Target Validation and Druggability

SMR

Hot Topics in Drug Discovery:

Finding the Next Lead

11 November 2009

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and

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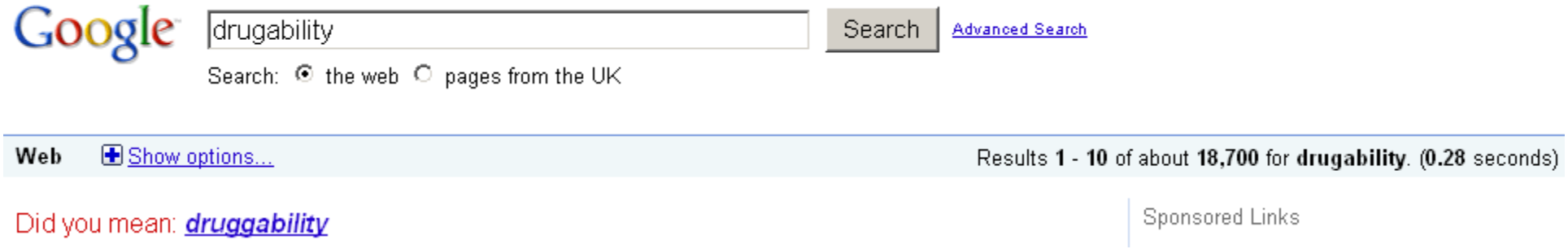
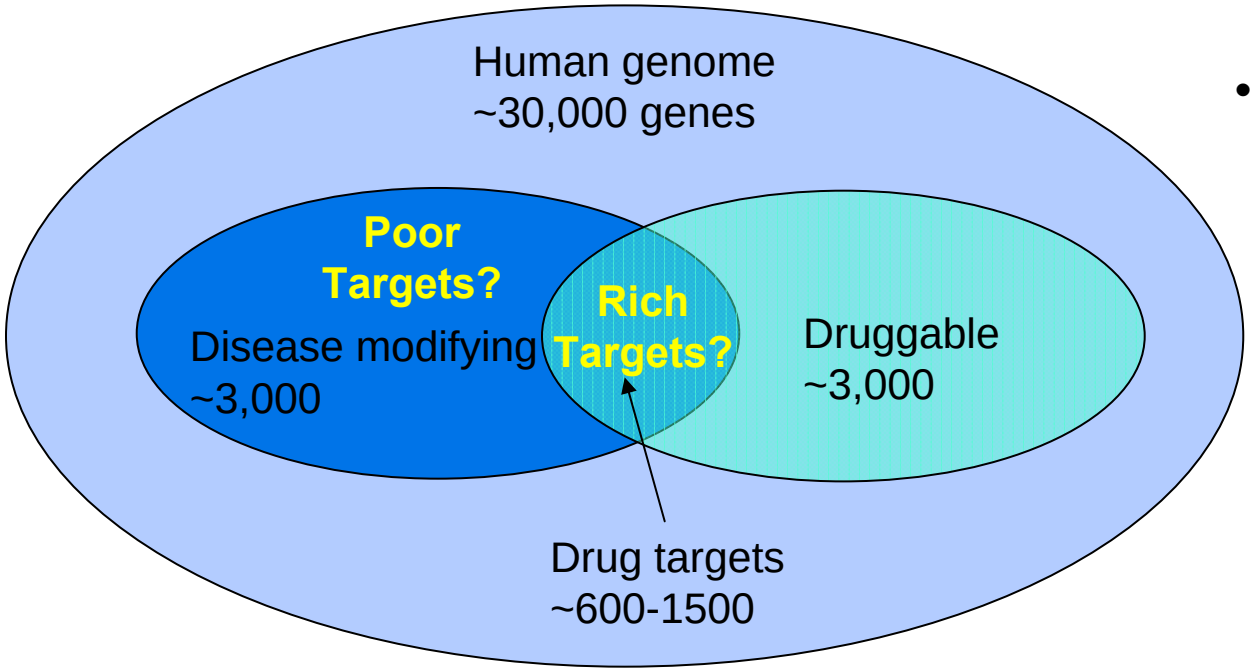
Overview

- The target universe
  - The “druggable genome”/proteome/targetome
- Target identification
  - Genetic approaches
  - Data-mining approaches
  - *in vitro* approaches
- Target validation
  - Biological approaches
  - Computational assessments of “druggability”



The Target Universe: Genes

- 30,000 genes from human genome
  - Initial estimate: 600–1500 are “druggable”
    - Capable of being affected by orally-available drug
    - ~300 so far have yielded products

