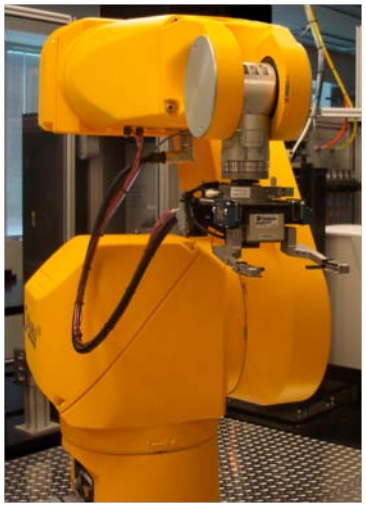
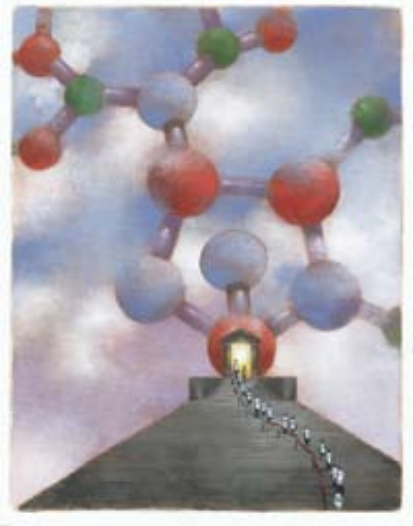


Scientific Presentation:

The Molecular Libraries Roadmap

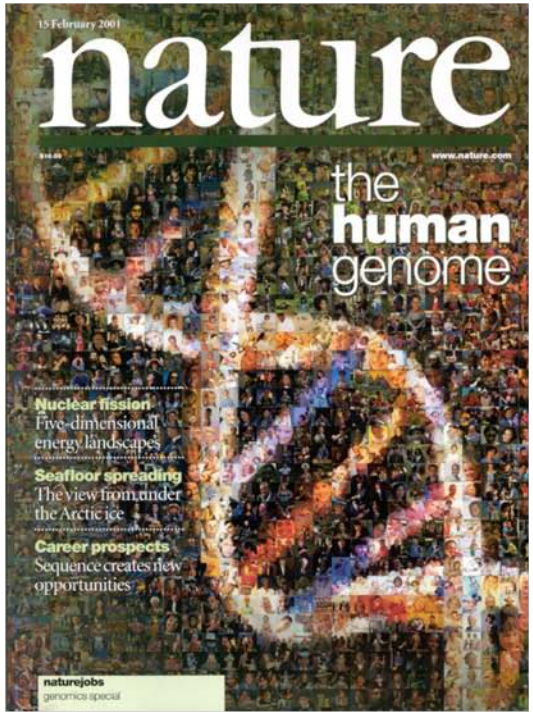


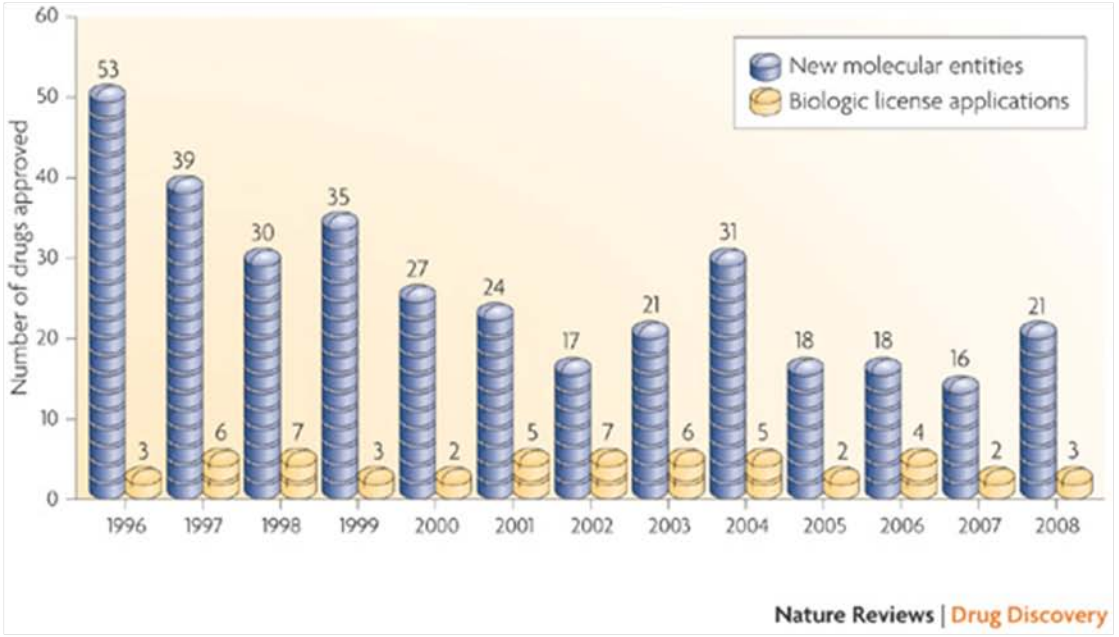
Christopher P. Austin, M.D.
Director, NIH Chemical Genomics Center
Senior Advisor to the Director for Translational Research, NHGRI

NIH Council of Councils
November 16, 2009

NIH Chemical Genomics Center

The best of times, the worst of times





How to translate the genome into biological insights and therapeutics?

Molecular Libraries Initiative: Rationale

- Urgent need to determine function of genes
  - Small molecules complementary to molecular genetic tools
  - Act on PROTEIN, most proximate to physiology
- Urgent need to catalyze development of therapeutics for rare and orphan diseases
  - >6000 rare diseases
  - Genetic basis of many known
  - Treatments available for <200

NIH Roadmap ACCELERATING MEDICAL DISCOVERY TO IMPROVE HEALTH

Home Page

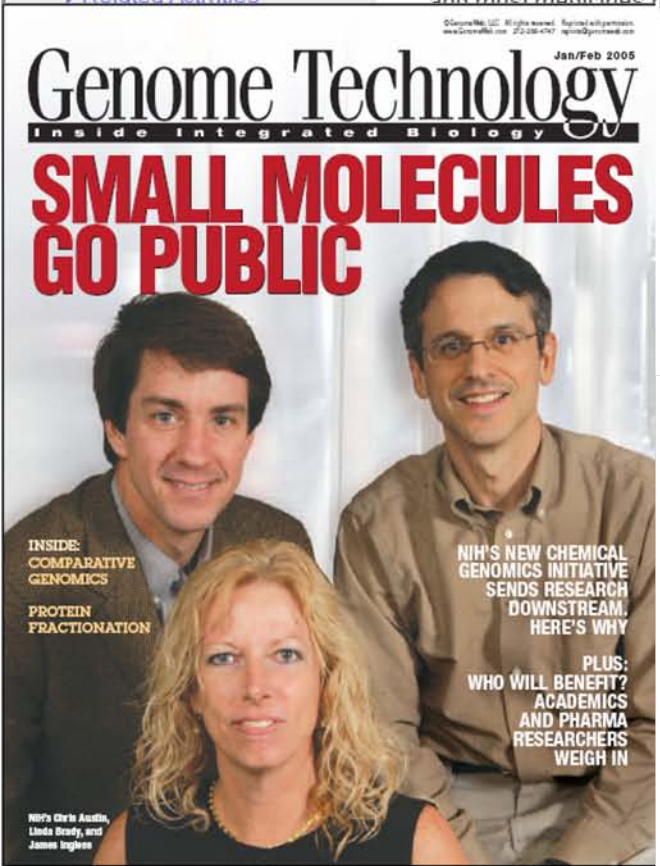
Molecular Libraries and Imaging

- Overview
- Implementation Group Members
- Funding Opportunities
- Funded Research
- Related Activities

Molecular Libraries and Imaging

OVERVIEW

Small molecules, often with molecular weights of 500 or below, have proven to be extremely important to researchers to explore function at the molecular, cellular, and in vivo level. Such molecules have also been proven to be valuable for treating diseases, and most medicines marketed today are from this class.



"...To empower the research community to use small molecule compounds in their research, whether as tools to perturb genes and pathways, or as starting points to the development of new therapeutics for human disease."

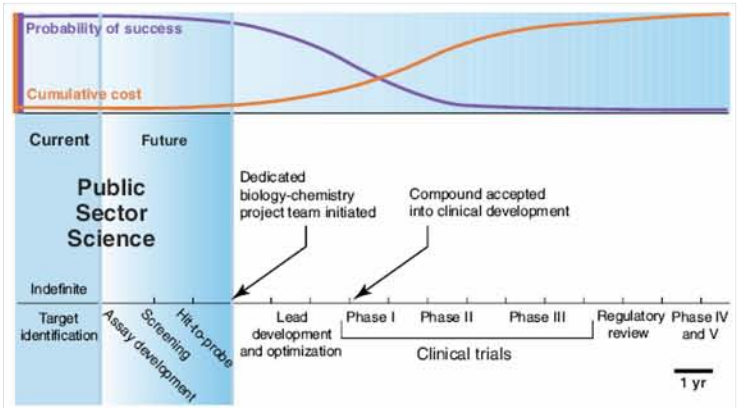
POLICY FORUM

MOLECULAR BIOLOGY

NIH Molecular Libraries Initiative

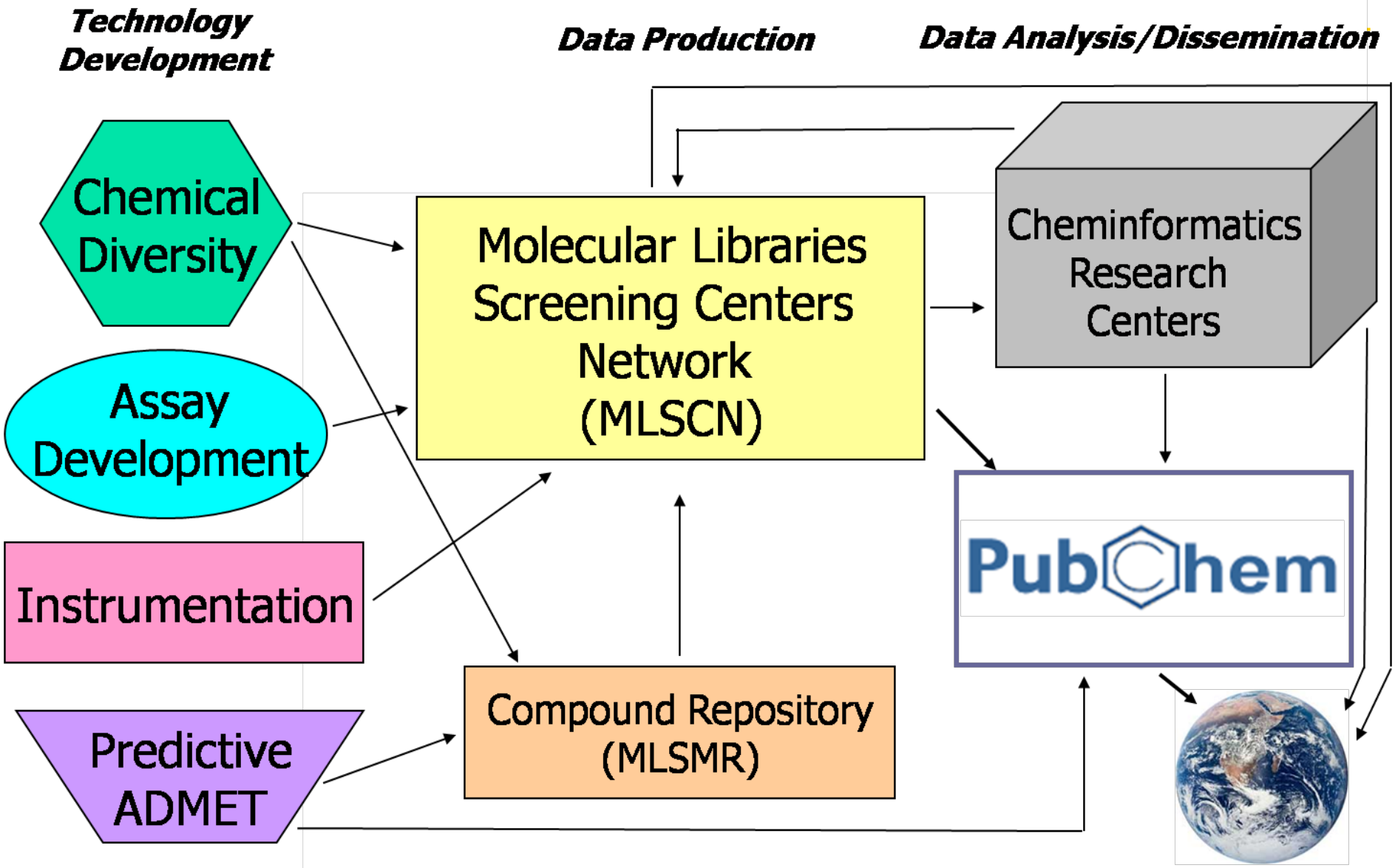
Christopher P. Austin,[1*] Linda S. Brady,[2] Thomas R. Insel,[2] and Francis S. Collins[1]

12 NOVEMBER 2004 VOL 306 SCIENCE www.sciencemag.org

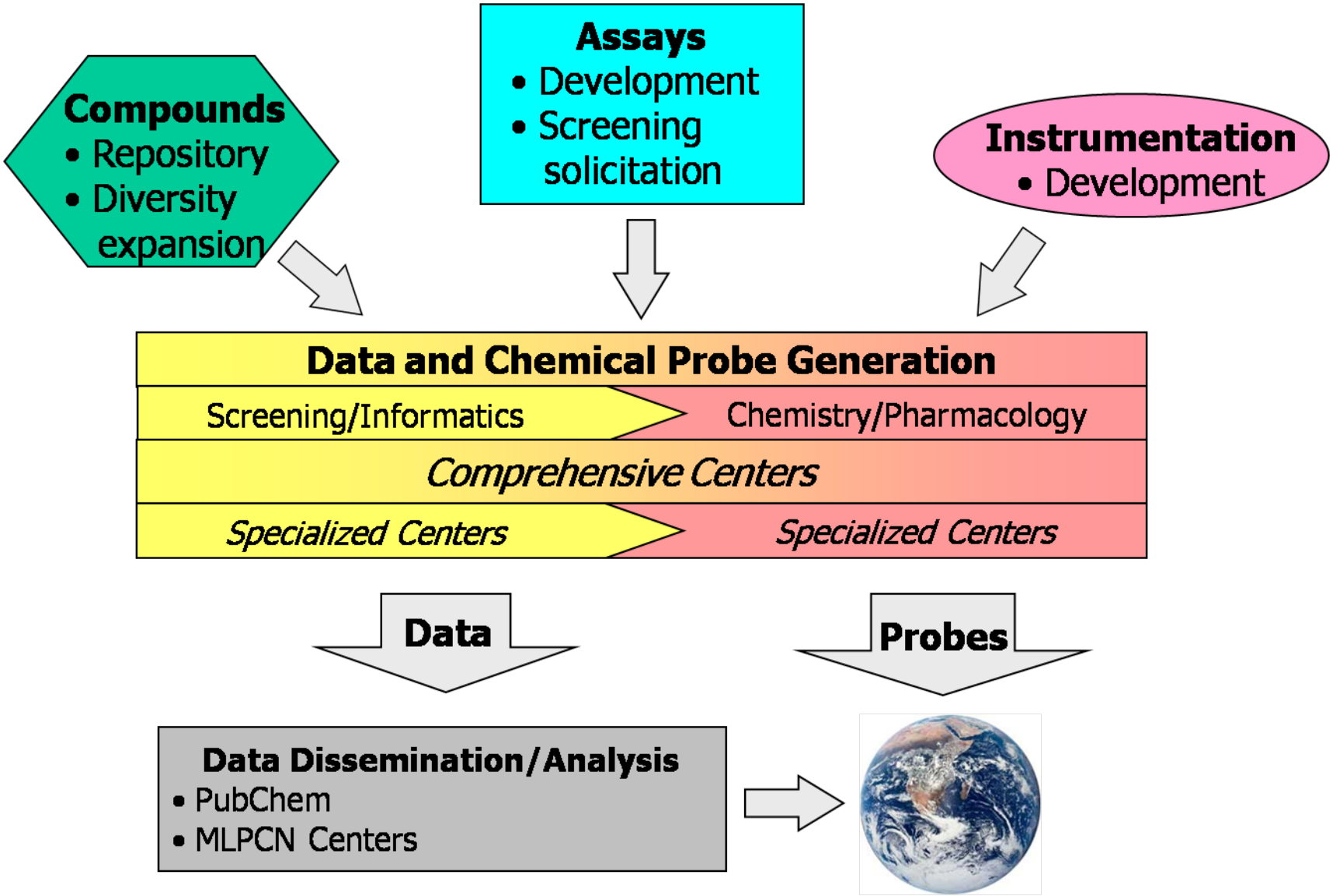


Interface of the MLI and drug development.

The Molecular Libraries Roadmap: Pilot Phase FY2004-2007



The Molecular Libraries Roadmap: Formal Phase FY2008-2013



http://mli.nih.gov

Home | MLP Overview | Access MLPCN Resources | Tech Development | Compound Repository | Data | Funding | MLP Probes | FAQ | Contacts

Pilot Program | Publications | News & Events | NIH Resources

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MLP

CARS

CARS Training

Assay Wiki

Assay Annotation

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type, hit enter

Related Links

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NIH Roadmap

Home

Overview

The Molecular Libraries Program (MLP; an NIH Roadmap Initiative) aims to enhance chemical biology efforts through High Throughput Screening (HTS) to obtain small molecule probes effective at modulating a given biological process or disease state. More ...

Molecular Libraries Probe Production Centers (MLPCN)

The flagship of the MLP is the Molecular Libraries Probe Production Centers, a network of national laboratories, whose aim is generate novel small molecule probes by performing HTS, secondary screens and medicinal chemistry. The assays for these probes are sourced from the scientific community.

Accessing the MLPCN

The scientific community can access the resources of the MLPCN through various funding initiatives that are part of the MLP. More ...

Small Molecule Probes

The small molecule probes already identified in the pilot phase of the MLP can be found here.

Access To Results

The MLPCN (as well as the pilot phase initiative) is a public resource and as such all the biological data generated as well as the identity of the small molecules (300,000+ compounds) is in the public domain. More ...

The Centers

Find details about the MLPCN centers here.

Other Supporting MLP Initiatives

The Molecular Libraries Program has other initiatives intended to further develop chemical biology. These initiatives are focused on technology development and contain future funding information. More ...

Further Development of Small Molecule Probes

NIH has many pre-existing and new efforts to further develop small molecule probes resulting from the MLPCN effort. More ...

Tech Development

- Assay Development
- Chemical Diversity
- Instrumentation

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- MLP
- CARS
- CARS Training
- Assay Wiki
- Assay Annotation

Search

type, hit enter

Related Links

- NIH Homepage
- NIH Roadmap

Assay Development

This Initiative is one component of the MLP and constitutes a major NIH effort to broaden access to rapid assay technologies. This program will fund the development and adaptation of biological assays for use in automated high throughput molecular screening (HTS). It is intended that this Initiative promote the development of automated screening projects that can be submitted to the MLPCN. High throughput molecular screening (HTS) is the automated, simultaneous testing of thousands of distinct chemical compounds in models of biological mechanisms. Active compounds identified through HTS can provide the starting point in the design of powerful research tools that allow pharmacological probing of basic biological mechanisms, and which can be used to establish the role of a molecular target in a disease process, or, its ability to alter the metabolism or toxicity of a therapeutic. The immense potential of HTS to impact our understanding of biological mechanisms is largely untapped because access to automated screening facilities and large compound libraries is limited in academic, government and non-profit research sectors. Many in vitro biological models are currently used to study biological pathways, the effects of genetic perturbations and to establish a disease association. These can be adapted to high throughput formats for the purpose of screening large collections of biologically active compounds. There are a number of characteristics that make an assay suitable for high throughput approaches. The assay must be robust, reproducible and have a readout that is amenable to automated analysis. In addition, it must be possible to miniaturize the assay, for example; to a 96-well plate (or higher density) format or flow-cytometric approach. Further, the assay protocol should be simple enough for automated handling. A broad range of models share many of these features, including; biochemical assays, cellular models and certain model organisms such as yeast or C. elegans. This initiative will support the development of innovative assays for use in both basic research and in therapeutics development programs, with an emphasis on novelty of assay approach and/or novel targets and mechanisms.

Funding Opportunities

For information about the current Assay Development PAR (PAR-08-024), please click on the embedded link or contact: **Mark Scheideler, Ph.D.** (National Institute of Neurological Disorders and Stroke)

Data Sharing

As the objectives of each Assay Development for HTS may be somewhat unique, so may the particular data sharing plans associated with them. Therefore, interested investigators are strongly encouraged to review the data sharing plans of each RFA in concert with the specific aims of the particular Announcement.

For information about the previous RFA's data sharing plan, see "Section V. of Assay Development for HTS.

Funded Research

To download details on the Assay Development Funded Projects and links to abstracts and publications, click here.
The table below contain a brief summary of the document.

Assay Development Funded Research

PI NAME	TITLE	PUBLICATIONS
Bezprozvanny, Ilya B	Screen for blockers of a CaV2.2-Mint-PDZ1 association	

129 R21s funded

Tech Development

Assay Development
Chemical Diversity
Instrumentation

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Chemical Diversity

The goals of this initiative are two-fold:

- to support the development of new methodologies related to natural products chemistry; and
- to support the production of chemical libraries in unexplored regions of "chemical diversity space" where there is reason to believe that novel bioactivities may be found.

Related to the first goal, during the Pilot Phase six R01 grants were made with MLP funds to support the development of new methodologies for natural products chemistry. The rationale behind this funding initiative is that over the years, a large proportion of approved drugs have originated from, or were inspired by, natural products (i.e., small molecules made by specialized pathways, often in plants, marine invertebrates, and microorganisms). This is not surprising, since natural products have evolved in order to enhance the survival of the organisms that produce them–in other words, for their bioactivites. However, in recent years, most pharmaceutical companies have moved away from natural products as leads for drug discovery and development. This often may be due in large part to the limitations of current methodologies for natural products chemistry, including production, isolation, purification, and characterization. This will enhance the availability of biologically active natural products for screening by the MLPCN as well as for drug discovery and development.

