Trends in Chemical Biology and Drug Discovery
BIOENG-510- Spring 2025

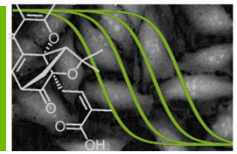
DRUG DISCOVERY, SCREENING COMPOUNDS FOR BIOACTIVITY

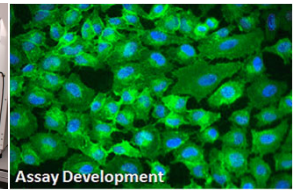
Biomolecular Screening Facility
(Plateforme Technologique de Criblage Biomoleculaire)

Thursday, May 15th 2025. Gerardo Turcatti

Biomolecular Screening Facility

From assay development to hits validation / expansion and hits to leads





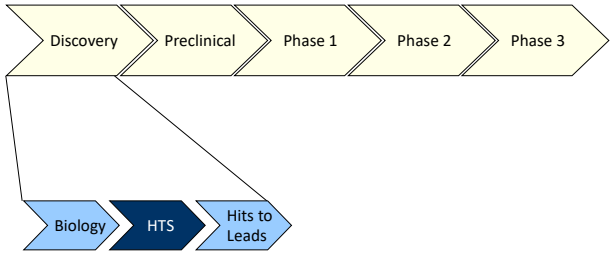
Since 2006, a Swiss multidisciplinary platform for drug discovery screening projects

Goals of the lecture

INTRODUCTION OF THE SCREENING PROCESS

- in the framework of Chemical Biology and DD
- the rational behind – importance of assay, compound libraries
- the linked quantitative analysis (including statistical validions, limitations…)
- the output follow-up

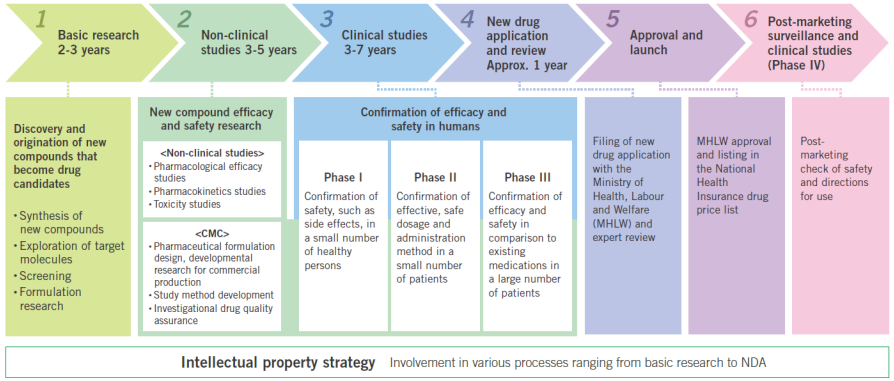
Traditional Drug Discovery Process



Drug Discovery

Drug discovery is the process whereby compounds with activity against a specified target or function are identified, evaluated and optimized for clinical applications

The Drug Research and Development Process



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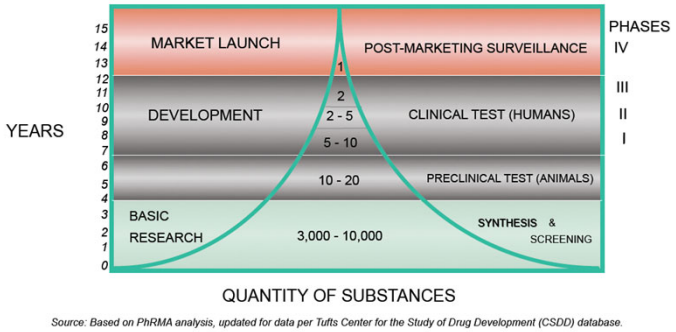
Time scale from discovery to drug approval



Source: Based on PhRMA analysis, updated for data per Tufts Center for the Study of Drug Development (CSDD) database.

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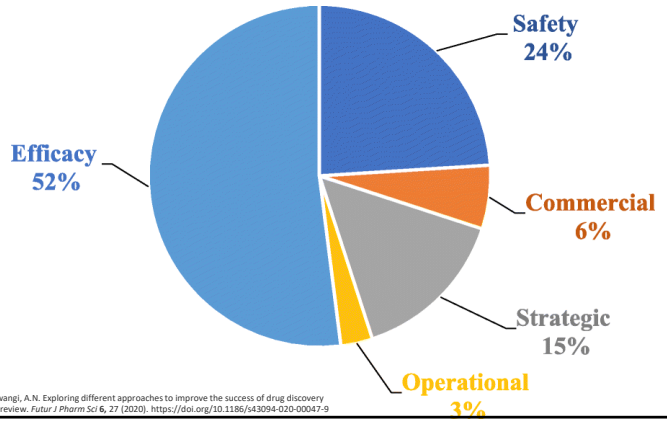
Causes of attrition in drug discovery and development



Kiriiri, G.K., Njogu, P.M. & Mwangi, A.N. Exploring different approaches to improve the success of drug discovery and development projects: a review. *Futur J Pharm Sci* **6**, 27 (2020). https://doi.org/10.1186/s43094-020-00047-9

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Some trends/strategies for accelerating the DD process

- Phenotypic screens by imaging
- Physiologically-relevant models
- Drug repurposing
- Drug combination
- Expansion of the chemical diversity to screen
- Artificial intelligence

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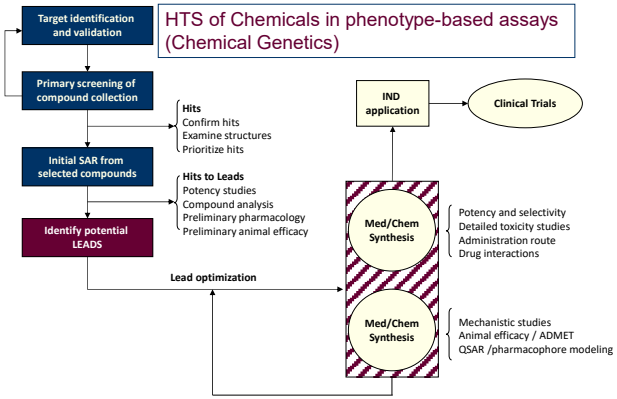
Strategy for preclinical drug discovery



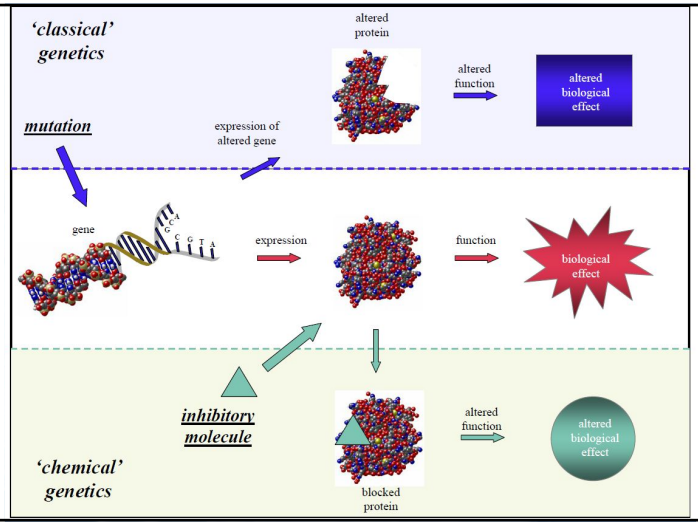
Chemical genetics

- An alternative to classic genetic approaches

Chemical genetics uses chemicals that alter specifically protein function in place of mutations (chemical interference)

