

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”

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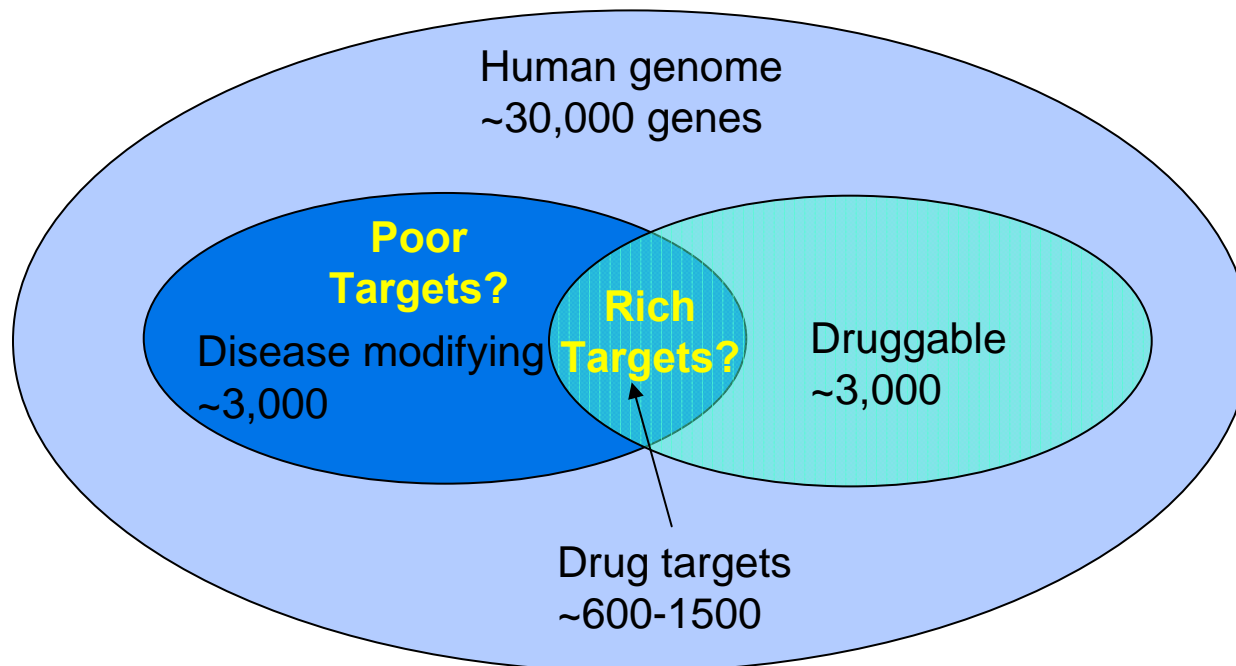
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Did you mean: [druggability](#)