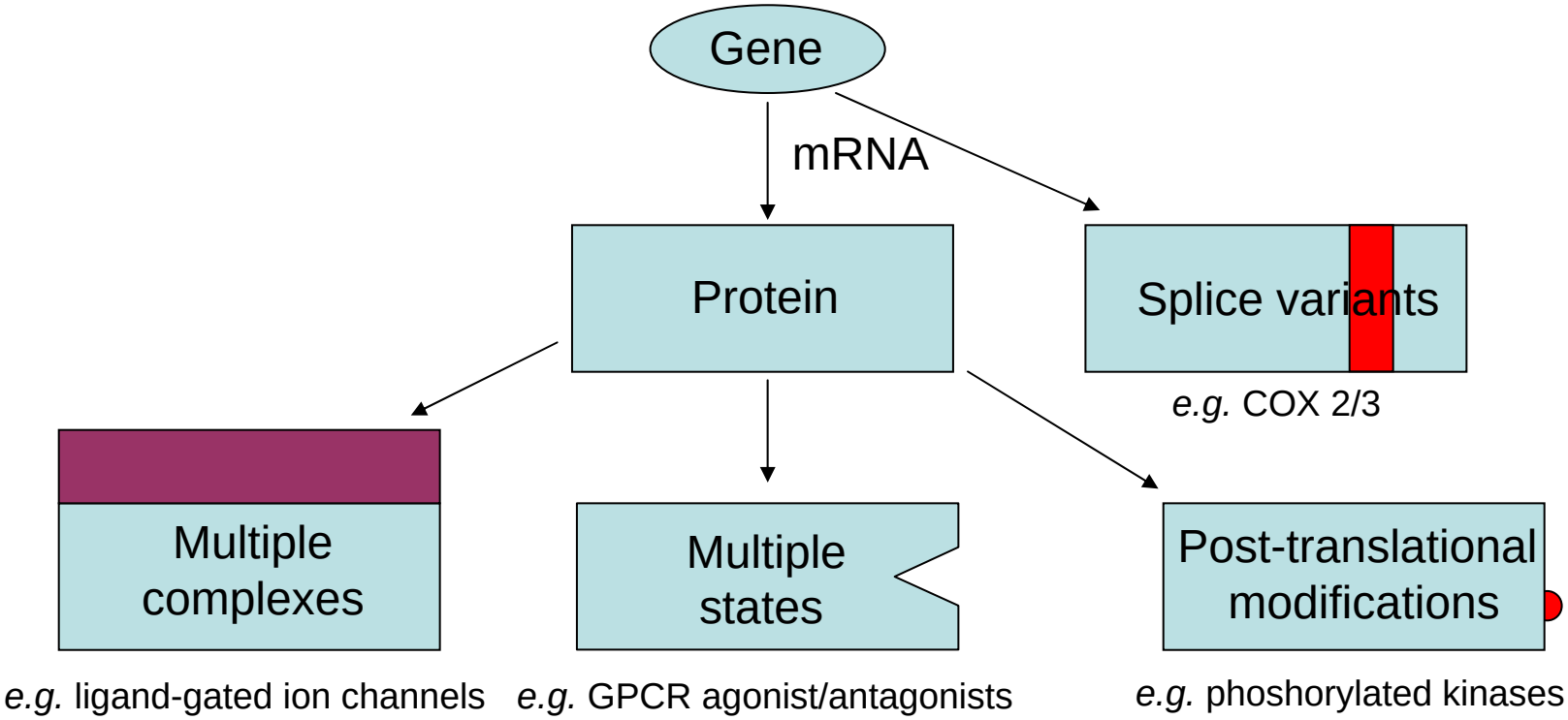


- No consideration of
  - non-human targets
    - Pathogens
  - non-oral routes
    - iv/inhaled
  - non-small molecule
    - Vaccines
    - Antibodies
    - Proteins
    - siRNA

Overington *et al.* (2006) Nature Rev Drug Disc, 5, 993

The Target Universe: Proteins

- One gene can give rise to many targets



- Some targets are combinations of proteins *e.g.* Gleevec
- Can be target/mode/location *e.g.* AChE in eye/CNS/NMJ

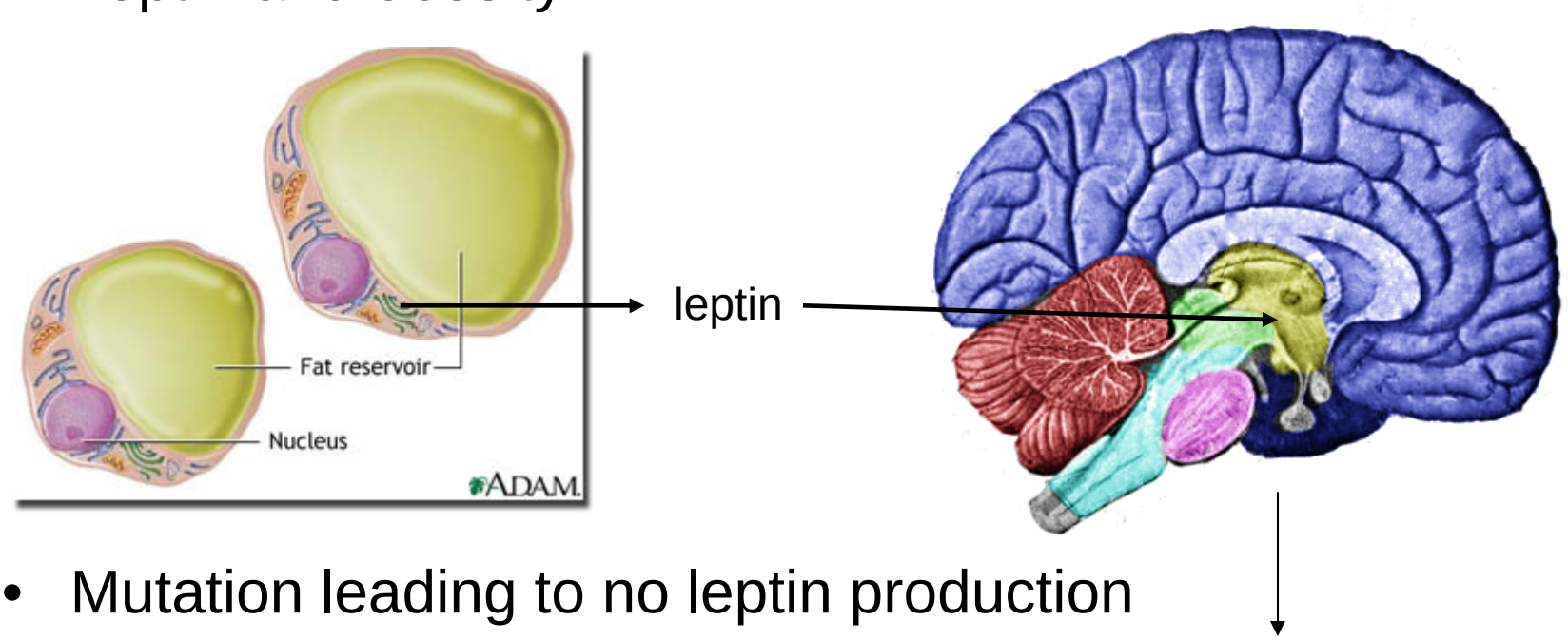
Kubinyi (2003) Nature Rev Drug Disc 2 665

