Methods: from disease models to therapy

(BIOENG-518)

Developing and validating screening assays in the frame of Drug Discovery

Duchenne Muscular Dystrophy

- Genetic disease / Dystrophin
- Primary therapies: Gene therapy / translation or splicing modifiers
- Secondary therapies: Drug Discovery including drug repurposing and small molecules screening (e.g. anti-inflammatory drugs –> gluco-corticosteroids derivatives)

DMD treatment options

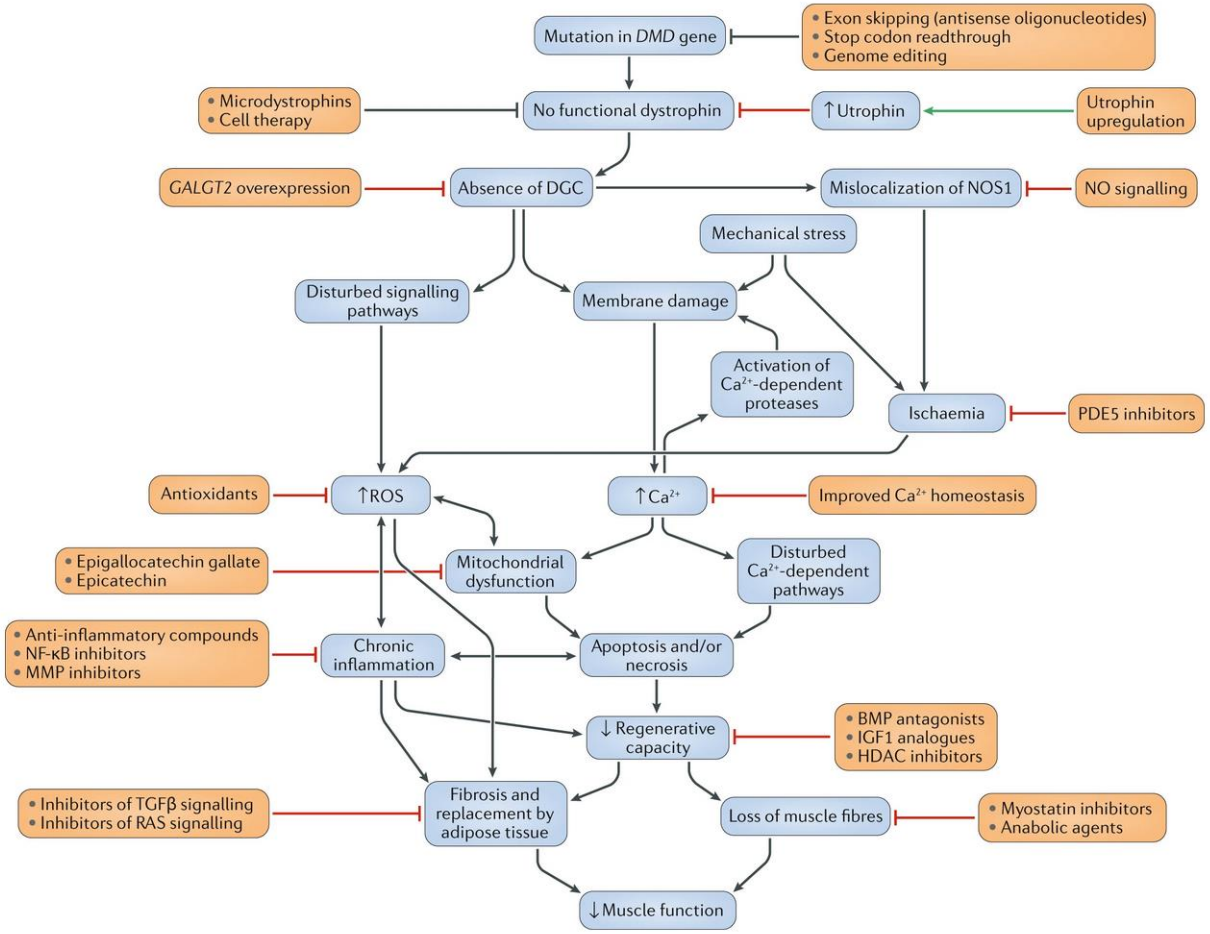
Heydemann & Siemionow, Biomedecines, 2023, 11, 830

Strategy	Pros	Cons	Specific Therapy	Pre-Clinical/Clinical Results	Refs
1. In vivo gene correction	If stem cells are repaired this may be a true cure. Mutation independent	May cause off-site mutations.Safe delivery to all muscle cells is not yet perfected.	SRP-9001. rAAV-rh74 delivery of micro-dystrophin, 1 injection.	NCT03375164, 03769116, 04626674. Patients had long-term gene expression and phenotype improvements. Phase 3 is now recruiting. Some patients developed antibodies to the virus.	[5,6]
			Pf-06939926. rAAV9 delivery of mini-dystrophin, 1 injection, 2 doses.	NCT0336502, NCT04281485, NCT05429372. Patients had gene expression, and an average 3.5-point increase in the NSAA score. A total of 40% of patients experienced vomiting and/or nausea. Phase 3 trial on hold due to a patient's death.	[7]
			SGT-001, rAAV9 delivery of micro-dystrophin, 1 injection, 2 doses.	NCT03368742. Variable dystrophin expression. Phenotype improvement in 6MWT and NSAA scores. Many severe adverse effects; liver and kidney injuries.	[8]
		Mutation specific	CRISPR/Cas9	Achieved 60% of normal dystrophin in a canine DMD model	[9]
2. In vivo mRNA correction	These have demonstrated clinical benefits.	Safe delivery to all muscle cells is not yet perfected. Some adverse drug reactions. Must be continually re-administered. Mutation specific	Read through; Ataluren	NCT01826487, NCT01557400. Reduces many of the disease symptoms, such as loss of ambulation and respiratory decline	[10,11,12]
			Exon 51 skipping; Eteplirsen	NCT02255552. Small, if any, improvements over the control group at 96 weeks post treatment. Delay in pulmonary decline.	[13,14,15]
			Exon 53 skipping; Vitolarsen	Achieved an average of 5.9% of normal dystrophin levels after 20 weeks of treatment	[16]
			Exon 51 skipping; Drisapersen	NCT01254019. Some benefit with post hoc statistics in the 6MWT, clinical trials terminated	[17]
			Exon 53 skipping; Golodirsen	NCT02310906. Decreased muscle function decline.	[18,19]
			Exon 45 skipping; Casimersen	NCT02500381. Confirmed safety.	[20,21]
3. Upregulation of supporting molecules	Will treat most DMD patients. Low side-effects.		Utrophin	NCT02858362. Study was halted due to lack of efficacy.	[22]
			Integrin-α7; SU9516	PC., Slows disease progression	[23]
			Integrin-α7; Obestatin	PC. Increased force production and other aspects of the mdx phenotype	[24]
			Sarcospan	PC, decreases mdx muscle pathology including cardiomyopathy	[25,26]
4. Enhancing muscle metabolism	FDA-approved for Type 2 diabetes		Increase pAMPK; Metformin	NCT01995032. No DMD reducing results.	[27,28]
			Increase PGC1α	NCT01856868. Some benefit for the patients.	[29]
5. Novel steroids	Fewer side-effects	May still decrease patient's immune response	Deflazacort	A retrospective patient study identified benefits of deflazacort over prednisone.	[30]
			Vamorolone	PC. Vamorolone reduces fibrosis, inflammation and cardiomyopathy in mdx mice with reduced side effects. NCT02760264, 02760277, 03038399. Improvement in muscle function over natural history values and fewer side-effects then with corticosteroids.	[31,32]
6. Repurposing pharmaceuticals	Less expensive. Already passed human safety trials.		Tamoxifen	NCT02835079. Lower decreases in muscle and respiratory functions.	[33]
			Simvastatin	PC. Reduced pathology and increased muscle function.	[34,35]
			Zidovudine (AZT)	PC. Reduced pathology and increased muscle function.	[36]
7. Cell Transplants		Requires immune suppression.	Myoblasts	NCT02196467. Local high-density cell injections with immune suppression. Dystrophin was detected at the injection site at 4-weeks post-injections.	[37]
	Additionally benefitted skeletal muscles.		Cardiospheres	NCT02485938. Coronary injections without immune suppression. At 12-months post treatment only the treated patients had reduced size of myocardial scars.	[38]
	No immune suppression is needed. Intraosseous, systemic delivery		Dystrophin expressing chimeric cells (DEC)	PC. Skeletal, cardiac and diaphragm muscle improvements up to 180 days post single injection.	[39,40,41,42,43,44,45]

DMD therapies

Fig. 1: Primary and secondary therapies for Duchenne muscular dystrophy.

From: Therapeutic developments for Duchenne muscular dystrophy



Verhaart & Aartsma-Rus, Nature Neurology Review, 2019, 15, 373

DMD: molecules in clinical trials

Compound	Mechanism of action	Trial phase	Refs
Fibrosis			
Coenzyme Q10	Electron acceptor for NADH and succinate dehydrogenase	Phase II/III	149, 150
Halofuginone	Inhibitor of collagen α1(I) chain and MMP2	Phase I/II (terminated)	151, 152
Tamoxifen	Oestrogen receptor modulator	Phase III	153,154,–155
Pamrevlumab	Anti-CTGF antibody	Phase II	156
Fibrosis and regeneration			
Givinostat	Histone deacetylase inhibitor	Phase II/III	125, 126, 157
Inflammation			
Prednisone, prednisolone and deflazacort (corticosteroids)	Immunosuppression	Phase III	8, 158, 159
Vamorolone	NF-κB inhibitor	Phase II and phase III (planned)	91, 92, 160, 161
Edasalonexent	NF-κB inhibitor	Phase III	162,163,164,–165
Cosyntropin	Melanocortin receptor activator	Phase II	166
Flavocoxid	NF-κB inhibitor	Phase I	167, 168
TAS-205	Haematopoietic prostaglandin D synthase inhibitor	Phase IIa	169, 170
Muscle growth and regeneration			
Domagrozumab	Myostatin-targeted antibody	Phase II (terminated)	113, 171
Taldithercept alfa	Anti-myostatin adnectin	Phase II/III	172
rAAV1.CMV.huFollistatin344	Myostatin inhibitor	Phase I/II	122, 123
Calcium homeostasis and fibrosis			
Idebenone	Antioxidant	Phase III	96, 173, 174
Rimeporide	Sodium–hydrogen exchanger 1 inhibitor	Phase I	175
Vasodilatation			
Lisinopril	ACE inhibitor	Phase II/III	150, 176
Metformin, L-citrulline or L-arginine	Increases NO signalling	Phase III	177, 178
Sildenafil or tadalafil	PDE5 inhibitor	Phase III (terminated)	108, 179
Spironolactone or eplerenone	Aldosterone inhibitor	Phase III	180,181,–182
Cardiomyopathy			
CAP-1002	Cardiosphere-derived cells	Phase II	183, 184
Ramipril	ACE inhibitor	Phase IV	185
Carvedilol	β-Blocker	Phase IV	185, 186
Ifetroban	Thromboxane A2 receptor antagonist	Phase II	187
Poloxamer-188NF	Membrane sealant	Phase II	188,189,–190
Nebivolol	β1 adrenergic receptor antagonist	Phase III	191, 192
Mitochondria			
Epigallocatechin gallate	NO–AMPK–SIRT1–PGC1α pathway	Phase II/III	193, 194
(+)-epicatechin	NO–AMPK–SIRT1–PGC1α pathway	Phase I/II	195
Osteoporosis			
Zoledronic acid	Bisphosphonate; inhibits bone resorption	Phase III	196, 197
Utrophin upregulation			
rAAVrh74.MCK.*GALGT2*	*GALGT2* overexpression	Phase I/II	198, 199
Ezutromid	Utrophin modulation	Phase II (terminated)	200,201,–202
Puberty delay			
Testosterone	Androgen receptor activation	Not stated	203, 204