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The Target Universe: Genes

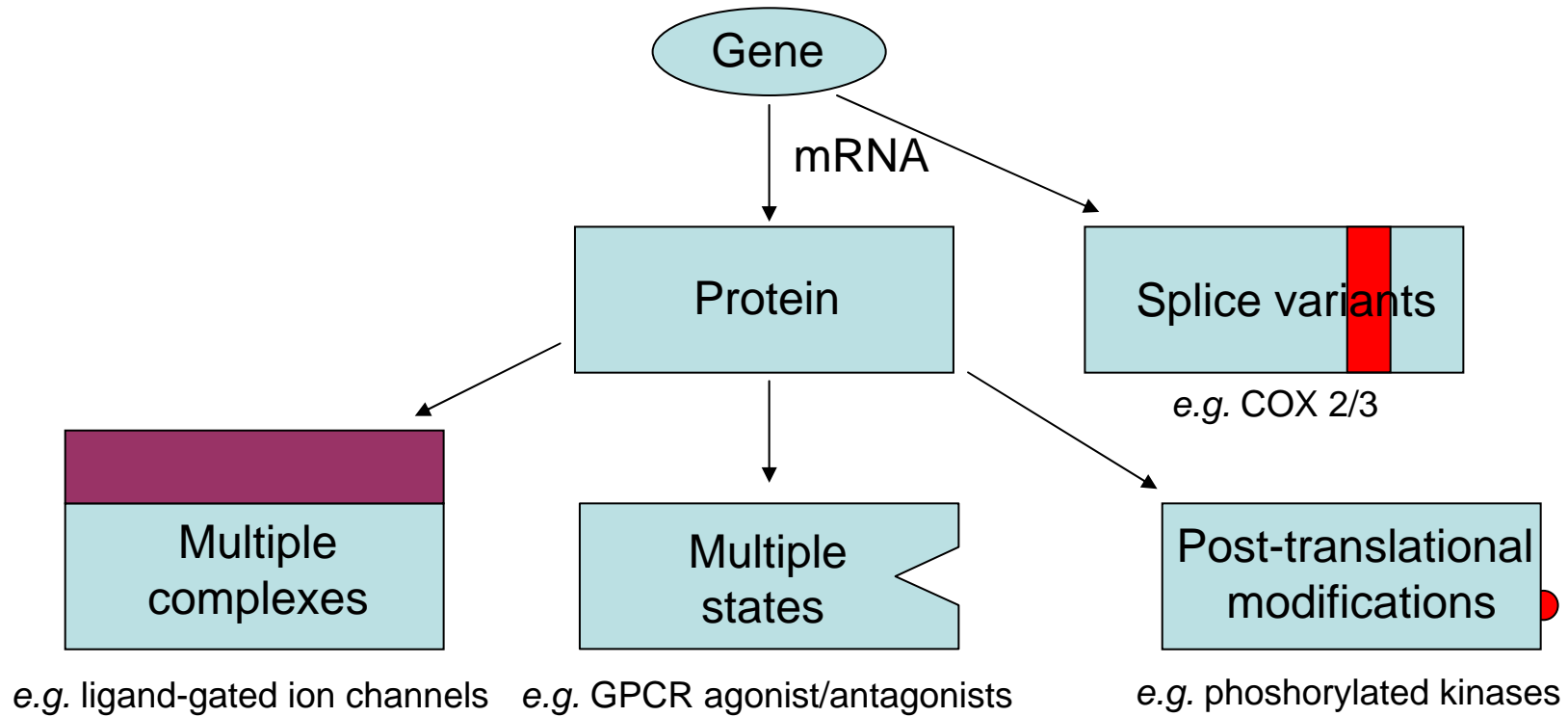
- 30,000 genes from human genome
 - Initial estimate: 600–1500 are “druggable”
 - Capable of being affected by orally-available drug
 - ~300 so far have yielded products



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

The Target Universe: Proteins

- One gene can give rise to many targets



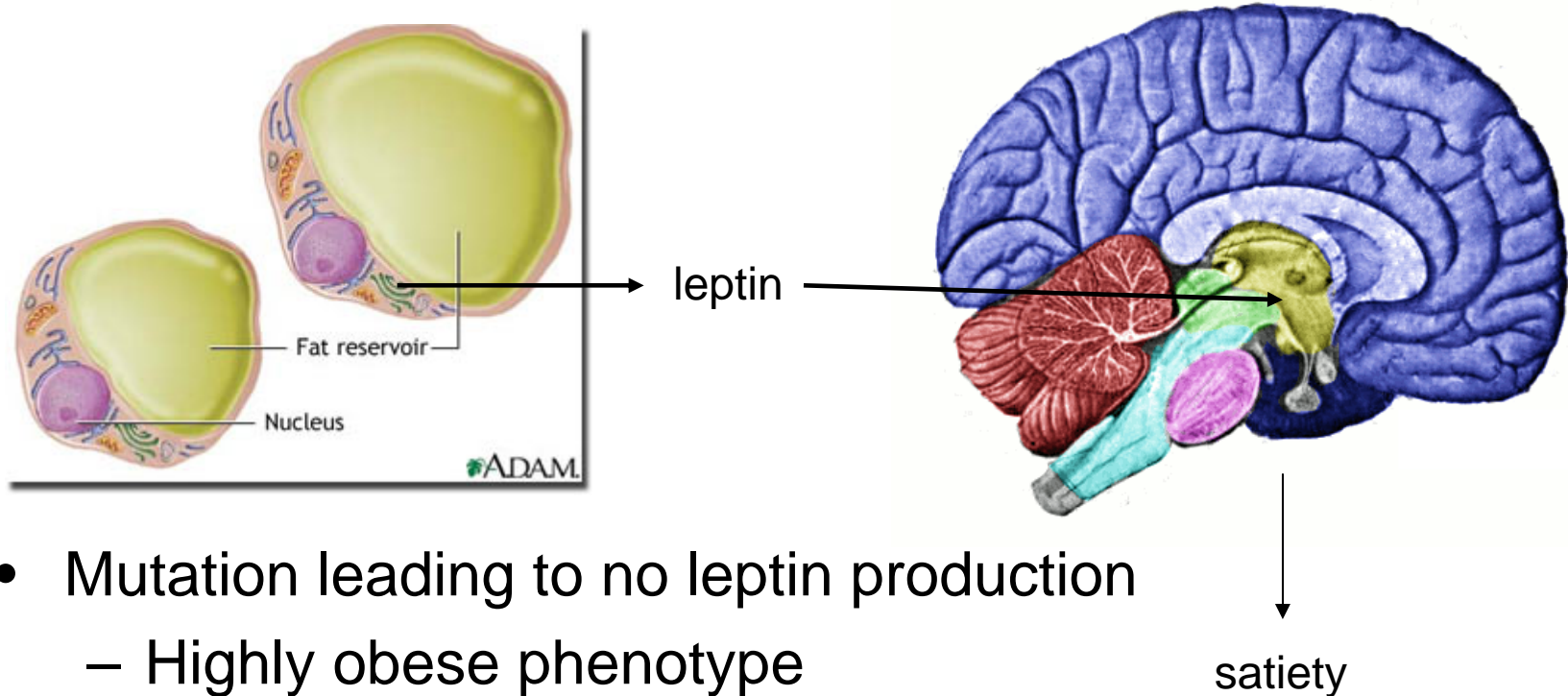
- Some targets are combinations of proteins e.g. Gleevec
- Can be target/mode/location e.g. AChE in eye/CNS/NMJ