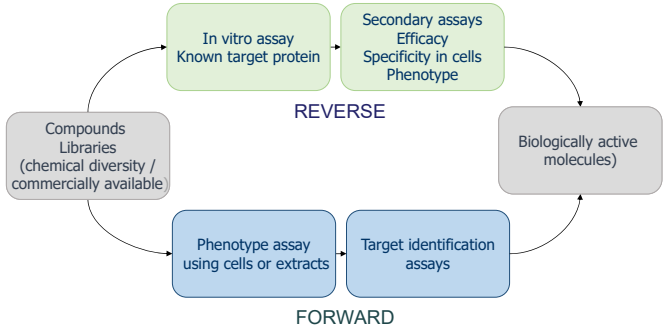
Reverse chemical genetics: traditional approach for searching a candidate drug. The target is known

Forward chemical genetics: Search of small molecules provoking specific phenotypes in the cell. These molecules can then be used for determining their protein targets.



Chemical genetics

Discovery of biologically active molecules by screening

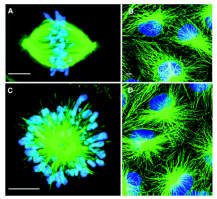


Chemical genetics

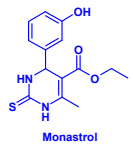
FORWARD
(systematic approach in academia) Mayer et al. (1999)
Science 286 971-4

Chemical: Monastrol
Phenotype caused: Inhibition of mitosis by collapsing the mitotic spindle
Target/mech identified: Eg5, a kinesin involved in maintaining the spindle structure.

Figure 2.for 4 hours with 0.4% DMSO (control) (**A** and **B**) or 68 µM monastrol (**C** and **D**). No difference in distribution of microtubules and chromatin in interphase cells was observed (B and D). Monastrol treatment of mitotic cells replaces the normal bipolar spindle (A) with a rosette-like microtubule array surrounded by chromosomes (C). Scale bars, 5 µm.



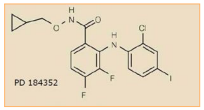
16 320 composés testés



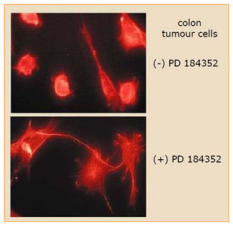
Monastrol

Chemical genetics

REVERSE
Study of the Biology of MEK1



Chemical: PD184352
*PD184352 was identified in high throughput in vitro (**target-based**) kinase **assay***
Highly potent and selective MEK1 inhibitor
Role of MEK1 for cell cycle progression was shown in cells
Colon tumor size was reduced in mice treated with PD 184352



NOTE:
The analogous (classical) reverse-genetic approach, *Mek1*-deficient mouse embryos died in early embriogenesis. In such cases, reverse chemical-genetic methods complement and extend (classical) reverse-genetic methods for studying specific gene product functions *in vivo*

J. S. Sebolt-Leopold, D. T. Dudley, R. Herrera, K. Van Becelaere, A. Wiland, R. C. Gowan, H. Tecle, S. D. Barrett, A. Bridges, S. Przybranowski, W. R. Leopold, A. R. Saltiel, *Nat.Med.* **1999**, 5 810-816.

Molecular Screening

Essential discipline in drug discovery

A pivotal role in Chemical Biology research

Molecular screening: tools, technologies and methodological approaches for the discovery of bioactive molecules and cellular mechanisms

Discovery of bioactive molecules and cellular mechanisms

Question linked to a disease or biological investigation directed to

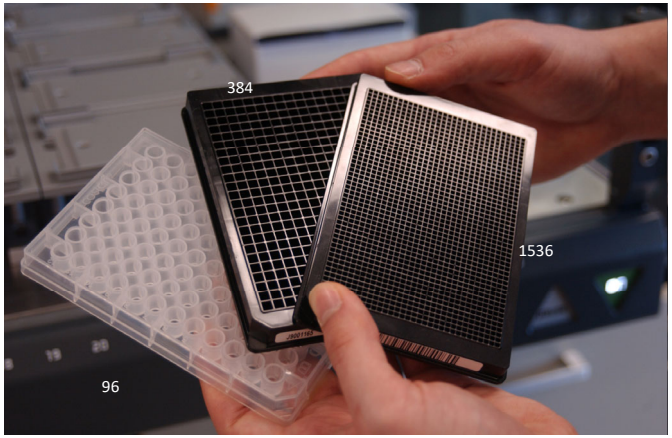
- A protein **target**
- Cell **signaling pathway**
- A cell **phenotypic** change

Development of a representative assay

- Biochemical, cellular or both
- The assay must include a precise **detection** method
- The assay should be **robust and reliable**
- The assay should allow analyzing tenths-hundred thousands compounds, therefore scalable up: **High Throughput Screening**

- Need for automation and miniaturization

Assay miniaturization



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Primary screening: the pillars

Screening allows the identification of active entities (compound, protein…)

Library
- Chemicals
- siRNA…

Assay (MICROPLATE)
- Target
- Cellular pathway
- Phenotype

Instrumentation
- Liquid handlers
- Readers

Data analysis and data management
of experimental results (LIMS)

Typical HT-Assays

TARGET KNOWN

IN VITRO BIOCHEMICAL TARGET- BASED ASSAYS

•Purified target protein : Typically enzymatic assays (i.e. kinases, MIF)
•*Enriched protein in* cell extracts (enzymatic assays; i.e. Telomerase) or membrane preparations (GPCRs; i.e. NK1)

IN VITRO

CELLULAR BIOLOGICAL TARGET- BASED ASSAYS

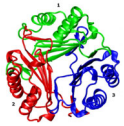
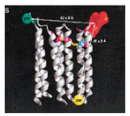
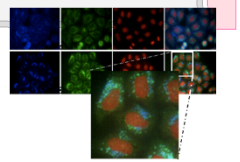
Cell-based assays for primary and secondary target-based screens:
Pathway investigation (i.e. Wnt, Notch); Membrane receptors (i.e. GPCR)

TARGET UNKNOWN

CELLULAR PHENOTYPE- BASED ASSAYS

Target discovery / validation, pathway investigation (i.e. lipids trafficking)

CELLULAR

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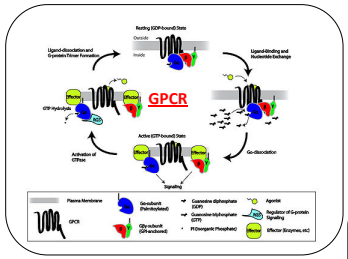
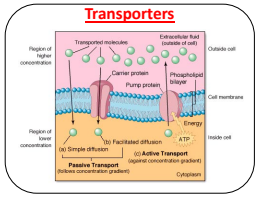
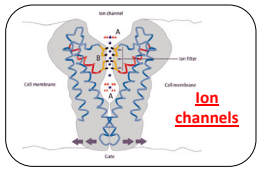
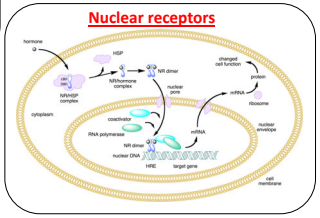
Targets families