The second Chemical Diversity Technology Development goal is being addressed through a series of P41 (Biotechnology Research Resource) grants. Under the terms of these grants, the grantees are providing novel libraries to the MLSMR, for HTS evaluation by the MLPCN. A key provision of this funding initiative is that the library designs are driven by biological rather than purely chemical considerations. Additionally, since the goal is to identify effectors of truly novel biological phenotypes and mechanisms, the compounds produced under this initiative represent chemotypes that are distinct from what is available commercially and in existing compound collections.

The projects funded under RFA-RM-05-013 and RFA-08-004 are designed to address such bottlenecks and to afford improved procedures.

Links to all current MLI funding opportunities: Funding Opportunities

For information about the current Pilot Scale Libraries for High-Throughput Screening (P41) RFA, see RFA-RM-08-003.

For information about the current New Methodologies for Natural Products Chemistry (R01) RFA, see RFA-RM-08-004.

For more information, please contact:

John M. Schwab, Ph.D.
National Institute of General Medical Sciences

>40 R01s and P41s funded

Tech Development

Assay Development
Chemical Diversity
Instrumentation

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MLP
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CARS Training
Assay Wiki
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Instrumentation

This Screening Instrumentation program is focused on development of innovative instrumentation for high throughput screening of synthetic chemical and natural product libraries such as the ones that will be registered and housed in the NIH-sponsored Molecular Libraries Screening Center Network. High throughput molecular screening (HTS) is the automated, rapid testing of thousands of distinct small molecules or probes in cellular models of biological mechanisms or disease, or in biochemical or pharmacological assays. Active compounds identified through HTS can provide powerful research tools to elucidate biological processes through chemical genetic approaches, or can form the basis of therapeutics or imaging agent development programs. HTS has experienced revolutionary changes in technology since the advent of molecular biology and combinatorial chemistry, and the incorporation of modern information management systems. Current HTS instrumentation allows screening of hundreds of thousands of compounds in a single day at a rate orders of magnitude greater than was possible a decade ago. However, there are still bottlenecks which currently limit HTS capacity, such as (a) compound collection maintenance, tracking, and disbursement, and (b) rapidity, accuracy, and content of assay instrumentation. This program sought proposals to develop HTS instrumentation that is not only faster and more efficient than currently available systems, but also substantially more sensitive with high levels of specificity, reproducibility, and accuracy. Eight R01 grants were awarded in 2005.

Funding Opportunities

For information about the current Instrumentation (R01) RFA, see RFA-RM-08-020 or contact one of the following:

Ajay, Ph.D
Division of Extramural Research
National Human Genome Research Institute
5635 Fishers Lane
Suite 4076, MSC 9305
Rockville, MD 20892-9305 (regular mail)
Rockville, MD 20852 (courier mail)
Telephone: (301) 496-7531

Brenda Korte, Ph.D
National Institute of Biomedical Imaging and Bioengineering
Co-Leader, MLI Instrumentation Project Team

>20 R01s funded

BioAssay | Compound | Substance

GO Advanced search

Chemical structure search | BioActivity analysis

New PubChem searches now display a "Selected Records" panel. more ...

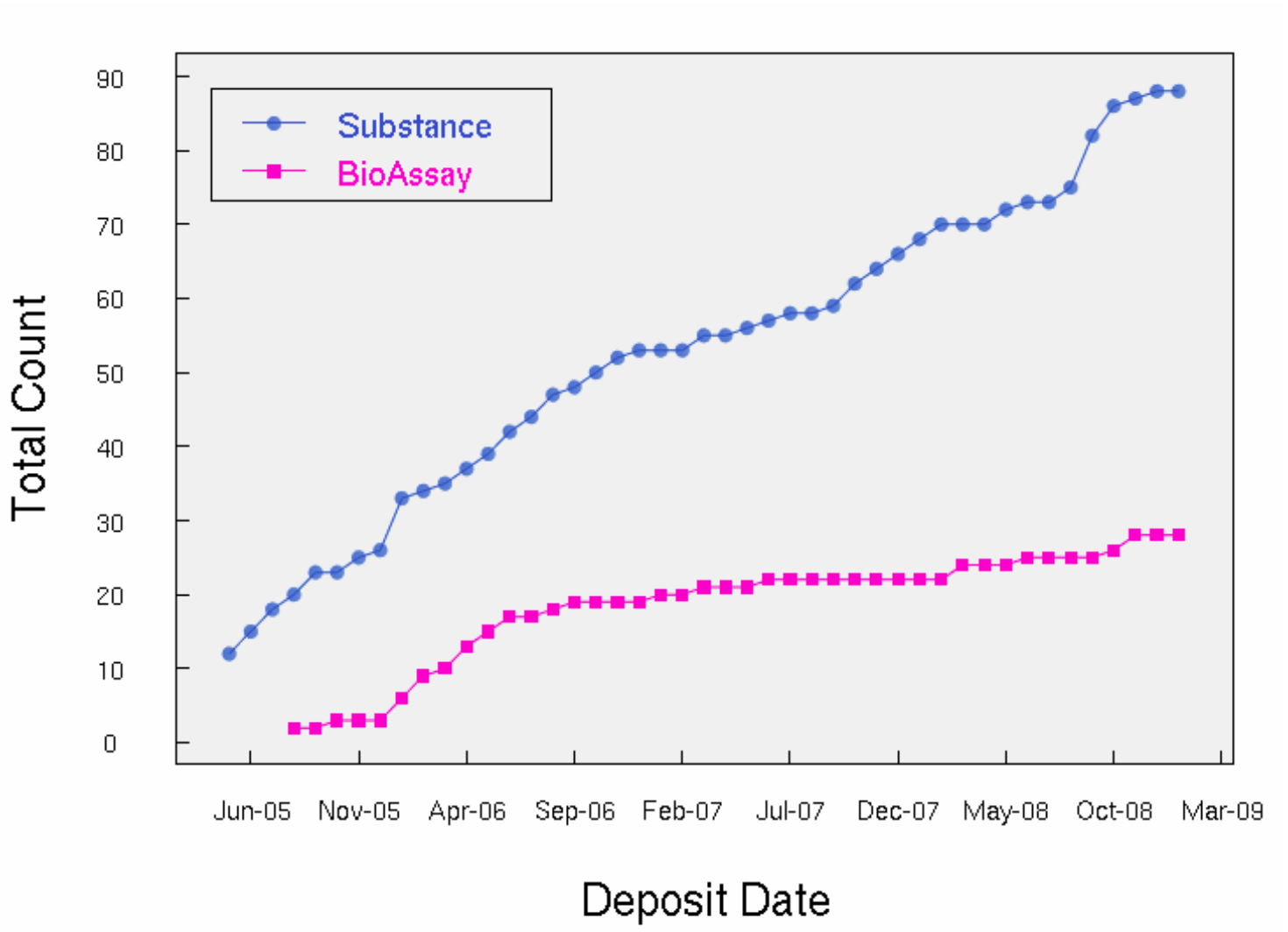
New Structures from the **Ganolix** are now available in PubChem.

more ...

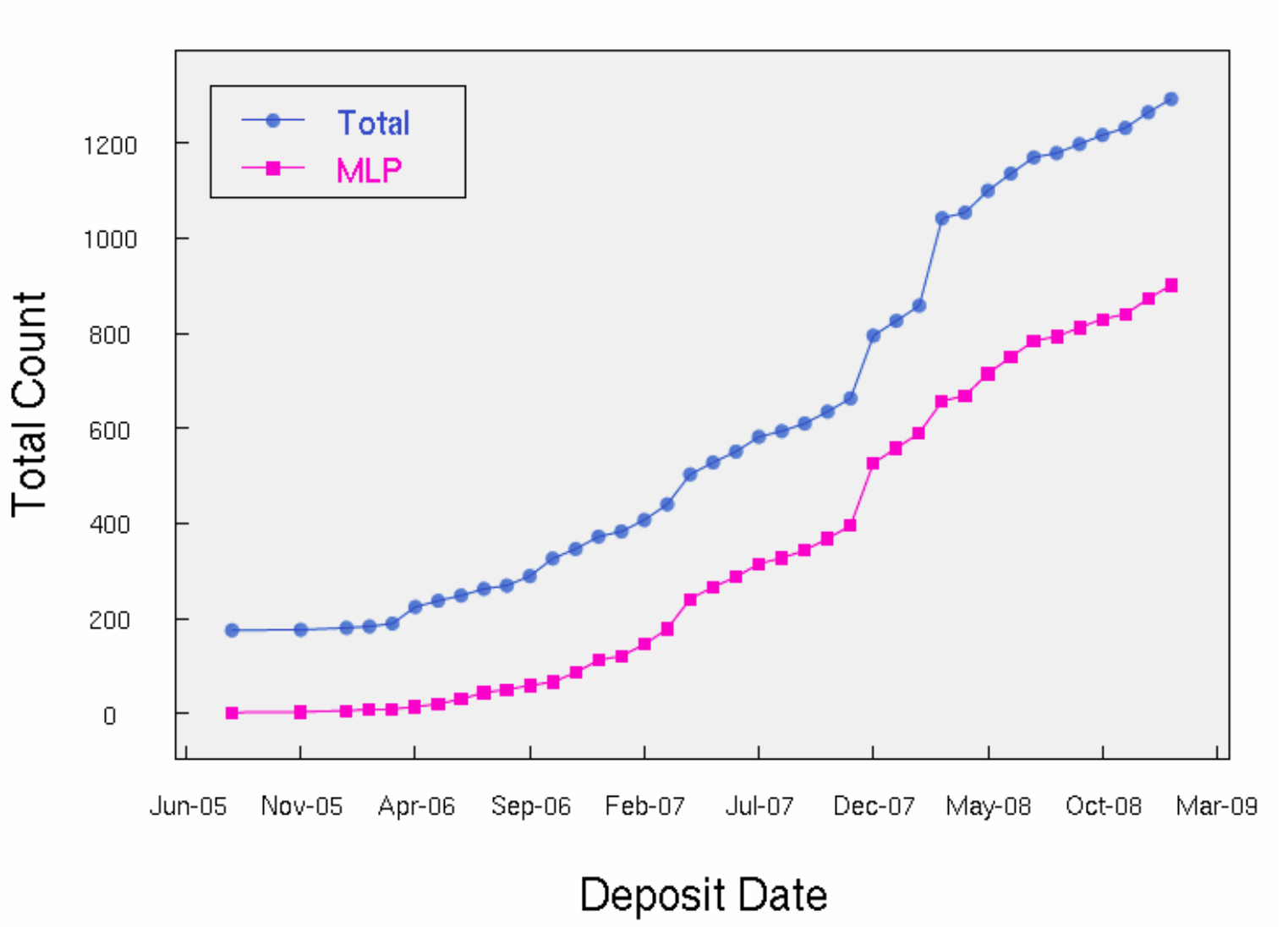
Bioactivity summary
Bioactivity datatable
Bioactivity structure-activity
Chemical structure search
3D conformer viewer
Chemical structure clustering
Deposition gateway
Structure download
PubChem FTP

National Center for Biotechnology Information
NLM | NIH | HHS

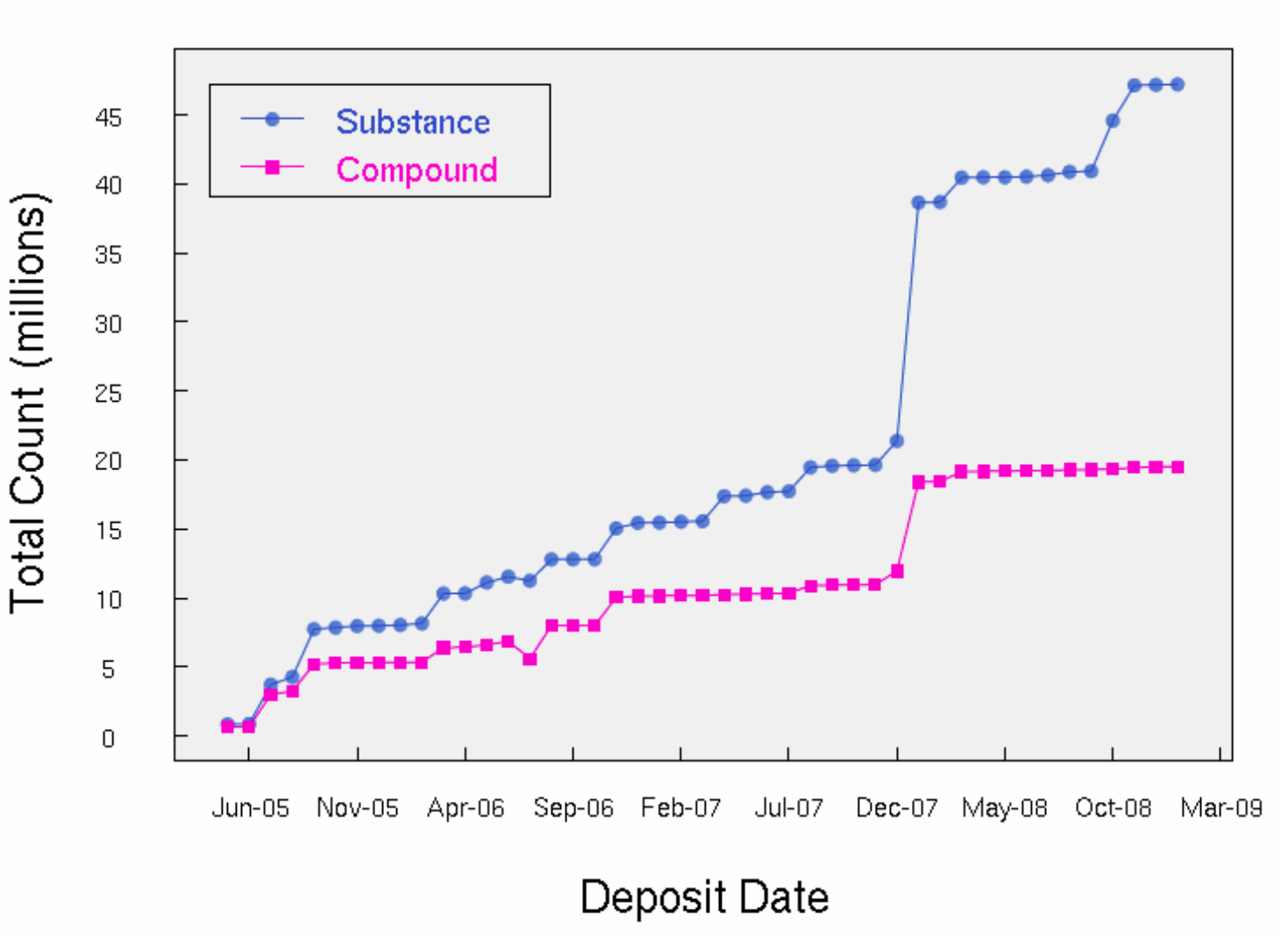
Growth In PubChem Contributing Organizations



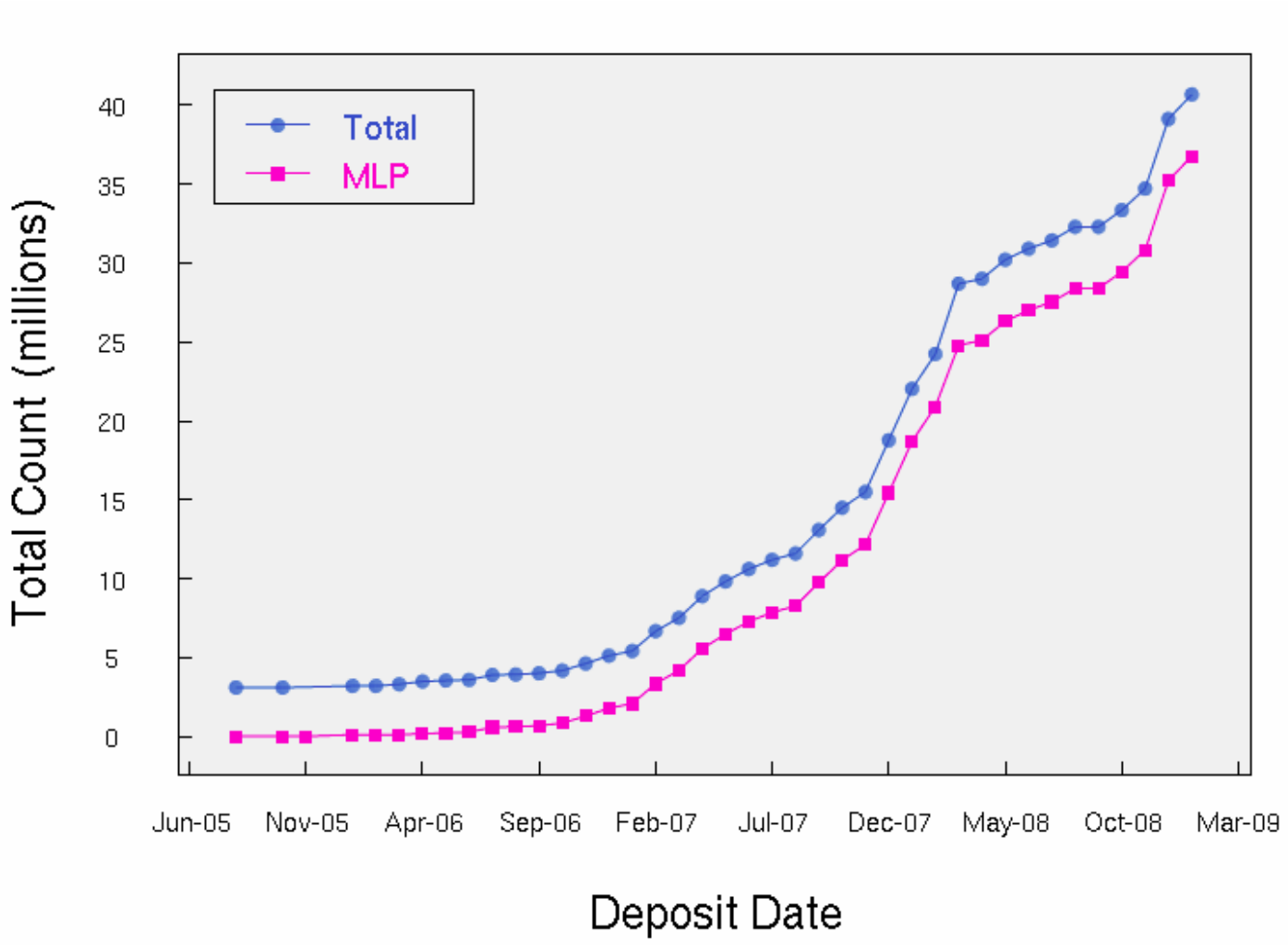
Growth In PubChem BioAssays



Growth In PubChem Substances / Compounds



Growth In PubChem Tested Substances



NIH MOLECULAR LIBRARIES
SMALL MOLECULE REPOSITORY

BioFocus
A Galápagos Company

A Roadmap Initiative

- Home
- MLSMR Project
  - Compound Identification
  - Quality Control
  - Sample Storage
  - Sample Arrays
  - Informatics
- MLPCN Centers
- MLSMR Contacts
- Submit Compounds

Registered Users Login

Welcome

NIH Molecular Libraries Small Molecule Repository collects samples for high throughput biological screening and distributes them to the NIH Molecular Libraries Probe Production Centers Network. Learn more.

MLSMR is a key component of the Molecular Libraries Initiative, an NIH Roadmap project supporting New Pathways to Discovery in the 21st century. The project is funded in whole with Federal funds from the National Institutes of Health, Department of Health and Human Services, under Contract No. HHS-N-278-2004-41001C.