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

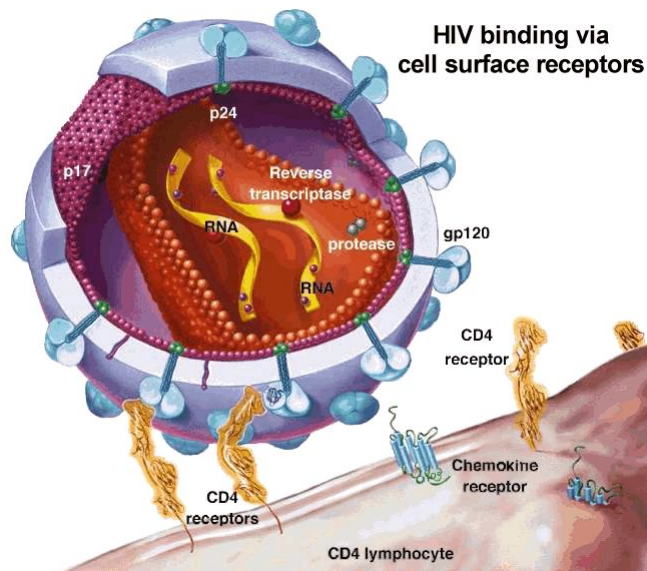
- Leptin and obesity



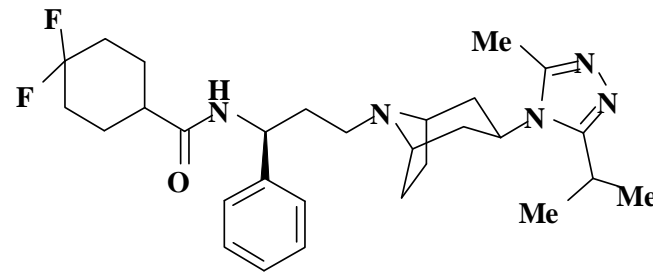
- Mutation leading to no leptin production
 - Highly obese phenotype
- Developed leptin analogue
 - Worked in patients with this mutation
 - but not in the majority of remaining obese patients

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

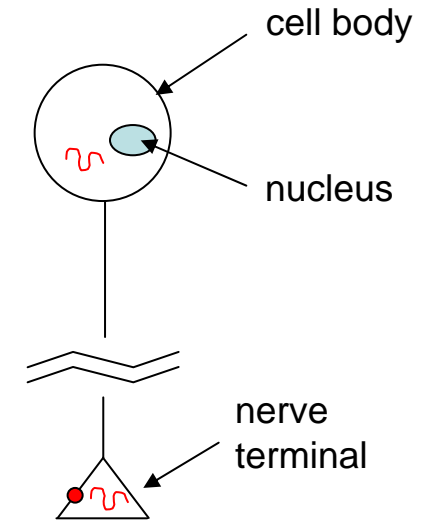


Changes in Gene Expression

- 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) ↑

Targets from Automated Data-Mining

- e.g. STAT3 involvement in COPD

Am J Physiol Lung Cell Mol Physiol 2005;288(6):L1040-8. ISSN:1040-0605 [Get Document From Digital Library]

Oncostatin M causes VEGF release from human airway smooth muscle: synergy with IL-1beta.

Faffe, DS; Flynt, L; Mellema, M; Whitehead, TR; Bourgeois, K; Panettieri, RA; Silverman, ES; Shore, SA

Vascular endothelial growth factor (VEGF), a potent angiogenesis factor, likely contributes to airway remodeling in asthma. We sought to examine the effects and mechanism of action

VEGF, a potent angiogenesis factor, likely contributes to airway remodeling in asthma

The STAT3 inhibitor piceatannol decreased both OSM-induced VEGF release...