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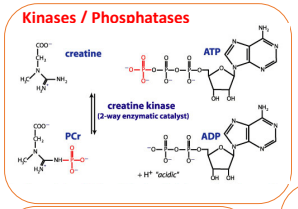
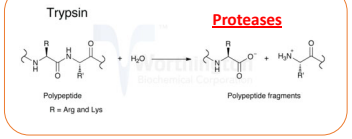
Targets families: enzymes

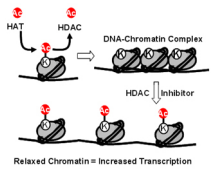
Kinases / Phosphatases



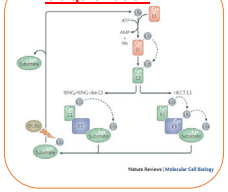
Proteases



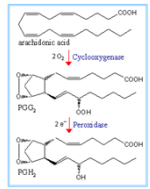
Epigenetic



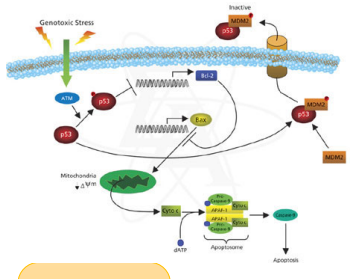
Ubiquitination



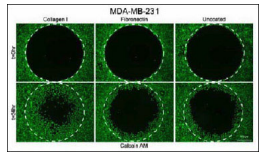
Metabolic (AA, NO…)



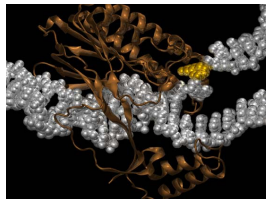
Pathway regulation



Cell proliferation / migration



Protein - protein /DNA interactions

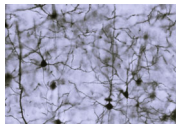


Micro-organisms
Bacteria
Fungis
Yeasts

Protein secretion (cytokines…)
ELISA / RIA assays

Complex assays, increasing the biological relevance

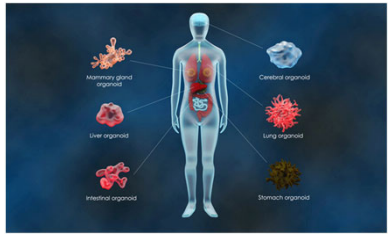
Primary cells like neurons,cardiomyocytes
- typically 2- 3 weeks of culture after isolation
- limited amount of cells available

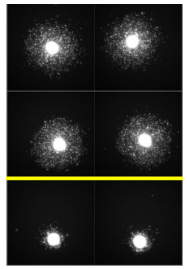


Whole organisms like worms, nematodes, zebrafish
- low number of organisms per well
- technical issue to «manipulate»
- low drug permeability

Complex assays, increasing the biological relevance

- Relevant cellular models
- 3D cell culture
- Spheroids
- Organoids
- Phenotypic readout
- Supervised / unsupervised analysis
- Deep learning analysis





Assay Development for Screening

- Large diversity of targets
- Different types of assays
- Huge kinds of readouts

… but single strategy

➢ Which parameters are impacting the assay?
➢ How to control them for ensuring high assay quality (quantitative analysis, reproducibility)?

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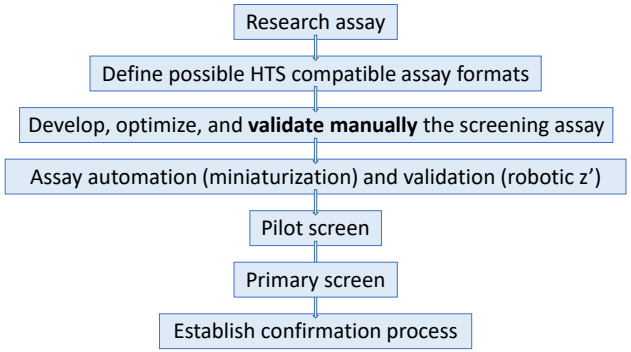
Typical workflow



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Assay design: research *vs.* screening

Table 1 Differences in allowed parameters between laboratory "bench top" and HTS assays

Parameter	Bench top	HTS
Protocol	May be complex with numerous steps, aspirations, washes	Few (5–10) steps, simple operations, addition only preferred
Assay volume	0.1 ml to 1 ml	<1 µl[a] to 100 µl
Reagents	Quantity often limited, batch variation acceptable, may be unstable	Sufficient quantity, single batch, must be stable over prolonged period
Reagent handling	Manual	Robotic
Variables	Many—for example, time, substrate/ligand concentration, compound, cell type	Compound[b], compound concentration
Assay container	Varied—tube, slide, microtiter plate, Petri dish, cuvette, animal	Microtiter plate
Time of measurement	Milliseconds to months Measurements as endpoint, multiple time points, or continuous	Minutes to hours Measurements typically endpoint, but also pre-read and kinetic
Output formats	Plate reader, radioactivity, size separation, object enumeration, images interpreted by human visual inspection	Plate reader—mostly fluorescence, luminescence and absorbance
Reporting format	"Representative" data; statistical analysis of manually curated dataset	Automated analysis of all data using statistical criteria

[a]Special reagent dispensers required. [b]Ideally available in milligram quantity with analytical verification of structure and purity.

HTS compatibility

- Homogeneous assay preferred (mix and measure)
- Limited number of steps
- Incubation time and temperature (RT preferred)
- Reproducibility

- Resistance to interferences
- Sensitivity (saving reagent amount)
- Miniaturization
- Content versus throughput

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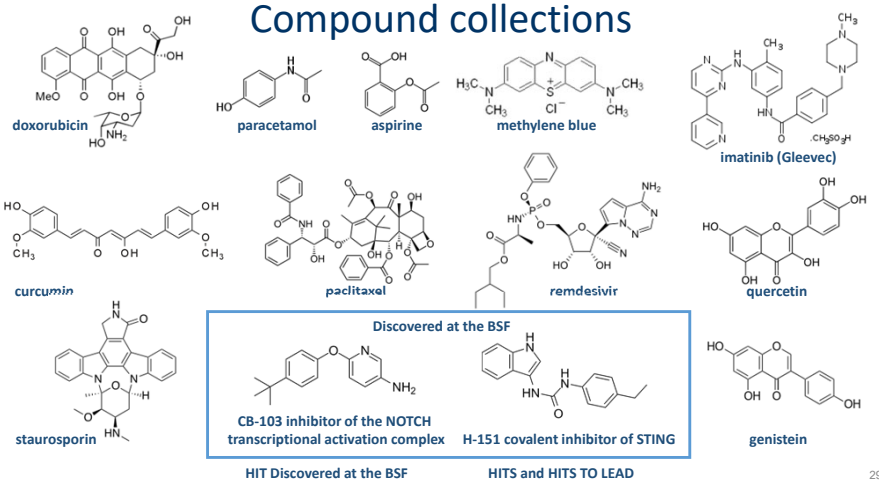
Compound collections



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About chemical libraries

- **Size of a collection**
  - Pharma: 1-2 Mio compounds
  - Academic: 20'000 to 200'000 compounds
  - BSF: about 100'000 compounds since 2015
- **Concentration of molecules**
  - 2-**10** mM in DMSO anhydrous
  - In general compounds are tested at **10** µM in an assay
  - Assessment of DMSO tolerance in cellular assays
- **Chemical diversity**

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The rule of five (Ro5)

Rule of five (RO5) is a rule to evaluate drug-likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was formulated by C.A. Lipinski in 1997, based on the observation that most orally administered drugs are relatively small and moderately lipophilic molecules.