Target Identification

- Genetic evidence: identification of genes that
  - cause disorder if mutated
    - or increase risk of disorder if mutated
  - protect against disorder if mutated
  - show changes in expression in disease states
  - occur in pathogens but have no human homologue
- Data-driven evidence: identification of proteins that
  - play an important role in disease-associated pathway
- Experimental evidence: identification of targets by
  - chemogenomic screening of small-molecule tools

Mutations Causing Disease

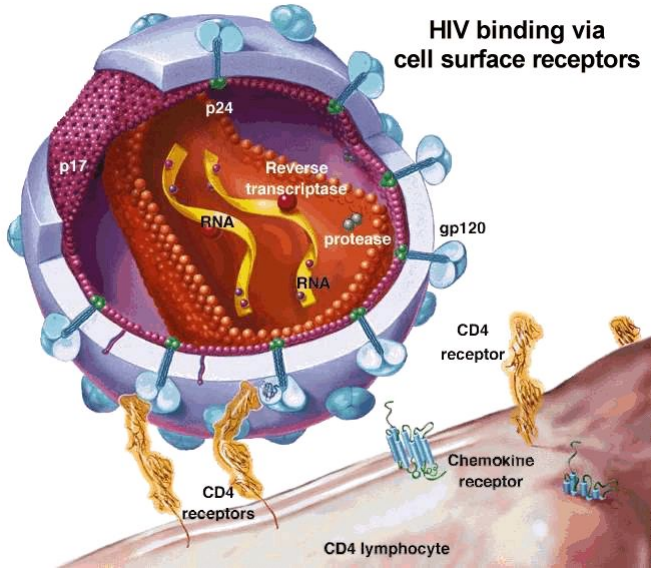
- Leptin and obesity



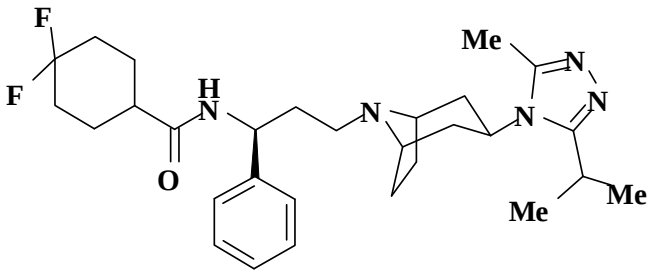
- Mutation leading to no leptin production
  - Highly obese phenotype
- Developed leptin analogue
  - Worked in patients with this mutation
    - but not in the majority of remaining obese patients

Mutations Protecting Against Disease

- CCR5 and HIV infection
  - 32 base pair deletion in CCR5 gene
  - Receptor does not express on T cell surface
  - No deleterious phenotype
  - Remarkably resistant to HIV infection
    - CCR5 is a co-receptor for viral cell penetration

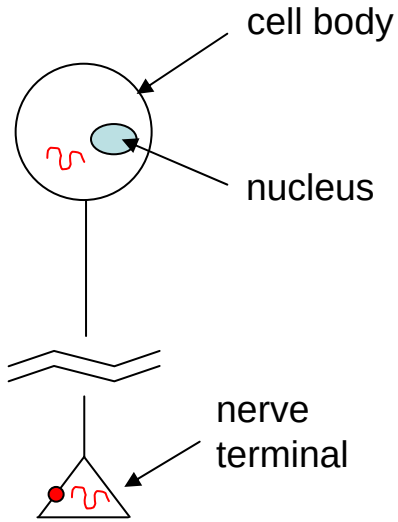


Maraviroc: CCR5 antagonist



Changes in Gene Expression

- Compare normal and disease tissue
  - Levels of
    - protein: harder (antibodies)
    - mRNA: easier (hybridisation)