OSM may contribute to airway remodeling observed in chronic airway disease.

Expert Opin Ther Targets 2007;11(7):869-80. ISSN:1744-7631 [Get Document From Digital Library]

STAT3 and suppressor of cytokine signaling 3: potential targets in lung inflammatory responses.

G...

The authors review the progress in understanding how STAT3 and SOCS3 regulate the lung inflammatory response

response. There are numerous lung disease, idiopathic pulmonary fibrosis, presence of pathogens and the injured lung, the actioning as a transcription factor, STAT3 signaling (SOCS) family, including SOCS3.

Recently, data on the activation and function of STAT3 and SOCS3 in the lung during the acute inflammatory response are emerging, suggesting that these molecules can be potential targets for regulating pulmonary inflammatory responses. The authors review the progress in understanding how STAT3 and SOCS3 regulate the lung inflammatory response.

Lung Cancer 2009;63(3):341-7. ISSN:0169-5002 [Get Document From Digital Library]

Stat3 downstream genes serve as biomarkers in human lung carcinomas and chronic obstructive pulmonary disease.

In both cancer and COPD, the STAT3 gene was up-regulated

Several STAT3 down-regulated genes also showed differential expression patterns in carcinoma and COPD

ns. Both diseases are related to each other and can (7)Stat3C bitransgenic model was generated formation in the lung. A group of Stat3 downstream genes (including SOCS3) were identified by Affymetrix GeneChip analysis and confirmed by real-time PCR for lung cancer diagnosis. To determine which human lung cancers (squamous cell carcinomas) and lung tissue with up-regulated in human adenocarcinomas, but not in those with a history of smoking and not up-regulated in those with adenocarcinoma and COPD. These studies support a concept of shared gene expression patterns in lung cancer and COPD. These studies support a concept of shared gene expression patterns in lung cancer and COPD.

diagnosis and prognosis in mice and humans.

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

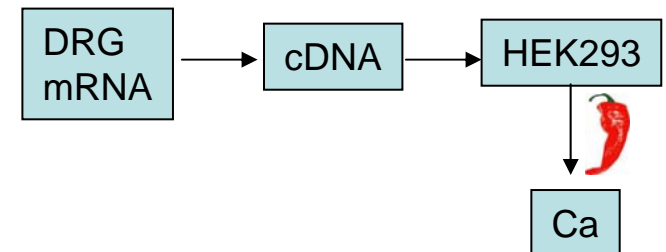
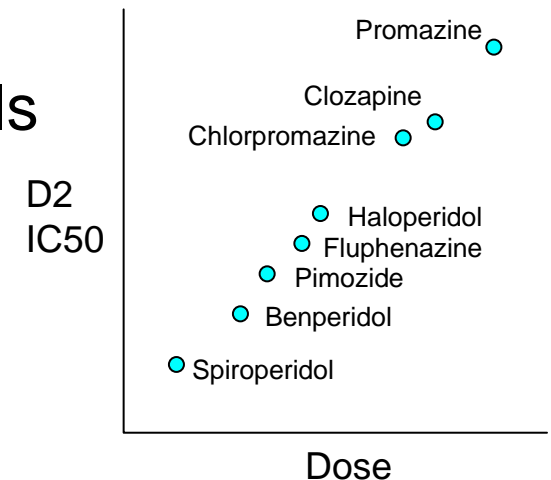
- e.g. D2 role in schizophrenia
- D2 potency vs efficacy