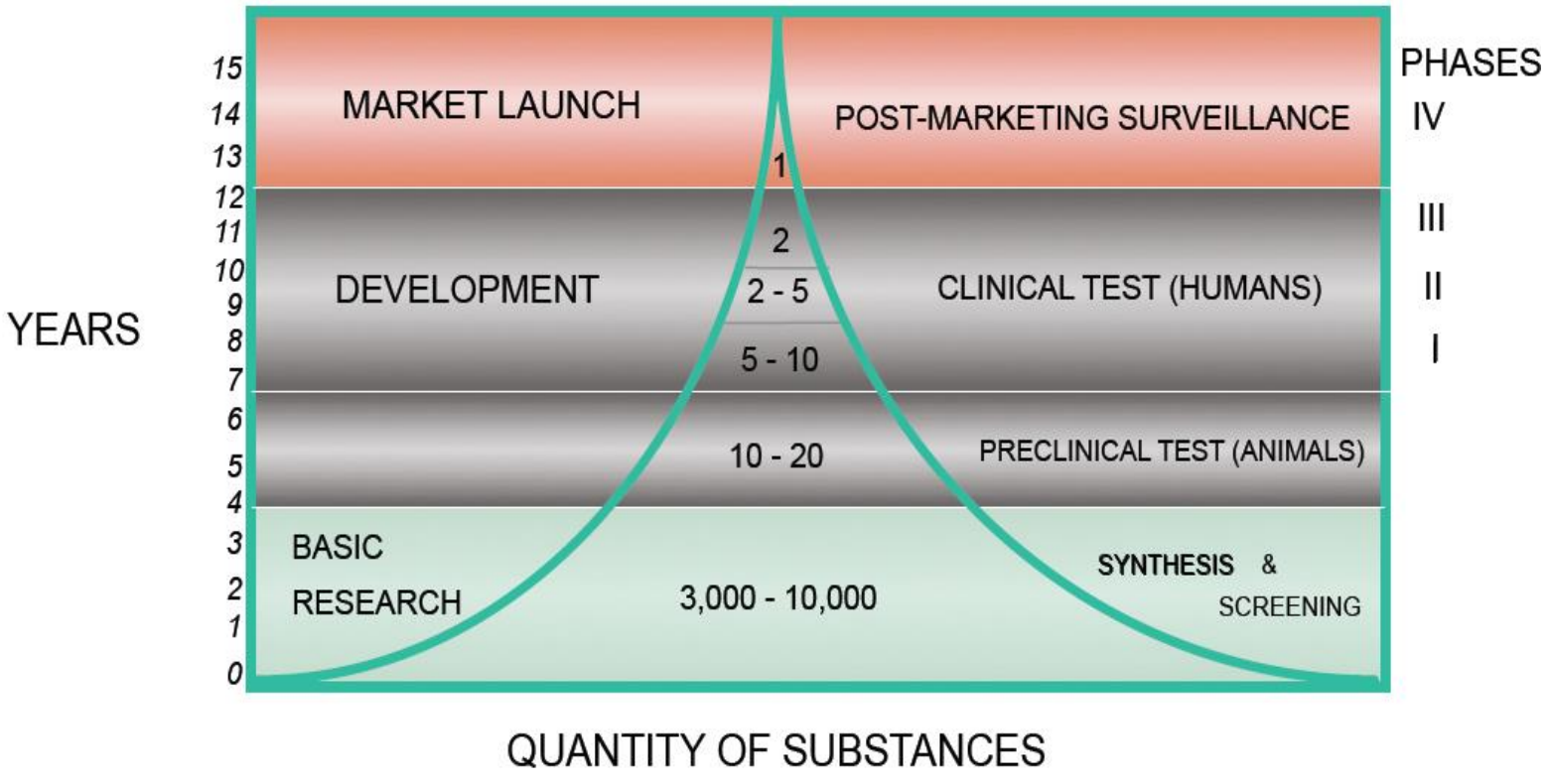
AAV, adeno-associated virus; ACE, angiotensin-converting enzyme; CTGF, connective tissue growth factor; *GALGT2*, the gene encoding β1,4 *N*-acetylgalactosaminyltransferase 2; MCK, minimized mouse creatine kinase promoter; MMP2, matrix metalloproteinase 2; NF-κB, nuclear factor-κB; NO, nitric oxide; PDE5, phosphodiesterase 5.

Verhaart & Aartsma-Rus, Nature Neurology Review, 2019, 15, 373

Goals of the course

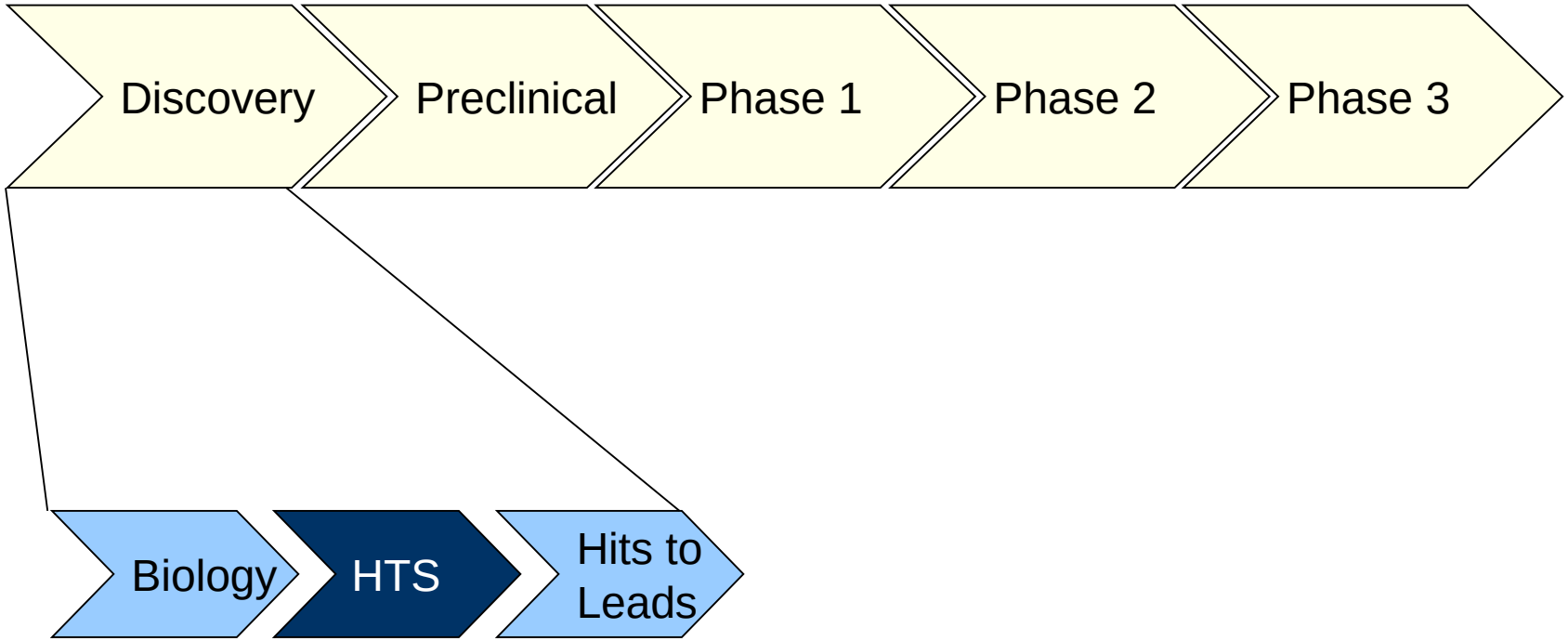
- To understand screening activity, including:
  - the general frame in DD
  - the rational behind – importance of assay
  - the linked quantitative analysis (including statistical validity, limitations…)
  - the output follow-up
- For practical part : to handle different kinds of experiments related to screening assays and to analyze generated data

Discovery and Development of a Drug



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

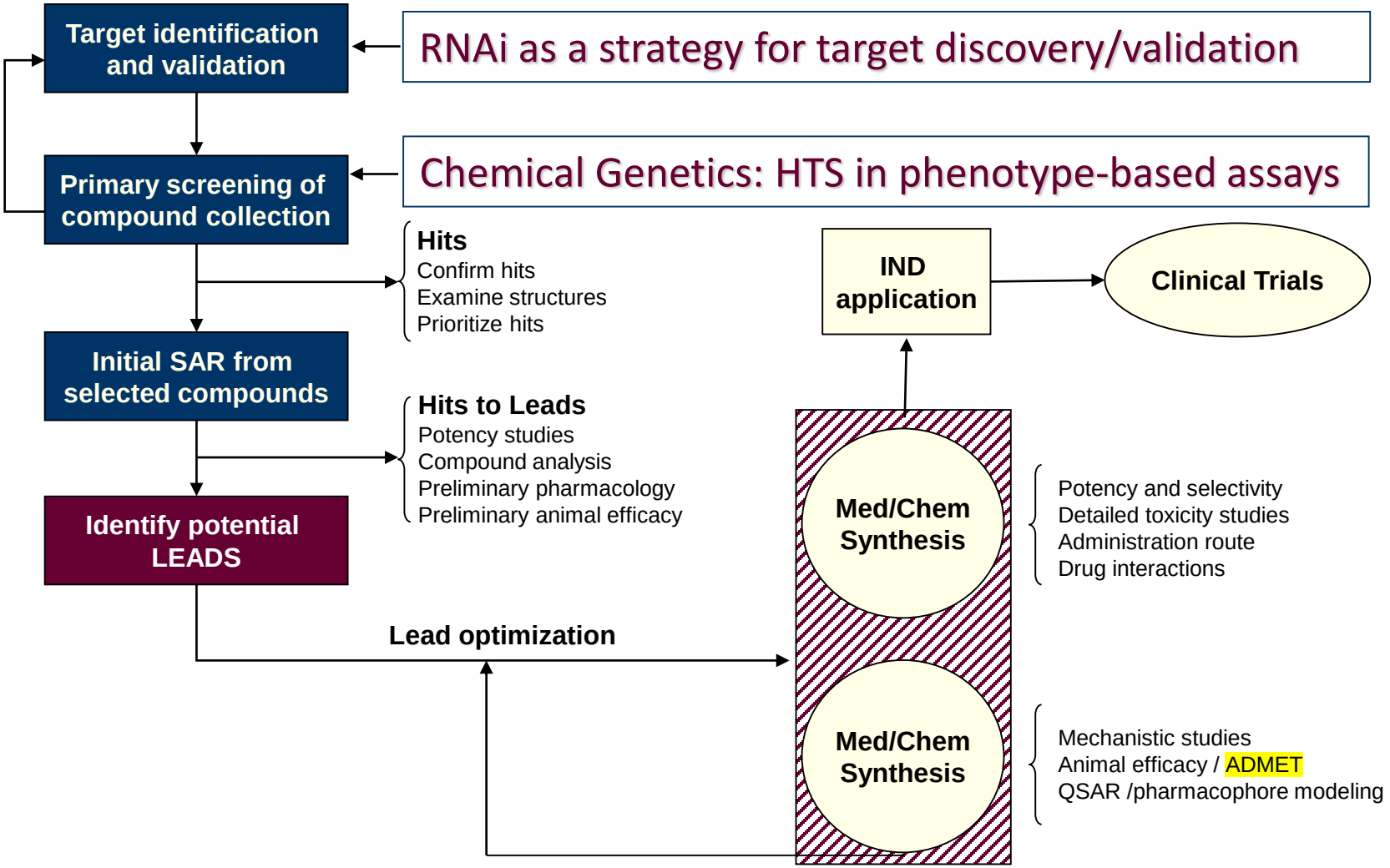
Traditional Drug Discovery Process



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

Strategy for preclinical drug discovery



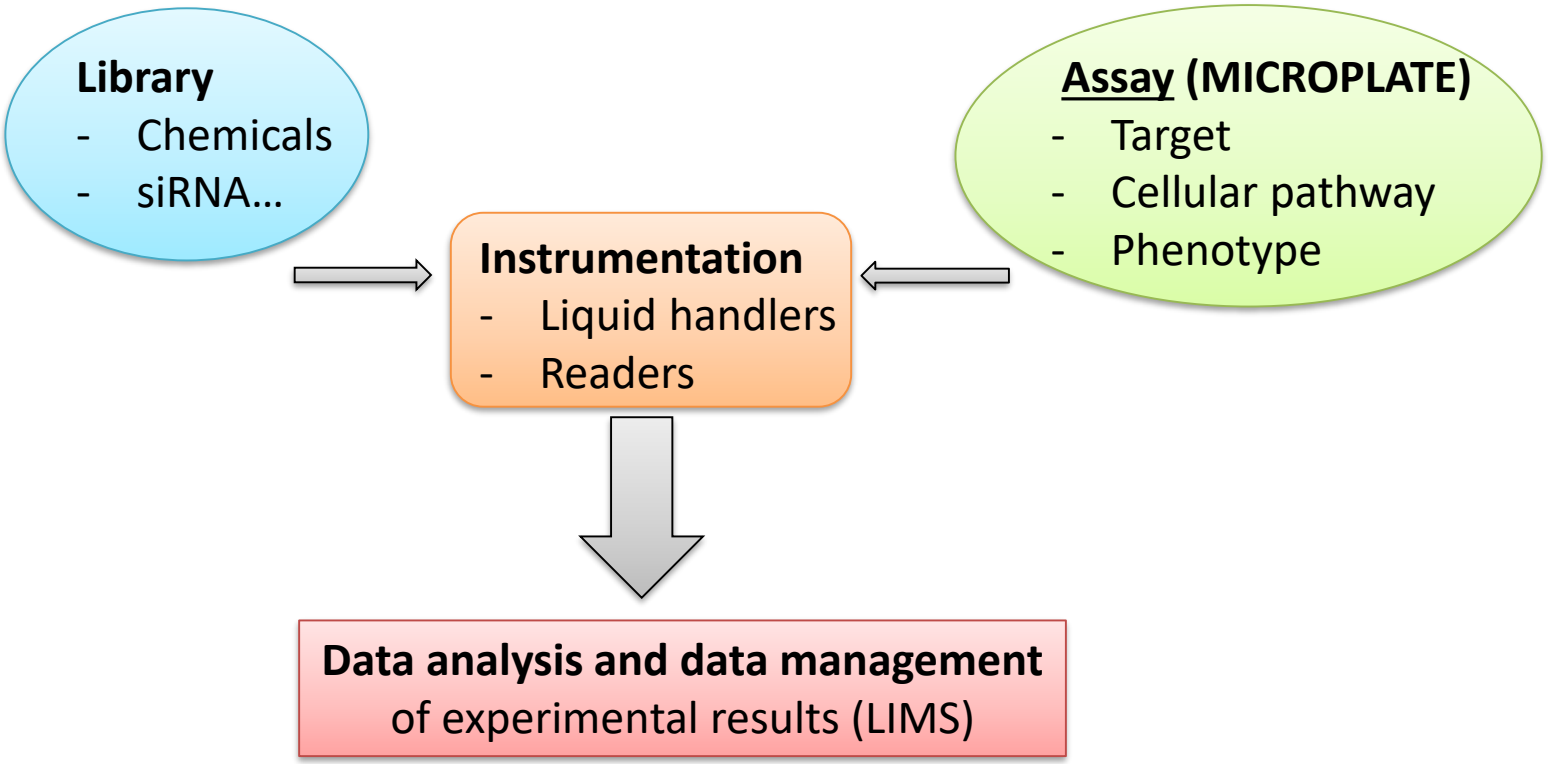
The major goal of a screening is to identify an active entity (chemical, siRNA…) against :