- Its **molecular weight** is **less than 500**.
- The compound's lipophilicity, expressed as a quantity known as **log*P*** (the logarithm of the partition coefficient between water and 1-octanol), **is less than 5**.
- The **number of groups in the molecule that can donate hydrogen atoms** to hydrogen bonds (usually the sum of hydroxyl and amine groups in a drug molecule) is **less than 5**.
- The **number of groups that can accept hydrogen atoms** to form hydrogen bonds (estimated by the sum of oxygen and nitrogen atoms) is **less than 10**.

The rules, apply only to absorption by passive diffusion of compounds through cell membranes; compounds that are **actively transported** through cell membranes by **transporter proteins are exceptions** to the rule. Lipinski's rule states that, in general, **an orally active drug has no more than one violation of the criteria**

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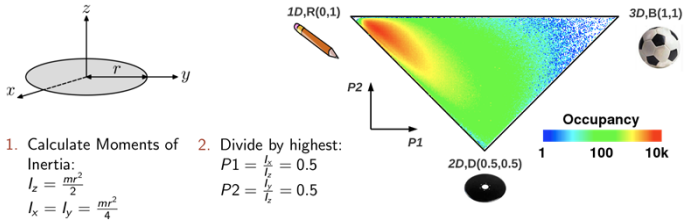
Adding another descriptor: Molecular shape



1. Calculate Moments of Inertia:
   $I_z = \frac{mr^2}{2}$
   $I_x = I_y = \frac{mr^2}{4}$
2. Divide by highest:
   $P1 = \frac{I_x}{I_z} = 0.5$
   $P2 = \frac{I_y}{I_z} = 0.5$

Description of the triangular molecular shape-triangle as proposed by Sauer and Schwarz.[1, 2] A) Example of the calculation of the Px-, Py-, Pz-, P1- and P2-descriptors for a solid disc. B) (P1,P2)-space showing the occupancy of the currently chemical space of commercially available compounds. The sharp corners of the triangle represent the three different possibilities of 1D-, 2D- and 3D-molecules. These points are located at (0,1), (0.5,0.5) and (1.0,1.0) respectively.

In addition to the 'rule of five', shape has been used at the BSF as a criteria for selecting the first chemical diverse collection of 54'000 compounds

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Descriptors used for selecting the Chemical Diverse Collection (CDC) at BSF

Descriptor	Meaning
P1	1st Relative PMI-Shape descriptor.[1]
P2	2nd Relative PMI-Shape descriptor.[1]
F-sp3	Fraction of sp^3-carbon atoms relative to carbon count.[2]
MW	Molecular weight.
HAC	Heavy-atom count.
HBA	H-bond acceptor atom count (*no multi-valency*).
HBAm	H-bond acceptor site count (*with multi-valency*).
HBD	H-bond donor atom count (*no multi-valency*).
HBDm	H-bond donor site count (*with multi-valency*).
logP	Octanol:water partition coefficient.

[1] As reported by Sauer and Schwarz.[1, 2]
[2] As reported by Lovering et al.[3]

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Compounds structure similarity and activity towards a specific target

- Are always similar structures active against a given target?
- Are different structures able to interact with a given target?
- What is considered a similar structure?

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Chemical structure-activity

Transduction of the epinephrine signal: the β adrenergic pathway

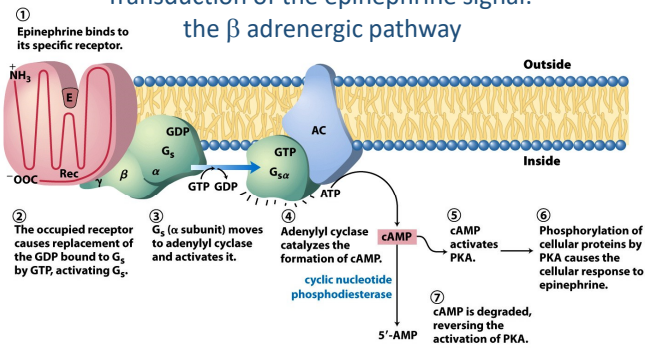

Figure 12-4a
Lehninger Principles of Biochemistry, Fifth Edition
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GPCRs constitute the largest family of proteins targeted by approved drugs: approximately 35% of approved drugs target GPCRs

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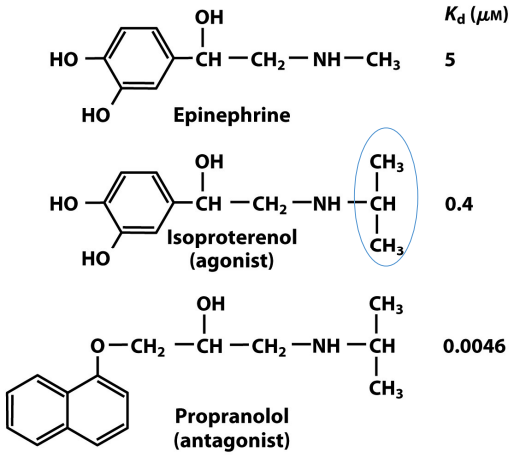

Figure 12-3
Lehninger Principles of Biochemistry, Fifth Edition
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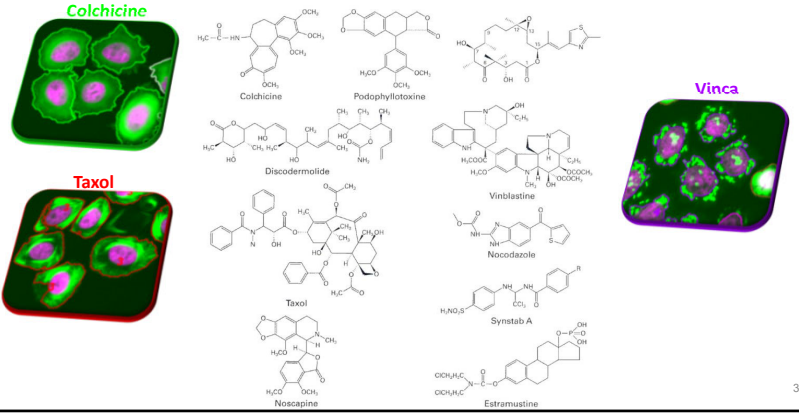
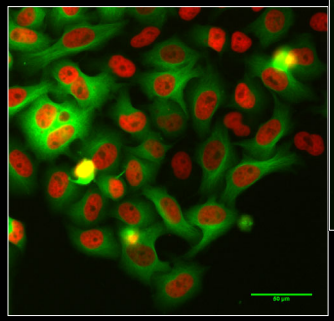
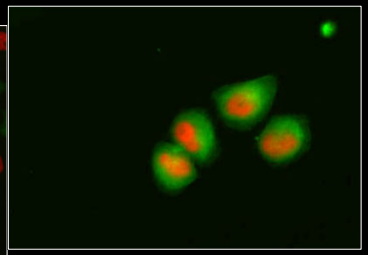
Chemical structure-activity

Chemical diversity: ***Different structures can interact with the same target**: i.e. tubuline*



Model used





Imaged every 15’