- 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



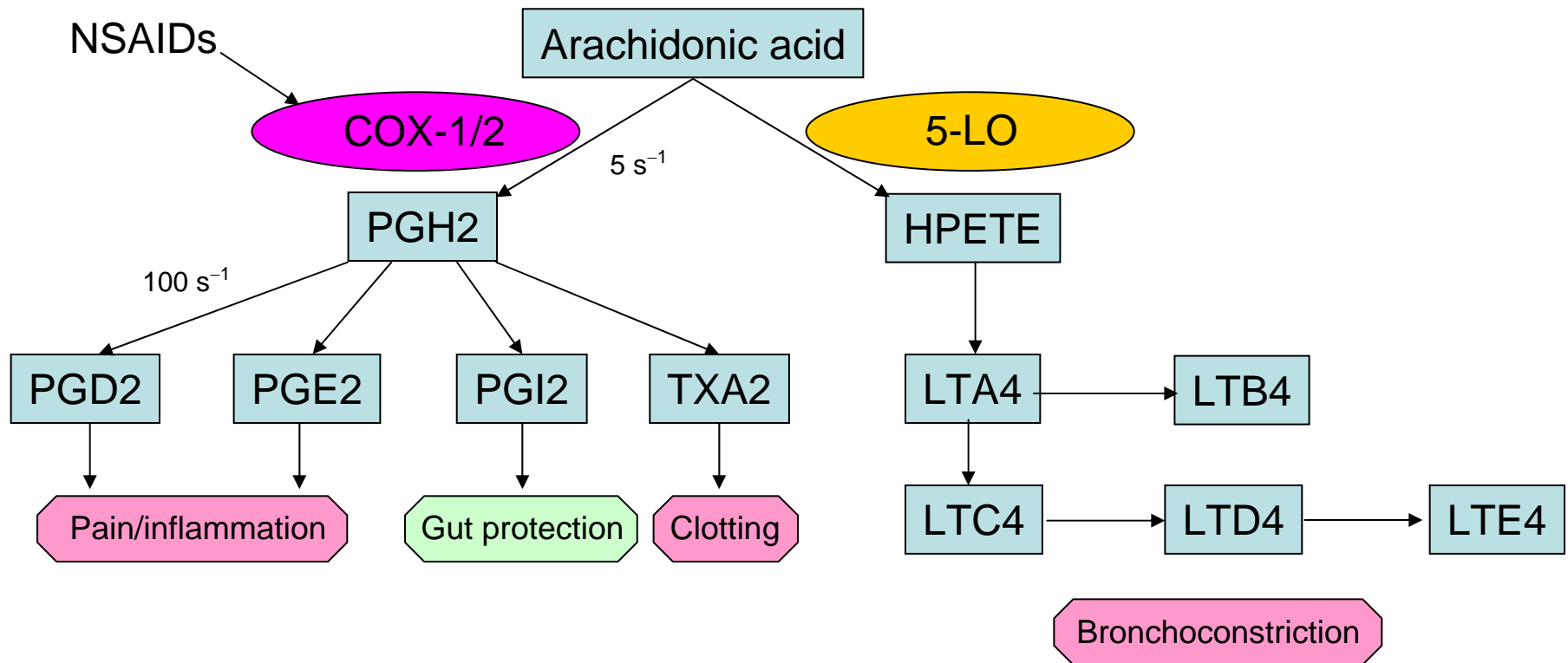
Target Validation

- 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

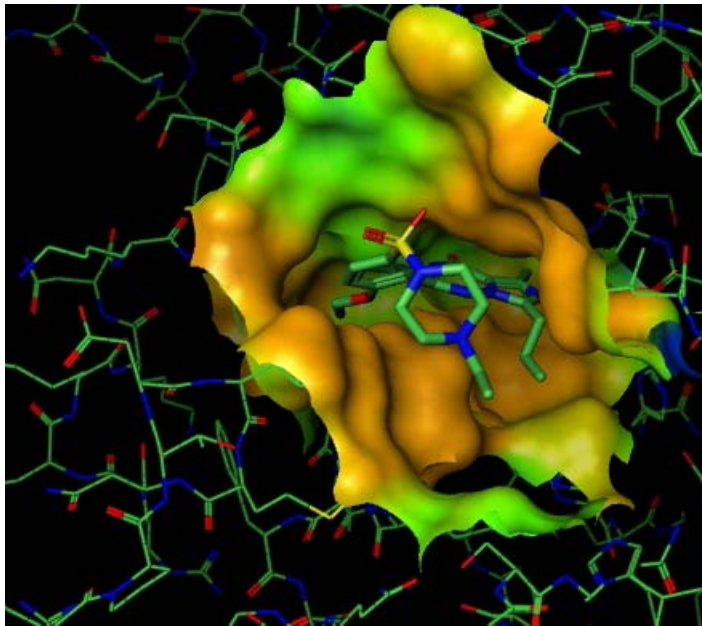


- Rate-determining step
- Functional degeneracy e.g. COX-1/2
- Pathway consequences e.g. COX/5-LO