- a biological target
- a pathway
- a phenotype

Chemical Biology
Systems Biology
Drug Discovery

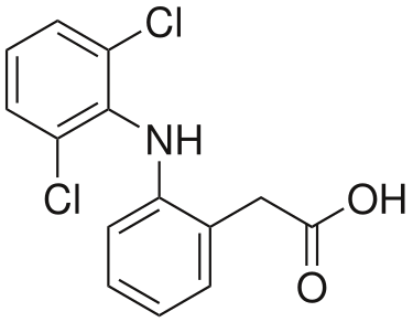
Primary screening: the players

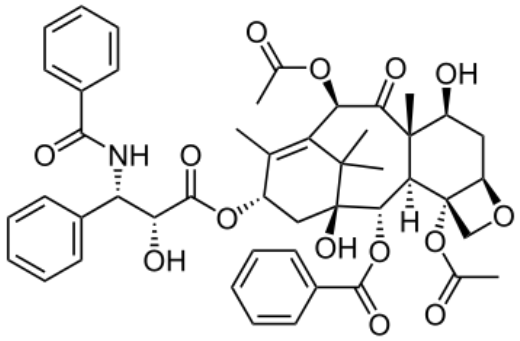
Screening allows the identification of active entities (compound, protein…)



Chemical Libraries

- Bioactive compounds
- Repurposing collection
- Diversity based libraries
- Focused (PPI, kinases, GPCR, nucleosides…)
- Natural products
- Swiss Chemical Collection (academic)
- (Fragments based)





Descriptors used for selecting the Chemical Diverse Collection of BSF-ACCESS

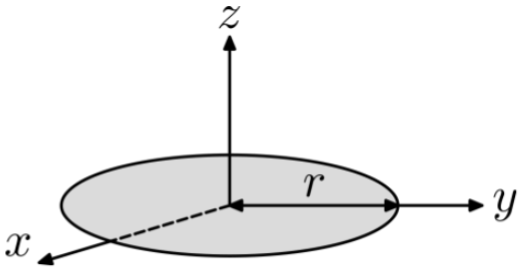
Descriptor	Meaning
P1	1st Relative PMI-Shape descriptor.[1]
P2	2nd Relative PMI-Shape descriptor.[1]
F-sp3	Fraction of sp^3-carbon atoms relative to carbon count.[2]
MW	Molecular weight.
HAC	Heavy-atom count.
HBA	H-bond acceptor atom count (*no multi-valency*).
HBAm	H-bond acceptor site count (*with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

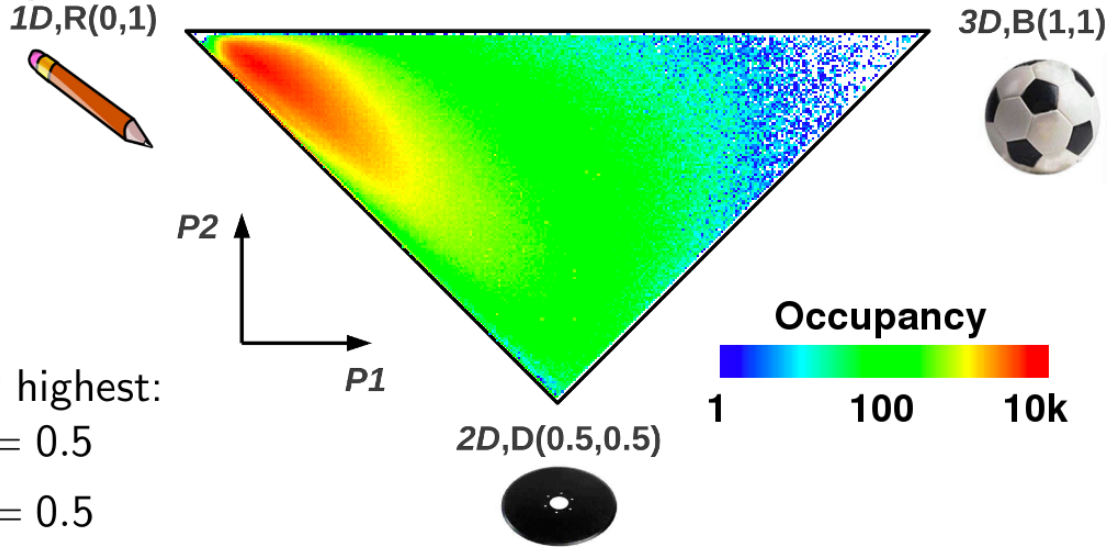
[1] As reported by Sauer and Schwarz.[1, 2]

[2] As reported by Lovering et al.[3]

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.

Molecular shape as a descriptor for selecting the CDC of ACCESS





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 a chemical diverse collection of 54'000 compounds

The rule of five

The medicinal chemist **Christopher Lipinski** and his colleagues analysed the physico-chemical properties of more than 2,000 drugs and candidate drugs in clinical trials, and concluded that a compound is more likely to be membrane permeable and easily absorbed by the body if it matches the following criteria:

- 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**.

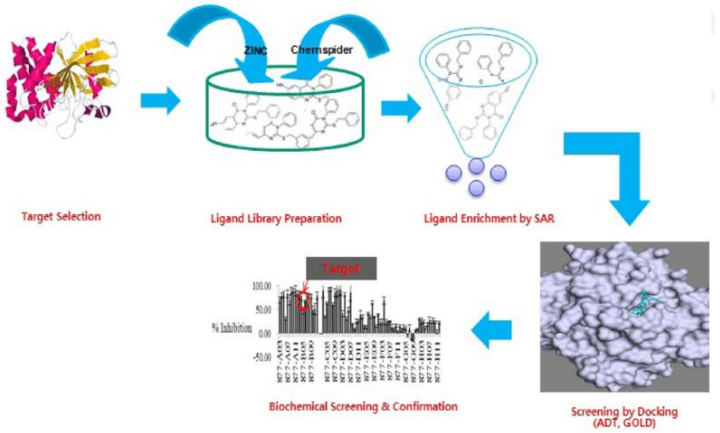
- The **number of groups in the molecule that can donate hydrogen atoms** to hydrogen bonds (usually the sum of hydroxyl and amine groups in a drug molecule) is **less than 5**.

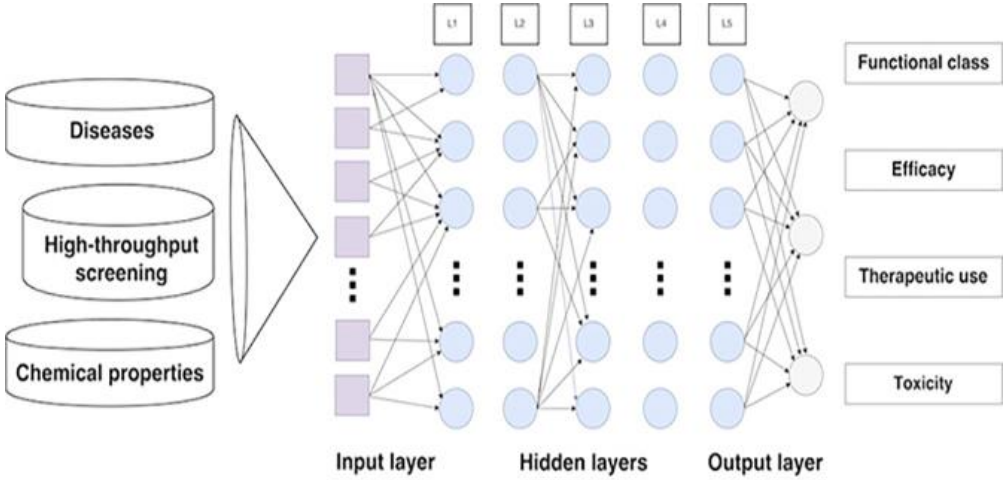
- The **number of groups that can accept hydrogen atoms** to form hydrogen bonds (estimated by the sum of oxygen and nitrogen atoms) is **less than 10**.

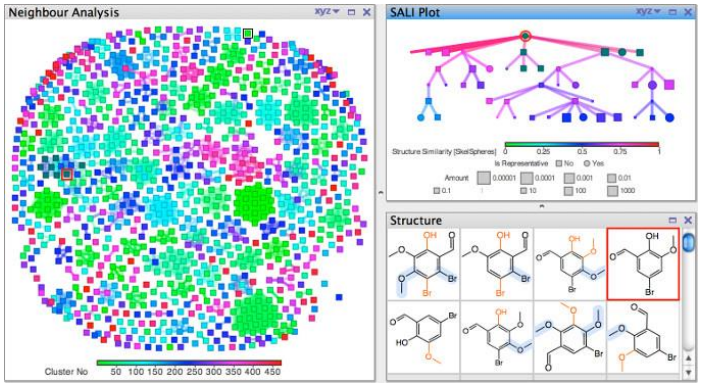
The rules, based on the 90-percentile values of the drugs' property distributions, apply only to absorption by passive diffusion of compounds through cell membranes; compounds that are actively transported through cell membranes by transporter proteins are exceptions to the rule. Due in no small part to their simplicity, the Lipinski criteria are widely used by medicinal chemists to predict not only the absorption of compounds, as Lipinski originally intended, but also overall drug-likeness.
Lipinski's rule states that, in general, an orally active drug has no more than one violation of the criteria.

Trends in chemical selection

- Rational (pre)selection by:
  - Virtual screening
  - Structural similarity search
  - Deep learning



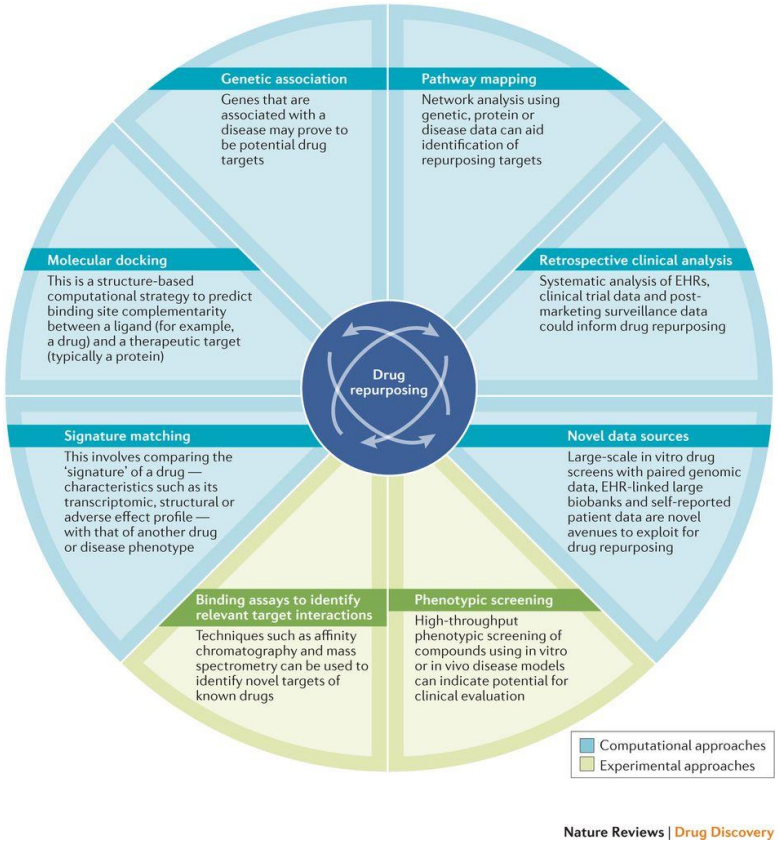




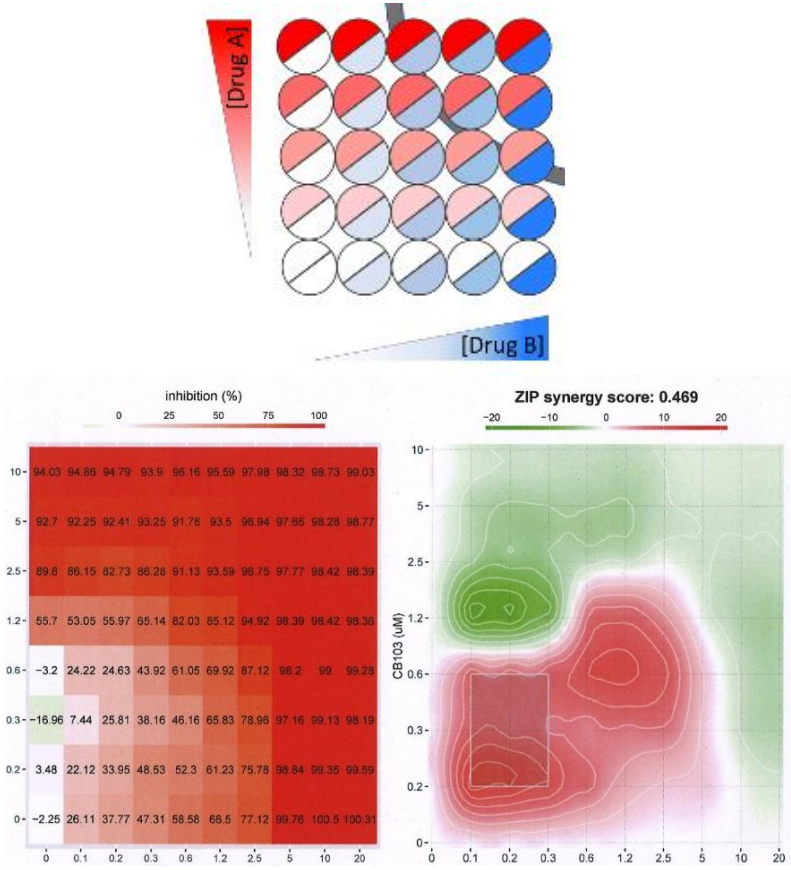
=> decrease of screened compounds number

Trends in chemical selection

- Drug repurposing



- Drug combination



Drug repurposing examples

Table 2

Some examples of repurposed drugs for neuropsychiatric disorders.