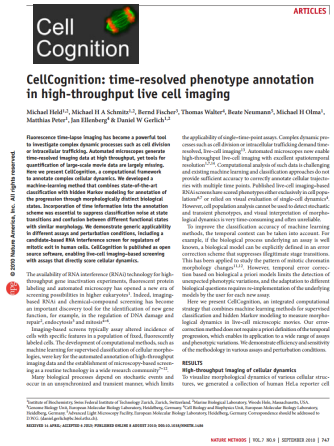
INCell 2200, obj. 20x/0.75 Time-lapse

RFP-H2B: Histones (nucleus)

GFP-Tubulin : β-tubulin (microtubules)

Hela cells in Fluorobrite medium

Analysis software used



Cell Cognition

ARTICLES

CellCognition: time-resolved phenotype annotation in high-throughput live cell imaging

Michael Held[1,2], Michael H A Schmitz[1,2], Bernd Fischer[3], Thomas Walter[4], Beate Neumann[3], Michael H Olma[1], Matthias Peter[4], Jan Ellenberg[4] & Daniel W Gerlich[1,2]

Fluorescence time-lapse imaging has become a powerful tool to investigate complex dynamic processes such as cell division or intracellular trafficking. Automated microscopes generate time-resolved imaging data at high throughput, yet tools for quantification of large-scale movie data are largely missing. Here we present CellCognition, a computational framework to annotate complex cellular dynamics. We developed a machine-learning method that combines state-of-the-art classification with hidden Markov modeling for annotation of the progression through morphologically distinct biological states. Incorporation of time information into the annotation scheme was essential to suppress classification noise at state transitions and confusion between different functional states with similar morphology. We demonstrate generic applicability in different assays and perturbation conditions, including a candidate-based RNA interference screen for regulators of mitotic exit in human cells. CellCognition is published as open source software, enabling live-cell imaging–based screening with assays that directly score cellular dynamics.

The availability of RNA interference (RNAi) technology for high-throughput gene inactivation experiments, fluorescent protein labeling and automated microscopy has opened a new era of screening possibilities in higher eukaryotes[1]. Indeed, imaging-based RNAi and chemical-compound screening has become instrumental for the rapid identification of new gene functions, for example, in the regulation of DNA damage and repair[2], endocytosis[3] and mitosis[4–6].

Imaging-based screens typically assay altered incidence of cells with specific features in a population of fixed, fluorescently labeled cells. The development of computational methods, such as machine learning for supervised classification of cellular morphologies, were key for the automated annotation of high-throughput imaging data and the establishment of microscopy-based screening as a routine technology in a wide research community[7–11].

Many biological processes depend on stochastic events and occur in an unsynchronized and transient manner, which limits the applicability of single-time-point assays. Complex dynamic processes such as cell division or intracellular trafficking demand time-resolved, live-cell imaging[12]. Automated microscopes now enable high-throughput live-cell imaging with excellent spatiotemporal resolution[7,8,13]. Computational analysis of such data is challenging and existing machine learning and classification approaches do not provide sufficient accuracy to correctly annotate cellular trajectories with multiple time points. Published live-cell imaging–based RNAi screens have scored phenotypes either exclusively in cell populations[6,9] or relied on visual evaluation of single-cell dynamics[4]. However, cell population analysis cannot be used to detect stochastic and transient phenotypes, and visual interpretation of morphological dynamics is very time-consuming and often unreliable.

To improve the classification accuracy of machine learning methods, the temporal context can be taken into account. For example, if the biological process underlying an assay is well known, a biological model can be explicitly defined in an error correction scheme that suppresses illegitimate stage transitions. This has been applied to study the pattern of mitotic chromatin morphology changes[14,15]. However, temporal error correction based on biological a priori models limits the detection of unexpected phenotypic variations, and the adaptation to different biological questions requires re-implementation of the underlying models by the user for each new assay.

Here we present CellCognition, an integrated computational framework for the automated annotation of methods for supervised classification and hidden Markov modeling to measure morphological dynamics in live-cell microscopic movies. Our error-correction method does not require a priori definition of the temporal progression, which enables its application to a wide range of assays and phenotypic variations. We demonstrate efficiency and sensitivity of the methodology in various assays and perturbation conditions.

RESULTS

High-throughput imaging of cellular dynamics

To visualize morphological dynamics of various cellular structures, we generated a collection of human HeLa reporter cell

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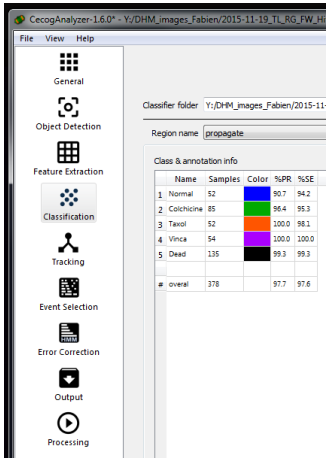
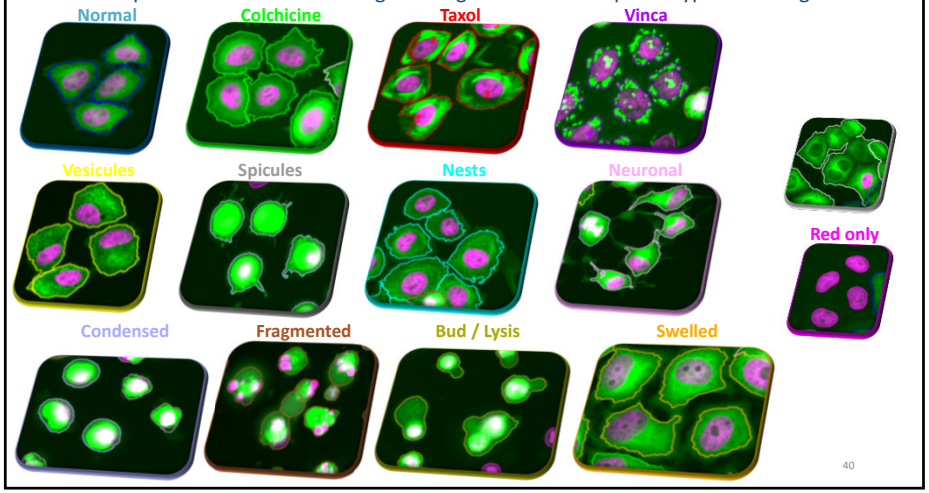
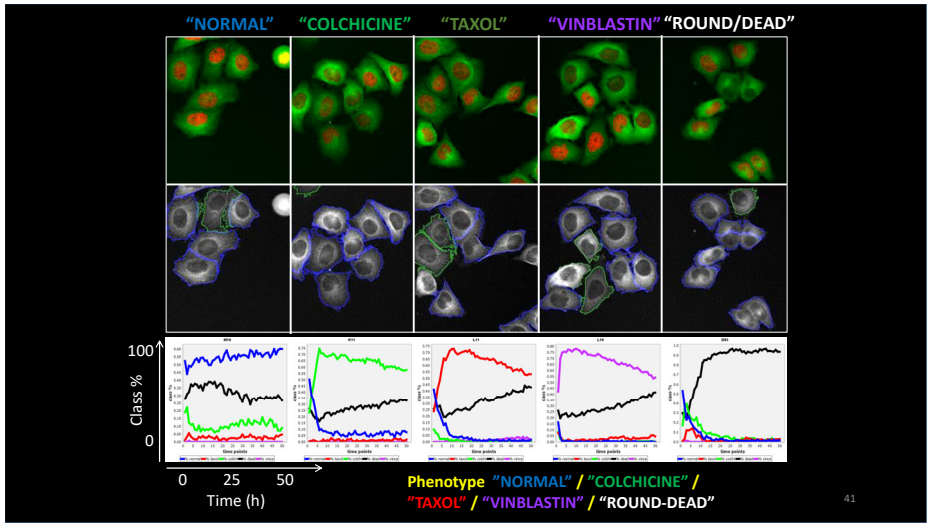