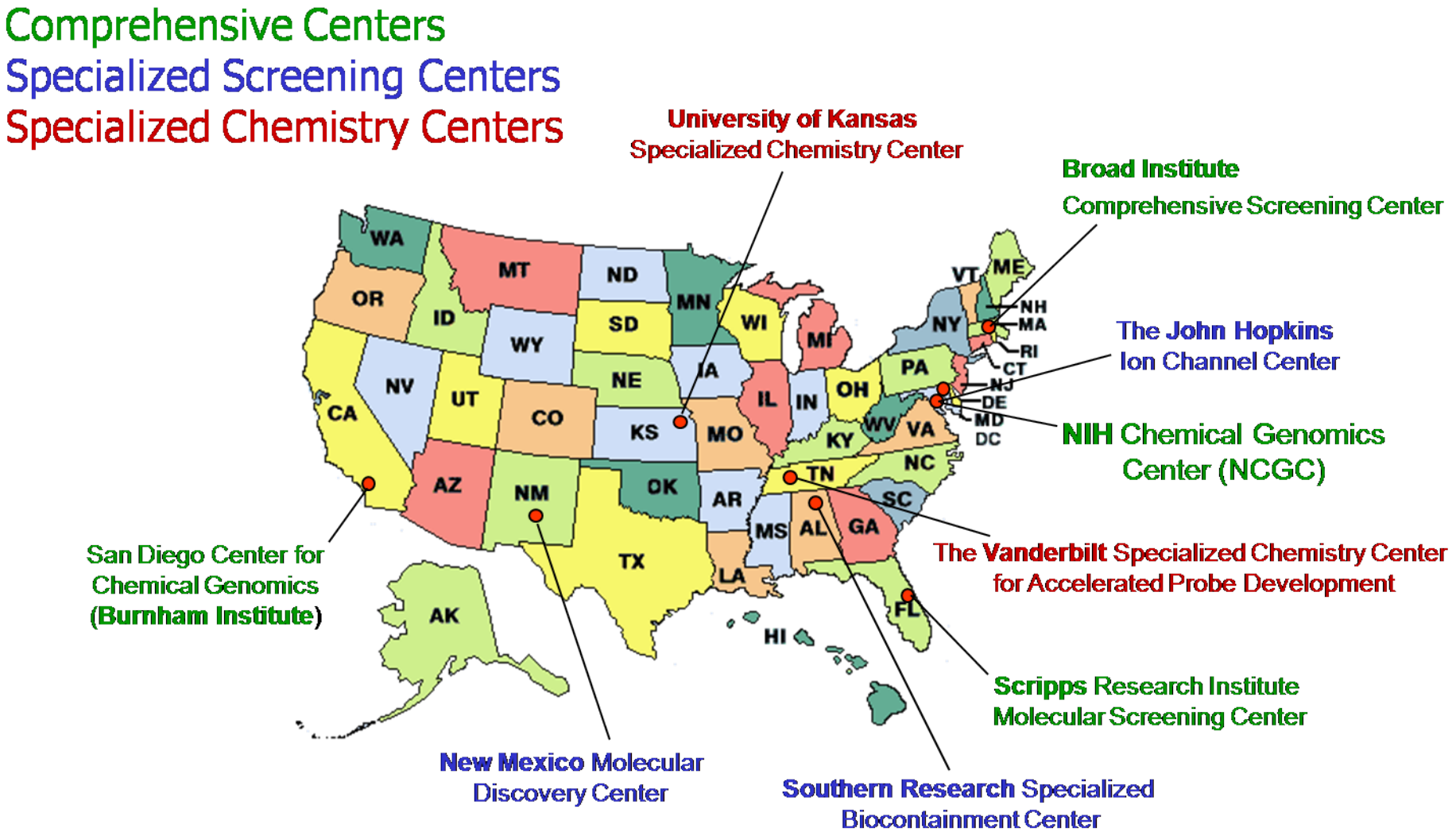
In the news:

Behind the Scenes at the NIH Molecular Libraries Small Molecule Repository

The NIH Molecular Libraries Small Molecule Repository is now selling the NIH Clinical Collection

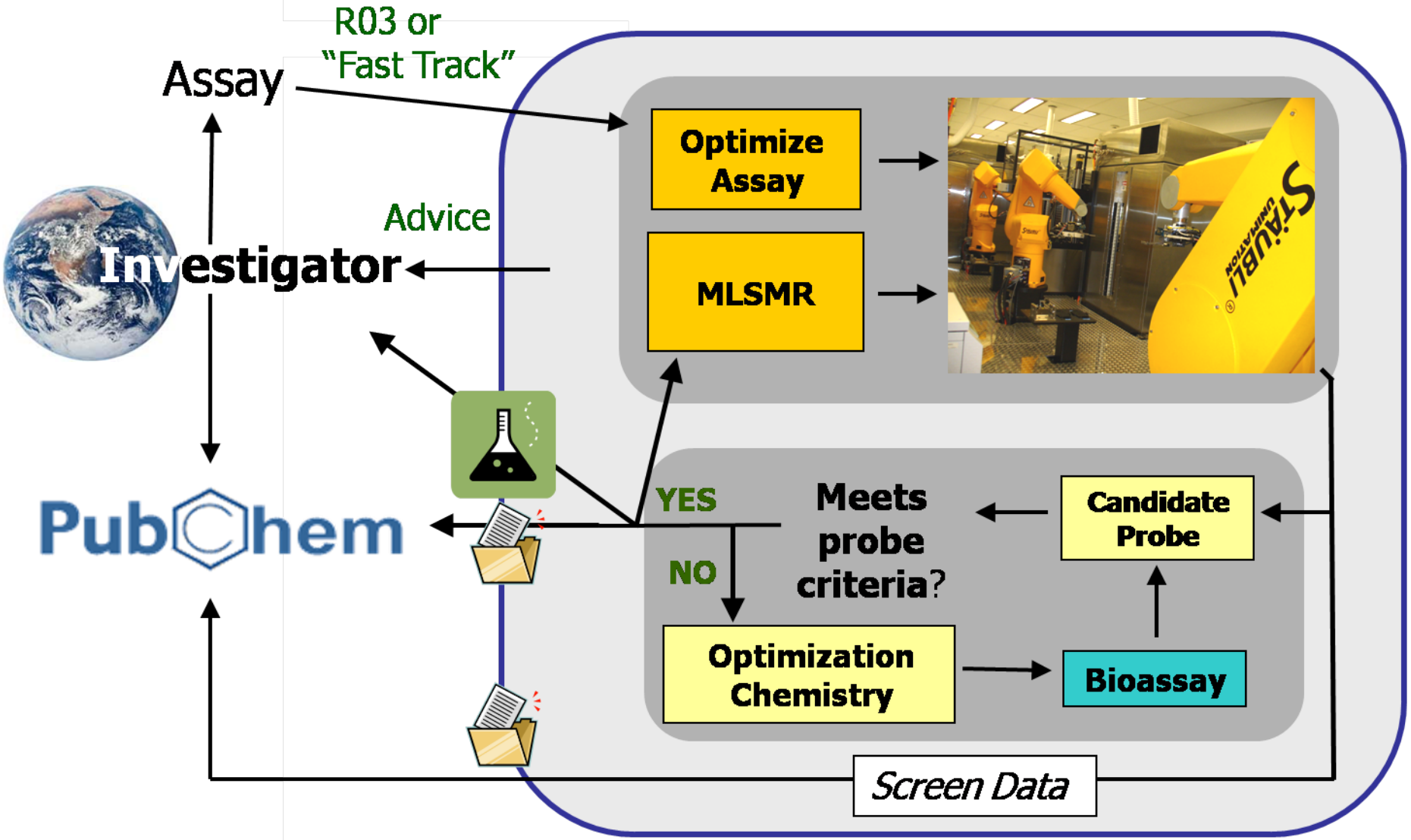
BioFocus, a Galapagos company operates MLSMR in South San Francisco.

The Molecular Libraries Probe Production Network (MLPCN)



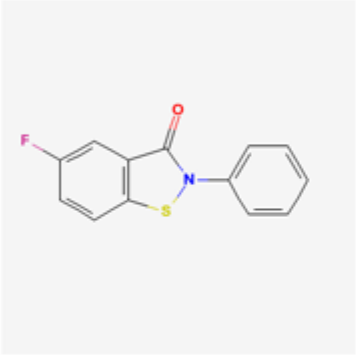
NIH Chemical Genomics Center

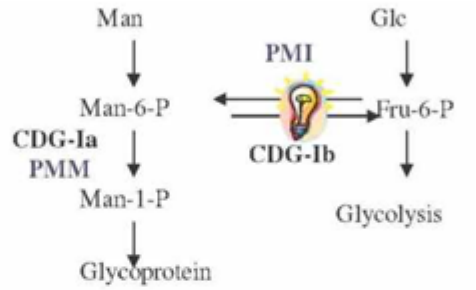
MLPCN Operation



The Excel Probe Report Web Table contains information on all probes: Probe Report Web Table (updated November 2, 2009)
To download a list of upcoming probe report titles in Excel format click here: Upcoming Probe Reports (updated November 2, 2009)

Latest Reports



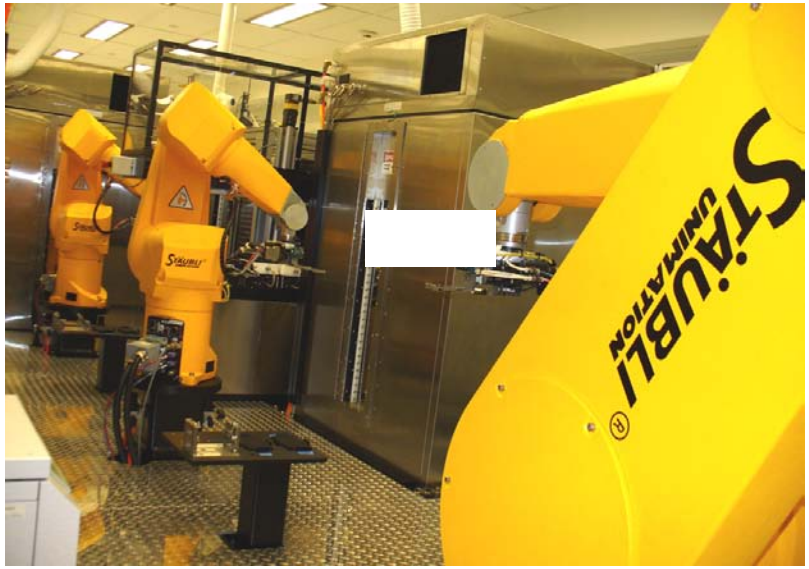


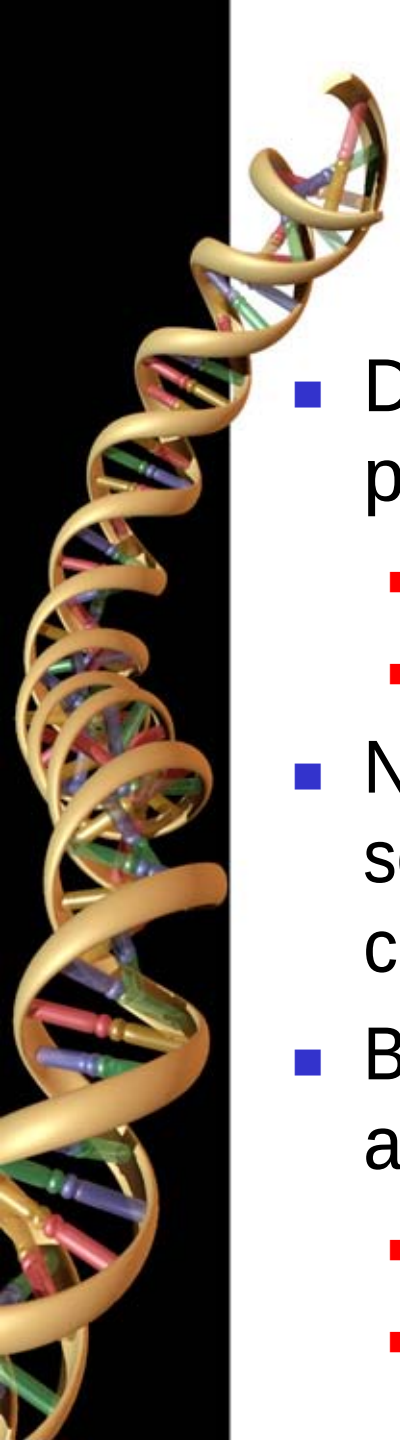
Mannose Metabolism. Mannose-6-P can be derived from fru-6-P via PMI. Alternatively, Man-6-P is derived from Man via hexokinase. Man-6-P is then converted into Man-1-P by PMM. Defects in this enzyme cause CDG-Ia and defects in PMI cause CDG-Ib. Our idea is that providing Man to PMM-deficient CDG-Ia cells along with the desired inhibitor of PMI will drive more Man-6-P into the glycosylation pathway rather than sending it toward glycolysis.

Probe Target and Type:	**Therapeutic Inhibitors of Phosphomannose Isomerase**
Assay Center:	**John Reed, Burnham Center for Chemical Genomics**
Chemistry Center:	**John Reed, Burnham Center for Chemical Genomics**
Assay Provider:	**Hudson Freeze, Burnham Institute for Medical Research**
Specific Aim:	**To develop novel phosphomannose isomerase (PMI) inhibitors for further characterization and therapeutics of Congenital Disorders of Glycosylation (CDG).**
IC50/EC50:	**1,300 nM**
AntiTarget and Selectivity:	**PMM2 []**
Chemical Probe (Pubchem Id):	**57287553**
Pubchem Summary BioAssay ID:	**1545**
Publications (PubMed Ids):	**Unavailable (see probe report for details)**
Probe Report:	**Click to Download**
Date Submitted:	**4/21/2009**

NIH Chemical Genomics Center

- Founded 2004 as part of ML Roadmap
- 75 scientists
- Over 100 collaborations with investigators worldwide
  - 70% NIH extramural
  - 10% NIH intramural
  - 20% Foundations, Research Consortia, Pharma/Biotech
- Focus on novel targets, rare/neglected diseases



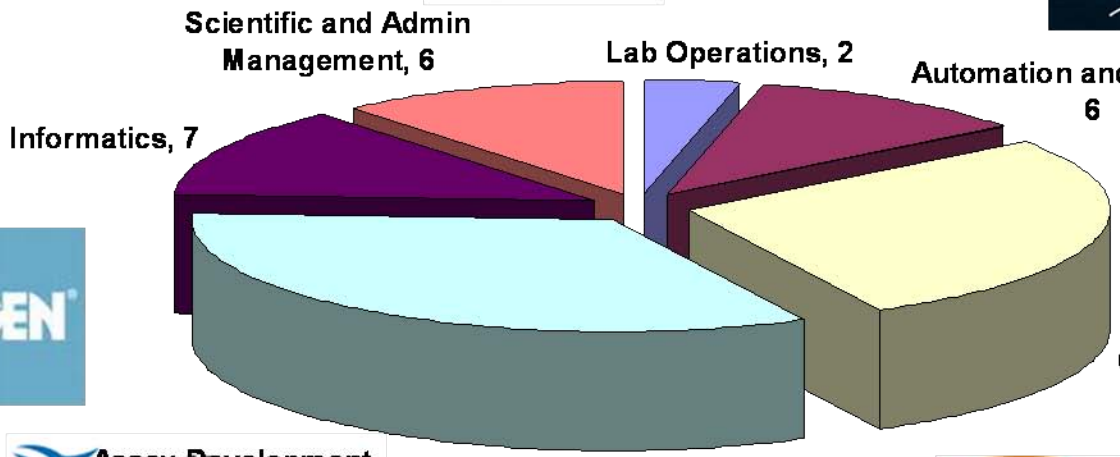


NIH Chemical Genomics Center: Mission

- Develop chemical probes of proteins, pathways, and cellular processes
  - Unprecedented targets and neglected diseases
  - Starting points for drug development
- New paradigms for assay development, screening, cheminformatics, and medicinal chemistry
- Broadly profile chemical space for biological activity
  - Therapeutics
  - Toxicology

NCGC
NIH Chemical Genomics Center

NCGC Staff

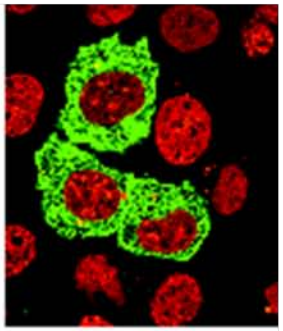


Amphora
Lilly
LARGE SCALE BIOLOGY
CELERA
an Applera Corporation Business
Pfizer
HGS
NORTHROP GRUMMAN
DEFINING THE FUTURE
ARQULE
AMGEN
Caliper LifeSciences
Johnson & Johnson
gsk GlaxoSmithKline
MERCK
Pharmacopeia
BD
BD Biosciences
Lexicon pharmaceuticals
NCGC
NIH Chemical Genomics Center

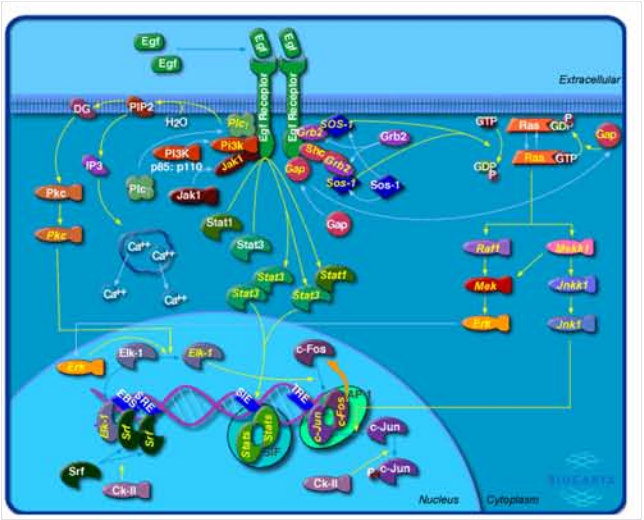
Range of screening assays performed

Extent of reductionism

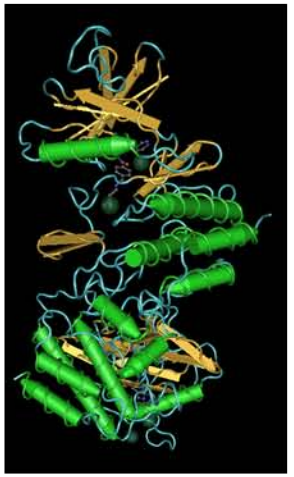
Phenotype
(Image-based HCS, GFP, etc)



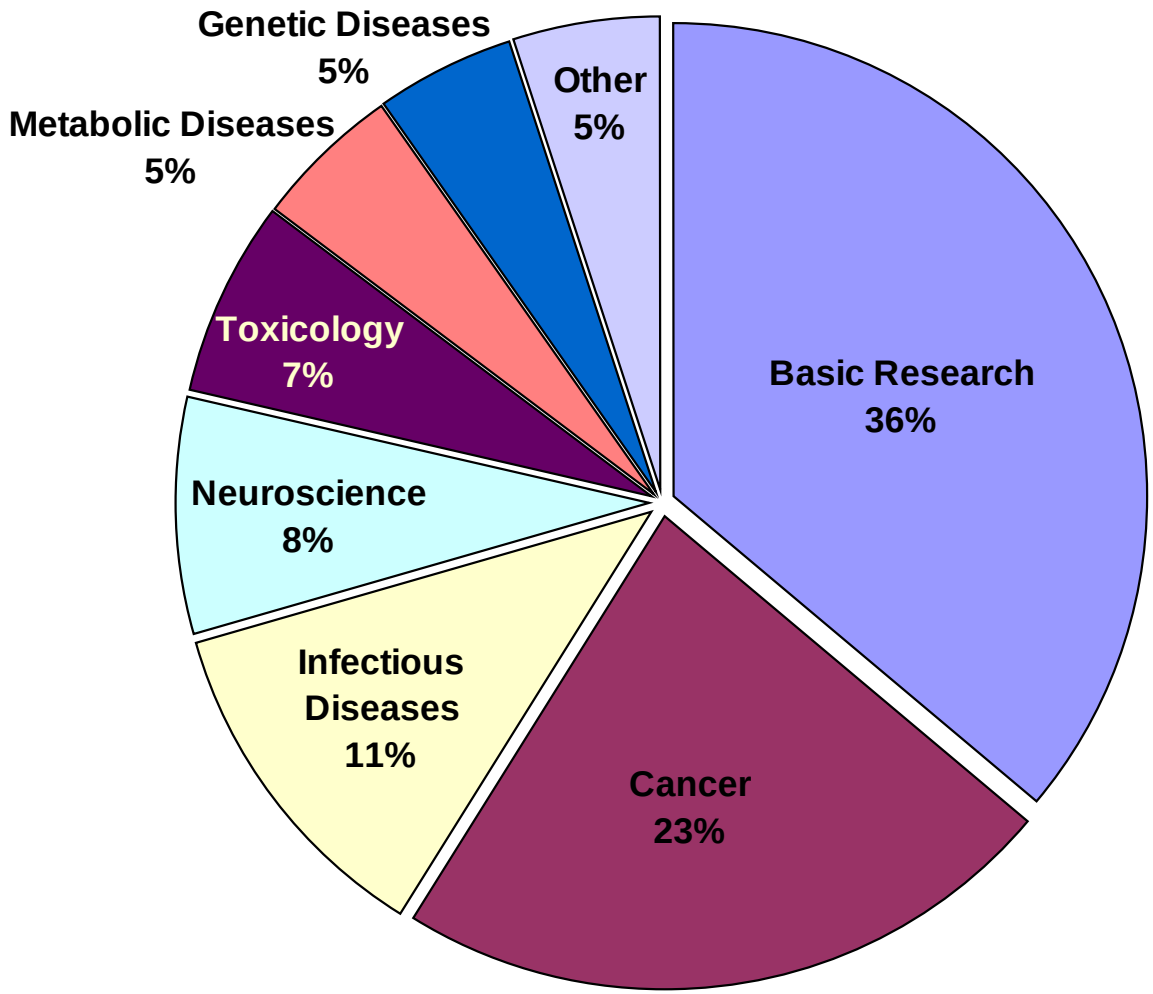
Pathway
(Reporters, e.g., luciferase, β-lactamase)



Protein
(Enzyme readouts, interactions, etc)