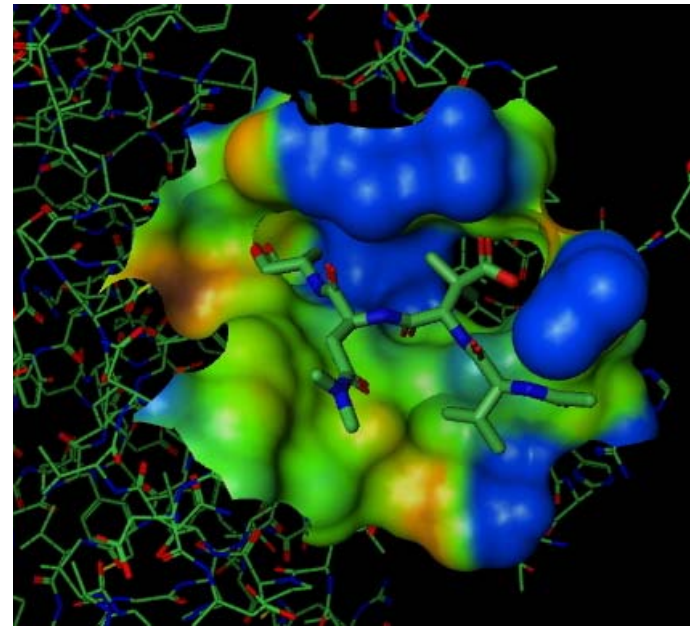
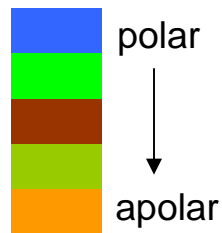
Understand the System: Receptors