Supervised Machine Learning: CellCognition software phenotypes teaching:





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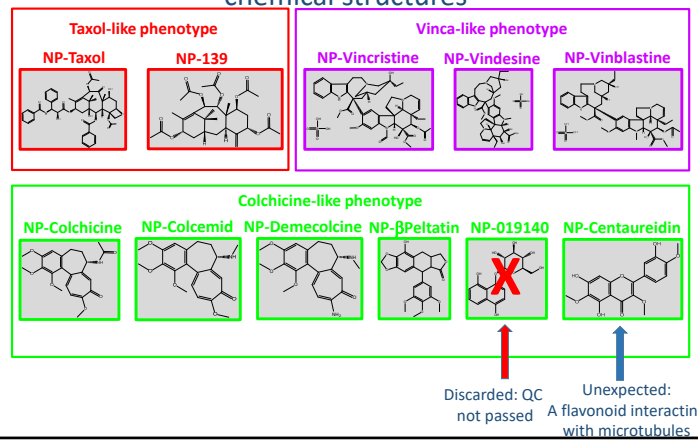
Cellcognition phenotypes and corresponding chemical structures



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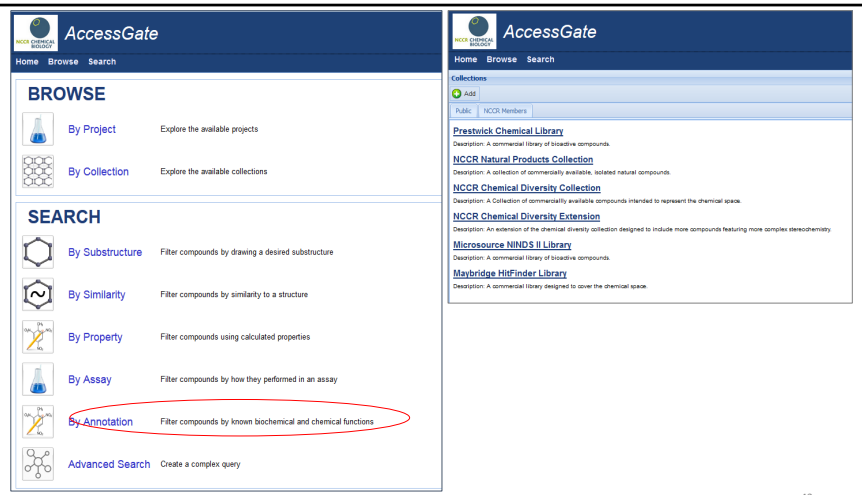

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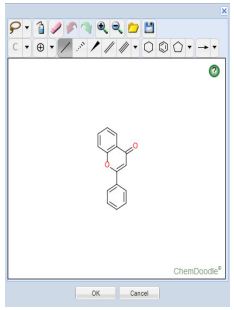
Search by structure-annotation



Flavone motif in BSF collections?

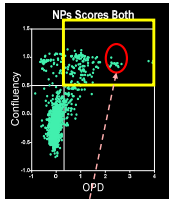
254 compounds

Flavone motif in the NP's set

144 compounds

Hits in my screening belonging to the cluster of 'microtubule perturbers'

1 compound



Cluster of tubulin modulators

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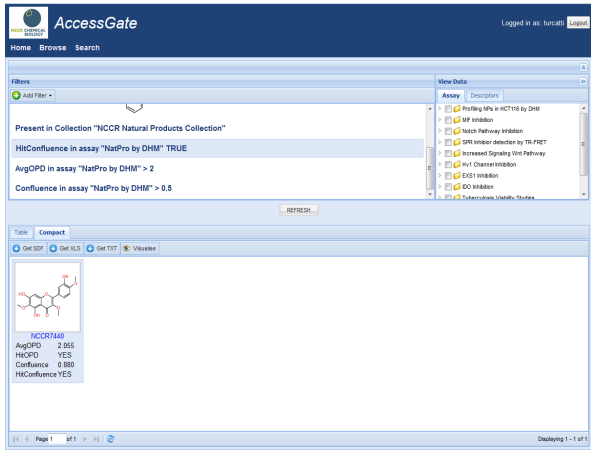
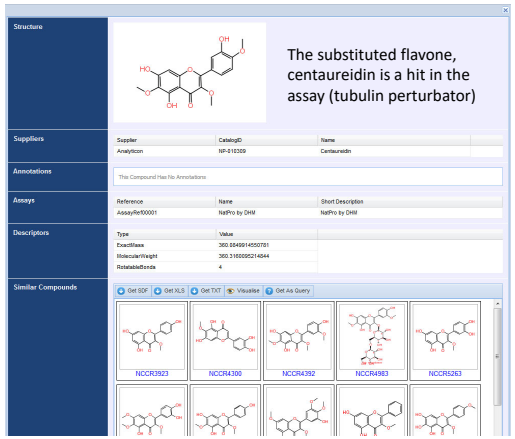
Is any flavone a hit in the cluster of tubulin perturbers or modulators?

Advanced query:
- flavone present in the NP's collection
- Is there any hit from the screen of NPs ?

(by confluence (>0.5) and has a value OPD (related to refractive index change of cells) >2)





The substituted flavone, centaureidin is a hit in the assay (tubulin perturbator)

No annotation in MeSH !
Then: Literature search

CENTAUREIDIN, A CYTOTOXIC FLAVONE FROM *Polymnia fruticosa*, INHIBITS TUBULIN POLYMERIZATION

Not everything is annotated even if published:

Annotations to be performed with BSF validated screening results

Instrumentation - Automation

Automation of fluidic handling

- Compound Management & control
- Assay automation for screening

Reading signal

- Multimode readers
- Imaging using automated microscopes

Main Principles of Compound Management

Automated storage system for tubes and plates Capacity > 200'000 compounds

- Ensure compound stotage & accessibility
- Maintain compound integrity
- Enable efficient compound usage
- Flexibility to adapt to new demands
- Ability to maintain a dynamic collection
- Avaialbility an integrity of inventory data

LC-MS system for checking chemical integrity (+ access to NMR if needed)

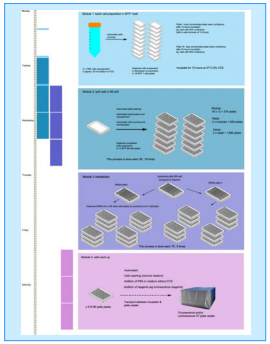
Assay automation for High Throughput Screening

Automatisation of the whole process, signal acquisition, data processing

*High Throughput cellular screen

64'000 compounds
3 replicas + intraplate controls
720 384-well plates

230'400 data points



Robotic Automatisation

Fluidic handling workstation

Atomation Instrumentation

Three integrated robotic systems for compound management tasks and screening (classic pipetting and non-contact dispensing)