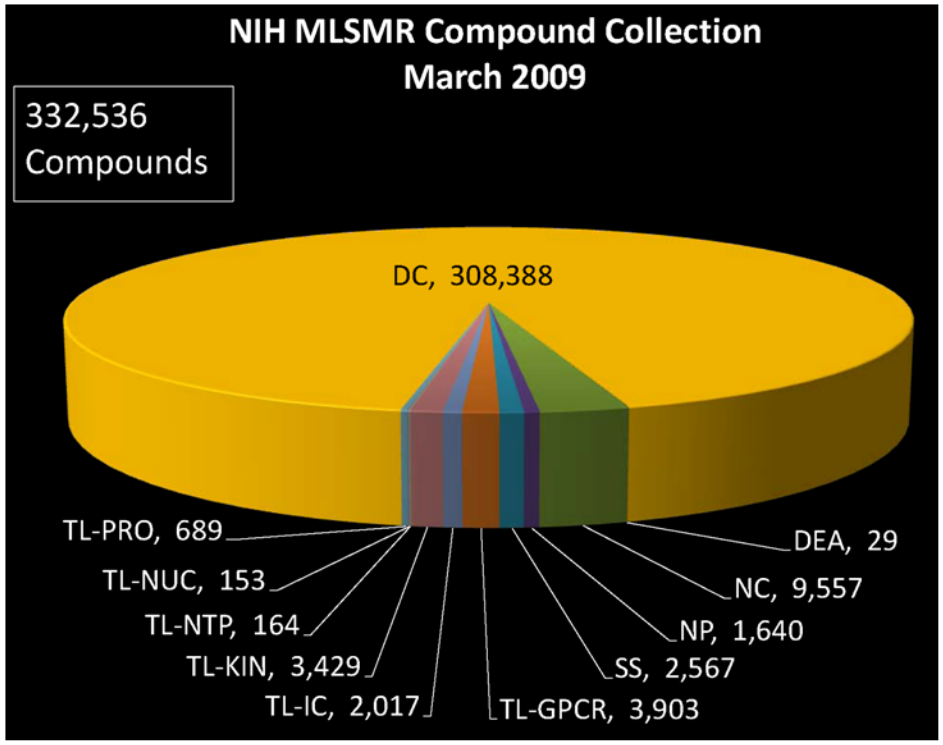


Disease areas of NCGC assays



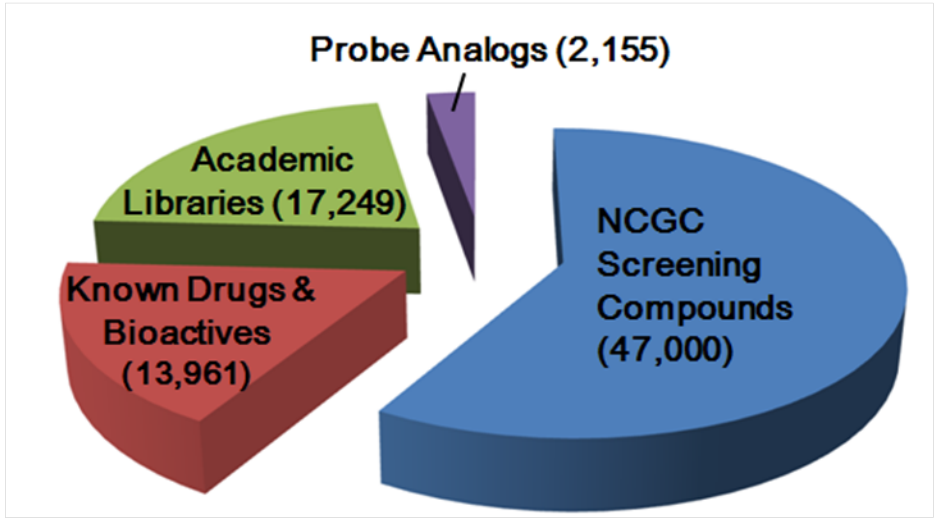
NIH Chemical Genomics Center

NCGC Screening Collections



MLSMR

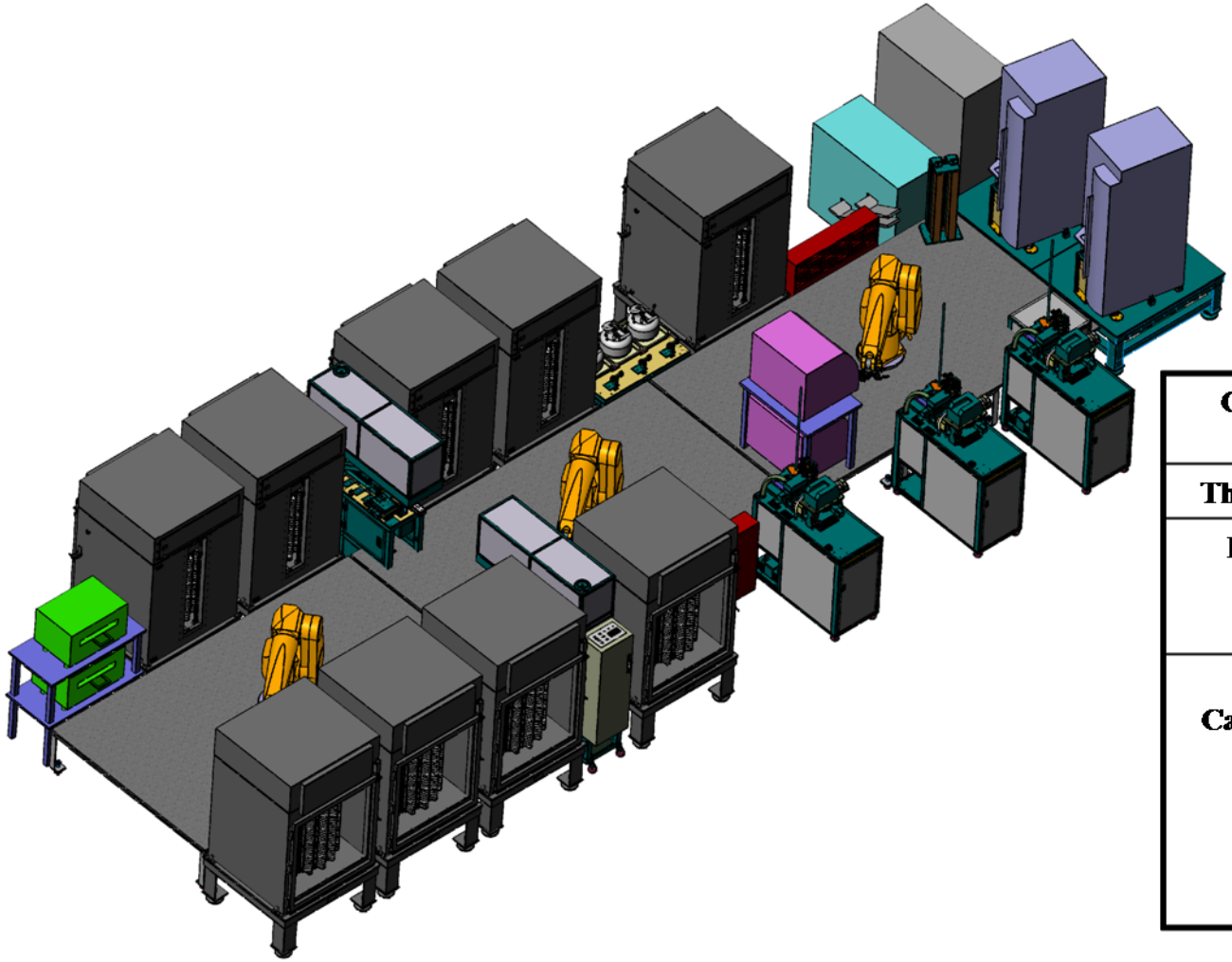
DC = Diversity Compounds
NC = Non-commercial
TL-KIN = Kinase Targeted Library
TL-GPCR = GPCR Targeted Library
TL-IC = Ion Channel Targeted Library
TL-PRO = Protease Targeted Library
TL-NUC = Nuclear Receptor Targeted
TL-NTP = National Toxicology Program
SS = Known Bioactives
NP = Natural Products
DEA = DEA Controlled Substances



- NCGC Screening Compounds
  - Diversity Libraries (54.8%)
  - Focused Libraries (3.7%)
- Known Drugs & Bioactives
  - Specialty Libraries (11.9%)
  - Other Commercial Suppliers (2.0%)
  - Toxic Collection (3.5%)
- Academic Libraries
  - BU CMLD (2.4%)
  - UP CMLD (1.7%)
  - KU CMLD (0.9%)
  - Torrey Pines (Combinatorial) (8.1%)
  - U. Georgia (Fractionated NPs) (2.6%)
  - U. Michigan (Fractionated NPs) (5.7%)
  - U. Wisconsin (Peptides) (0.1%)
  - UCSF (0.1%)
- Probe Optimization Analogs
  - NCGC Internal Synthesis (0.6%)
  - Commercial Suppliers (2.1%)

NCGC
NIH Chemical Genomics Center

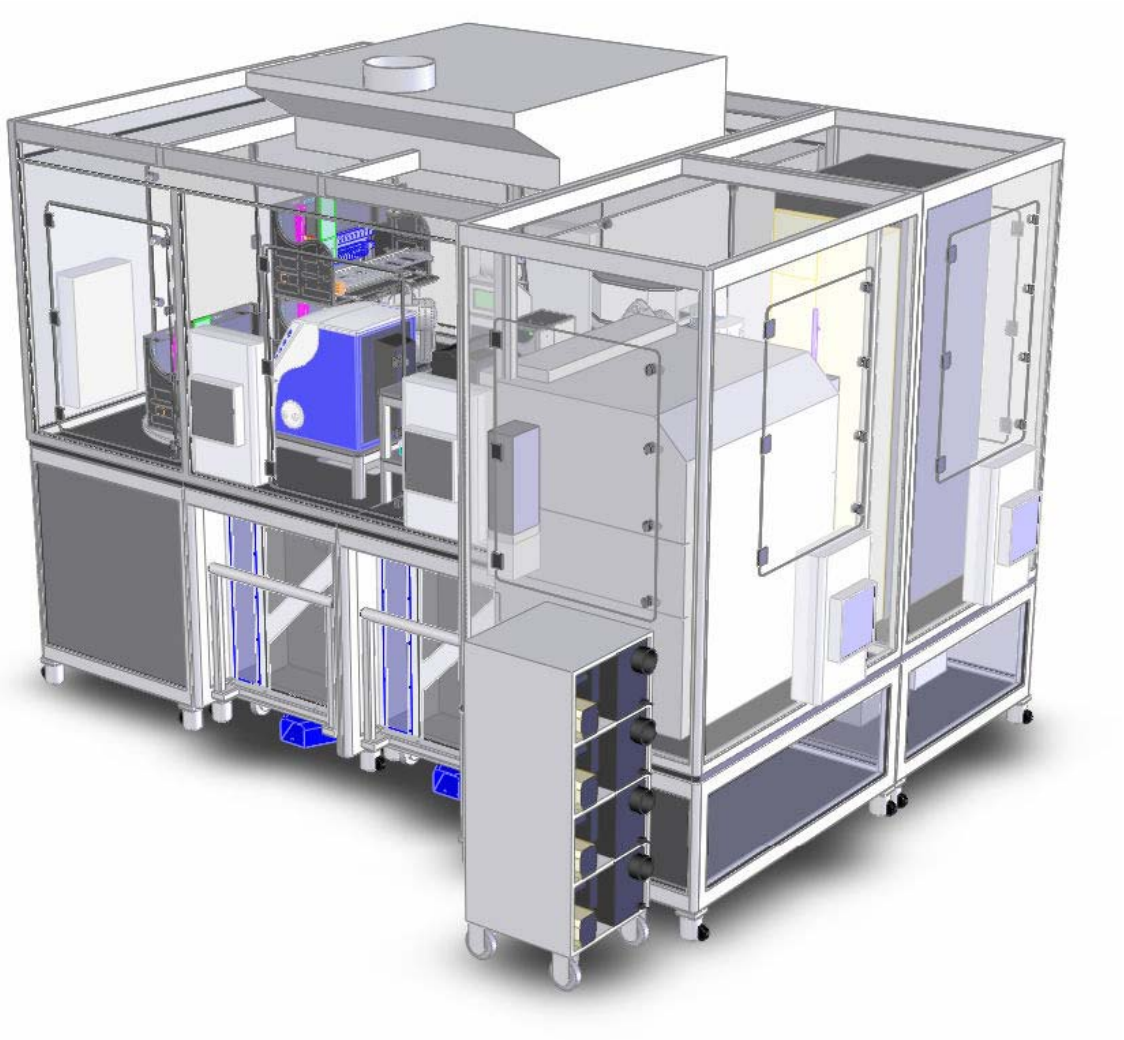
NCGC Screening System 1: BSL1/Kalypsys



Capacity:	**3.0MM Assay Wells 5.0MM Compound Wells**
Throughput:	**1400 plates/day**
Readers:	**ViewLux (2) Acumen (2) Envision (2)**
New Capabilities:	**Automatic Loading and Unloading stations using commercially available plate stackers Dispense Inspection Systems using integrated CCD cameras**

NIH Chemical Genomics Center

NCGC Screening System 2: BSL2/HRE



Capacity:	**370K Assay Wells 370K Compound Wells**
Throughput:	**~240 plates/day**
Readers:	**ViewLux Acumen Hamamatsu**
New Capabilities:	**Modular approach to HTS Three docking stations to quickly facilitate changing of both compound and assay plate storage Flexible scheduling software to allow for complex assay methods BSL2 rated to further diversify the assays capable of being run by NCGC**

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NCGC Screening System 3: BSL3/Beckman

Quantitative High-Throughput Screening (qHTS)

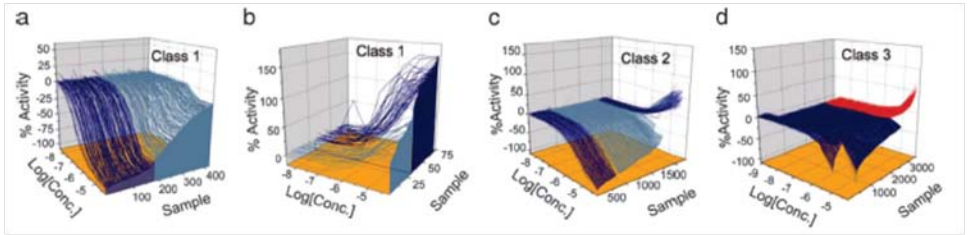
- Conventional HTS done at single concentration
  - typically 10 uM
- qHTS assays compounds at multiple concentrations
  - No known bioactivity: 7 concs
  - Known bioactivity: 15 concs
  - Range = 2nM – 100uM
  - 1536-well plate format, assay volume ~5 uL, ~1000 cells/well
  - Concentration-response curve generated for each compound from primary screen
- Produces robust ***activity profiles*** of all compounds
  - Dramatically reduced FP and FN
  - 4-6 months saved compared to conventional HTS
- Informatics pipeline for data processing, curve fitting & classification, extraction of SAR

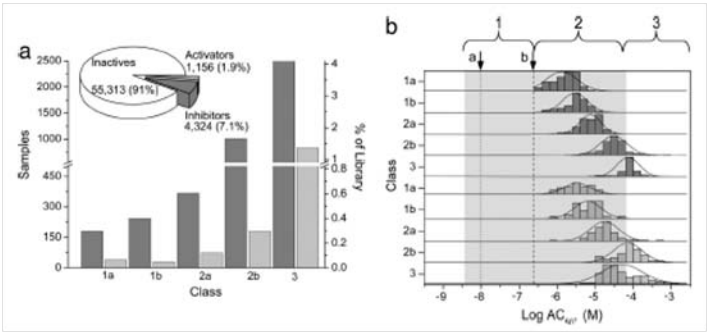
Quantitative high-throughput screening: A titration-based approach that efficiently identifies biological activities in large chemical libraries

James Inglese*, Douglas S. Auld, Ajit Jadhav, Ronald L. Johnson, Anton Simeonov, Adam Yasgar, Wei Zheng, and Christopher P. Austin

NIH Chemical Genomics Center, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892-3370

Communicated by Francis S. Collins, National Institutes of Health, Bethesda, MD, May 31, 2006 (received for review April 12, 2006)





PNAS | August 1, 2006 | vol. 103 | no. 31 | 11473–11478

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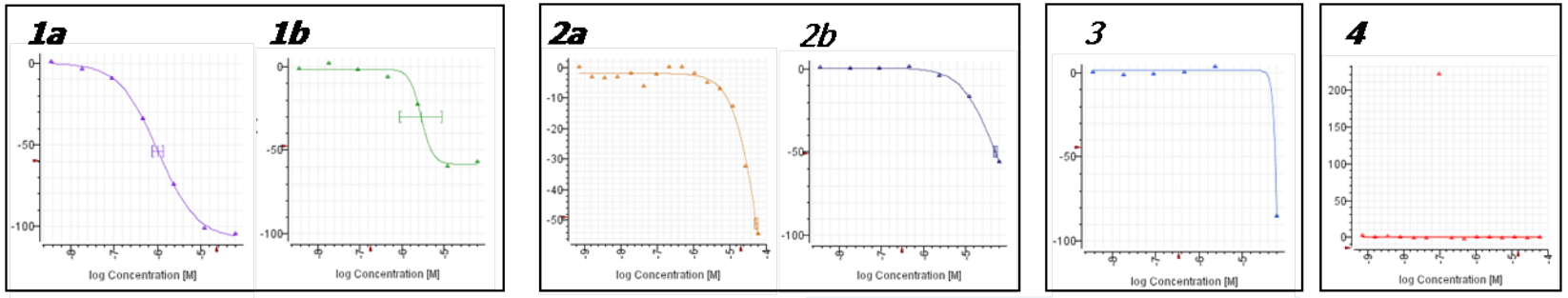
qHTS curve classification criteria

Curve Class	Description	Efficacy	r^2	Asymptotes	Inflection
1*	Complete curve (a) Partial curve (b)	> 80% (a) ≤ 80% (b)	≥ 0.9	2	yes
2†	Incomplete curve	> 80% (a) < 80% (b)	> 0.9 (a) < 0.9 (b)	1	yes
3	Single pt activity	> Min‡	NA	1	no
4	Inactive	NA	NA	0	no

NOTES: *AC_{50} derived from data; †AC_{50} extrapolated from data; ‡Min is > 3 SD from the mean activity of the sample field at the highest tested concentration

Examples

Lower confidence data



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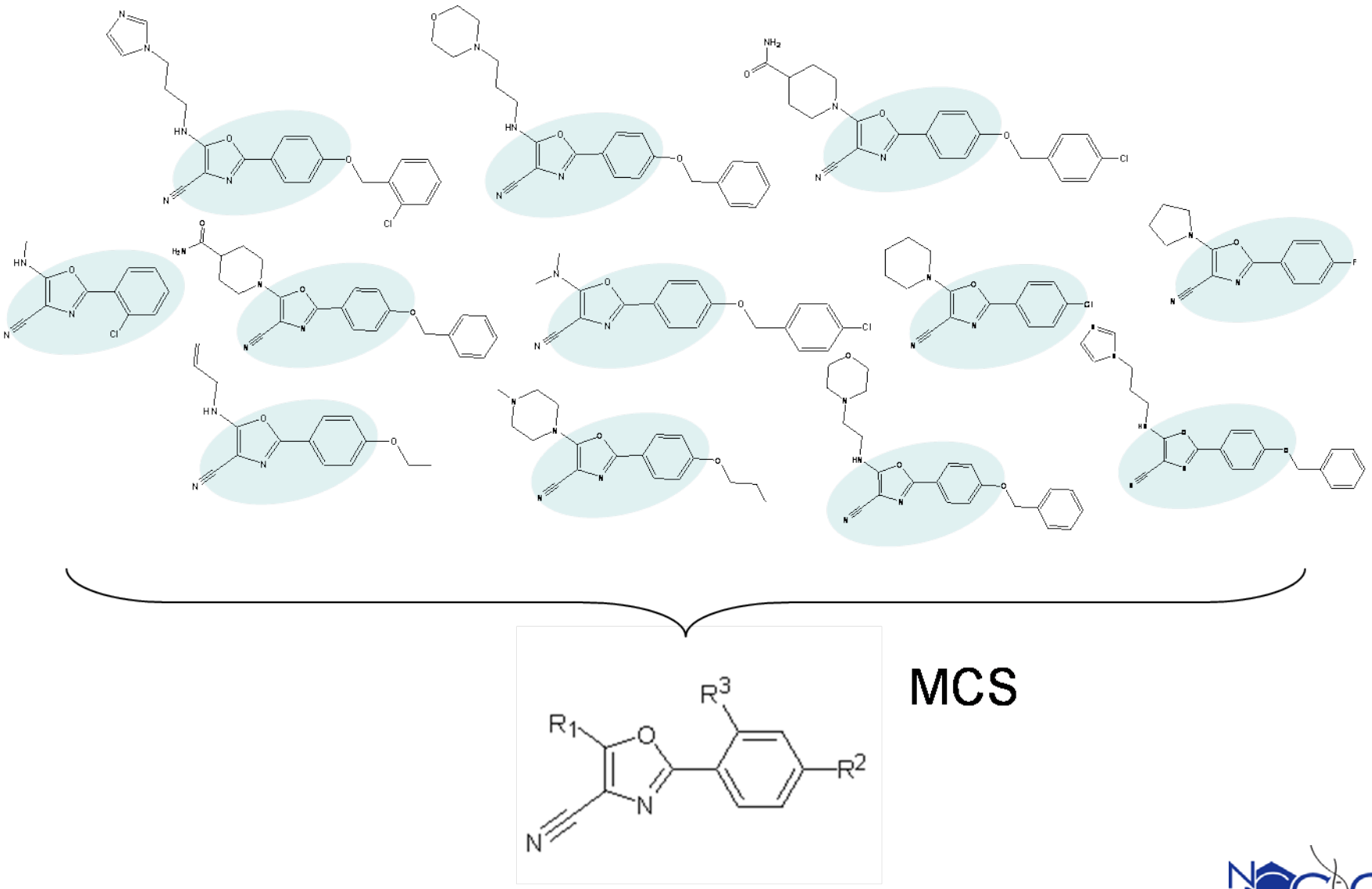
Derivation of nascent SAR from qHTS



MCS

Classes 1a, b and 2 ►12 cpds

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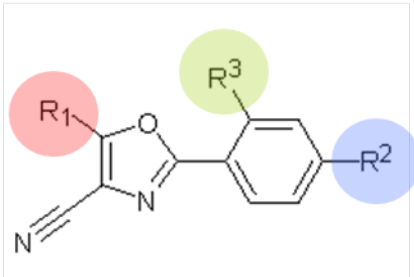
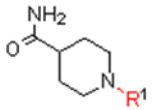
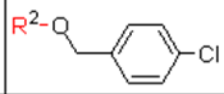
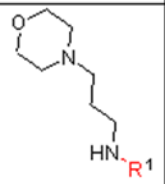
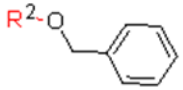
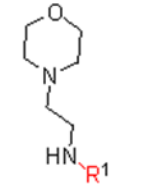
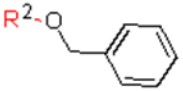
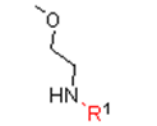
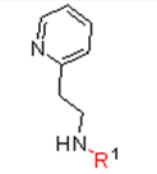
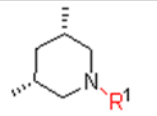
Structure-Activity Relationship (SAR) Report