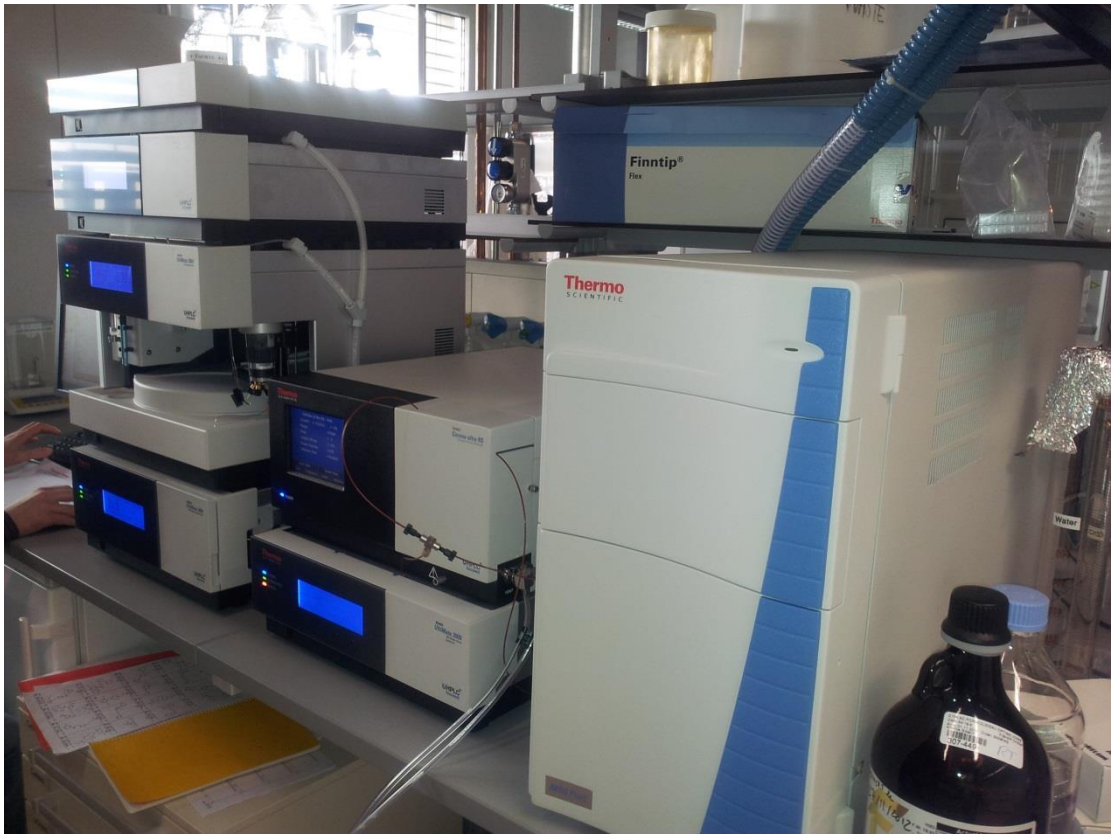
Drugs (alphabetic order)	Actions/classes	First intervention	New intervention	References
Amantadine	Anticholinergic-like agent	Influenza	Parkinson's disease, ADHD	[6, 7]
Amphotericin B	NSAID*	Antifungal	Bipolar disorder	[120]
Arbaclofen	GABA agonist	Cerebral palsy	Fragile X syndrome	[116, 121–123]
Atomoxetine	NSRI**	Parkinson's diseases	ADHD	[124]
Dexmecamylamine	Nicotinic receptor modulator	Hypertension	Depression	[125, 126]
Galantamine	Acetylcholinesterase inhibitor	Polio, paralysis	Alzheimer's disease	[127]
Mecamylamine	Nicotinic receptor antagonist	Hypertension	ADHD Depression	[128–131]
Mifepristone	Glucocorticoid receptor type II antagonist	Pregnancy termination	Psychotic major depression, Cushing's syndrome	[132–135]
Ropinirole	D2 agonist	Hypertension	Parkinson's disease, idiopathic restless leg syndrome	[136–138]
Tamoxifen	Estrogen receptor	Breast tumor	Bipolar disorder Mania	[139]
Valsartan	Angiotensin receptor blocker	Hypertension	Alzheimer's disease	[140]

DMD

* NSAID is nonsteroidal anti-inflammatory drug.
** NSRI is norepinephrine-selective reuptake inhibitor.

Chemicals storage and QC

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

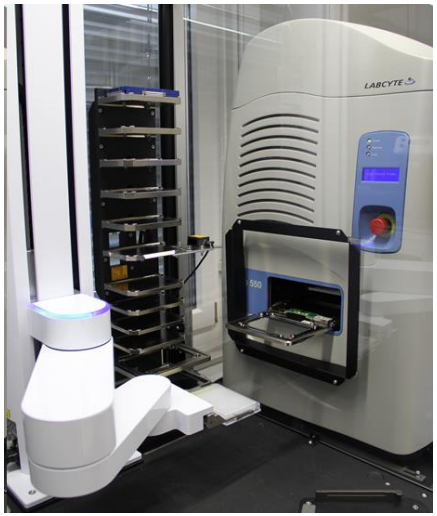


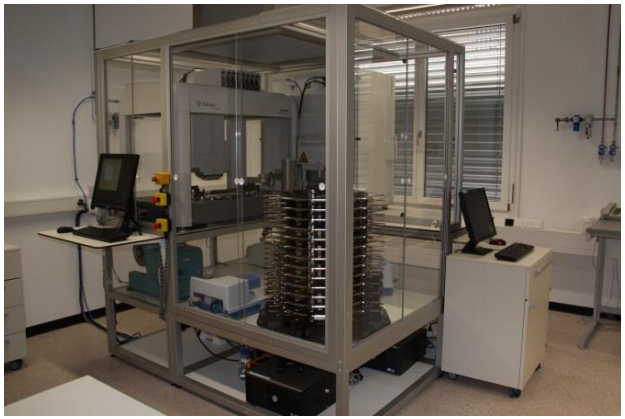
LC-MS system for checking chemical integrity (+ access to NMR if needed)

Instrumentation : robotic devices

Integrated system (driven by a scheduler) including:

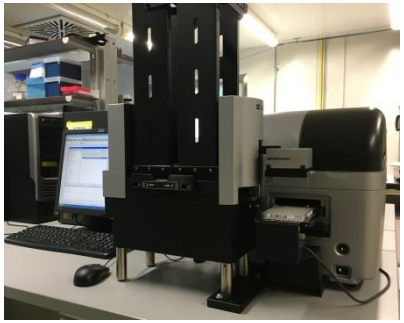
- a robotic arm
- a liquid handler (conventional pipettors or acoustic dispenser) & dispensers
- centrifuge/ peeler / sealer / (washer / shaker)
- hotels and cell incubator



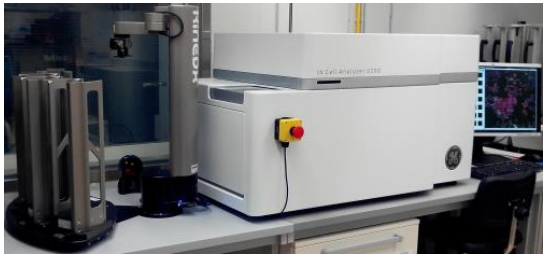


Open access instrumentation

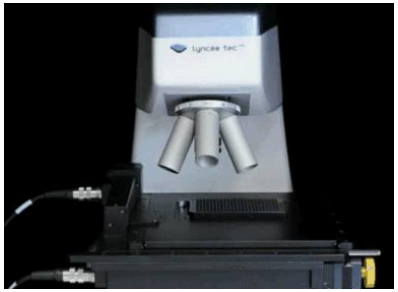
Multimode microplate readers
Biotek: NeoHTS, SynergyH1
Tecan: Spark, Infinite F500



Automated fluorescence microscopes
GE InCell Analyzer 2200
Molecular Devices ImageXpress



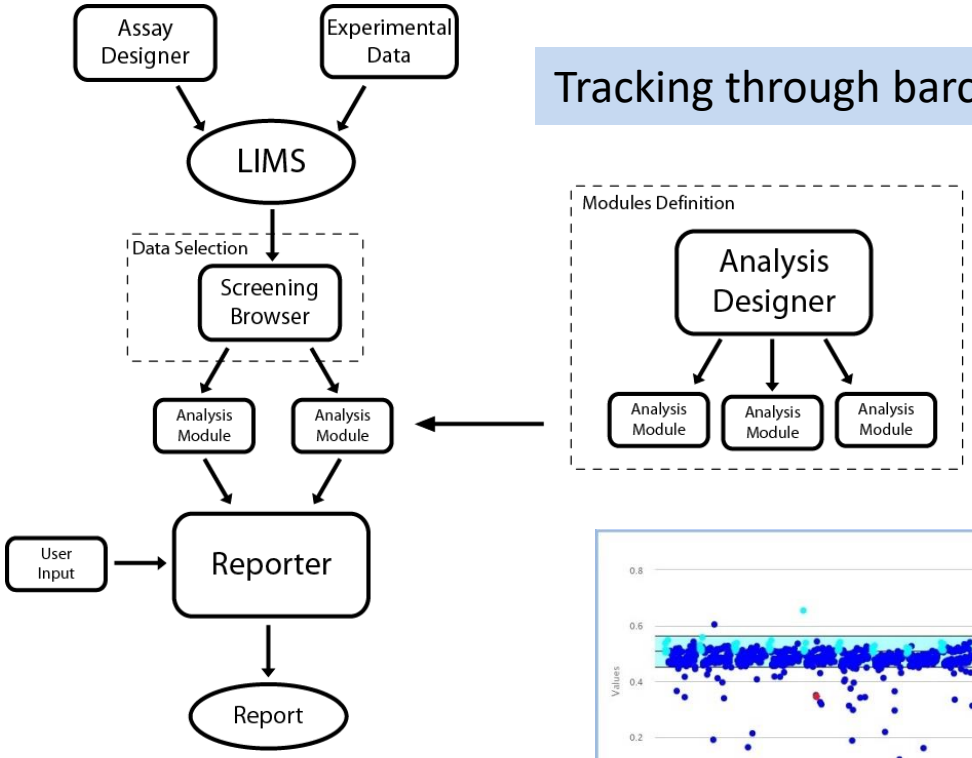
Digital Holographic Microscope
(LyncéeTec SA)



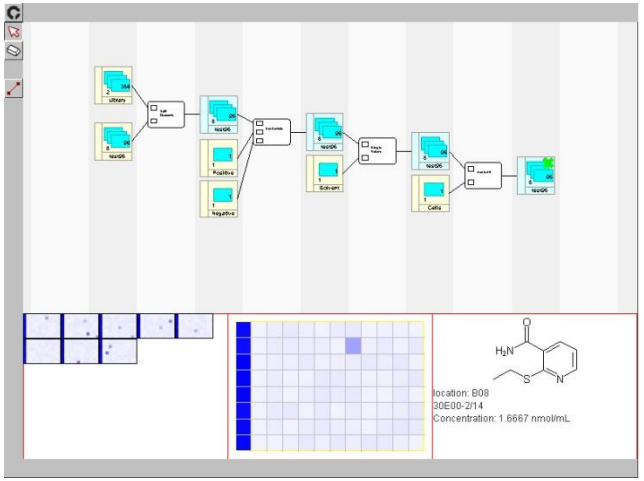
Dispensers & Washers
(Multidrop, Microflow, EL405/406)

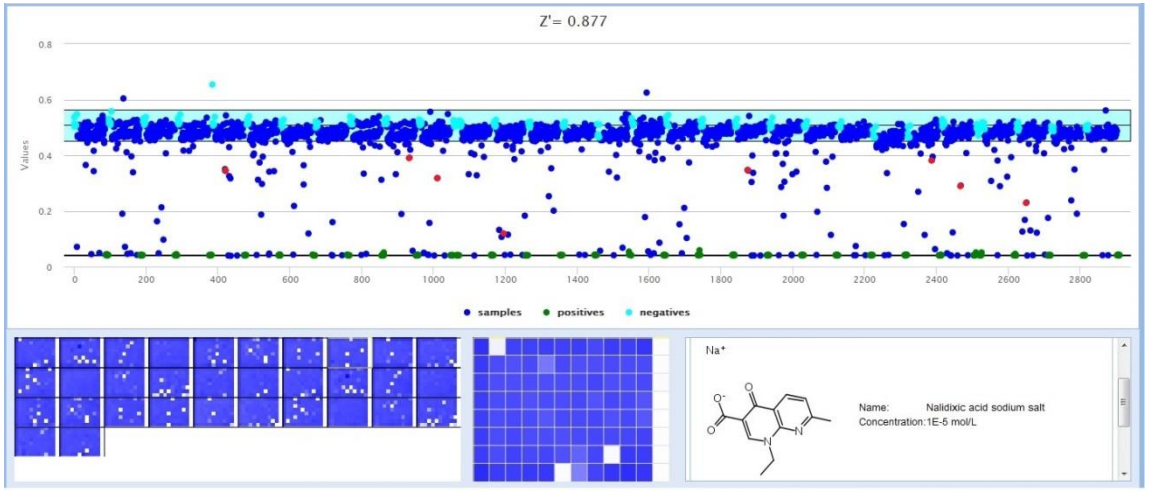
Cell culture
(laminar flow)