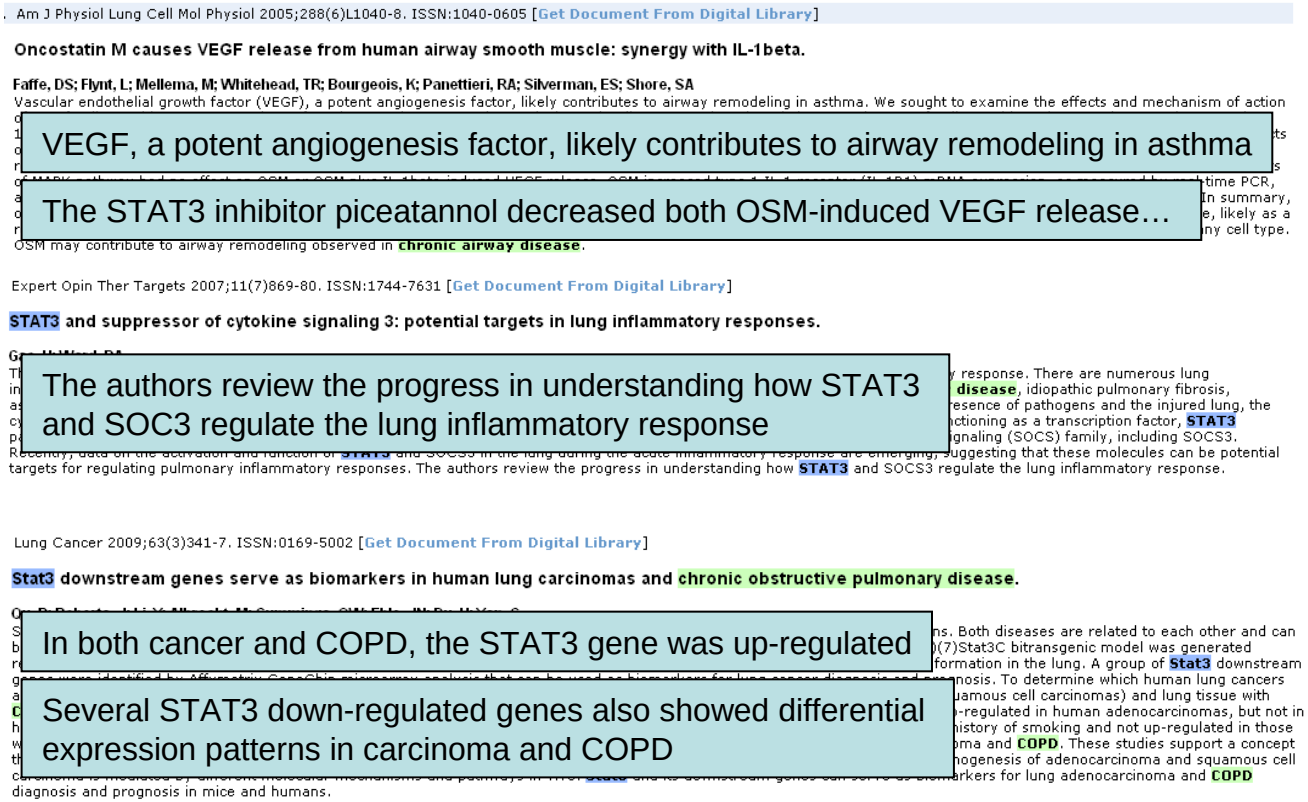


  - Changes imply a target/pathway involved in disease
    - or involved in response to disease
    - *e.g.* wound healing
      - histone methylases (PcGs, Eed, Suz12, Exh2) ↓
      - histone demethylases (Jmjd3, Utx) ↑

Shaw & Martin (2009) EMBO Rep 10 881

Targets from Automated Data-Mining

- *e.g.* STAT3 involvement in COPD



Loose association

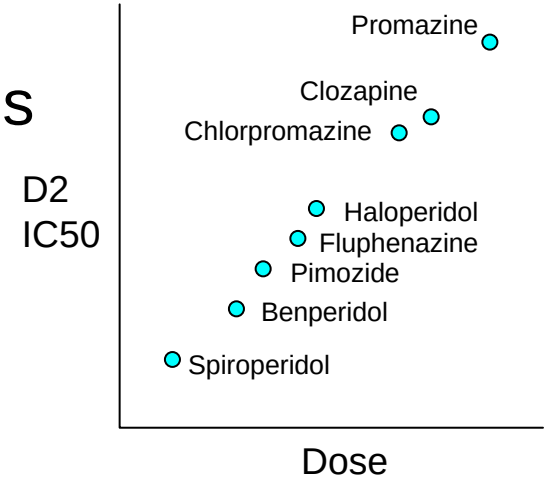
Another loose association

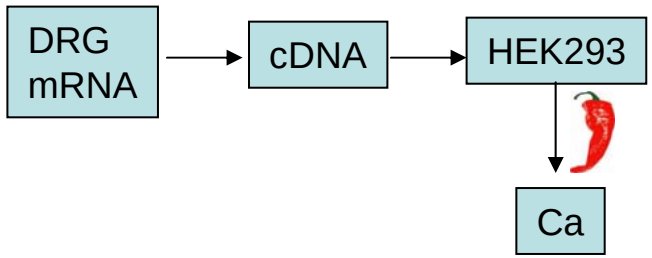
Direct association

STAT3 could either be a target or a biomarker for COPD

Targets from Small Molecules

- Define target by means of chemical tools
  - Launched products
    - *e.g.* D2 role in schizophrenia
    - D2 potency *vs* efficacy
  - Natural products
    - *e.g.* role of capsaicin in pain
      - Identified TrpV1 as target
  - Designed small-molecule subsets
    - Selective probes to ascertain protein roles
    - *e.g.* histone modification enzymes





Seeman et al. (1976) Nature 261 717
Caterina et al. (1997) Nature 389 816
Edwards et al., (2009) Nature Chem Biol 5 436