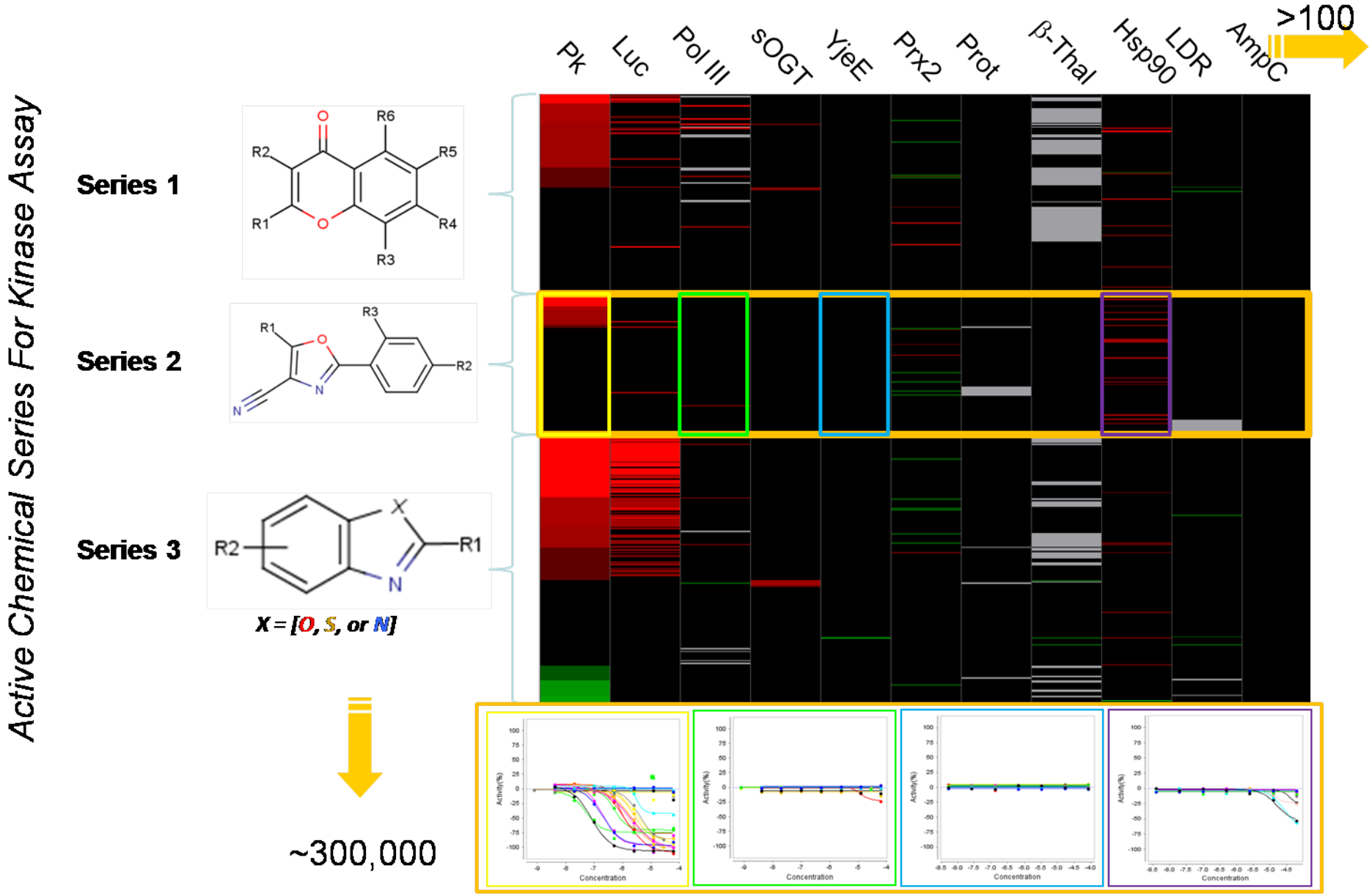


2-phenyloxazole-4-carbonitrile series

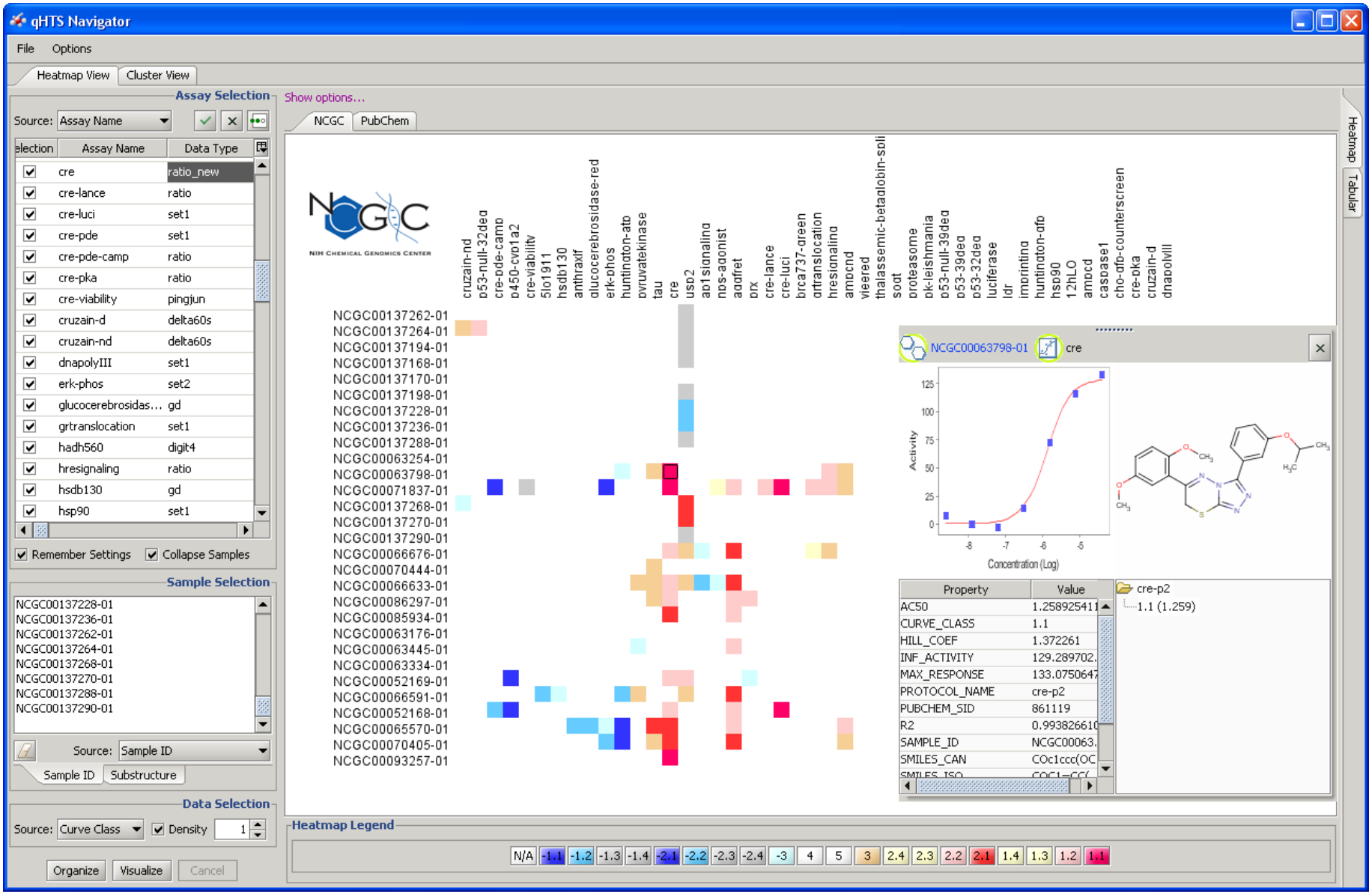
- The SAR report is a ‘map’ to enabling chemical optimization of a lead series

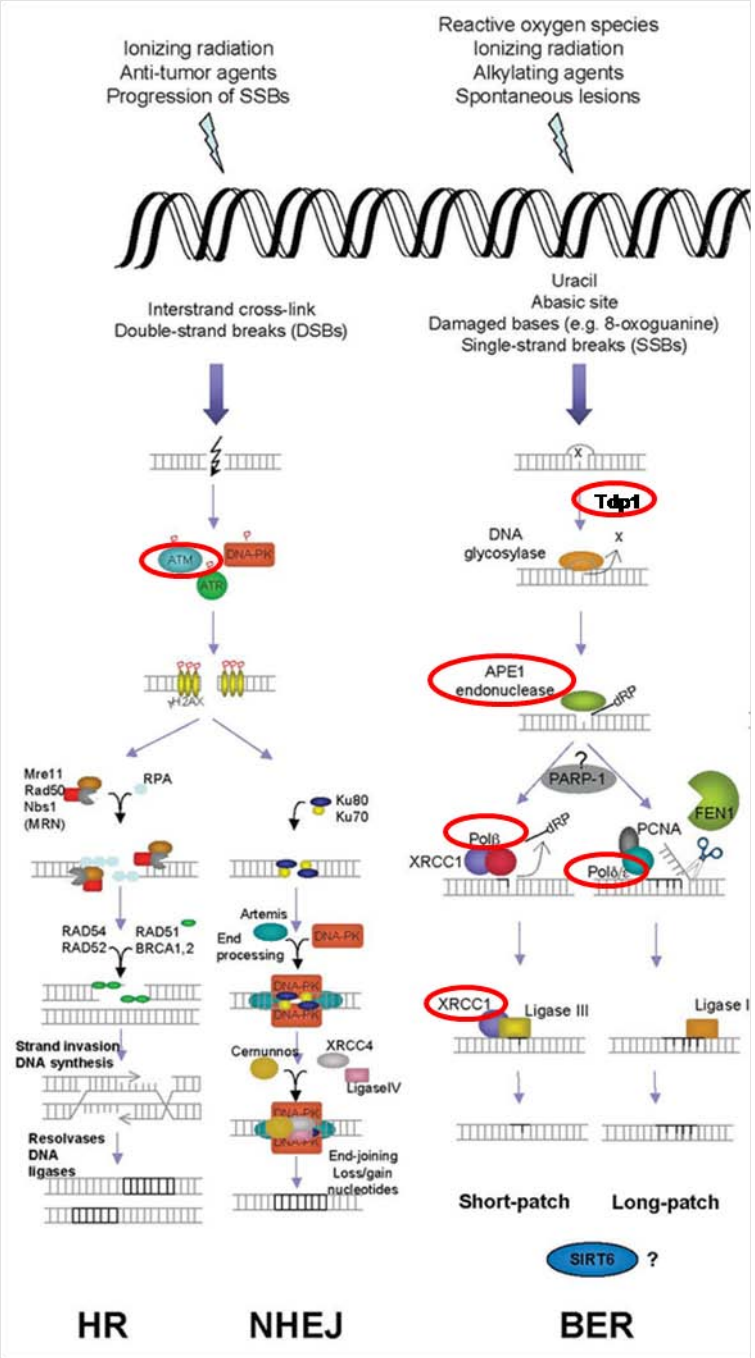
#	R1	R2	R3	NCGC ID	Curve Class	Rank	AC50 (uM)	Act Max Conc	hill Coeff
1			H	NCGC00067413-01	1.1	1/20	0.08	-92	1.1
2			H	NCGC00067270-01	1.1	5/20	1.9	-93	1.1
10			H	NCGC00067494-01	2.1	12/20	4.5	-96	1.4
21		F	H	NCGC00023889-01	3	20/20	42	-32	1.6
30		H	F	NCGC00039456-01	4		inactive	1	
39		H	Cl	NCGC00052762-01	4		inactive	1	

Electronic counterscreens across >100 assays



NCGC Chemical Genomics Browser





Trans-NIH nature of Molecular Libraries Roadmap brings about synergies

- NCGC has projects on many aspects of DNA repair from different areas of science
  - Yossi Shiloh, Tel Aviv University
    - ATM *(Rare disease)*
  - Yves Pommier, NCI
    - Tdp1 *(Cancer)*
  - David Wilson, NIA
    - Ape1 *(Aging)*
  - Roger Woodgate, NICHD
    - DNA Pol ε, ι *(Development)*
  - Sam Wilson, NIEHS
    - DNA Pol β *(Environmental toxicology)*
  - Shunichi Takeda, Kyoto University
    - XRCC1 *(Aging, toxicology)*
  - Structural Genomics Consortium
    - RecQ1 helicase *(Basic research)*
    - Bloom helicase *(Basic research)*

Targeting the Spliceosome Complex

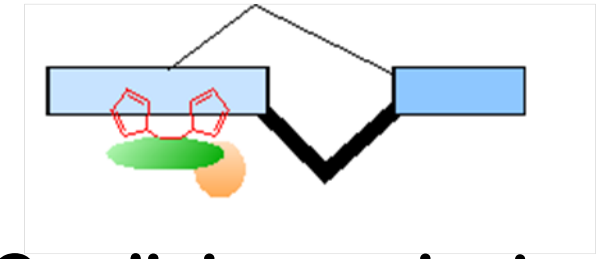
Review TRENDS in Biochemical Sciences Vol.30 No.8 August 2005
Full text provided by www.sciencedirect.com

The spliceosome: a novel multi-faceted target for therapy

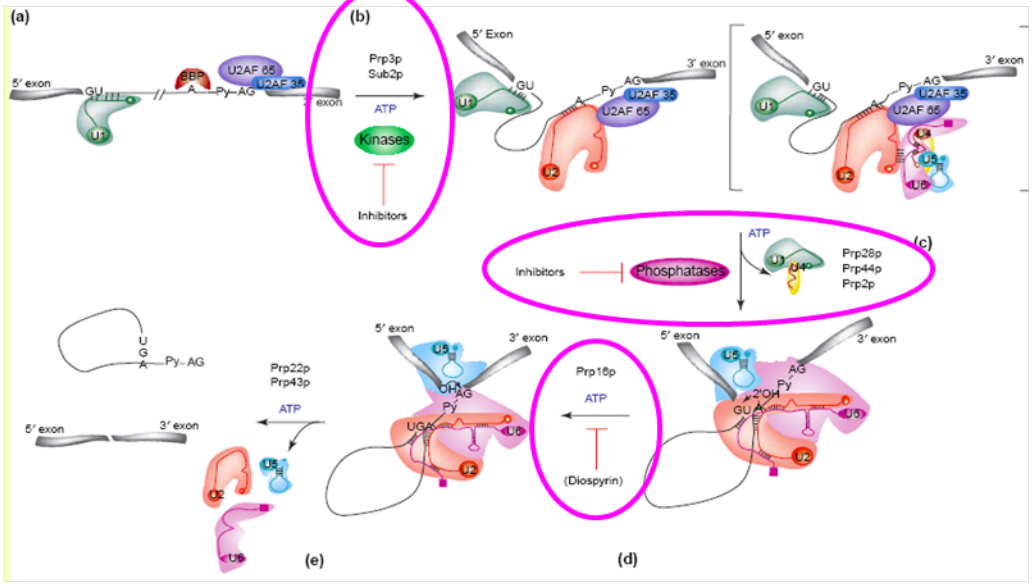
Jamal Tazi, Sébastien Durand and Philippe Jeanteur

Institut de Génétique Moléculaire de Montpellier (IGMM), UMR 5535, IFR 122, Centre National de Recherche Scientifique (CNRS), 1919, route de Mende, 34293 Montpellier, France

Cis- acting small molecules



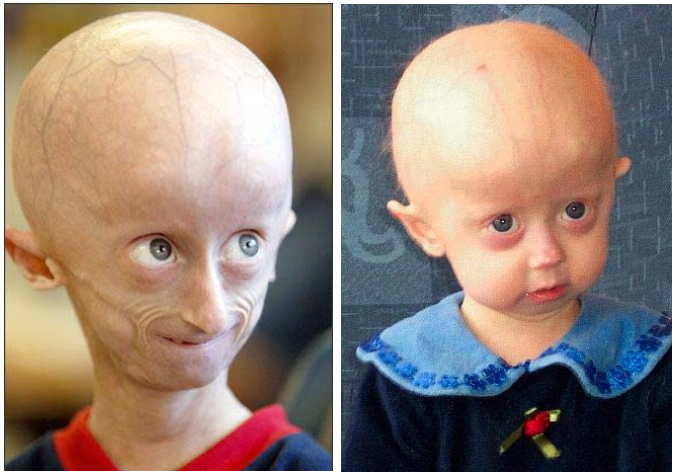
Trans- acting small molecules



NCGC splicing projects

- Progeria
  - Tom Misteli, NCI
- Spinal Muscular Atrophy
  - Elliot Androphy, U. Mass
- B-thalassemia
  - Ryzard Kole, UNC

Hutchinson-Gilford Progeria Syndrome



Pre-mature aging syndrome

1 in 4 million affected

Disease onset 12-24 months

Life expectancy 10-15 years

Aging symptoms, cardiovascular defects, atherosclerosis

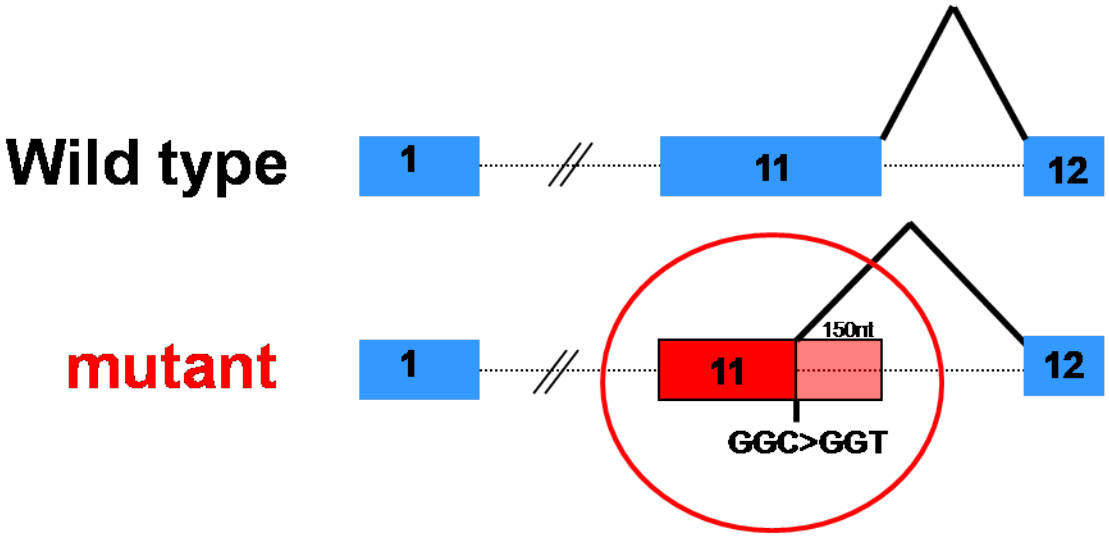
CAUSE: Spontaneous point mutation in lamin A

