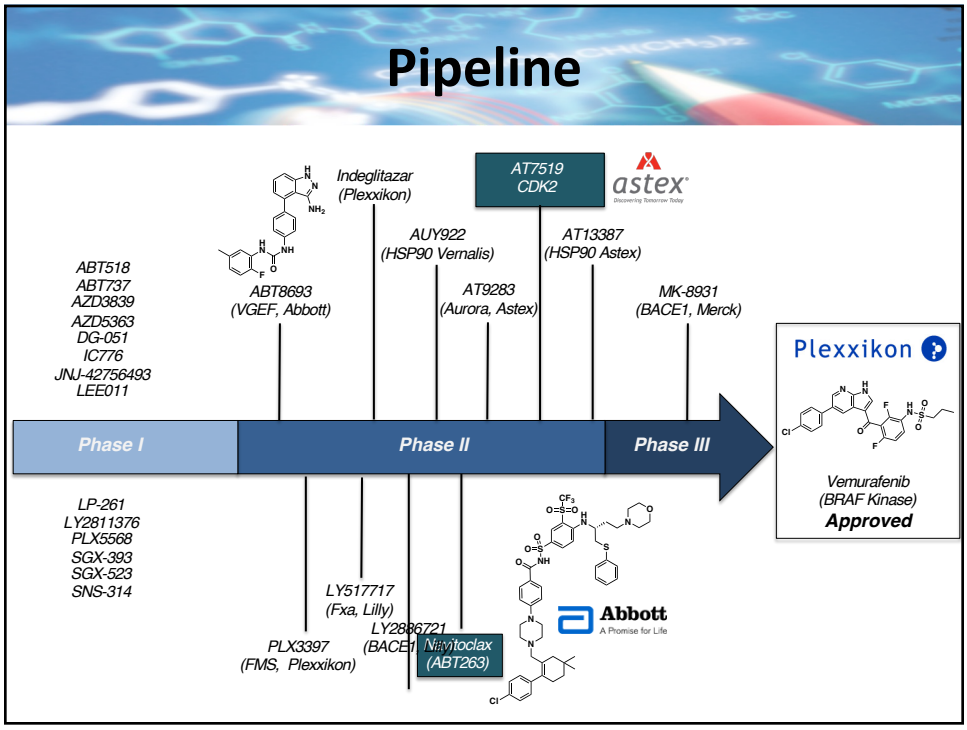
Pipeline
Indeglitazar
(Plexxikon)
AT7519
CDK2
astex
AUY922
(HSP90 Vernalis)
AT13387
(HSP90 Astex)
ABT518
ABT737
AZD3839
AZD5363
DG-051
IC776
JNJ-42756493
LEE011
ABT8693
(VGEF, Abbott)
AT9283
(Aurora, Astex)
MK-8931
(BACE1, Merck)
Plexxikon
Phase I
Phase II
Phase III
Vemurafenib
(BRAF Kinase)
Approved
LP-261
LY2811376
PLX5568
SGX-393
SGX-523
SNS-314
LY517717
(Fxa, Lilly)
PLX3397
(FMS, Plexxikon)
LY2886721
(BACE1, Lilly)
Nivitoclax
(ABT263)
Abbott
A Promise for Life

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