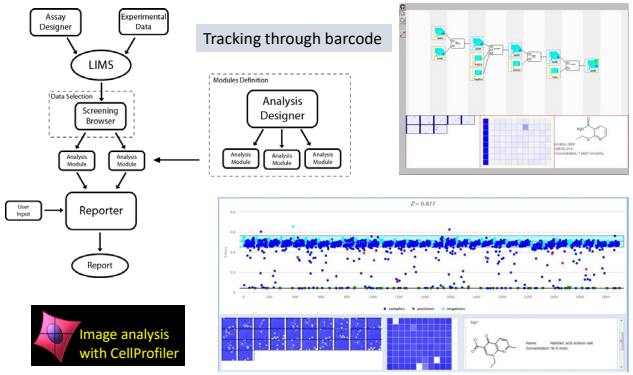
- Liquid handlers (pipettors, dispensers)
- Plate: centrifuge, peeler, sealer
- Washer, shaker, bar-code labelers and readers
- Articulated arms, conveyers
- Peripherials: automated incubators, fridge

Beckman workstation:
siRNA transfections, chemical screens and compound management

Caliper workstation:
Chemical screening and compound management

Labcyte workstation:
Acoustic dispensing. Plating compounds, cherry picking

BSF LIMS for large data set management, analysis and visualization



Tracking through barcode

Image analysis with CellProfiler

ASSAY VALIDATION

Is the manually validated assay suitable for HTS

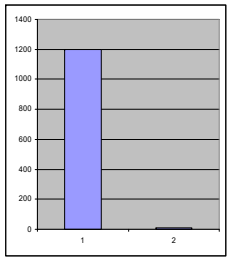
Is the assay statistically significant, reliable, robust ?
Calculate Z'- factor (Zhang et al., (1999) *J. Biomol. Screen* 4: 67-73)

Do we have 'tools' for assay validation ?
Positive controls, known inhibitors

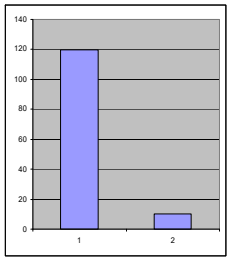
Can it be automatized for screening large collections of chemical compounds ?
Z'-factor automatic screen
IC50 for a well characterized inhibitor

ASSAY VALIDATION: Are signal to background or signal to noise good criteria for an assay?

Experimental conditions (I)
Mean Signal / Mean Background = 120
S/N = (Mean Signal – Mean Bkg)/SD Bkg = 406



Experimental conditions (II)
S/B = Mean Signal / Mean Background = 12
S/N = (Mean Signal – Mean Bkg) / SD Bkg = 241

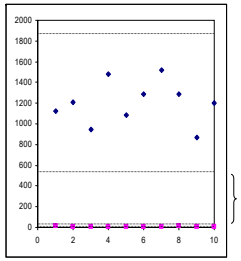


S/B Does not contain any information about data variation

S/N Indication about degree of confidence for assigning a value as real.
Not all the information needed for evaluating the quality of an assay

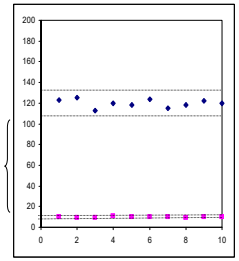
Z' – factor as an statistical tool for assay quality assessment

Experimental conditions (I)
Mean Signal / Mean Background = 120
S/N = (Mean Signal – Mean Bkg)/SD Bkg = 406



Screening window

Experimental conditions (II)
S/B = Mean Signal / Mean Background = 12
S/N = (Mean Signal – Mean Bkg) / SD Bkg = 241

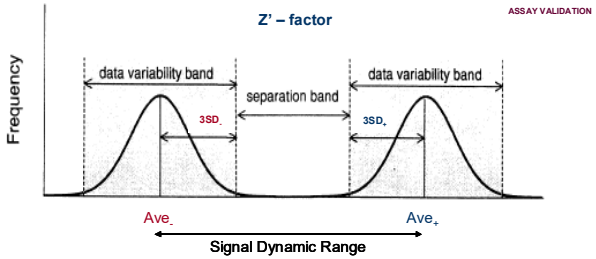


Z' = 0.43

Z' = 0.88

Z' – factor

ASSAY VALIDATION

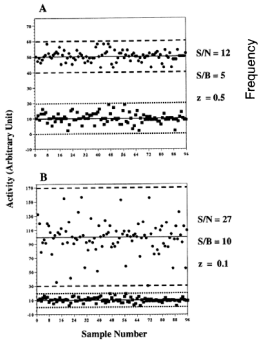


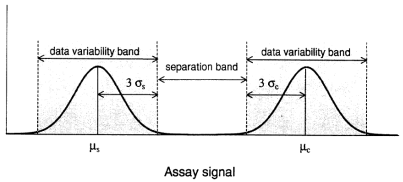
$$Z' = \frac{\text{Separation Band}}{\text{Signal Dynamic Range}} = \frac{|Ave_+ - Ave_-| - (3SD_+ + 3SD_-)}{|Ave_+ - Ave_-|} = 1 - \frac{(3SD_+ + 3SD_-)}{|Ave_+ - Ave_-|}$$

- Z' factor is an indicator of assay quality without the intervention of testing compounds
- It is a statistical characteristic of any given assay NOT LIMITED TO HTS

Zhang et al., (1999) *J. Biomol. Screen* 4: 67-73

Assay validation: statistical analysis



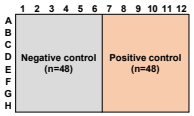


Coefficient of variation
CV (%) = 100*σ/μ

$$Z' = 1 - \frac{(3\sigma_{c+} + 3\sigma_{c-})}{|\mu_{c+} - \mu_{c-}|}$$

$1 \geq Z' > -\infty$

Z' = 1 : perfect assay
1 > Z' > 0.5 : good assay
0.5 > Z' > 0 : poor assay
Z' = 0 : yes / no assay
Z' < 0 : screening not possible



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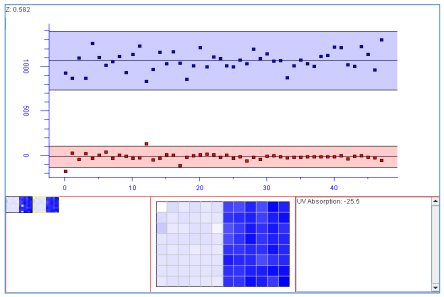
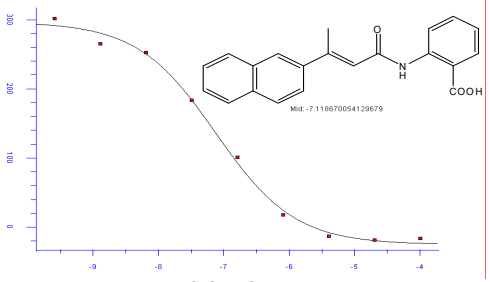
ASSAY VALIDATION: Z' CALCULATION