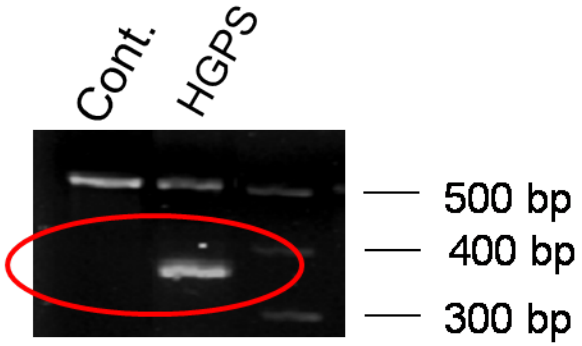
NATIONAL CANCER INSTITUTE

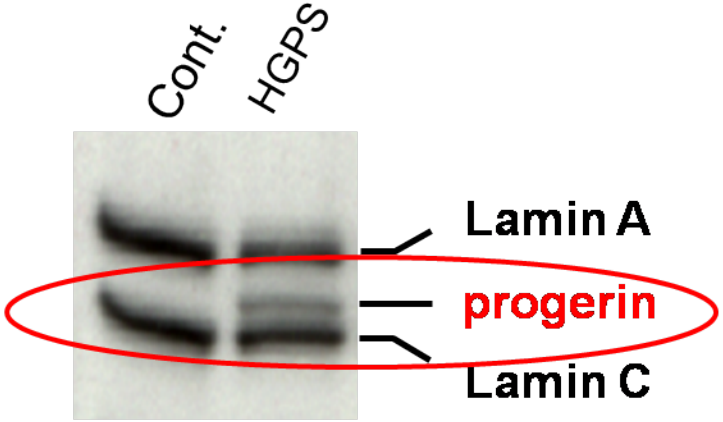
NCGC

NIH Chemical Genomics Center

HGPS is a splicing disease

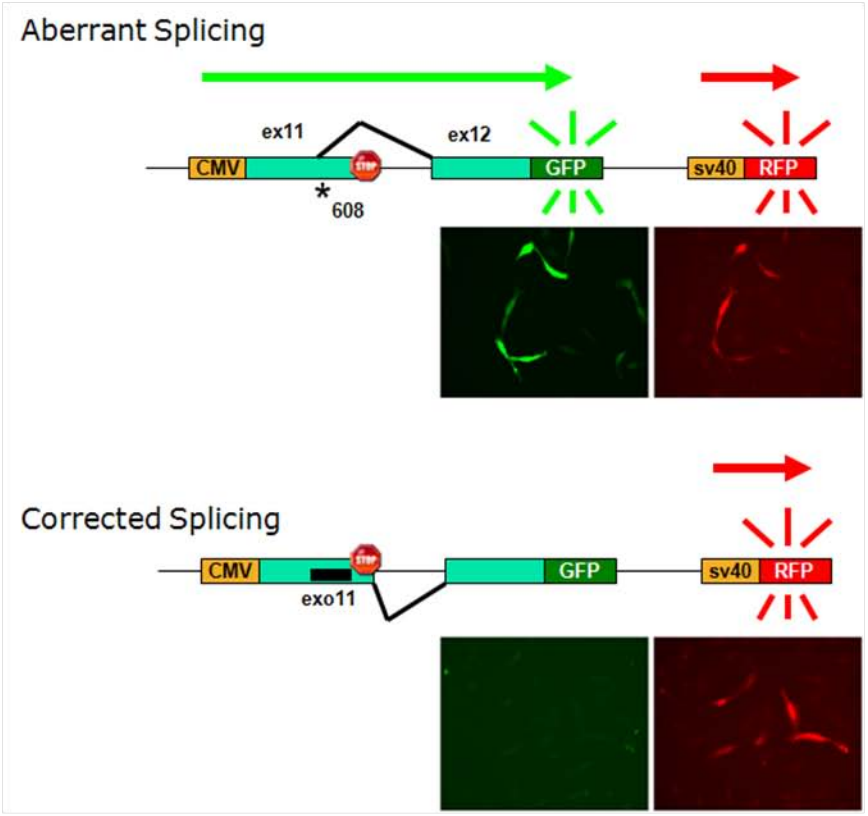


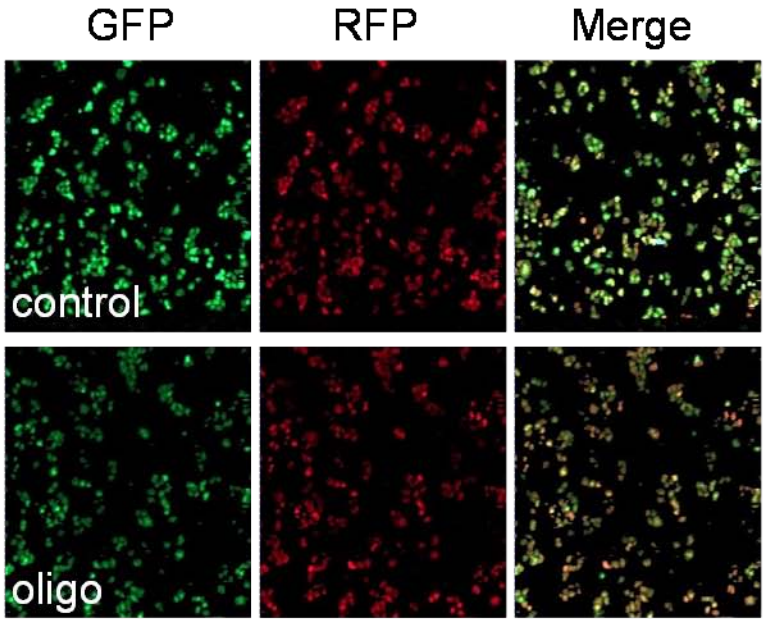


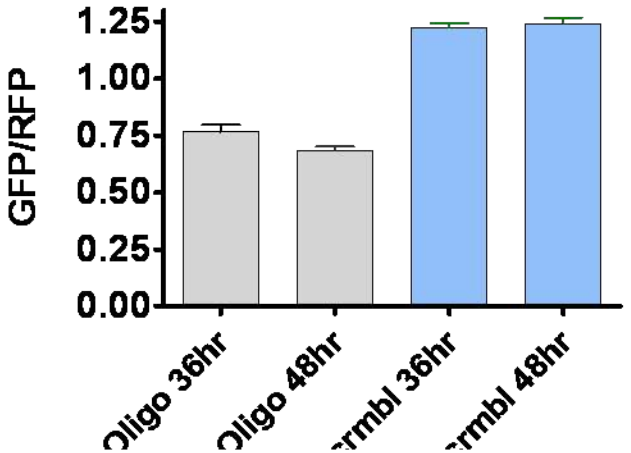


Eriksson et al., Nature, 2003
De Sandre-Giovannoli et al., Science, 2003

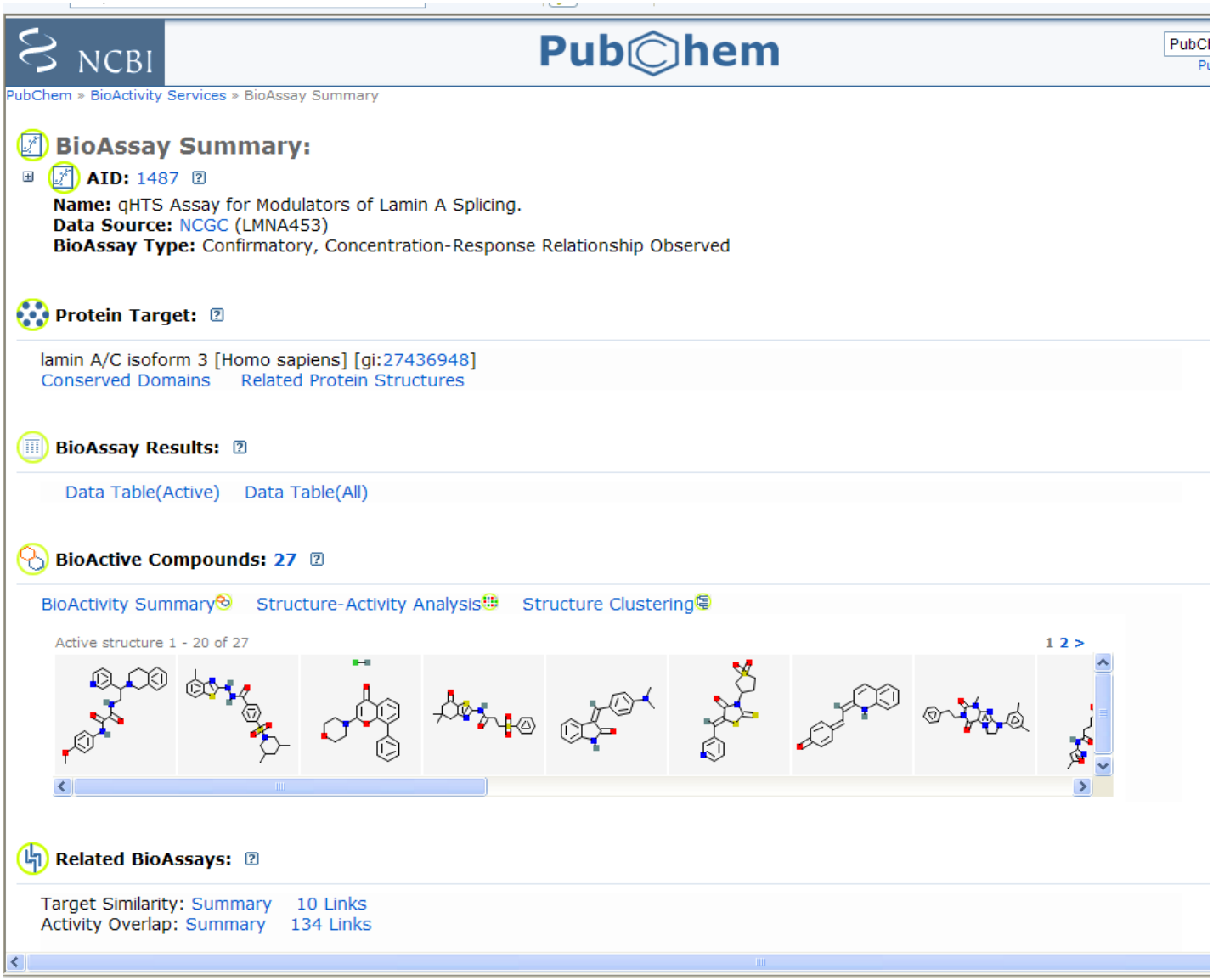
Primary Assay Principle and Read-out



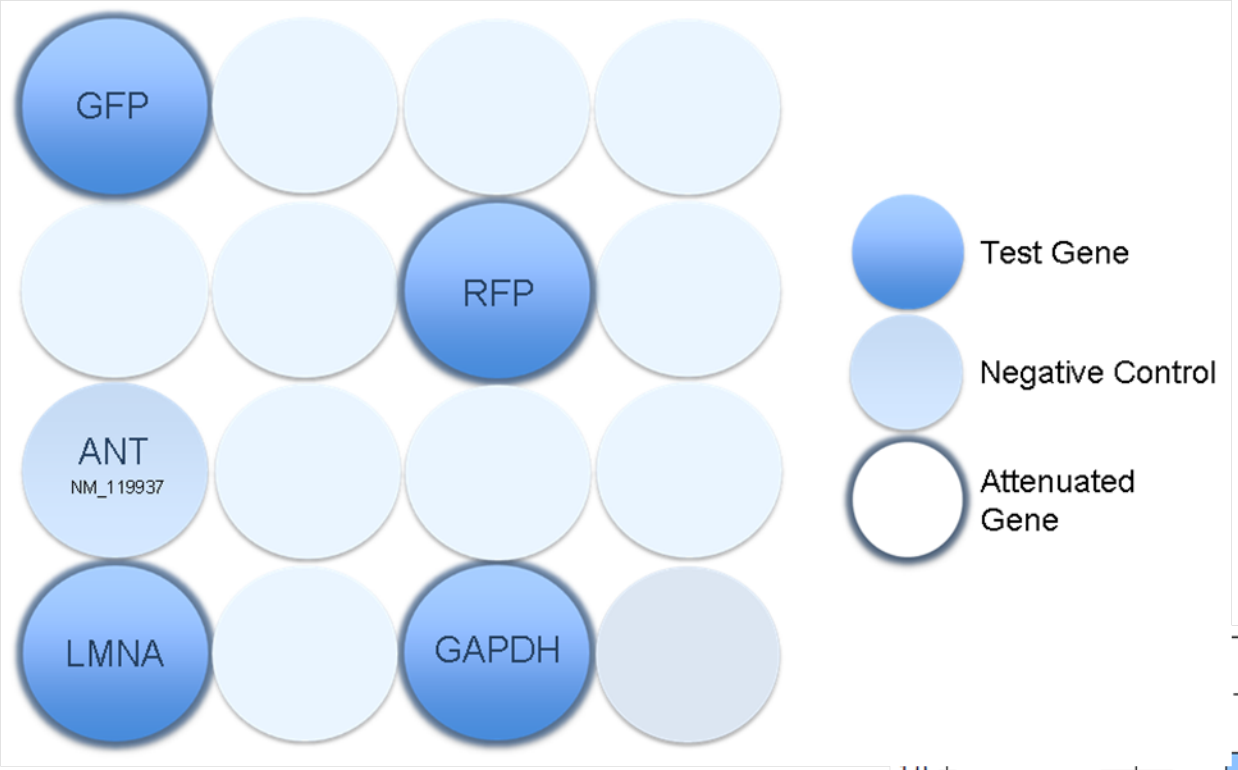




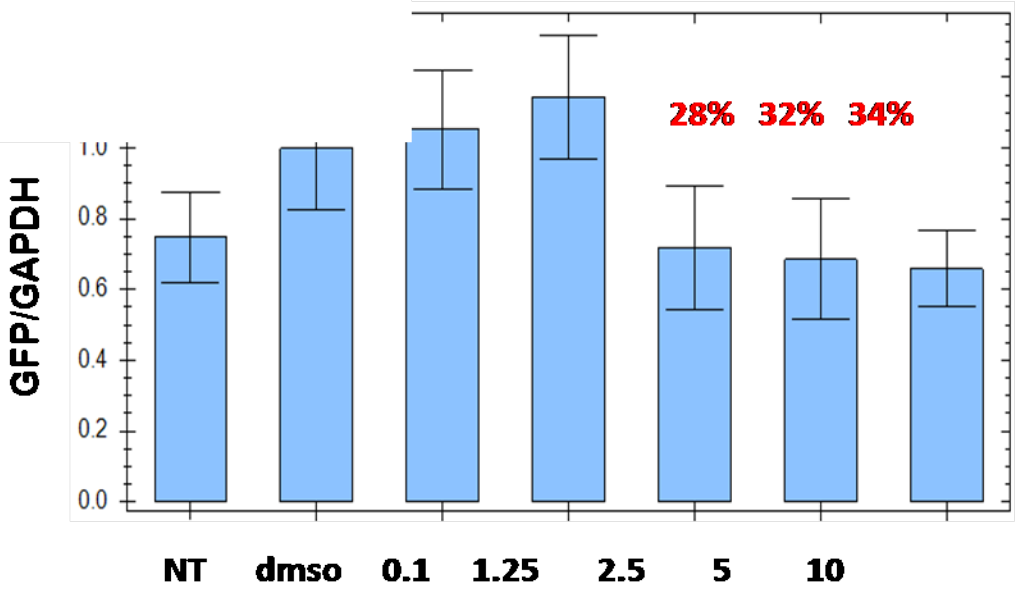
qHTS against ~200k compounds



Progeria qRPA (High-Throughput Genomics)



- Quinazoline-based compounds are active in RPA
- Cellular phenotypic effects under evaluation



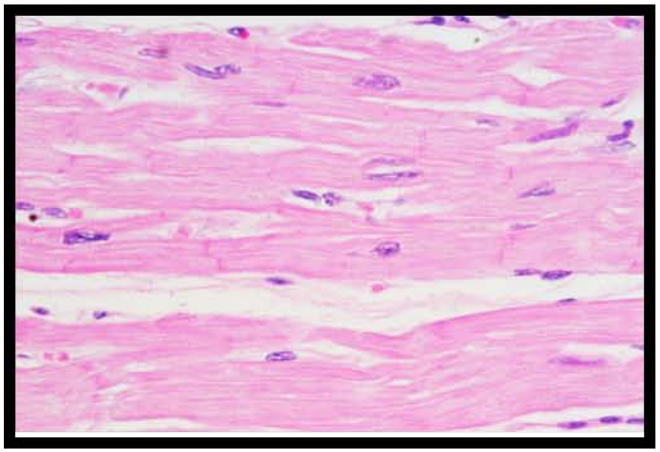
Selected cancer projects at NCGC

- **Targets**
  - Ape1
  - DNA polymerase beta
  - DNA polymerase eta
  - DNA polymerase iota
  - IκBα stabilization
  - JMJD2E
  - 12-lipoxygenase
  - MDR1
  - mTOR
  - Pyruvate kinase M2
  - RECQ1 helicase
  - Tdp1
  - Ubiquitin-specific protease 2
- **Protein-protein interactions**
  - BRCA1-BRCT interaction
  - CBFß / RUNX1 interaction
  - Hsp90-HOP1 interaction
  - Menin-MLL interaction
- **Pathways**
  - AP1
  - CREB
  - ERK
  - HRE
  - NFkB
  - p53
- **Phenotypic assays**
  - BRCA1 chemical genetic synthetic lethal
  - TS p53 chemical genetic synthetic lethal
  - Epigenetic modulators
  - Chordoma cell death
  - CLL cell death

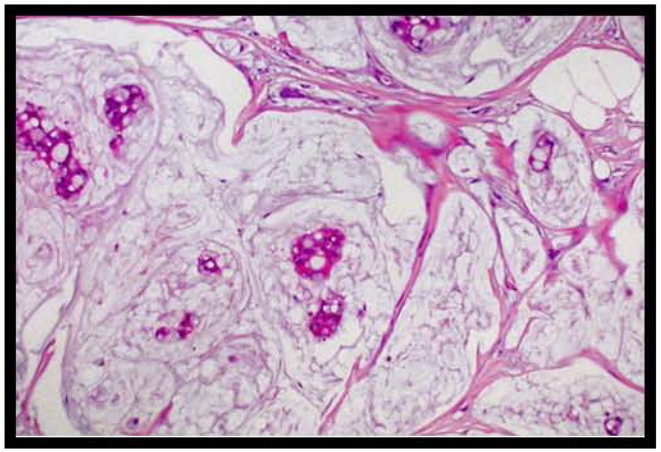
NCGC
NIH Chemical Genomics Center

Human Pyruvate Kinase Activators for Cancer

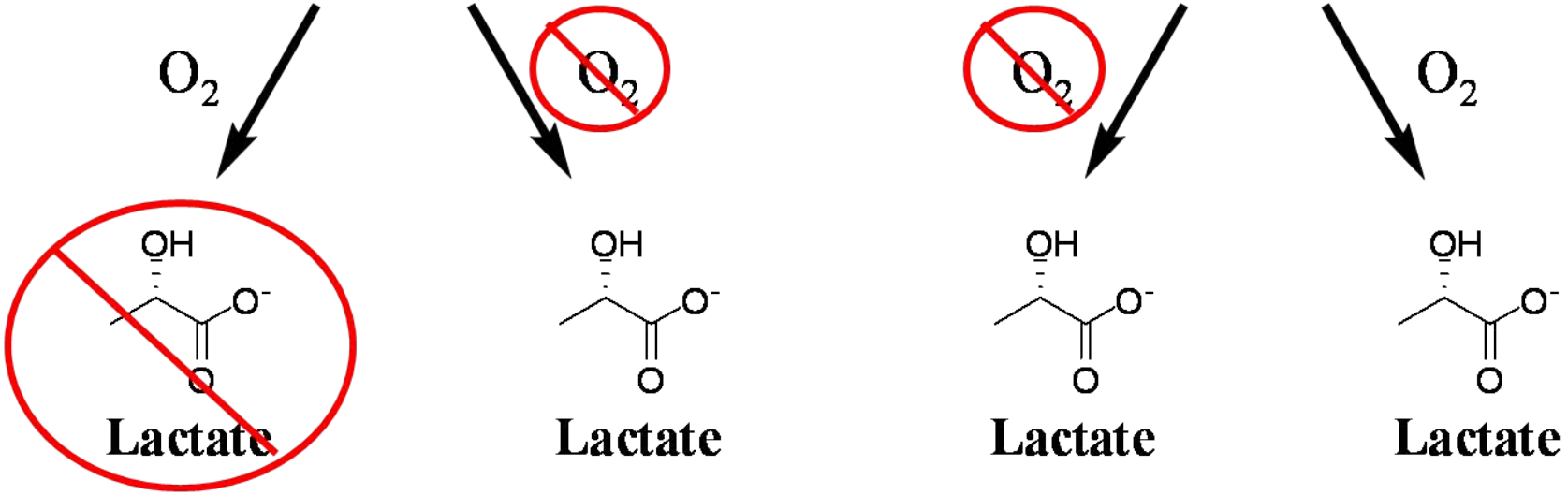
NCGC Collaboration with Lew Cantley, Harvard Medical School



healthy cells



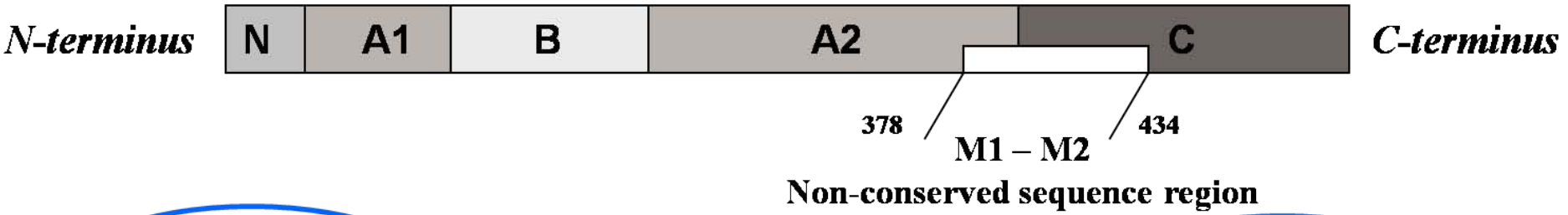
cancer cells



The Warburg Effect

Warburg, O. The Metabolism of Tumors (translated by F. Dickens). London: Constable & Co., Ltd., 1930.

Alternative splicing and expression of Pyruvate Kinase



Express PKM1

Liver (PKL) & red blood cell (PKR)

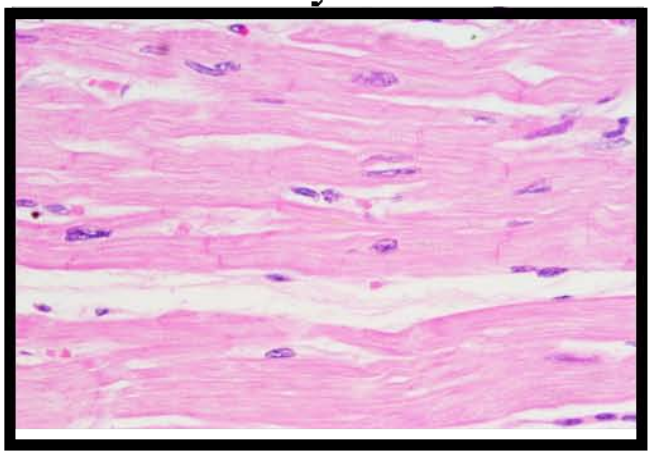
Express PKM2

(PKM gene)

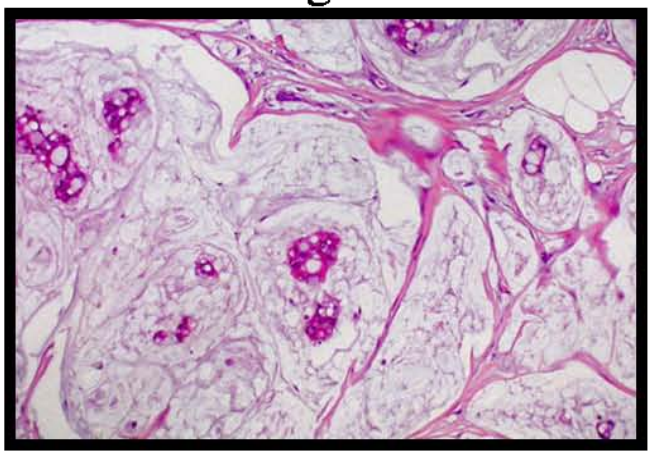
(PKL/R gene)

(PKM gene)

healthy cells



Proliferating/cancer cells



The Warburg Effect: A New Insight

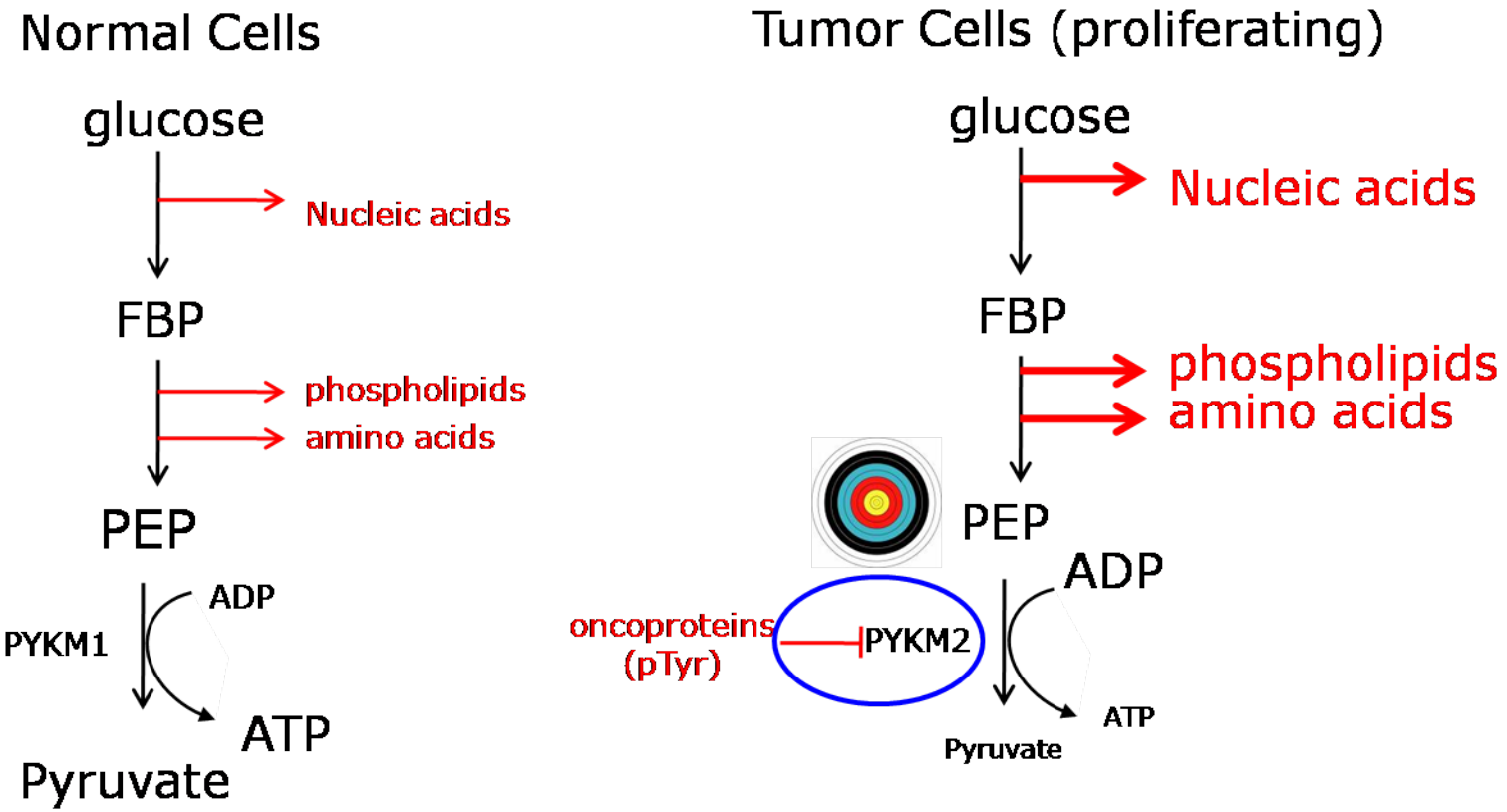
Nature February 2008

The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth

Heather R. Christofk[1], Matthew G. Vander Heiden[1,2], Marian H. Harris[3], Arvind Ramanathan[4], Robert E. Gerszten[4,5,6], Ru Wei[4], Mark D. Fleming[3], Stuart L. Schreiber[4,7] & Lewis C. Cantley[1,8]