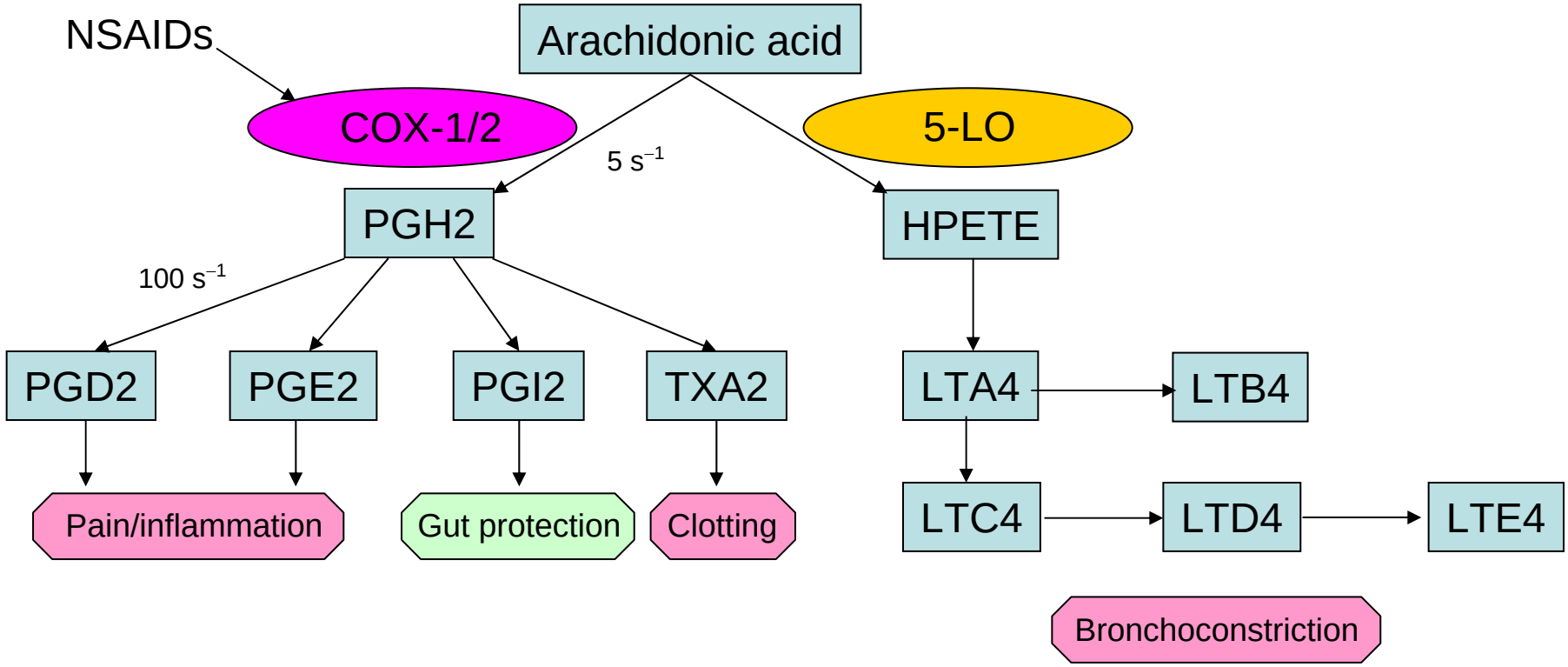
Target Validation

- Does target knock-out have the desired effects?
  - Disease-related phenotype, no deleterious effects
- Where in the body is the gene expressed?
  - Does expression vary with *e.g.* age/gender?
- Are there alternative pathways available?
- Can we prosecute a drug-discovery program?
  - Is the target druggable?
  - Is selectivity necessary/achievable?
  - Is there a suitable animal model system?
    - All of the above for animal model

Knock-ins/downs/outs

- Reduce expression of protein to assess involvement/tox
  - Knockout: whole organism
  - Knock-in: gene replaced with non-functional mutant
  - Knock-down *e.g.* siRNA: organ specific
- Can provide go/no-go decisions but
  - embryonic lethality can be uninterpretable
    - Knock-out can affect development
  - may not replicate effects of reversible antagonism
  - organism can compensate for knock-out

Understand the System: Enzymes

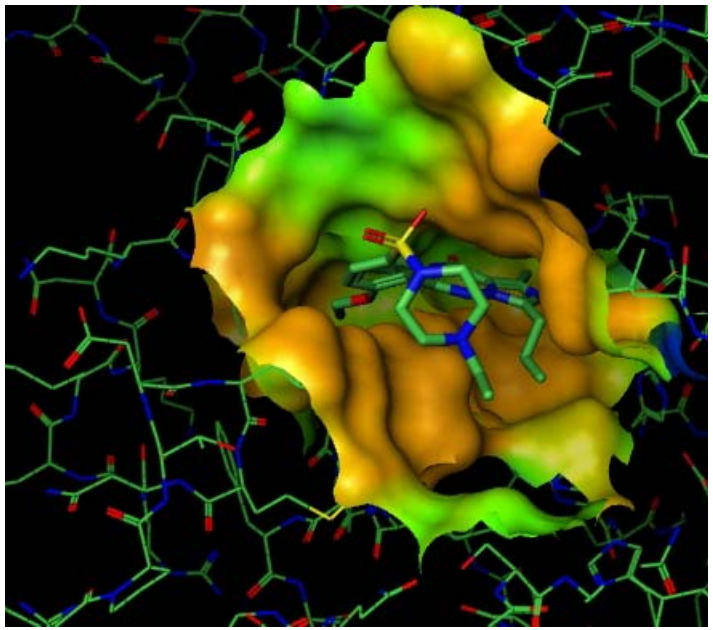


- Rate-determining step
- Functional degeneracy *e.g.* COX-1/2
- Pathway consequences *e.g.* COX/5-LO

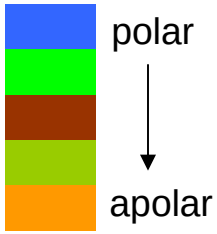
Understand the System: Receptors

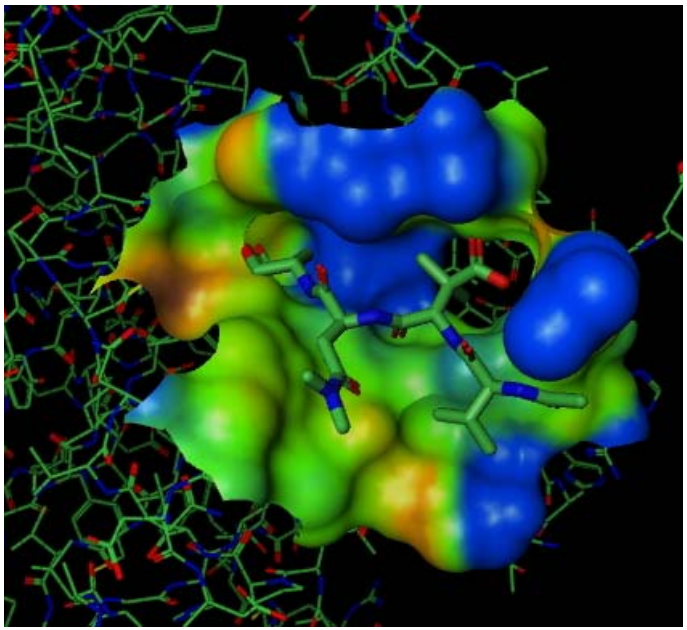
- Concentration and potency of endogenous ligands
- Peptidic GPCRs are notoriously difficult targets (NK1)
  - Endogenous ligand usually sub-nM
  - Occupies small fraction of receptors to give full response
  - Released in huge concentrations at synapses
  - Located in CNS
- What can we do?
  - Agonist projects
  - Systems with low concentrations of circulating hormone
    - AT_1, ET_A, CCR_5
  - Non-competitive and/or slow-offset modulation