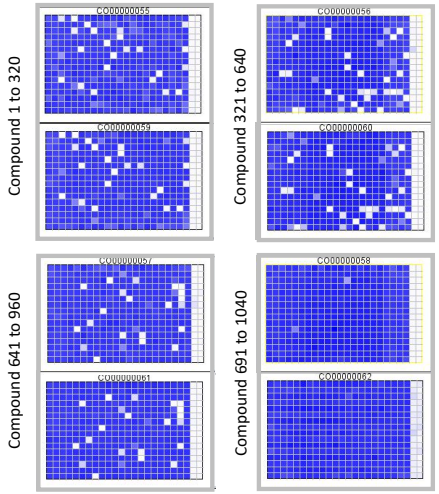


INHIBITION CURVE USING THE COMPOUND BIBR1532 (n=8; 2% final DMSO)



IC50 = 76 nM

REPLICATES
REPRODUCIBILITY



EXAMPLE
Cell viability assay
Read-out: Alamar Blue
Cells: *C. Glutamicum*
Plate format: 384 well plates
Replicates: 2
Collection: NINDS, 1040

60

Interplate assay quality (z' per plate)

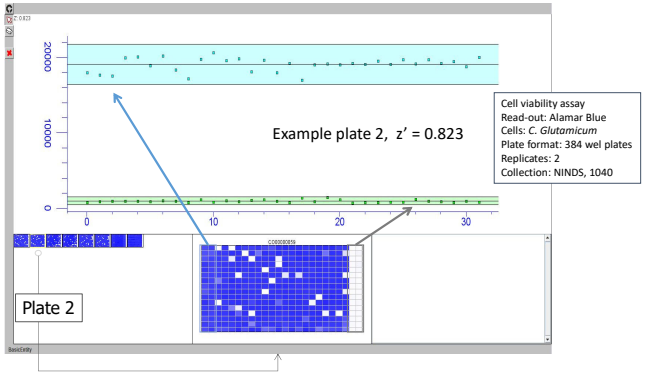


Plate	1	2	3	4	5	6	7	8
Z'	0.79	0.82	0.77	0.77	0.87	0.90	0.85	0.85

61

Intraplate assay quality (cumulated z' for all plates)

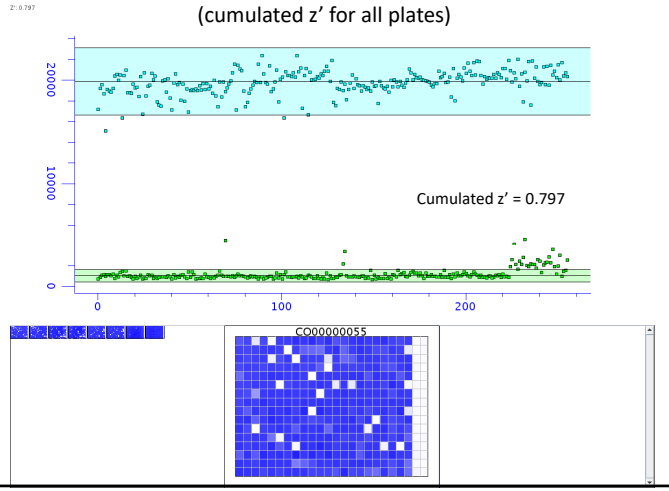


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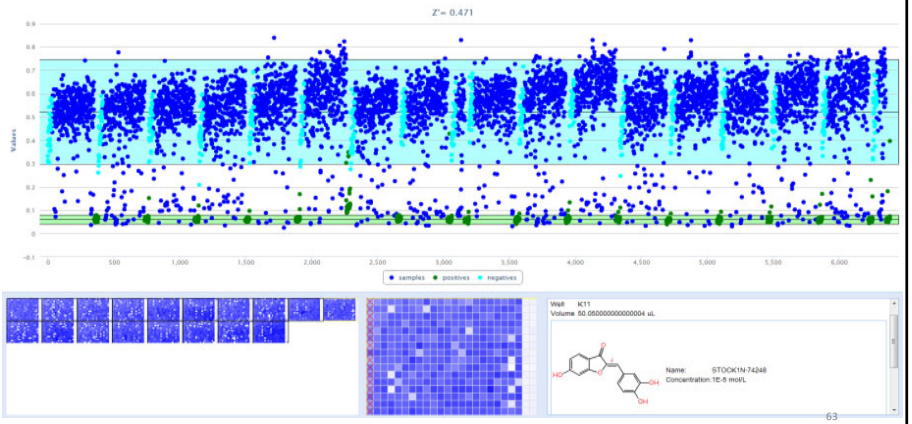
Image-based phenotypic screen using a collection of natural products

(purified extracts from plants and bacteria)



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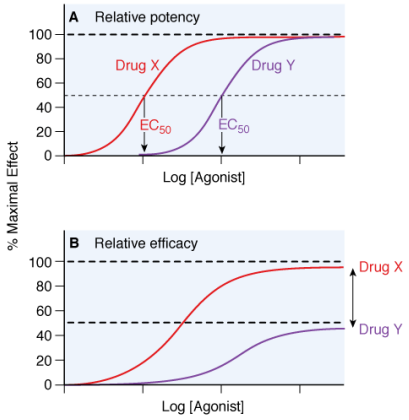
Dose responses: Potency and Efficacy



Medicinal chemistry / Hits to leads

- Confirmed hit (scaffolds, chemical series)
- Chemical tractability / complexity of synthesis
- Improve affinity / specificity / potency (SAR)
- Improve physico-chemical properties
- Lower toxicity
- IP: novelty / freedom to operate

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Early ADME/TOX characterization

- Solubility (logS) /stability
- Lipophilicity (logP, $logD_{7.4}$)
- Permeability (P_{app}) (PAMPA, Caco2 cells)
- P-gp efflux (cell excretion)
- Cytochromes P450 (3A4/2D6/2C9/1A2/2C19) (metabolic stability)
- Cytotoxicity
  - Control cell line(s)
  - HepG2 (hepatotox)
- Genotoxicity
  - Micronucleus
  - Ames tests
- Cardiotoxicity
- hERG channel (QT prolongation)
- **cardiomyocyte beating**

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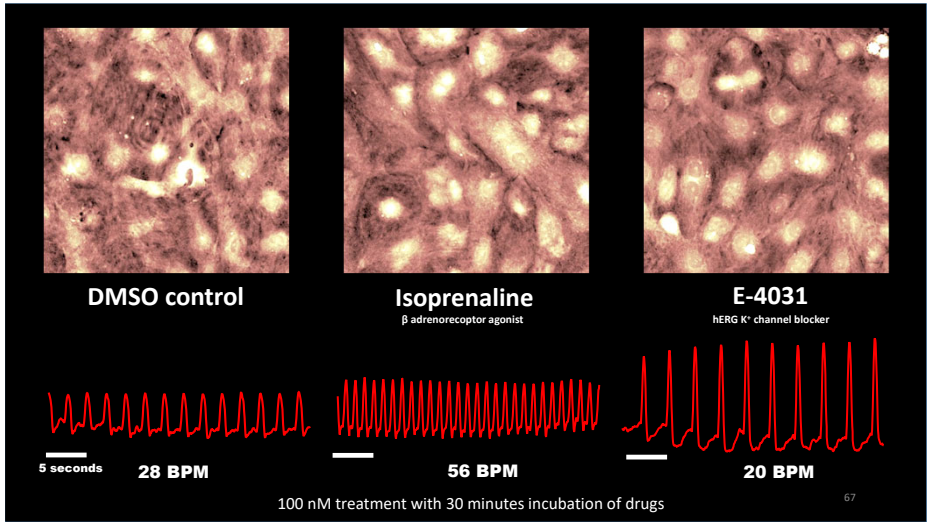

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