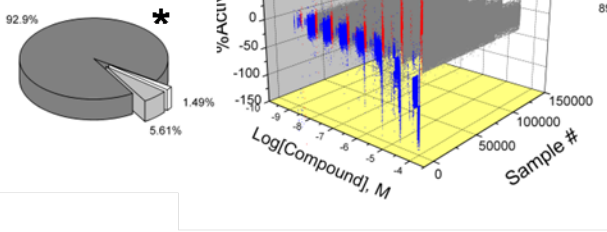
Pyruvate kinase M2 is a phosphotyrosine binding protein

Heather R. Christofk[1], Matthew G. Vander Heiden[1,3], Ning Wu[1], John M. Asara[2,4] & Lewis C. Cantley[1,4]

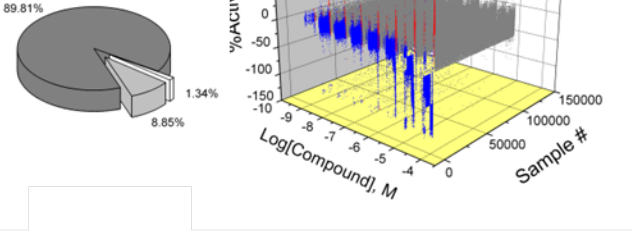


Summary of qHTS experiments against PK

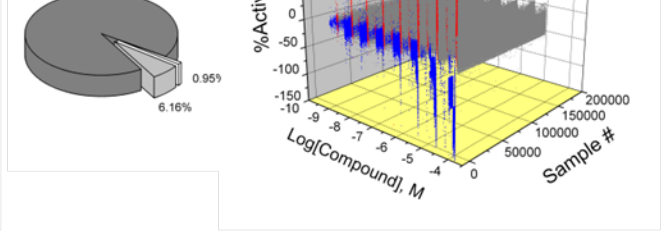
Human PK



Bacillus PK



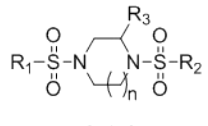
LmPK

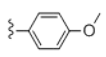
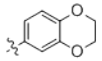
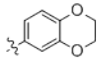
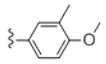
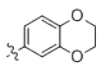
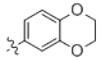
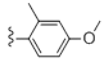
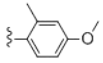
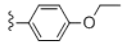
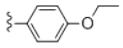
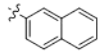
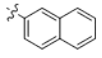
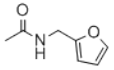


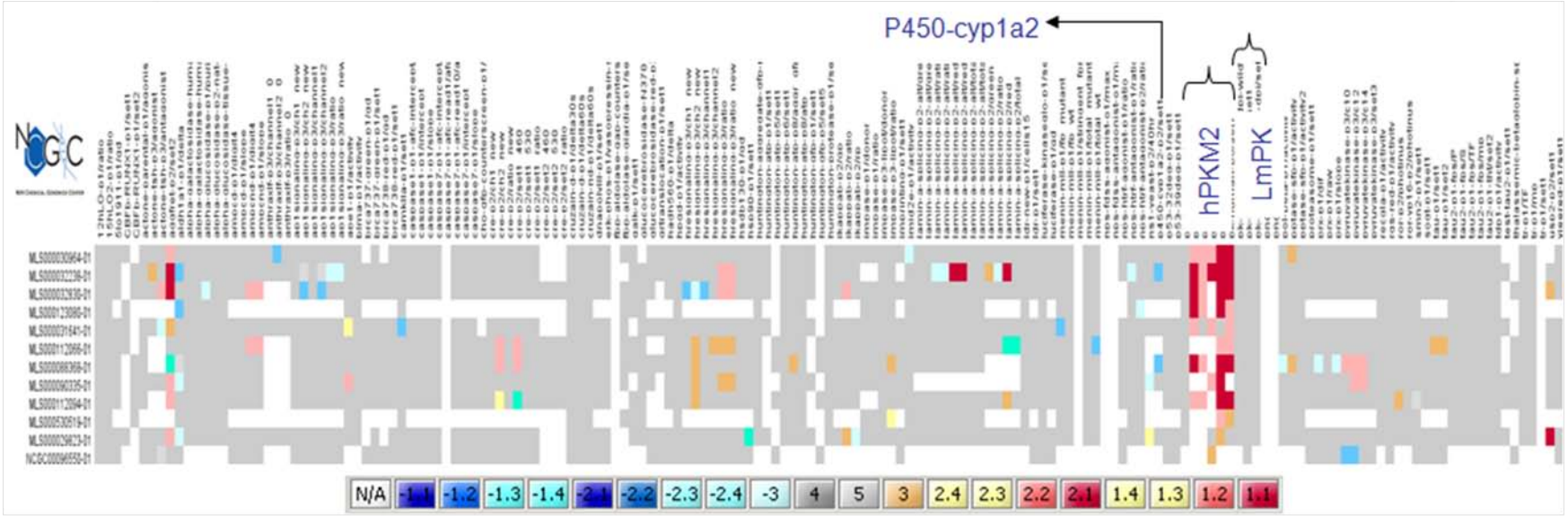
PK qHTS Assays				
Parameter	**Human-*PK***	***Bacillus-PK***	***Lm-PK***	
Compounds (total # tested)	107,610	107,610	150,452	
Sample-concentration measurements (sample wells)	1,053,184	1,037,696	1,264,384	
Total wells	1,207,296	1,187,328	1,471,488	
Plates Screened	786	773	875	
Plates Failed QC	50	3	7	
Plates Re-run for QC	163	163	0	
RZ'	0.67 +/- 0.12	0.68 +/- 0.08	0.76 +/- 0.06	
Signal / Background	11.4 +/- 9.0	6.9 +/- 1.4	3.3 +/- 0.6	
CV	10 +/- 4	12 +/- 5	7 +/- 3	
Screening System	Kalypsys	Kalypsys	Kalypsys	
Software for stat analysis	In-house client	In-house client	In-house client	

human PK: Human M2 isoform pyruvate kinase
bacillus-PK: *bacillus sterearothermophilus* pyruvate kinase
LmPK: *Leishmania mexicana* pyruvate kinase

qHTS data

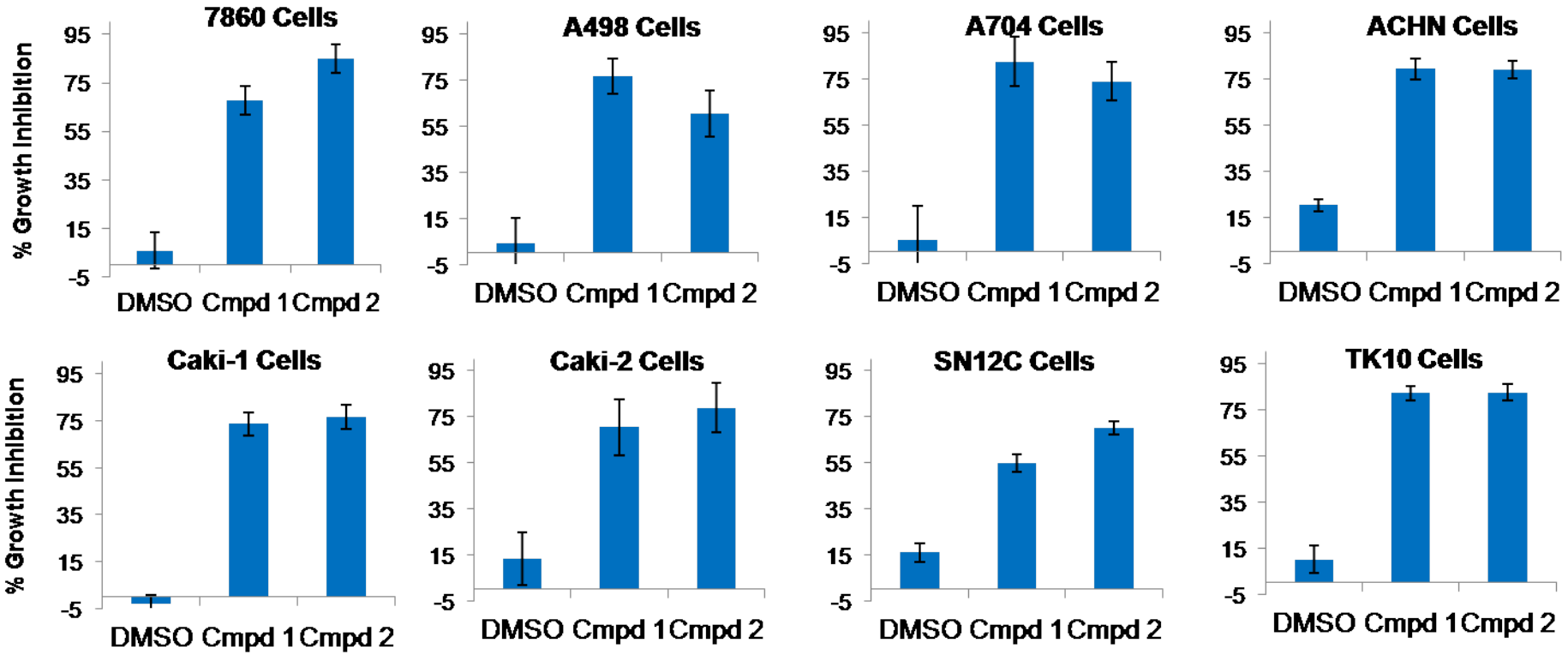


Sample ID	R_1	R_2	R_3	n	Curve Class	AC50 (uM)	Hill Coef
NCGC00030335-01			H	1	1.1	0.251	1.4
MLS0000322238-01			H	2	1.1	0.355	1.1
MLS000032830-01			H	1	1.1	2.51	1.0
MLS000123080-01			H	2	1.1	3.16	1.1
NCGC00028588-01			H	1	1.2	0.050	2.2
MLS000112094-01			H	2	2.1	10	1.8
NCGC00058483-01			H	2	2.1	12.59	1.0
MLS000112066-01			$-CH_3$	1	2.2	10	0.7
NCGC00049516-01				1	2.2	15.85	0.9
MLS000530519-01			H	0	2.2	31.62	3.6
NCGC00096550-01			H	1	3	1.26	2.1
MLS000029823-01				1	3	39.81	4.4



Pyruvate Kinase Activators: Current Status

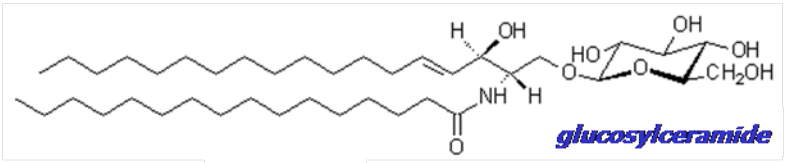
- >300 analogs prepared around both chemotypes
  Improved potency and solubility
- Cell-based antiproliferative activity demonstrated
- *in vivo* activity being evaluated



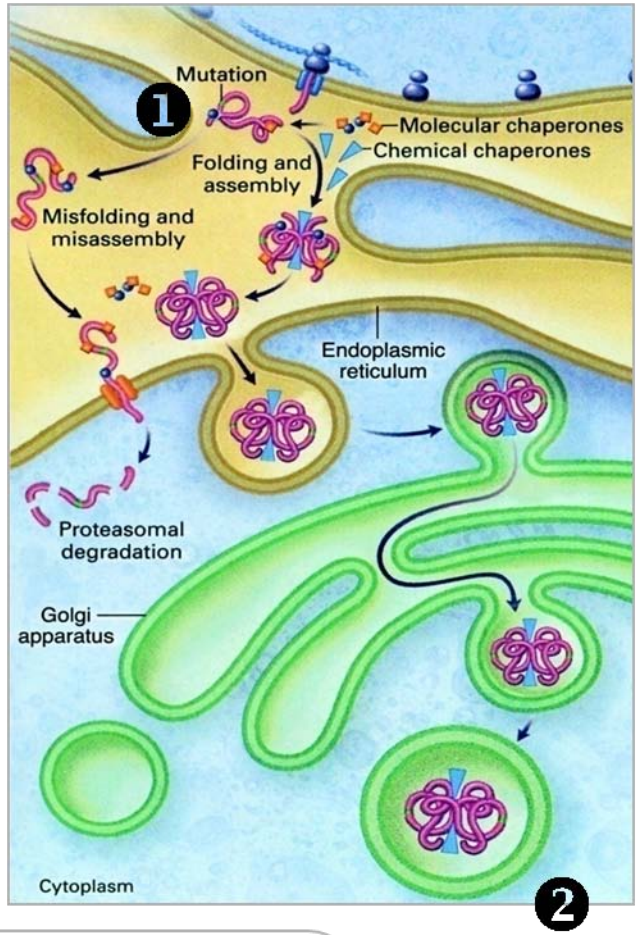
Ameliorating the Defect in Gaucher's Disease

NCGC Collaboration with Ellen Sidransky, NHGRI

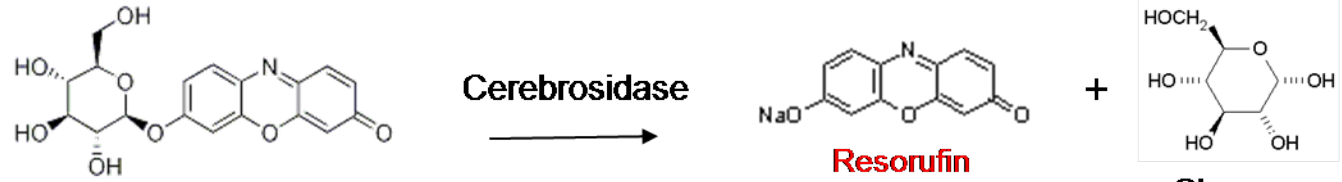
- Gaucher's Disease
  - Rare disease caused by mutations in enzyme glucocerebrosidase (GCS)



  - Current treatment: enzyme replacement
    - Limited efficacy, no BBB penetration, expensive
- Many mutations are missense, leading to trafficking defect
  - Pharmacological chaperones a therapeutic possibility



Fluorogenic substrate assay:



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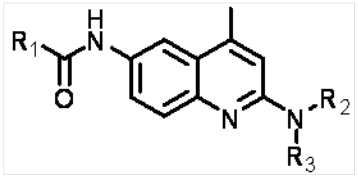
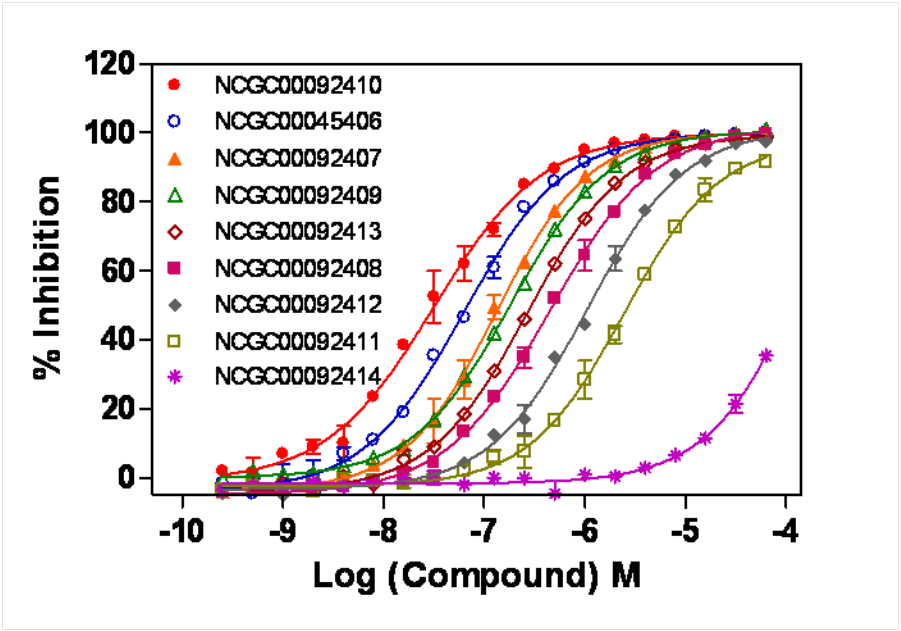
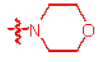
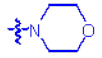
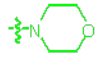
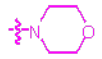
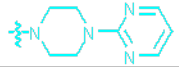
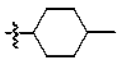
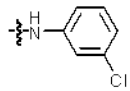
Structure–Activity Relationship (SAR) Data Derived from Primary qHTS

Parent compound





Comp. No.	R_1	NR_2R_3	qAC_{50} (μM)	Ki (μM)
NCGC00092410*			0.038	0.021
NCGC00045406			0.068	0.056
NCGC00092409*			0.189	0.121
NCGC00092407*			0.139	0.055
NCGC00092408*			0.468	0.184
NCGC00092412*			1.07	0.514
NCGC00092413*			0.278	0.120
NCGC00092411*			2.59	0.975
NCGC00092414*			Inactive	122

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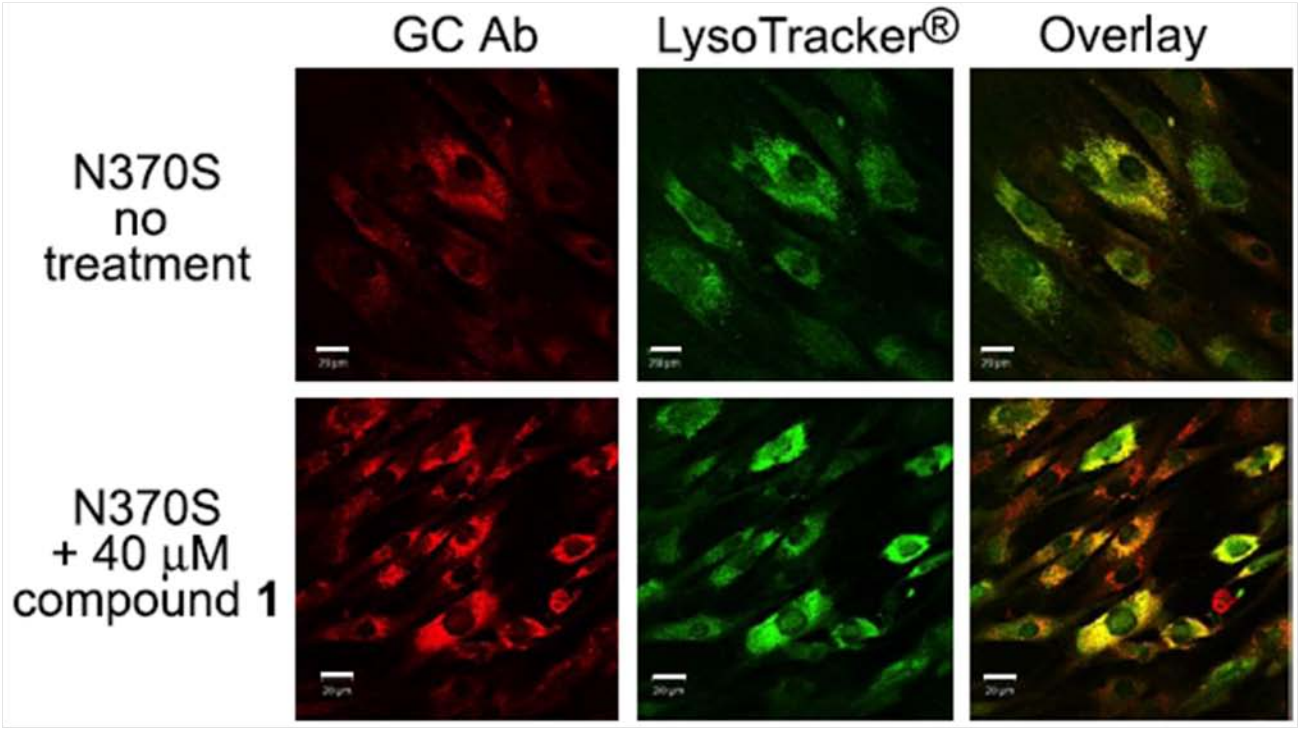
Three classes of glucocerebrosidase inhibitors identified by quantitative high-throughput screening are chaperone leads for Gaucher disease

Wei Zheng*, Janak Padia*, Daniel J. Urban†, Ajit Jadhav*, Ozlem Goker-Alpan†, Anton Simeonov*, Ehud Goldin†, Douglas Auld*, Mary E. LaMarca†, James Inglese*, Christopher P. Austin*‡, and Ellen Sidransky†‡

*NIH Chemical Genomics Center, National Human Genome Research Institute, National Institutes of Health, 9800 Medical Center Drive, MSC 3370, Bethesda, MD 20892-3370; and †Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Building 35 Rm1A213, 35 Convent Drive, Bethesda, MD 20892-3708

Communicated by Francis S. Collins, National Institutes of Health, Bethesda, MD, June 21, 2007 (received for review March 8, 2007)

13192–13197 | PNAS | August 7, 2007 | vol. 104 | no. 32



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Inhibitors of *Schistosoma mansoni* TGR

NCGC Collaboration with

David Williams
Department of Biological Sciences
Illinois State University
Normal, IL