BSF internal LIMS for large data set management, analysis and visualization



Tracking through barcode

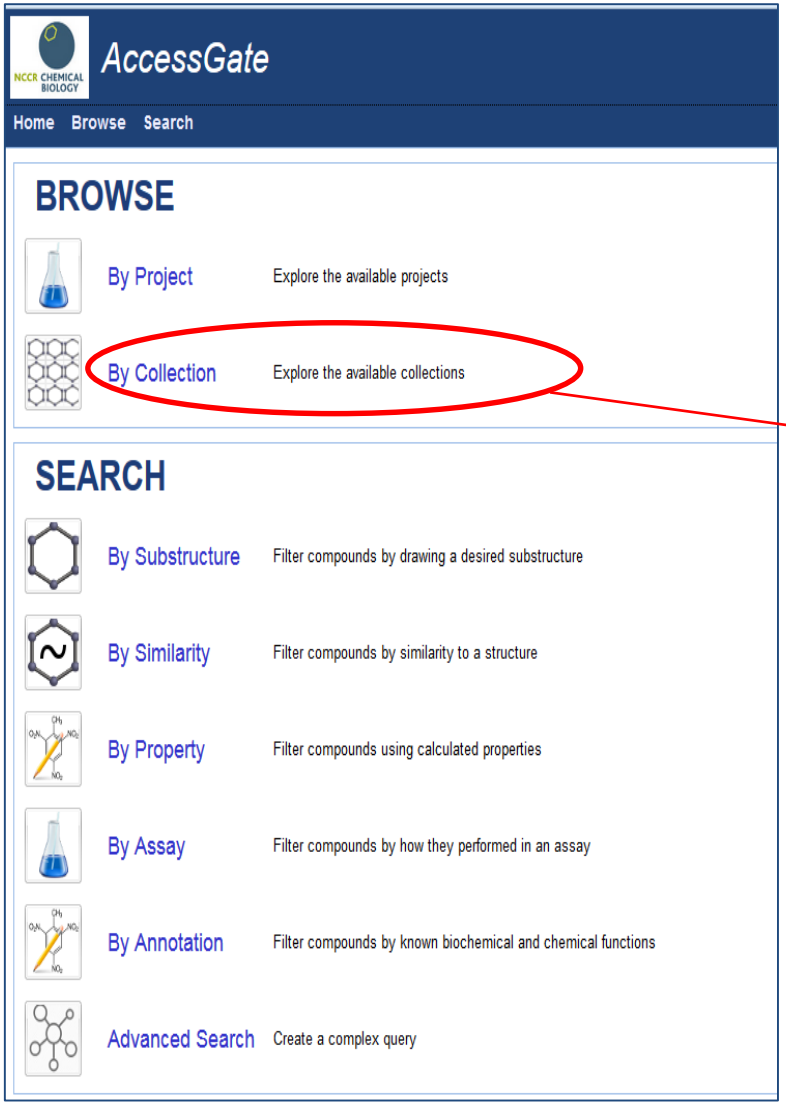


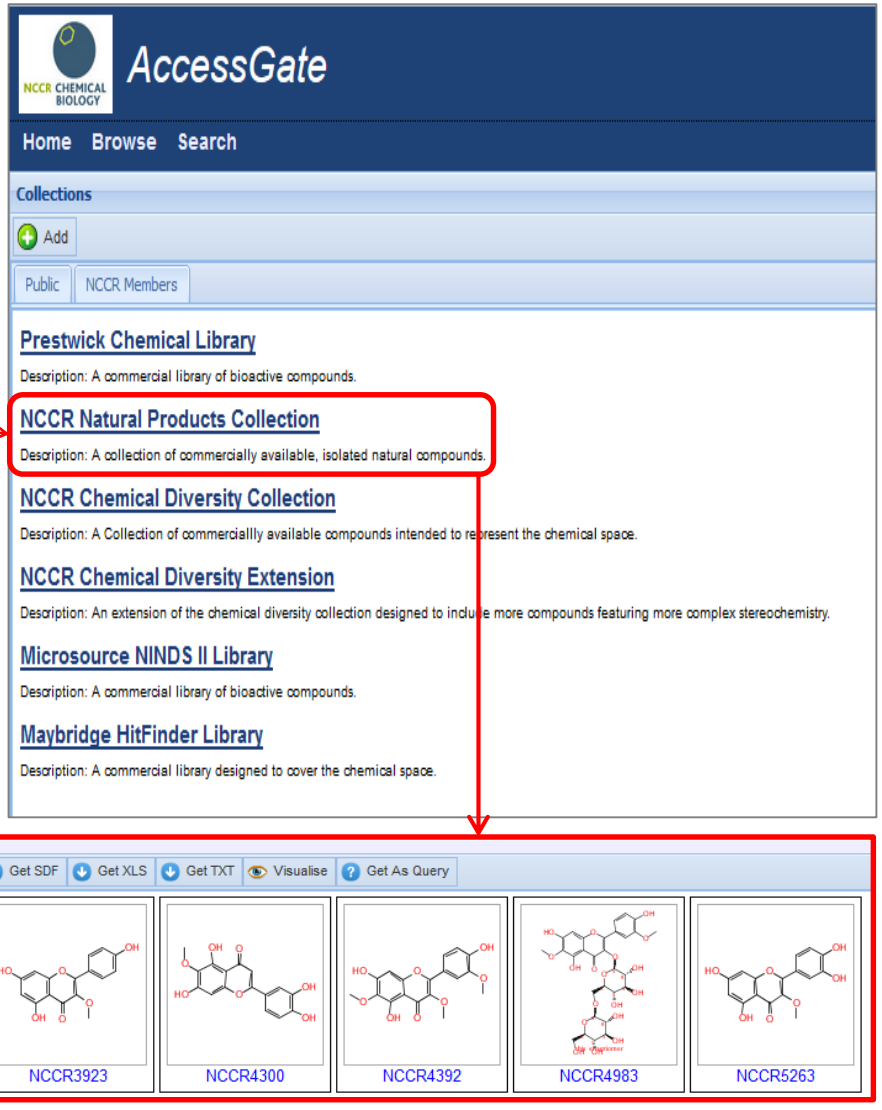


Data sharing platform-Webportal

- Web-based application for the transfer and the presentation of assay information and analyzed data
- Portal for the post-screen annotation of compounds
- Browsing of NCCR chemical collections
- Multilayered access to information

Data sharing platform-Webportal





Type of screening assays

Chemicals (SM & natural products)

TARGET KNOWN

IN VITRO BIOCHEMICAL TARGET- BASED ASSAYS

• Enzymatic assays : purified target protein (i.e. kinases, MIF…) or e*nriched protein in* cell extracts or membrane preparations (i.e. Telomerase)
• Protein-protein interactions

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

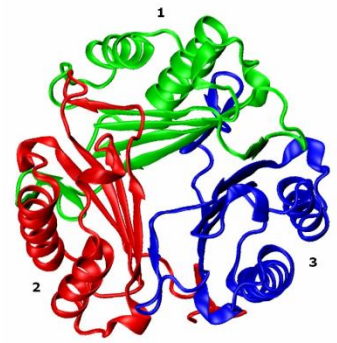
CELLULAR PHENOTYPE- BASED ASSAYS

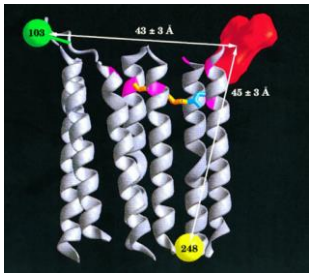
Target discovery / validation, pathway investigation (i.e. lipids trafficking, cell cycle…)