Druggability



PDE5: beautiful





CMV protease: ugly

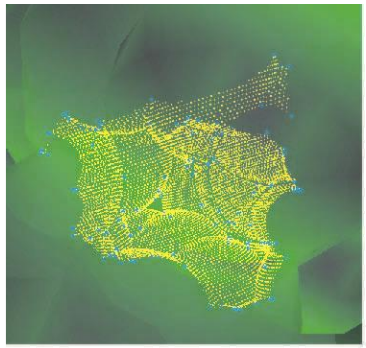
- A good pocket tends to be
  - the right size: accommodate drug-sized molecule
  - buried: increases interaction surface area
  - not too polar: allow drug-like properties in ligands

Practical approaches

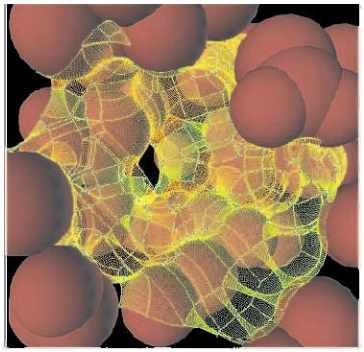
- Screen using representation of chemical space
- More active compounds = more druggable target
  - High throughput screen
    - Thorough
    - Expensive, with false positives and negatives
  - Screen fragment-based library
    - Cover chemical space more effectively
    - Need high-sensitivity assays/biophysical methods

Hajduk et al. (2005) J Med Chem 48 2518

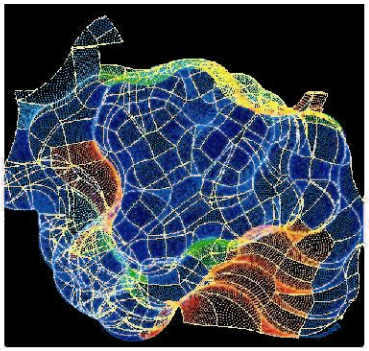
Analytical approaches



Define pocket

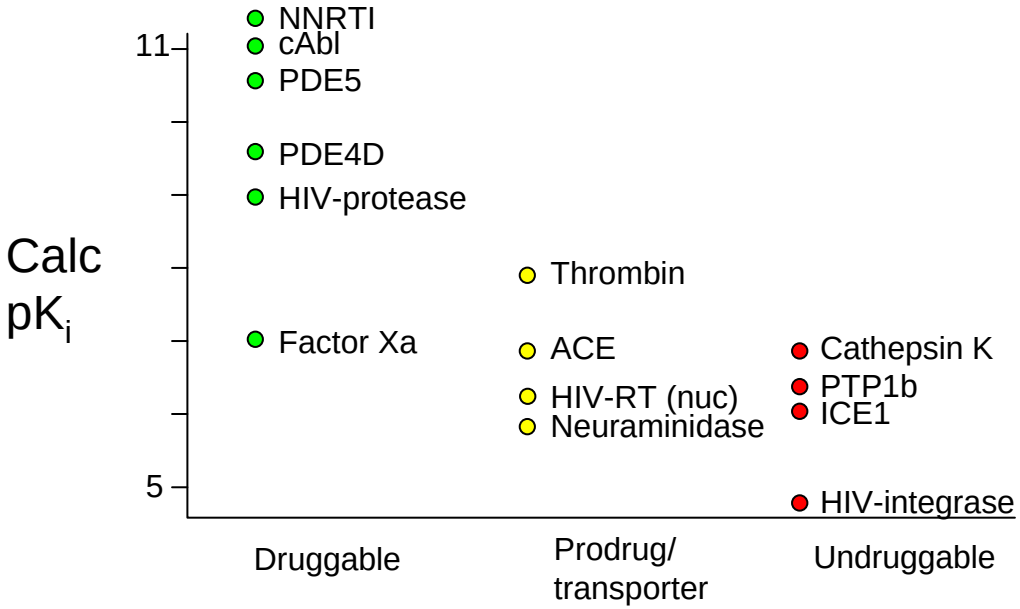


Compute non-polar SA



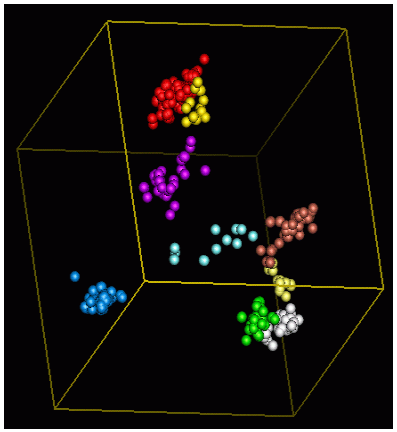
Compute curvature

Potency estimate

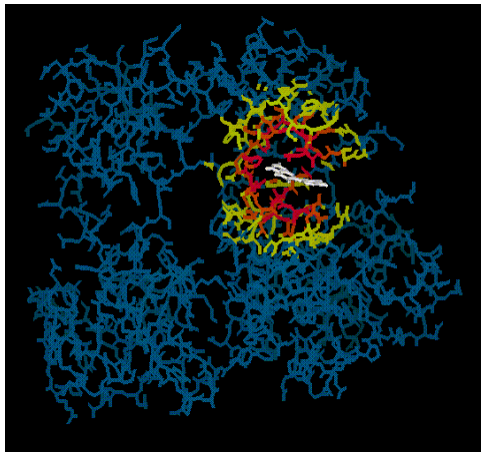


Hajduk et al. (2005) DDT 1675
Cheng et al. (2007) Nature Biotech 25, 71
Halgren (2009) J. Chem Inf Model 49 377