- 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



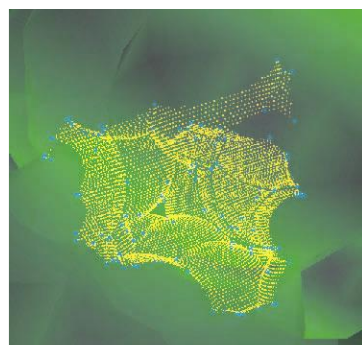
CMV protease: ugly

- 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

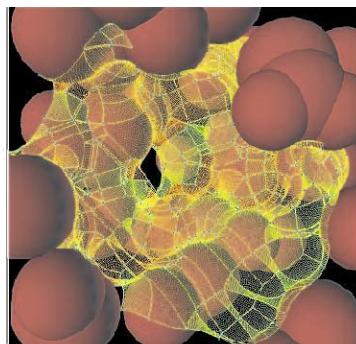
Practical approaches

- 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

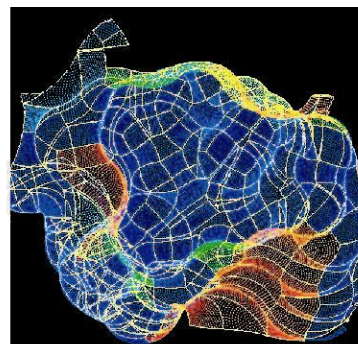
Analytical approaches



Define pocket



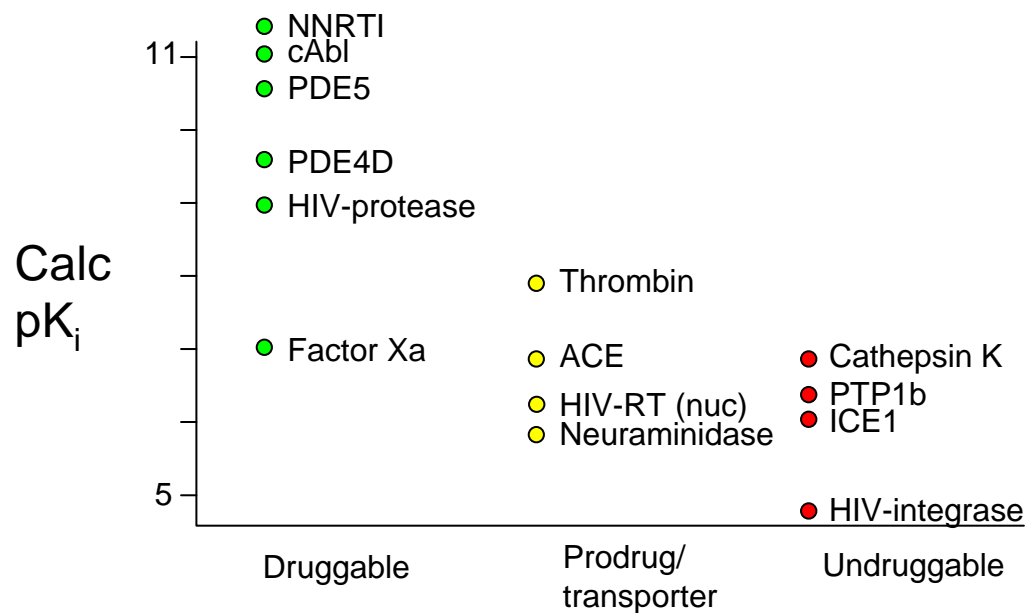
Compute non-polar SA



Compute curvature

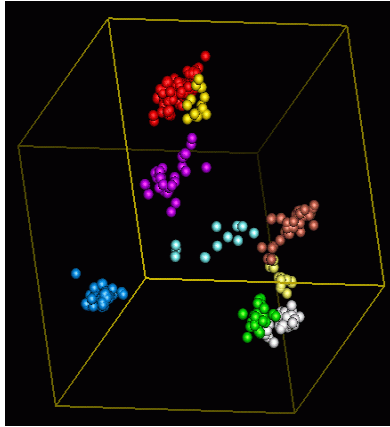


Potency estimate

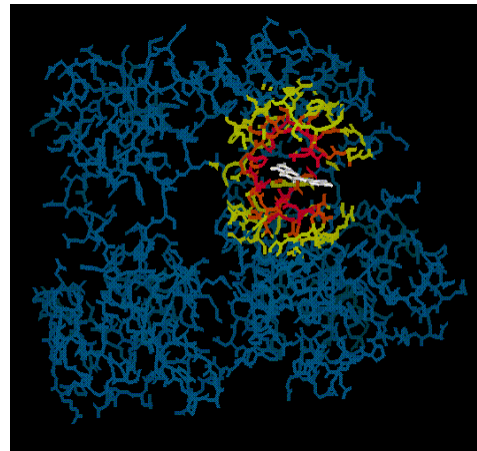


Hajduk et al. (2005) DDT 1675
 Cheng et al. (2007) Nature Biotech 25, 71
 Halgren (2009) J. Chem Inf Model 49 377

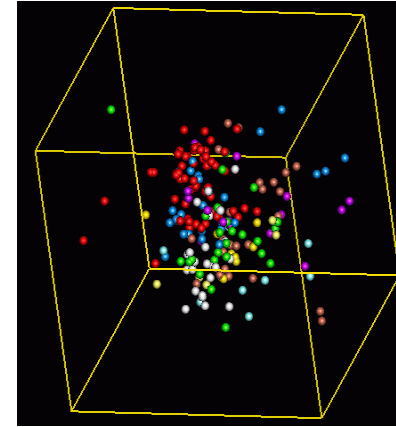
Selectivity: Site vs Whole Sequence



Kinase sequences



Identify ATP-site residues



ATP-site sequences

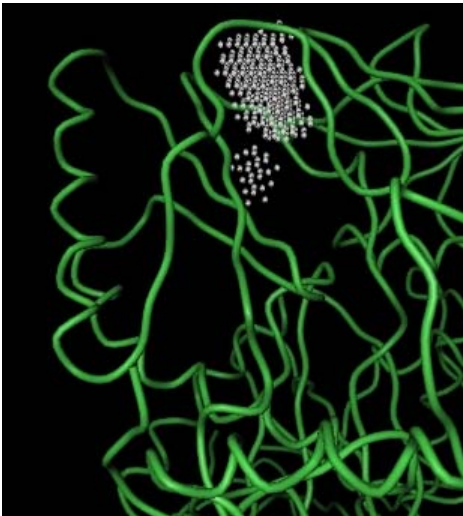
- Antifungals: broad spectrum and selectivity over human

Identity	Human	<i>C. albicans</i>	<i>A. fumigatis</i>
Total	25%	38%	35%
Site	15%	72%	78%

Caveats in Cavity Analysis

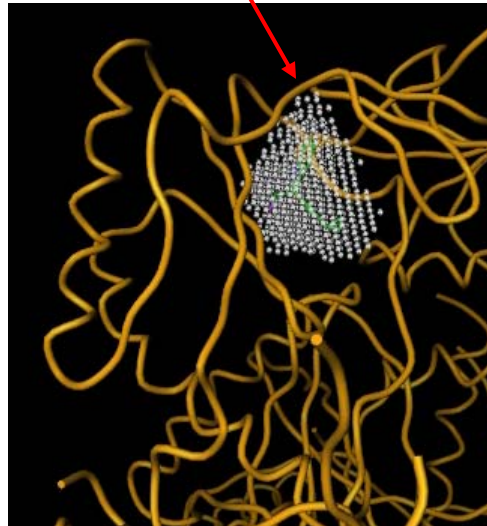
- Irreversible inhibitors do not need as much SA/burial
- Allosteric pockets induced by ligands
 - Cannot predict pocket properties from apo structures