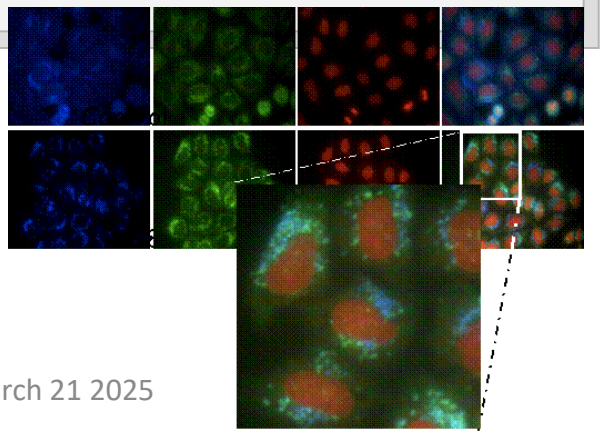
IN VITRO

CELLULAR

RNAi

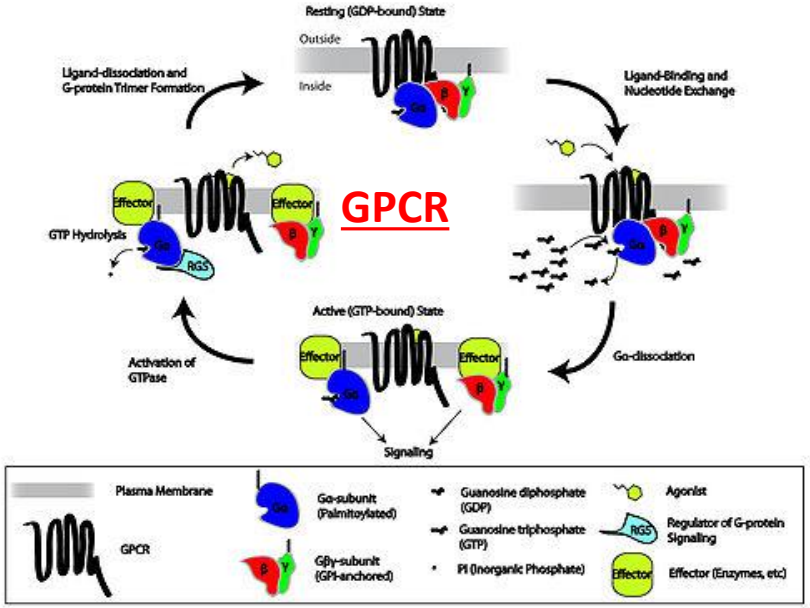




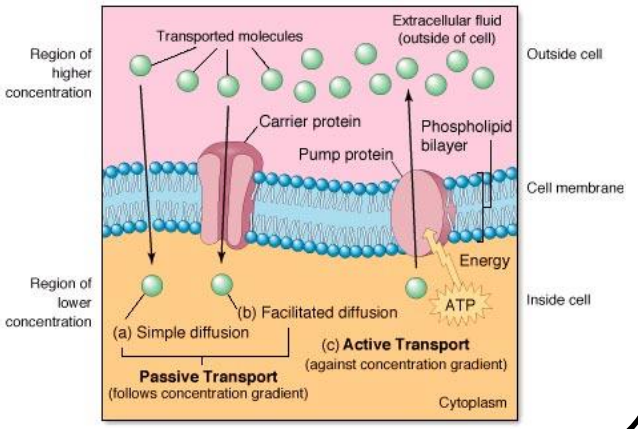


Targets families

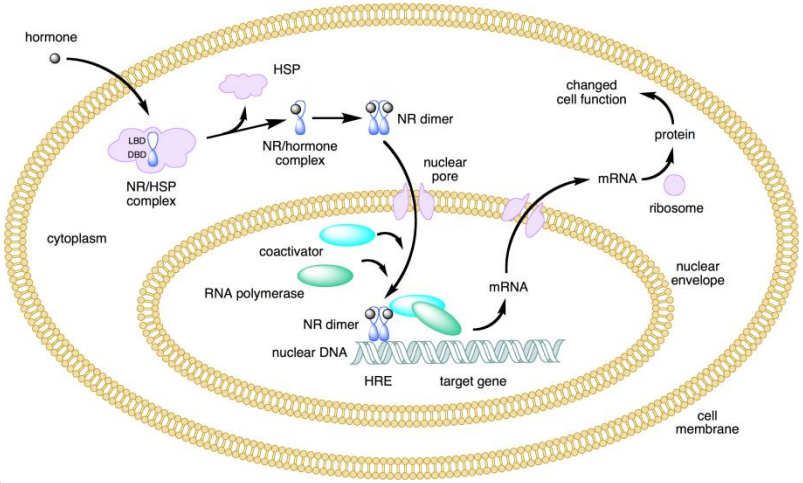
GPCR



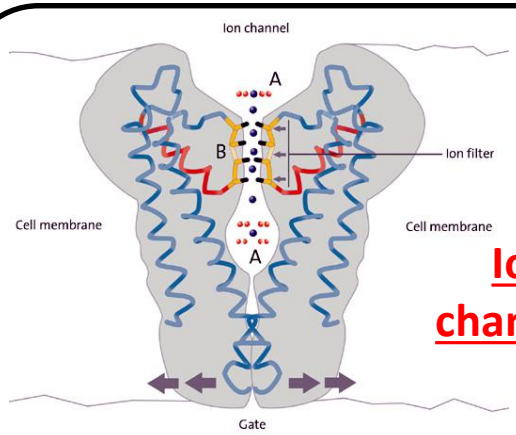
Transporters



Nuclear receptors

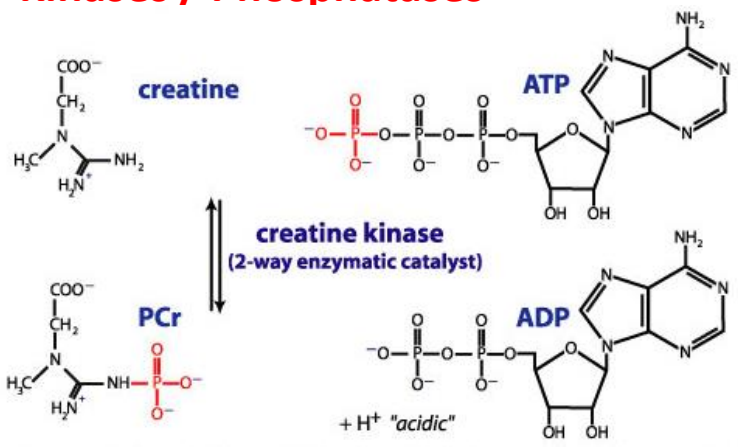


Ion channels

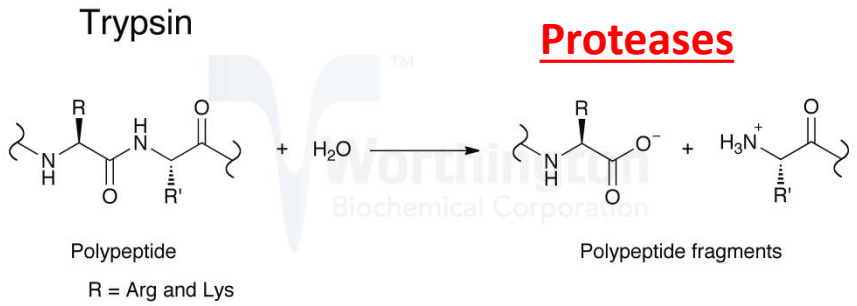


Targets families: enzymes

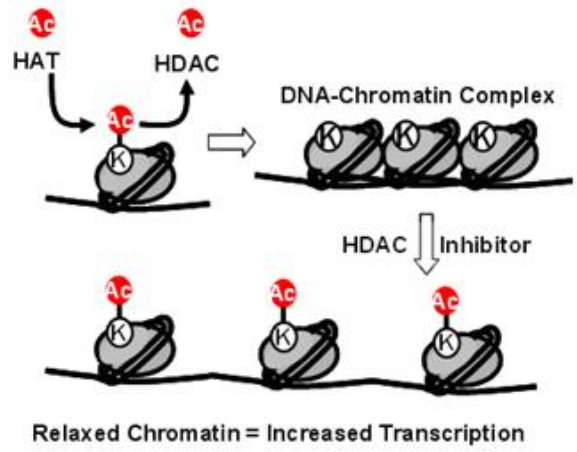
Kinases / Phosphatases



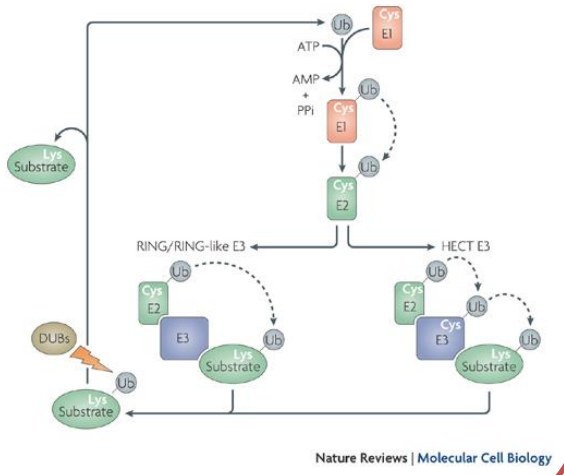
Proteases



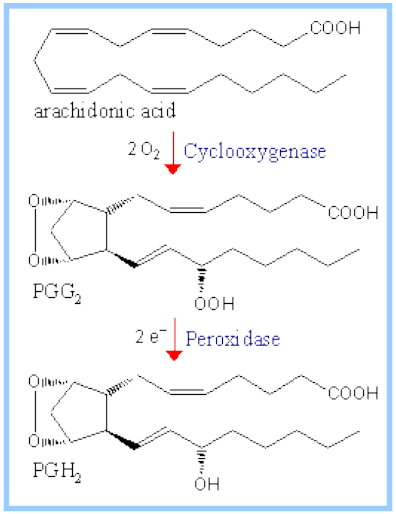
Epigenetic



Ubiquitination

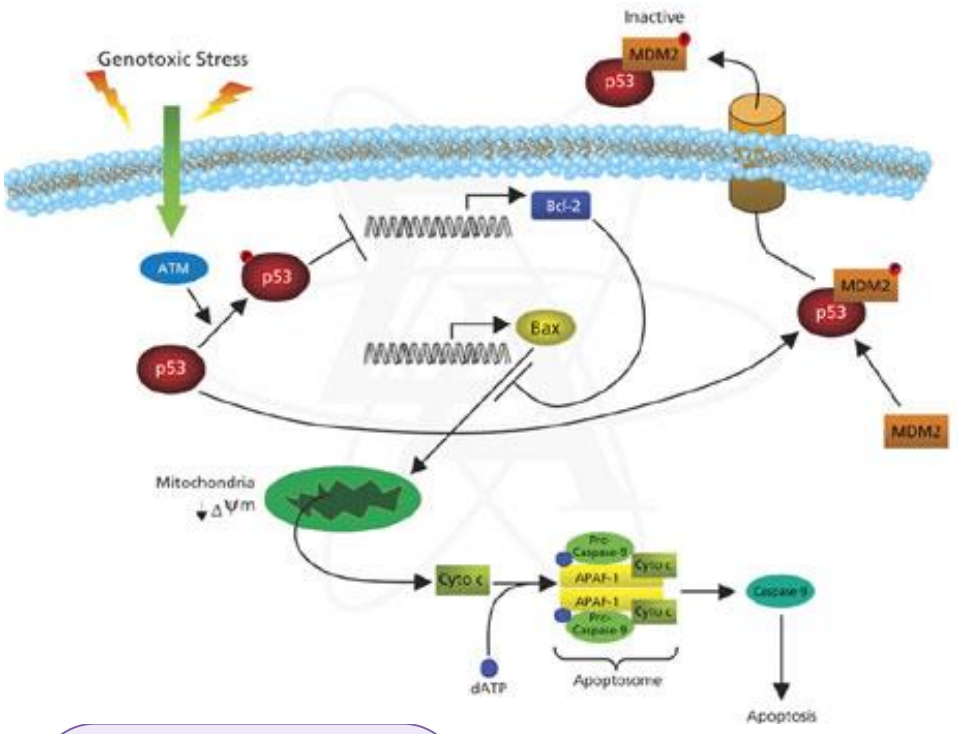


Metabolic (AA, NO…)

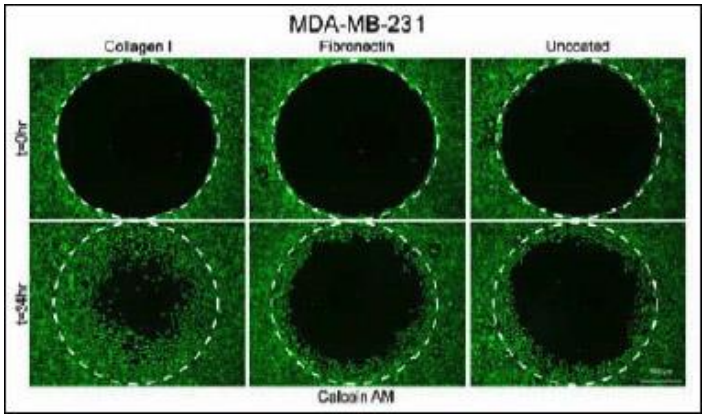


Other «Targets families»

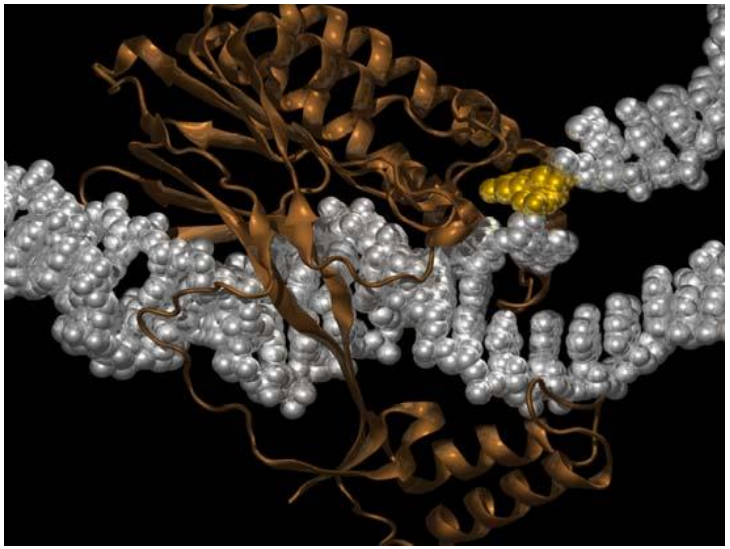
Pathway regulation



Cell proliferation / migration



Protein - protein /DNA interactions



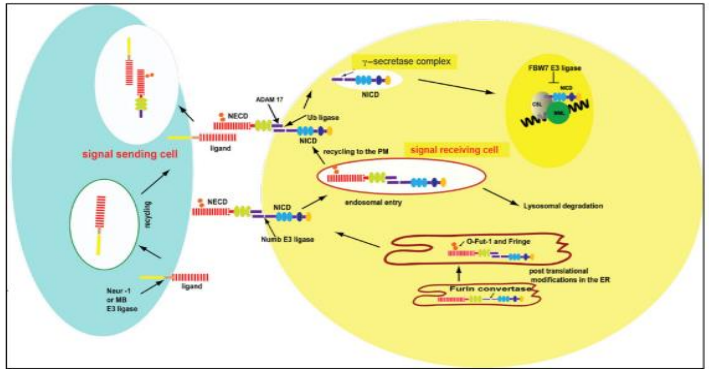
Micro-organisms
Bacteria
Fungis
Yeasts

Protein secretion (cytokines…)
ELISA / RIA assays

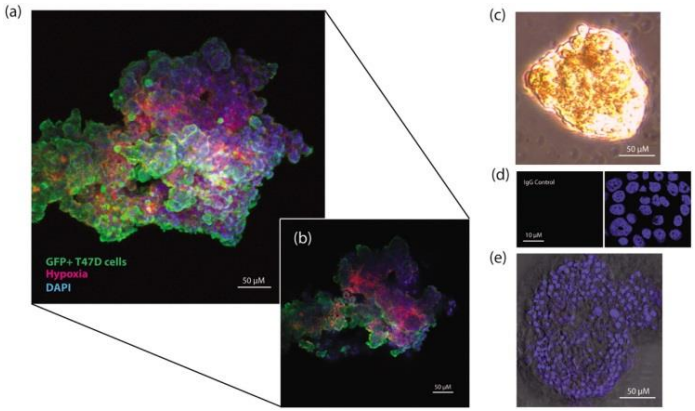
Examples of complex assays

Example of co-culture assay

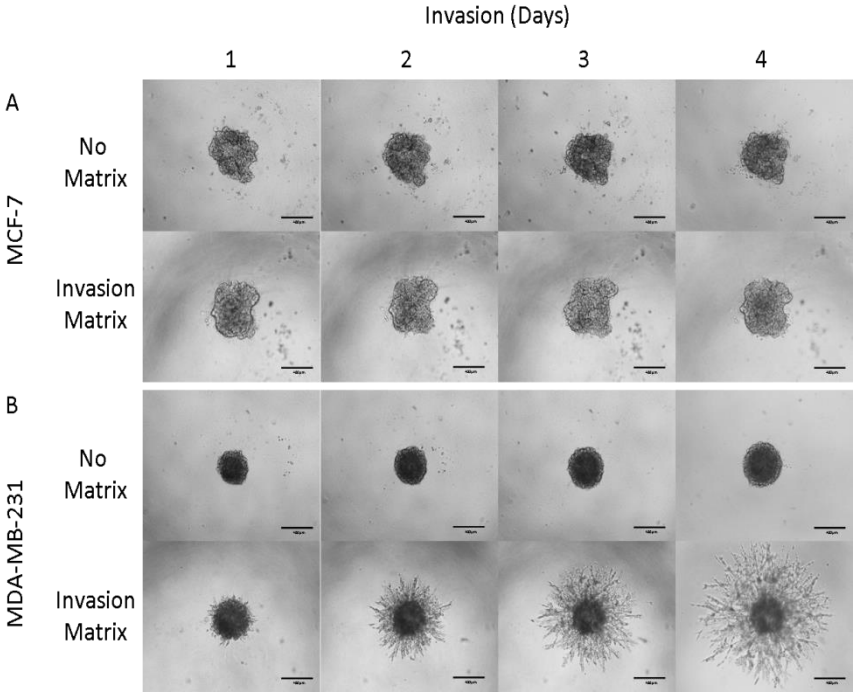
(EPFL- Radtke lab – Luc reporter gene)



Example of hypoxia in clusters

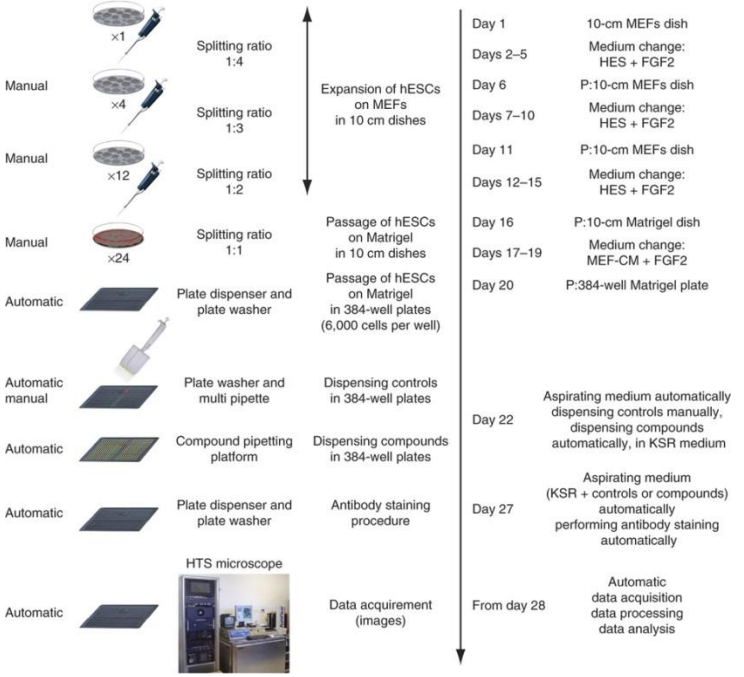


Example of 3D (trevigen)