ILLINOIS STATE UNIVERSITY

NCGC NIH CHEMICAL GENOMICS CENTER

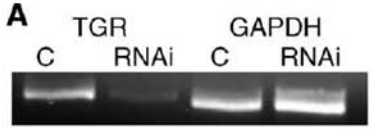
Identification of Target : 2006

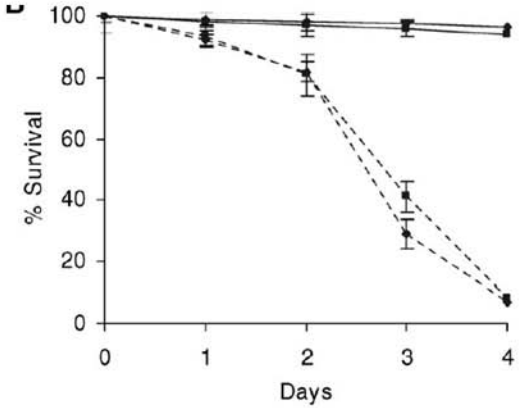
OPEN ACCESS Freely available online

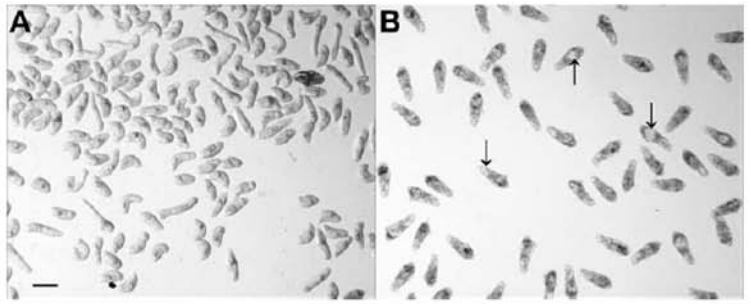
PLOS MEDICINE

Thioredoxin Glutathione Reductase from *Schistosoma mansoni:* An Essential Parasite Enzyme and a Key Drug Target

Angela N. Kuntz[1], Elisabeth Davioud-Charvet[2,3], Ahmed A. Sayed[1], Lindsay L. Califf[1], Jean Dessolin[2,4], Elias S. J. Arnér[5], David L. Williams[1*]

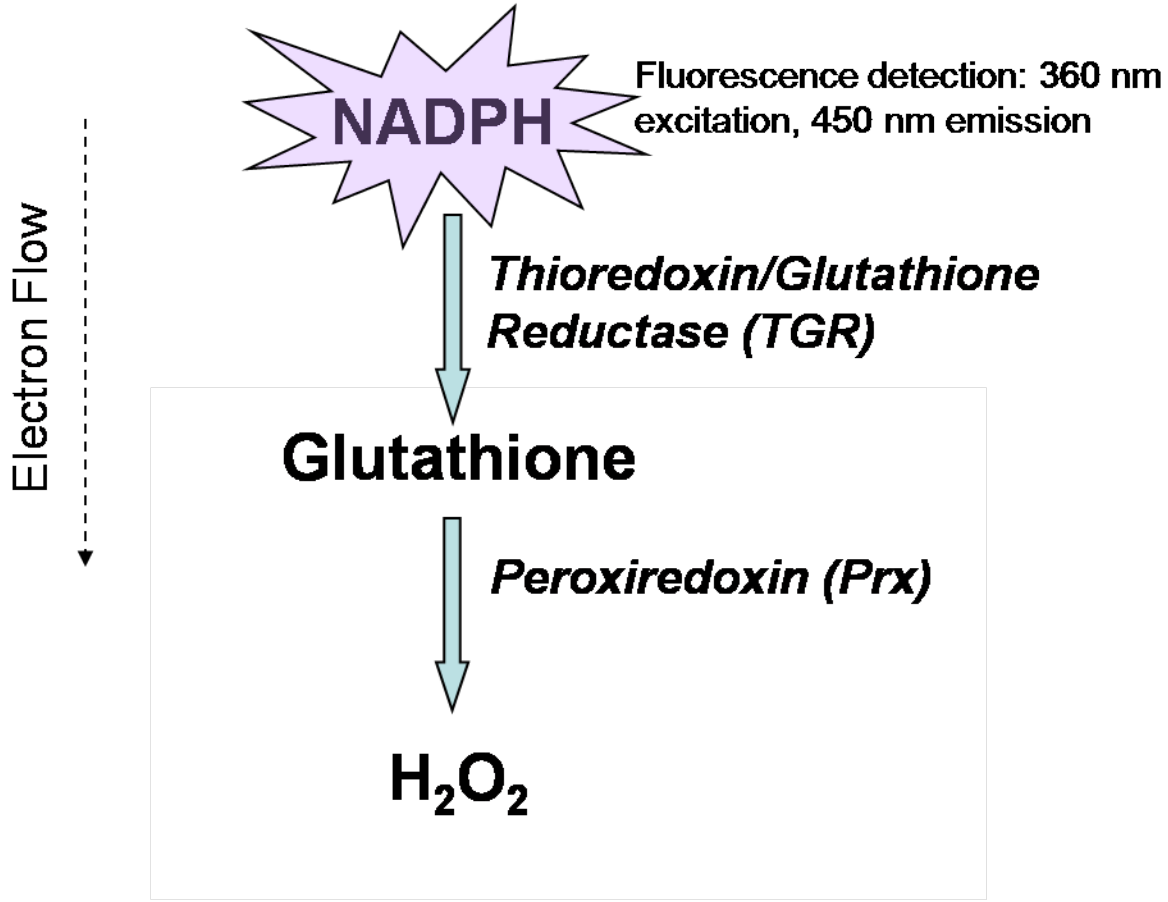






ILLINOIS STATE UNIVERSITY

Development of Assay: 2006



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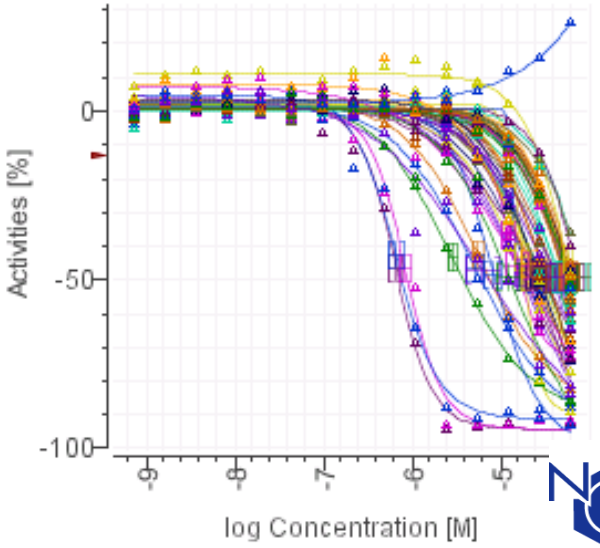
Quantitative HTS: 2007

- **70,000 compounds at 7 concentrations (qHTS)**
  - **Dose-response curve for all compounds (PNAS 103, 11473-8 (2006))**
  - **~10,000,000 data points (16 Time-Point Reads)**
  - **31 hours of robot time**
- **Results: 100 compounds with IC50 < 40 µM**
  - **71 compounds**
  - **6 different structural classes**



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Series Expansion & Target ID

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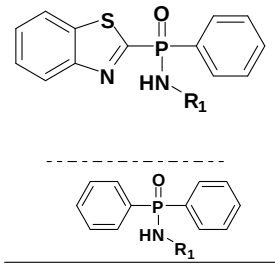
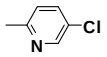
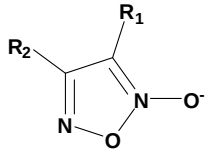
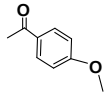
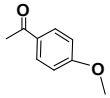
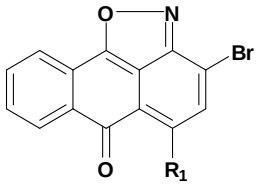
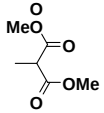
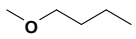
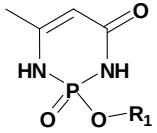
	Analogue #	R_1	R_2	qHTS	Confirmation	TGR Assay
Phosphinic Amide 	1		NA	NS	0.25	0.80
	2		NA	NS	23	12
	3		NA	0.037	0.025	0.025
	4		NA	>57	>57	>50
Oxadiazole 2-oxide 	5			NS	1.5	0.025
	6			24	ND	2.0
	7			9.2	1.5	0.020
	8			10	4.7	0.060
	9	-CN	-Ph	8.7	ND	1.8
Isoxazolone 	10		NA	0.53	>57	9.0
	11		NA	NS	0.12	0.080
	12		NA	NS	0.84	0.15
Phosphoramidite 	13	4-chlorophenyl	NA	NS	0.42	0.55
	14	naphthyl	NA	0.56	0.12	0.20
	15	phenyl	NA	NS	2.0	4.0
	16	4-ethoxyphenyl	NA	NS	0.18	0.6

- All retested compounds were found to be inactive against Prx2, leaving TGR as the sole target for all confirmed actives.
- Singleton hits 3, 10 and 14 were confirmed and successfully expanded to small series.
- The top active compounds were highly potent, with IC_{50}s approaching the limit of detection.

Simeonov et al,
PLoS Negl. Trop. Dis., **2**, e107 (2008).

NIH Chemical Genomics Center

Chemical probes: 2008

OPEN ACCESS Freely available online

PLoS NEGLECTED TROPICAL DISEASES

Quantitative High-Throughput Screen Identifies Inhibitors of the *Schistosoma mansoni* Redox Cascade

Anton Simeonov[1], Ajit Jadhav[1], Ahmed A. Sayed[2], Yuhong Wang[1], Michael E. Nelson[1], Craig J. Thomas[1], James Inglese[1], David L. Williams[2]*, Christopher P. Austin[1]*

1 NIH Chemical Genomics Center, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, United States of America, **2** Department of Biological Sciences, Illinois State University, Normal, Illinois, United States of America

Abstract

Schistosomiasis is a tropical disease associated with high morbidity and mortality, currently affecting over 200 million people worldwide. Praziquantel is the only drug used to treat the disease, and with its increased use the probability of developing drug resistance has grown significantly. The *Schistosoma* parasites can survive for up to decades in the human host due in part to a unique set of antioxidant enzymes that continuously degrade the reactive oxygen species produced by the host's innate immune response. Two principal components of this defense system have been recently identified in *S. mansoni* as thioredoxin/glutathione reductase (TGR) and peroxiredoxin (Prx) and as such these enzymes present attractive new targets for anti-schistosomiasis drug development. Inhibition of TGR/Prx activity was screened in a dual-enzyme format with reducing equivalents being transferred from NADPH to glutathione via a TGR-catalyzed reaction and then to hydrogen peroxide via a Prx-catalyzed step. A fully automated quantitative high-throughput (qHTS) experiment was performed against a collection of 71,028 compounds tested as 7- to 15-point concentration series at 5 μL reaction volume in 1536-well plate format. In order to generate a robust data set and to minimize the effect of compound autofluorescence, apparent reaction rates derived from a kinetic read were utilized instead of end-point measurements. Actives identified from the screen, along with previously untested analogues, were subjected to confirmatory experiments using the screening assay and subsequently against the individual targets in secondary assays. Several novel active series were identified which inhibited TGR at a range of potencies, with IC_{50}s ranging from micromolar to the assay response limit (~25 nM). This is, to our knowledge, the first report of a large-scale HTS to identify lead compounds for a helminthic disease, and provides a paradigm that can be used to jump-start development of novel therapeutics for other neglected tropical diseases.

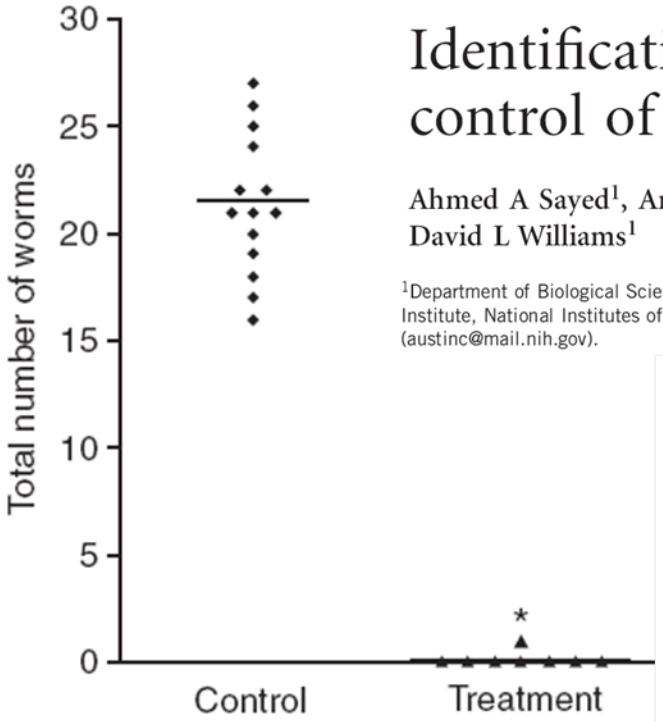
In vivo validation of mechanism: 2008

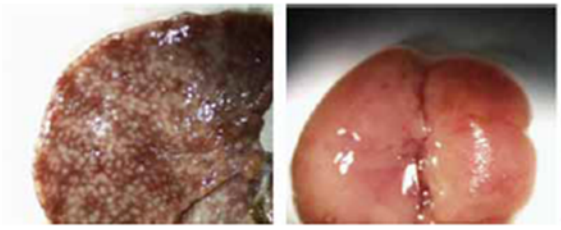
nature medicine

Identification of oxadiazoles as new drug leads for the control of schistosomiasis

Ahmed A Sayed[1], Anton Simeonov[2], Craig J Thomas[2], James Inglese[2], Christopher P Austin[2] & David L Williams[1]

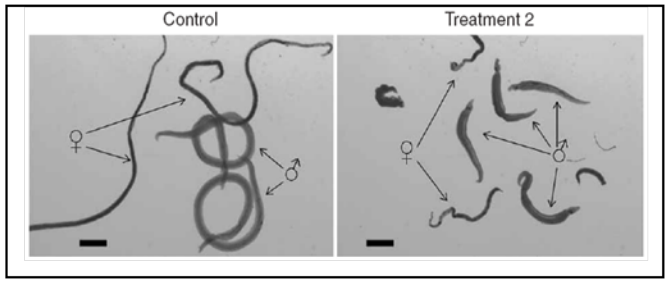
[1]Department of Biological Sciences, Illinois State University, Normal, Illinois 61790, USA. [2]NIH Chemical Genomics Center, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland 20892-3370, USA. Correspondence should be addressed to D.L.W. (dlwilli@ilstu.edu) or C.P.A. (austinc@mail.nih.gov).





Livers of treated mice

S. mansoni-infected mice treated with NCGC1597 @ 10 mg/kg IP for 5 days



Ex vivo killing of *S. mansoni* worms by NCGC1597

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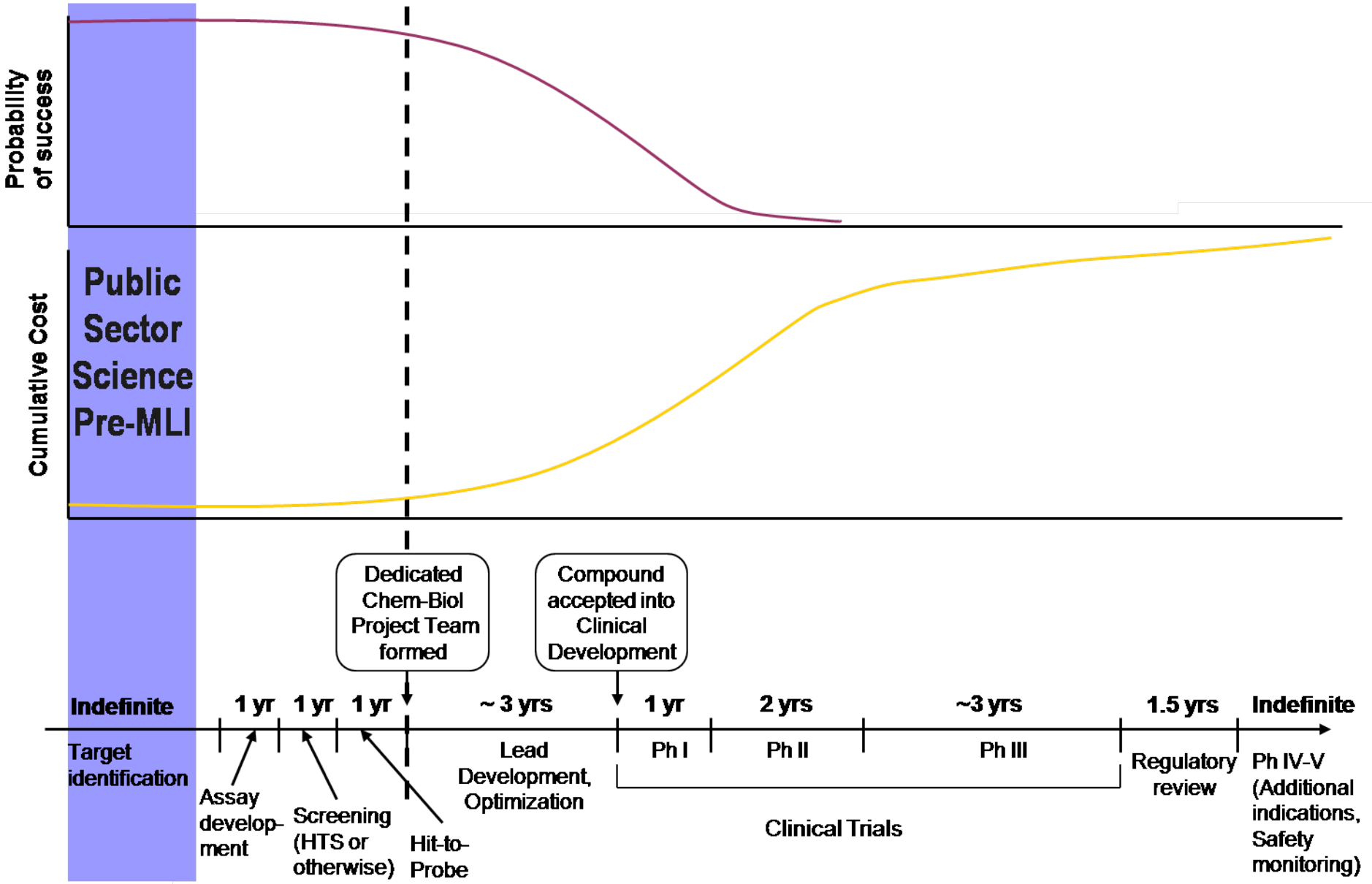
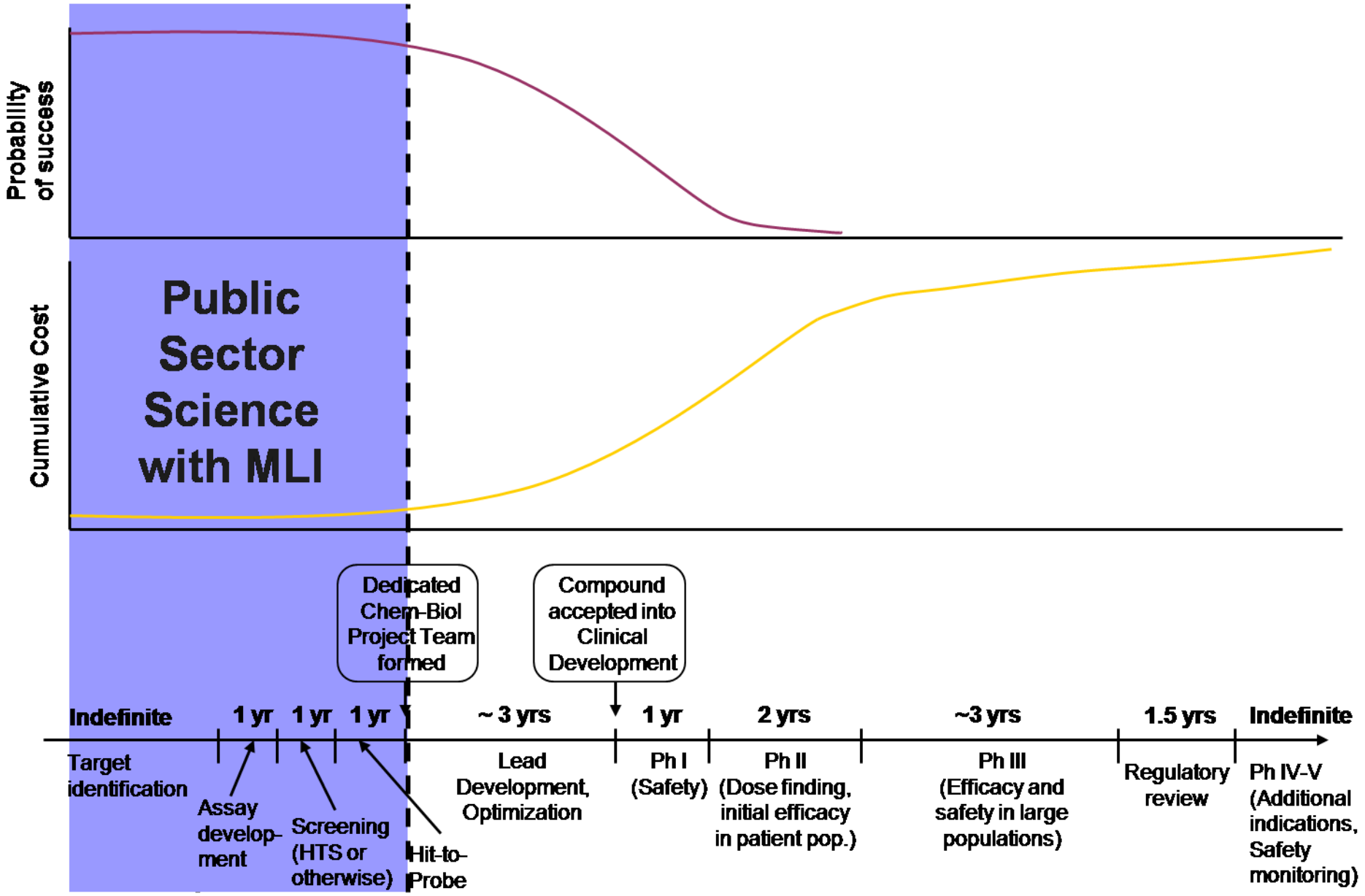
Probes are just the start of drug development



Probes are just the start of drug development



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How to cite this document:
Assay Guidance Manual Version 5.0, **2008**, Eli Lilly and Company and NIH Chemical Genomics Center.
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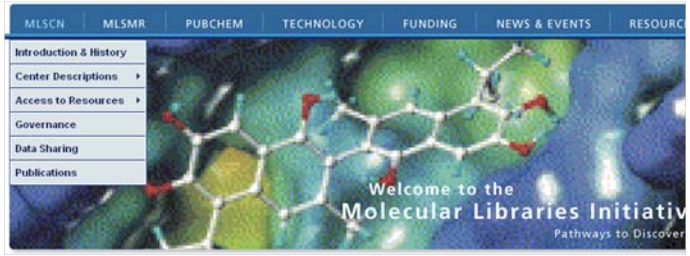

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