HIV reverse transcriptase



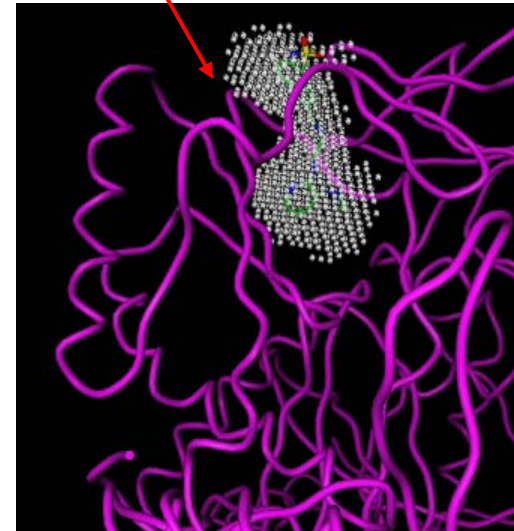
apo structure
293 Å³

loop movement



Efavirenz (MWt 316)
577 Å³

loop movement



Delavirdine (MWt 457)
823 Å³

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

TDR Targets Database

- Tropical Disease Pathogen genome database

The TDR Targets Database
Identification and ranking of targets against neglected tropical diseases

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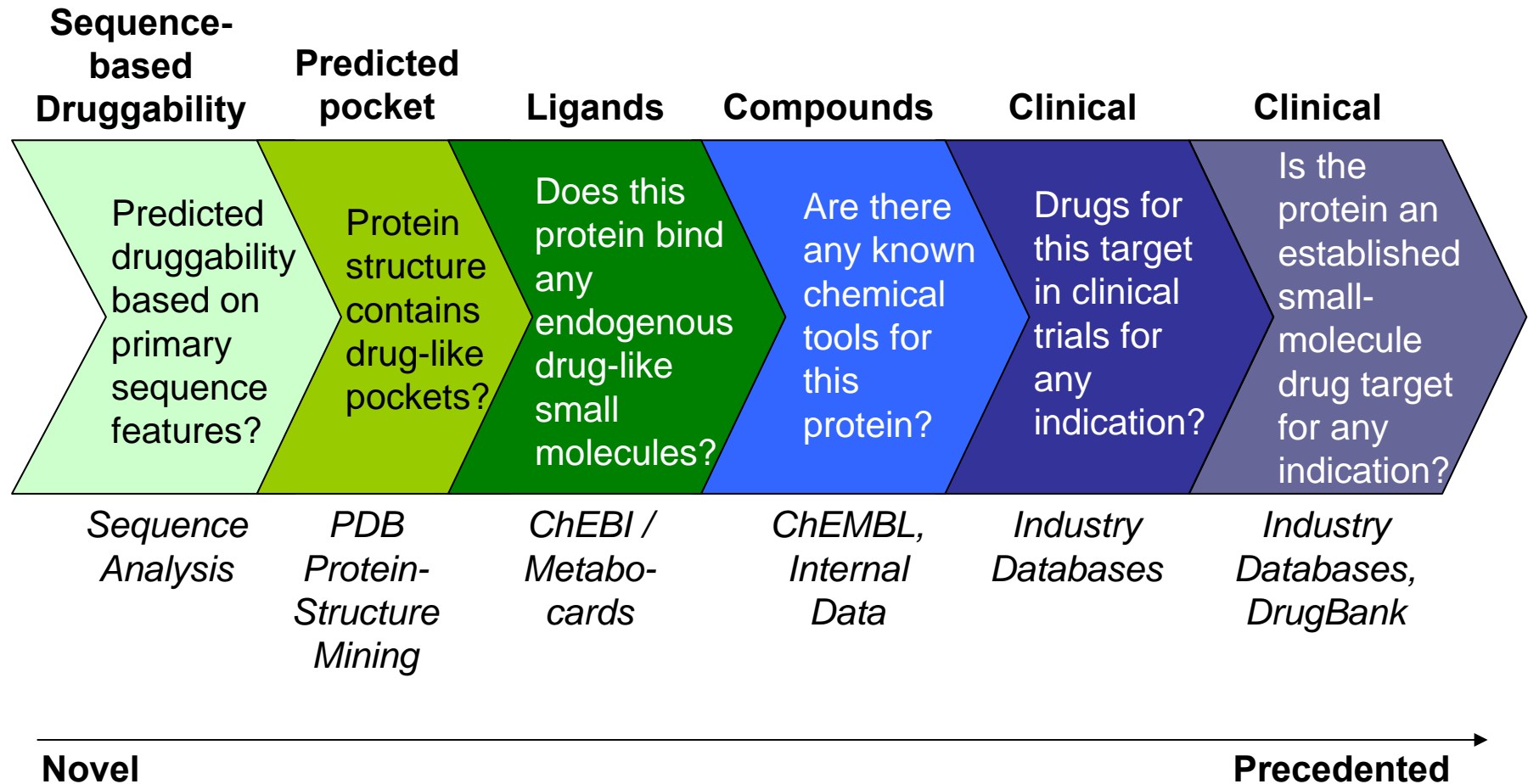
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91 records found | Showing page 1 of 4(records 1-25) | Number of records to display

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

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

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