Quail DF et al. J. Biomol. Screen. (2012) 17 : 1088-1095

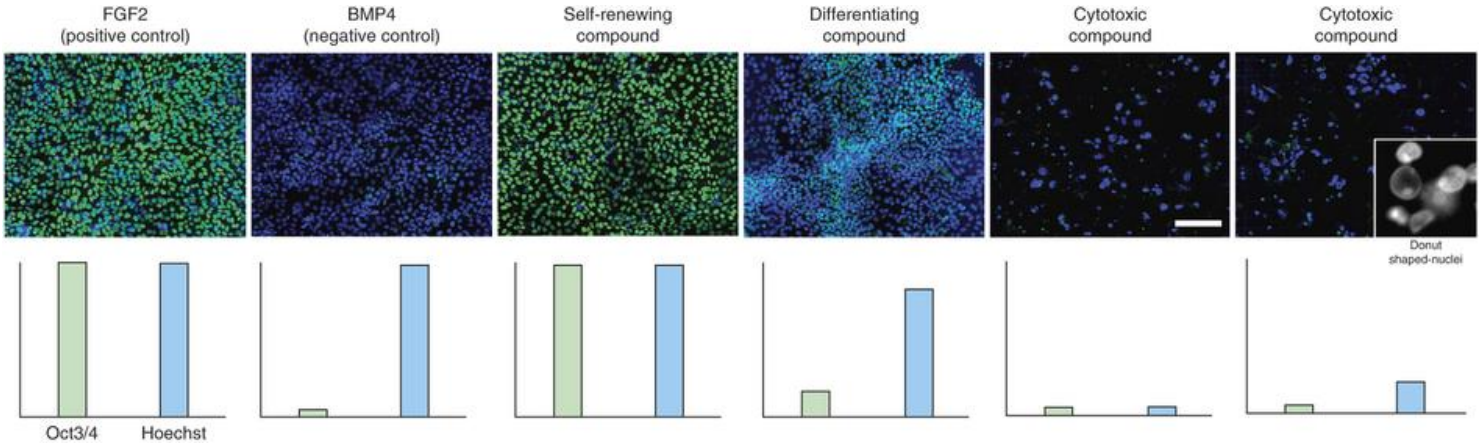
Examples of complex assays (II)



Screening for small-molecule regulators of hESC self-renewal, differentiation or death with phenotypic readout

- Stem cells
- 28 days protocol
- Microscopy reading

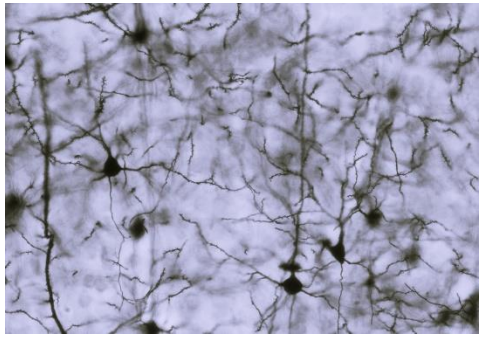
SC Desbordes & L Studer (2013)
Nat. Protocols 8, 111–130



Examples of complex assays (III)

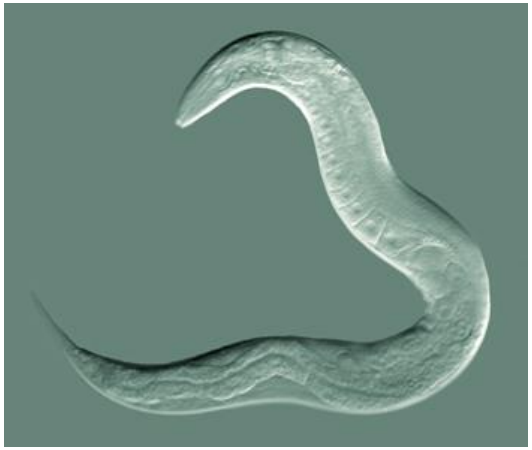
Primary cells like neurons,cardiomyocytes

- typically 2- 3 weeks of culture after isolation
- limited amount of cells available



Whole organisms like worms, nematodes, zebrafish

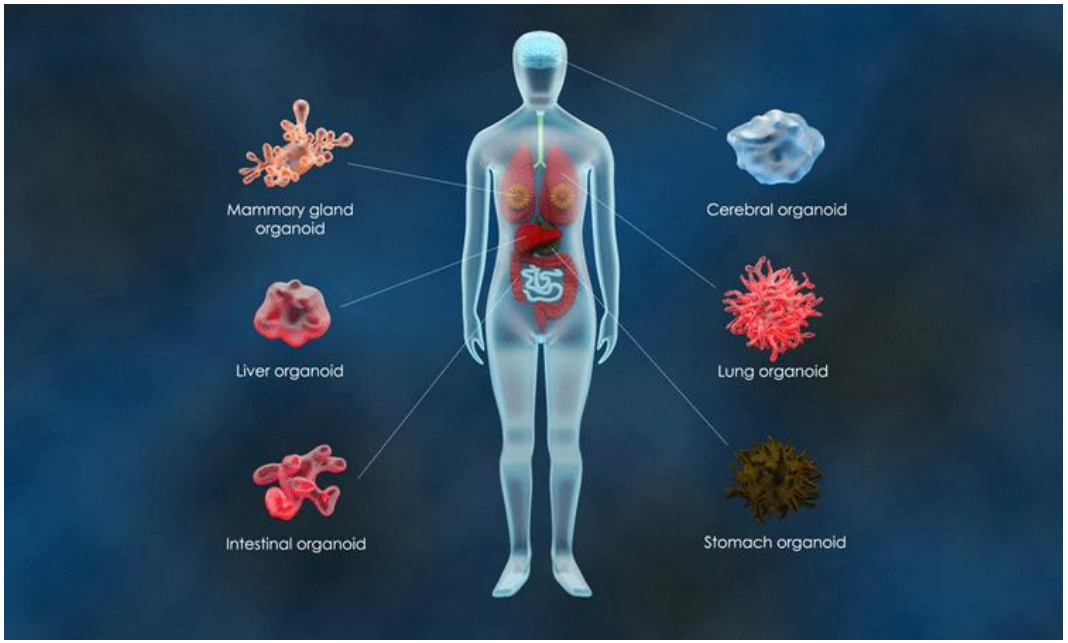
- low number of organisms per well
- technical issue to «manipulate»
- low drug permeability

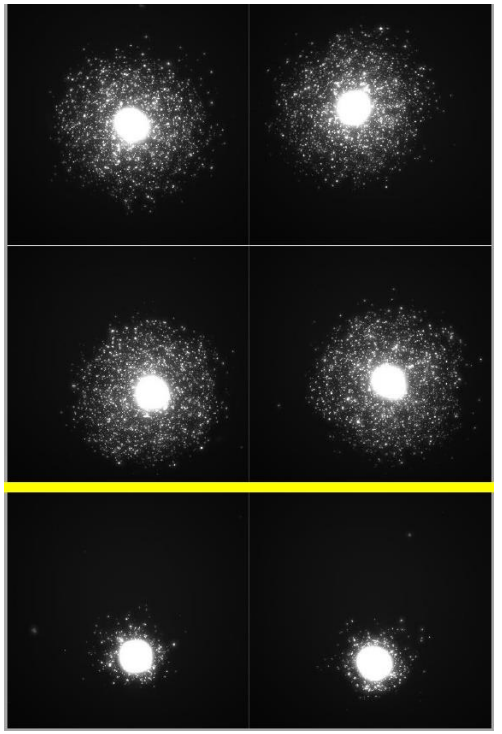


Trends in complex assays
-> to increase biological relevance

- Relevant cellular models
- 3D cell culture
- Spheroids
- Organoids

- Phenotypic readout
- Supervised / unsupervised analysis
- Deep learning analysis





Diversity of assays

- **Binding vs functionnal**
- **Cell based (or not)** : cell line, primary cells, co-culture assay, 3D , micro-environnement…
- **High content (or not)** : automated microscopy imaging (fluo, label-free), automated flow cytometry
- GPCR: binding, coupling at GTPase level, downstream effectors (cAMP, Ca2+, reporter genes…), internalization…
- Nuclear receptors: cell-based or not, binding (receptor–activator / receptor DNA), downstream effectors (cAMP, Ca2+, reporter genes…), internalization…
- Transporters : specific cell-based devices, R* binding / uptake…
- Ion channels : R*, fluo, electrophy…
- Enzymes: binding, activity, activation (R*, fluo & co…)

Assay Development for Screening

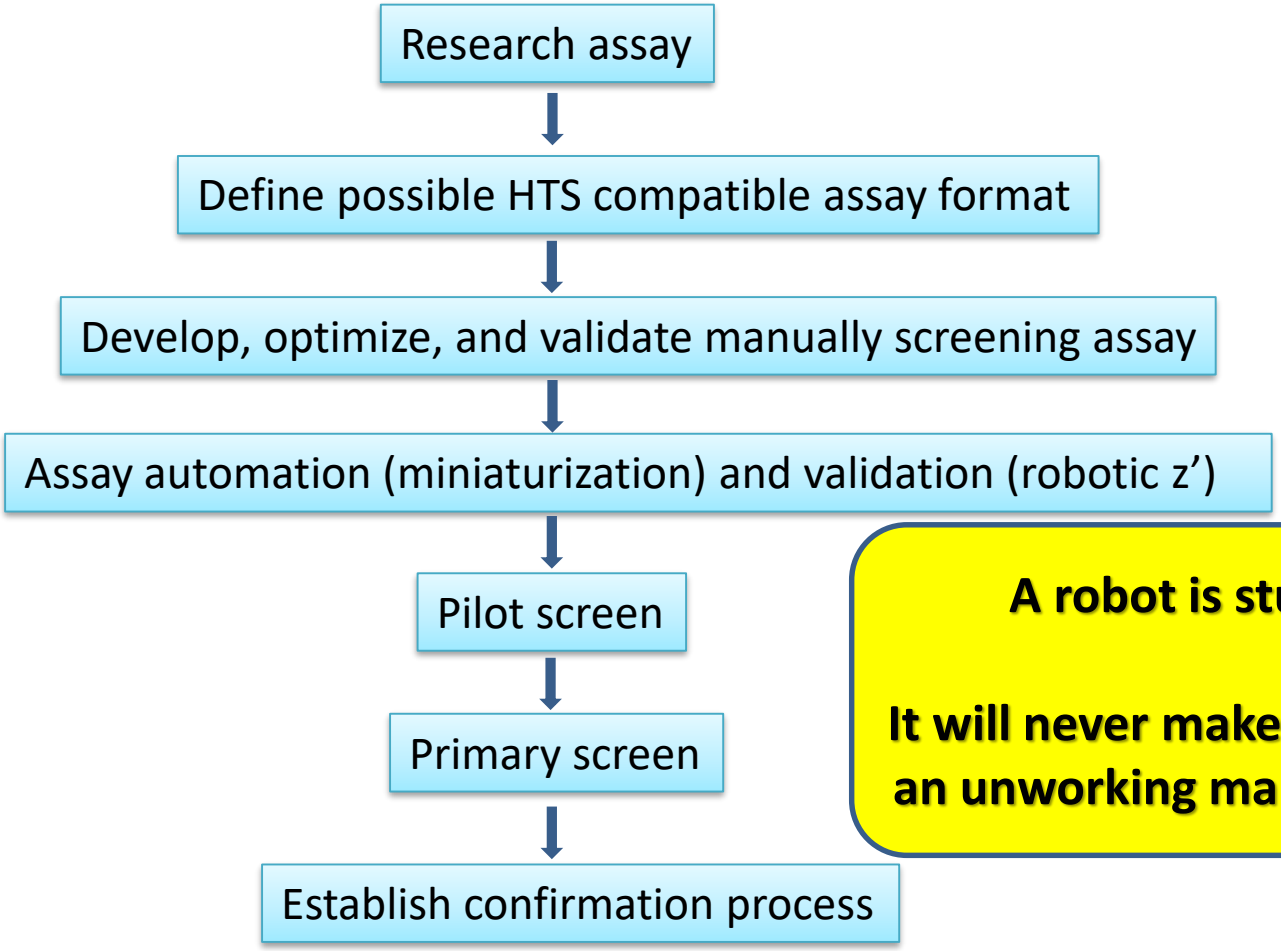
- Large diversity of targets
- Different types of assays
- Huge kinds of readouts

… but single strategy

➢ Which parameters are impacting the assay?

➢ How to control them for ensuring high assay quality (quantitative analysis, reproducibility)?

Typical workflow



A robot is stupid!

It will never make working…
an unworking manual assay

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	Minutes to hours
	Measurements as endpoint, multiple time points, or continuous	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.

The 5 minutes story: 5 X 40 = 200!