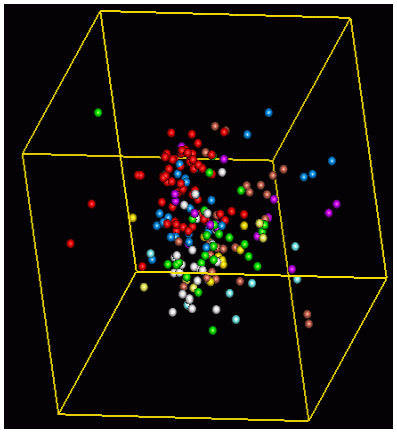
Selectivity: Site *vs* Whole Sequence



Kinase sequences



Identify ATP-site residues



ATP-site sequences

- Antifungals: broad spectrum and selectivity over human

Identity	Human	*C. albicans*	*A. fumigatis*
Total Site	25% 15%	38% 72%	35% 78%

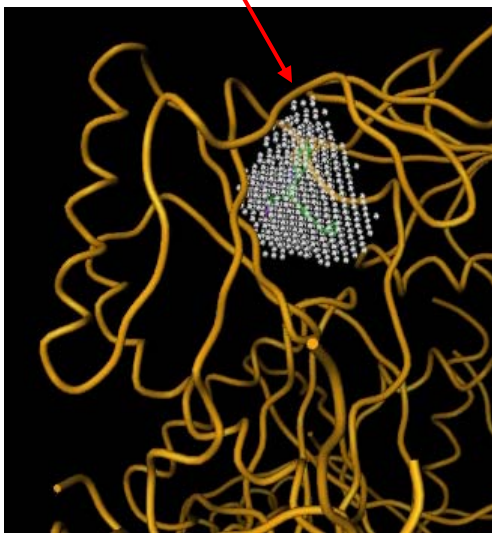
Caveats in Cavity Analysis

- Irreversible inhibitors do not need as much SA/burial
- Allosteric pockets induced by ligands
  - Cannot predict pocket properties from apo structures

HIV reverse transcriptase

apo structure
293 Å^3

loop movement



Efavirenz (MWt 316)
577 Å^3

loop movement

Delavirdine (MWt 457)
823 Å^3

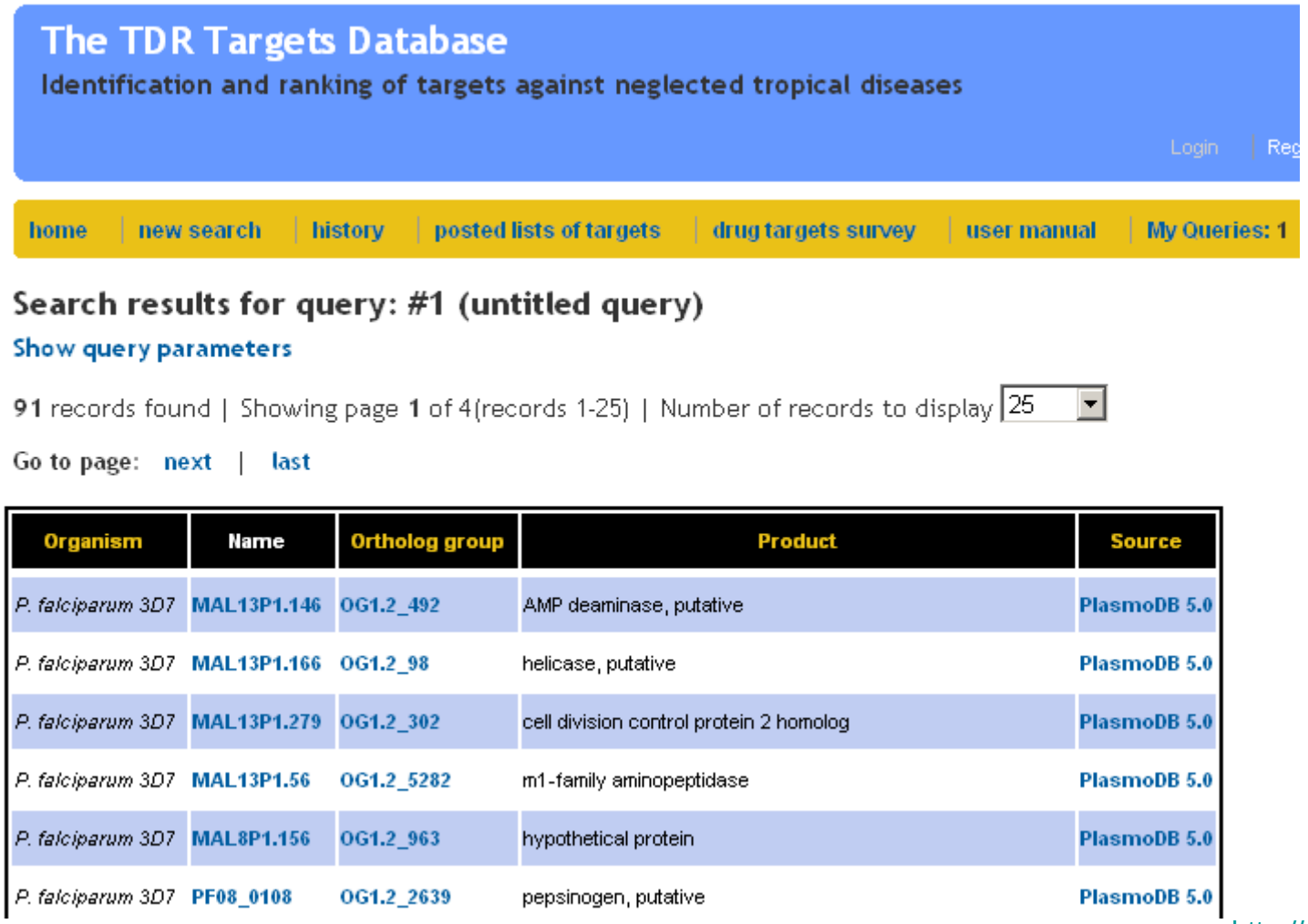
Empirical Approaches

- Homology-based
  - Sequence similarity to known druggable proteins
- Non-homology based
  - If structure available, calculate pocket properties
    - Volume, depth, curvature, accessibility, PSA…
    - Apply model based on 400 druggable pockets
  - Calculate sequence-based properties
    - #helices, size of protein…
    - Apply model based on 1400 known targets
- Combine all this information into one score

Agüero et al. (2007) Nature Drug Disc 7 900

TDR Targets Database

- Tropical Disease Pathogen genome database



The TDR Targets Database

Identification and ranking of targets against neglected tropical diseases

Login | Reg

home | new search | history | posted lists of targets | drug targets survey | user manual | My Queries: 1

Search results for query: #1 (untitled query)

Show query parameters

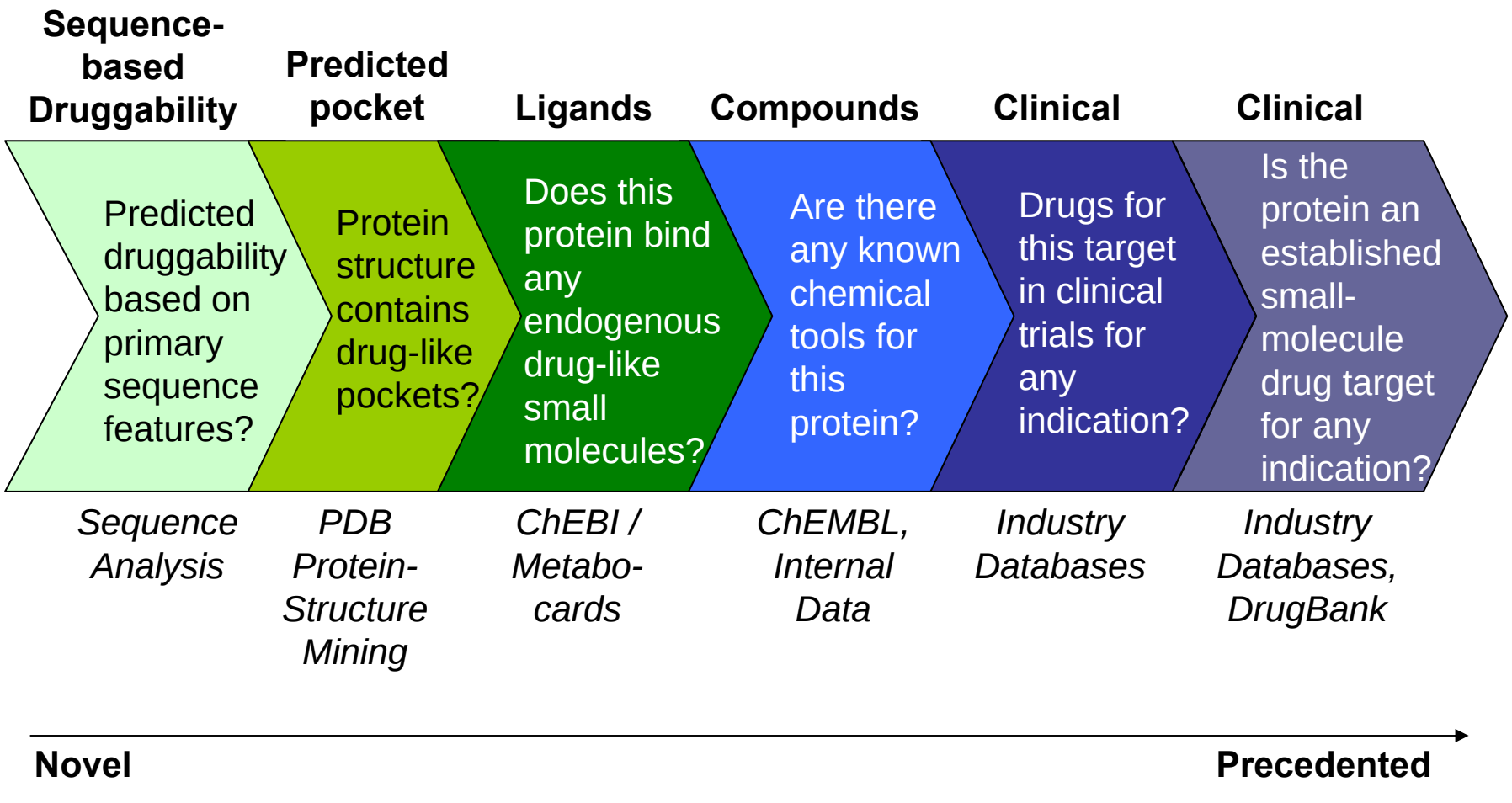
91 records found | Showing page **1** of 4(records 1-25) | Number of records to display 25

Go to page: next | last

Organism	Name	Ortholog group	Product	Source
P. falciparum 3D7	MAL13P1.146	OG1.2_492	AMP deaminase, putative	PlasmoDB 5.0
P. falciparum 3D7	MAL13P1.166	OG1.2_98	helicase, putative	PlasmoDB 5.0
P. falciparum 3D7	MAL13P1.279	OG1.2_302	cell division control protein 2 homolog	PlasmoDB 5.0
P. falciparum 3D7	MAL13P1.56	OG1.2_5282	m1-family aminopeptidase	PlasmoDB 5.0
P. falciparum 3D7	MAL8P1.156	OG1.2_963	hypothetical protein	PlasmoDB 5.0
P. falciparum 3D7	PF08_0108	OG1.2_2639	pepsinogen, putative	PlasmoDB 5.0

http://www.tdrtargets.org

Confidence in Druggability



Target Opportunity Universe

Confidence in druggability *vs* confidence in mechanism

High-risk but testable

New indications for cpds

Done

Novel biology

Novel opportunities for chemistry

Novel opportunities for biologicals

Campbell et al. (2009) DDT in press

Summary

- 2-3 targets per year yield launched drugs
- Many ways to associate a target with a disorder
- Many ways to further explore this link
  - Target confidence building rather than validation
- Reasons for optimism?
  - Technology is moving on
    - Molecular biology
    - Data mining
  - Seeds of change in culture
    - Pre-competitive research

Genes Unique to Pathogens

- Genomes of pathogens tend to be smaller
  - Less degeneracy: more proteins essential
- Some pathways not found in humans
  - *e.g.* cell wall synthesis, folate synthesis
- Easier to prioritise genome experimentally