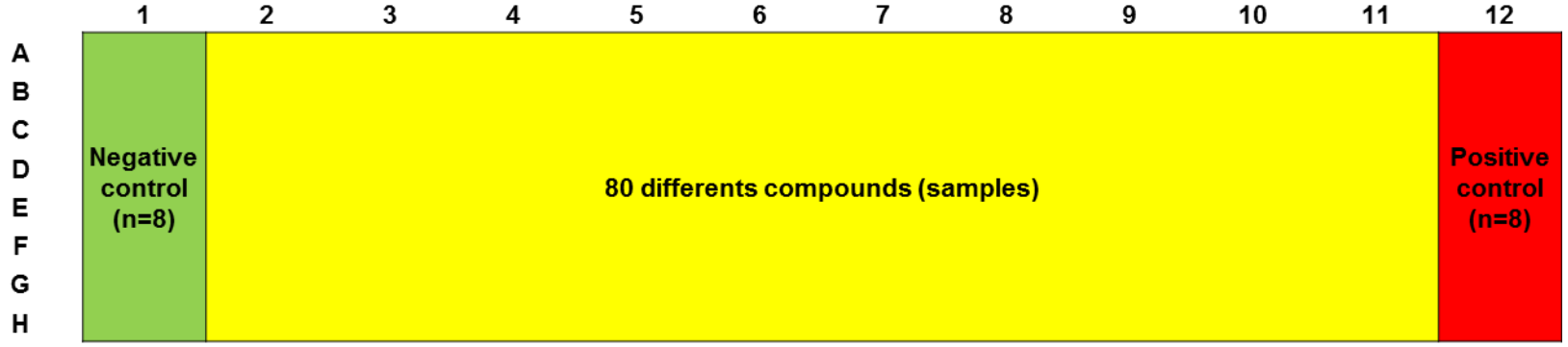
HTS compatibility

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

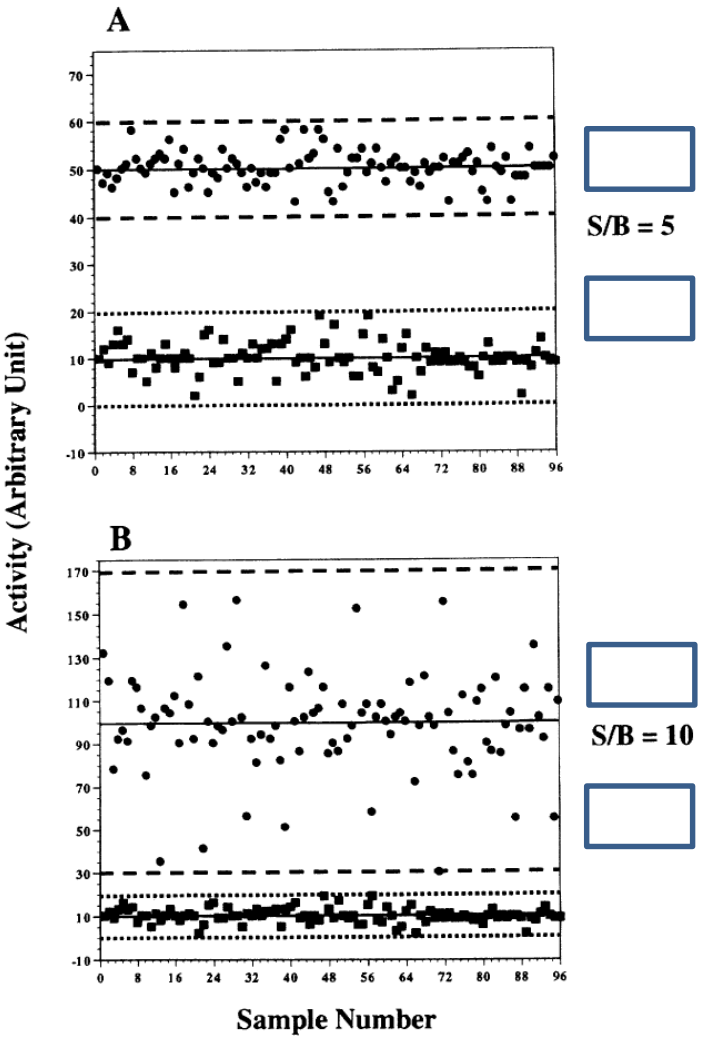
- Resistance to interferences
- Sensitivity (saving reagent amount)
- Miniaturization
- Content versus throughput

HTS plate configuration

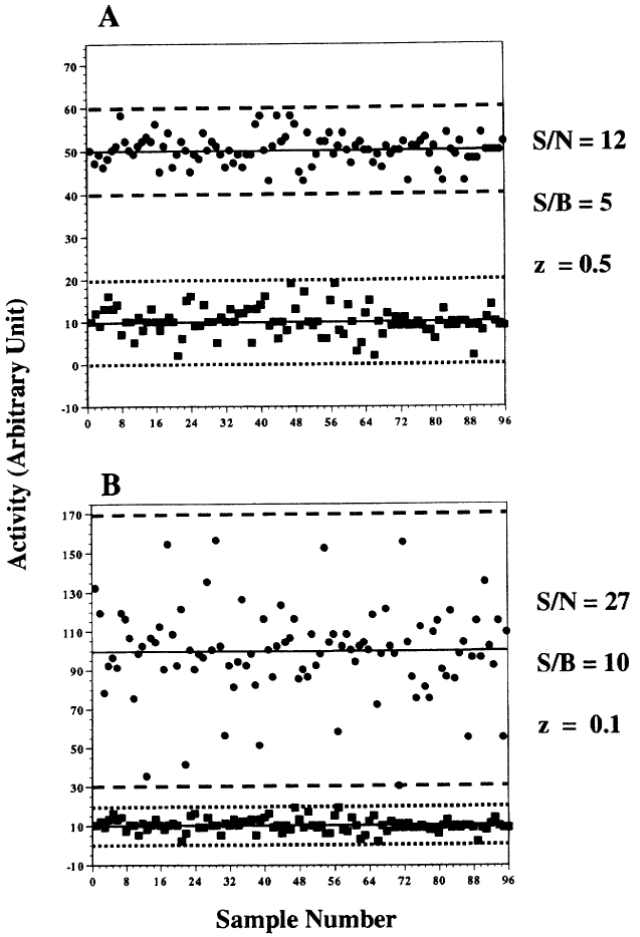
- Single standardized plate layout
- Each plate includes negative and positive controls (for data analysis and validation)
- Replicate on different plates
- 96W > 384W by quadrant

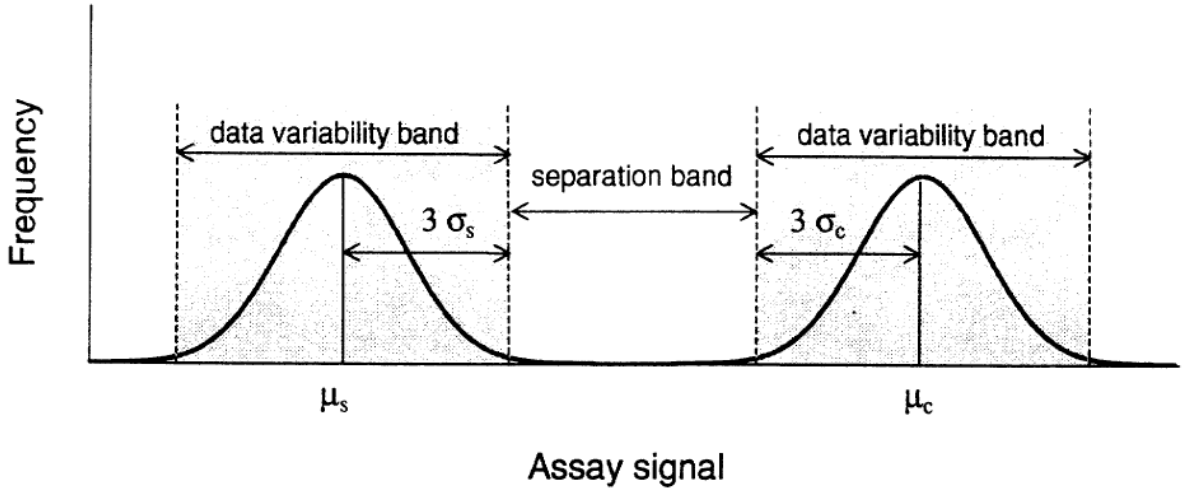


Which data set is the best?



Assay validation: statistical analysis





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

$1 \geq Z' > -\infty$

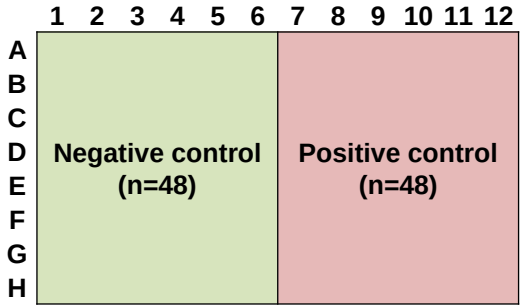
$Z' = 1$: perfect assay

$1 > Z' > 0.5$: good assay

$0.5 > Z' > 0$: poor assay

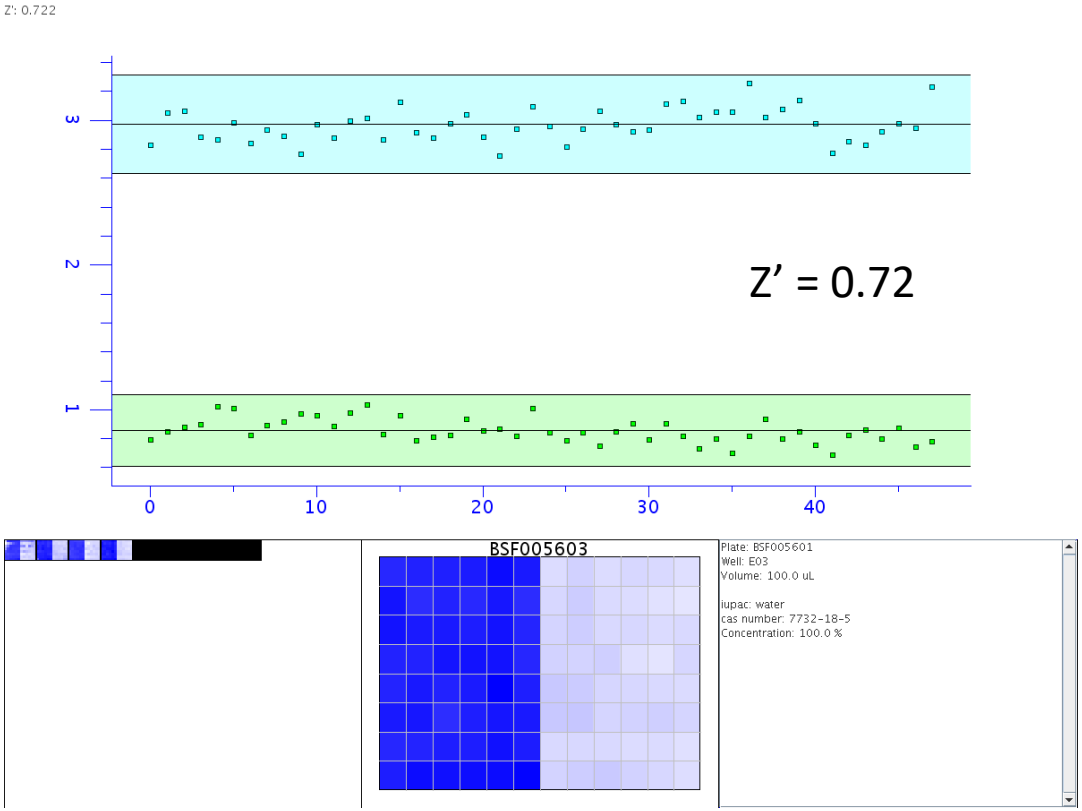
$Z' = 0$: yes / no assay

$Z' < 0$: screening not possible



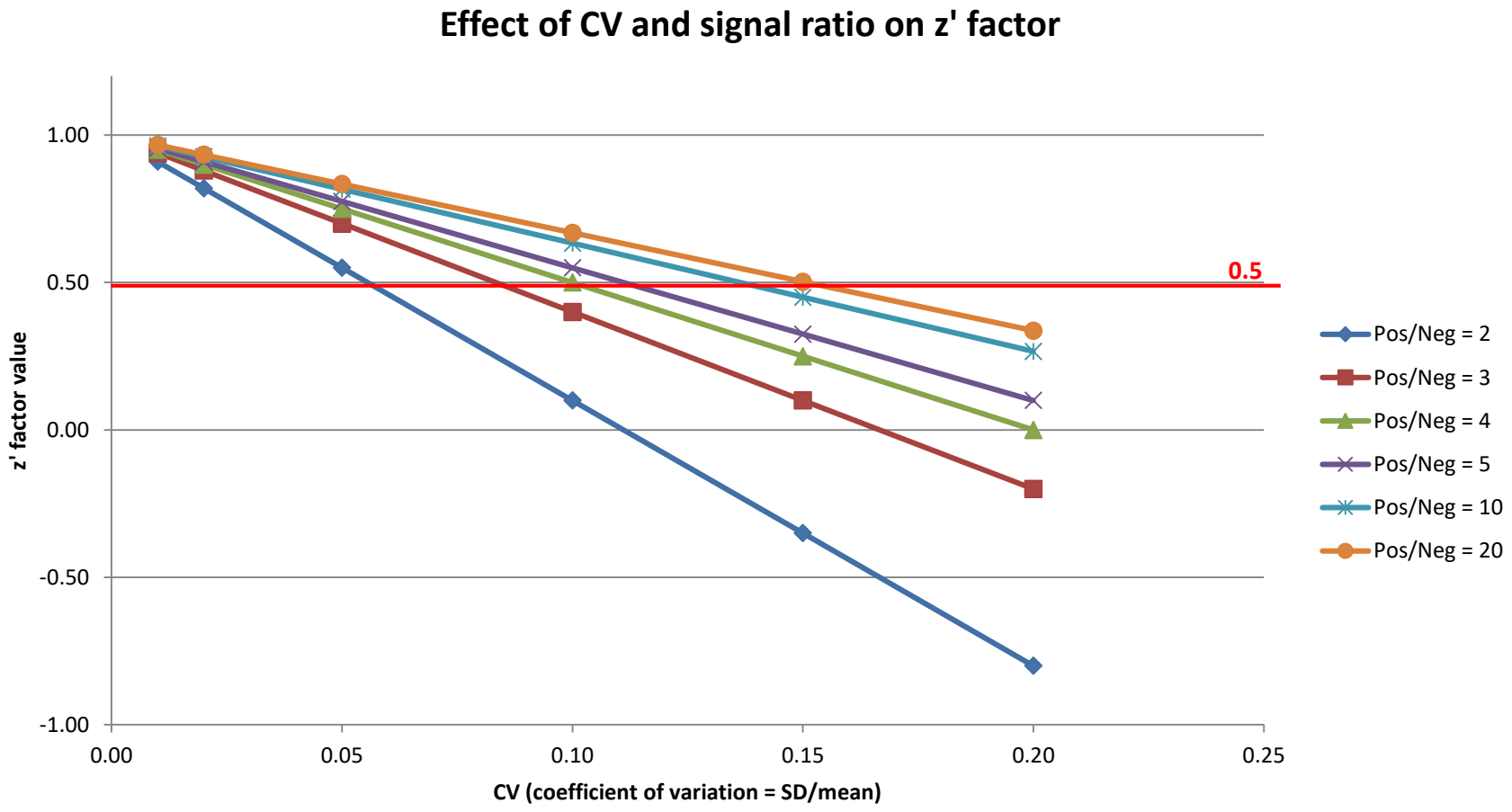
Coefficient of variation
CV (%) = 100*σ/μ

Example of z’ factor determination

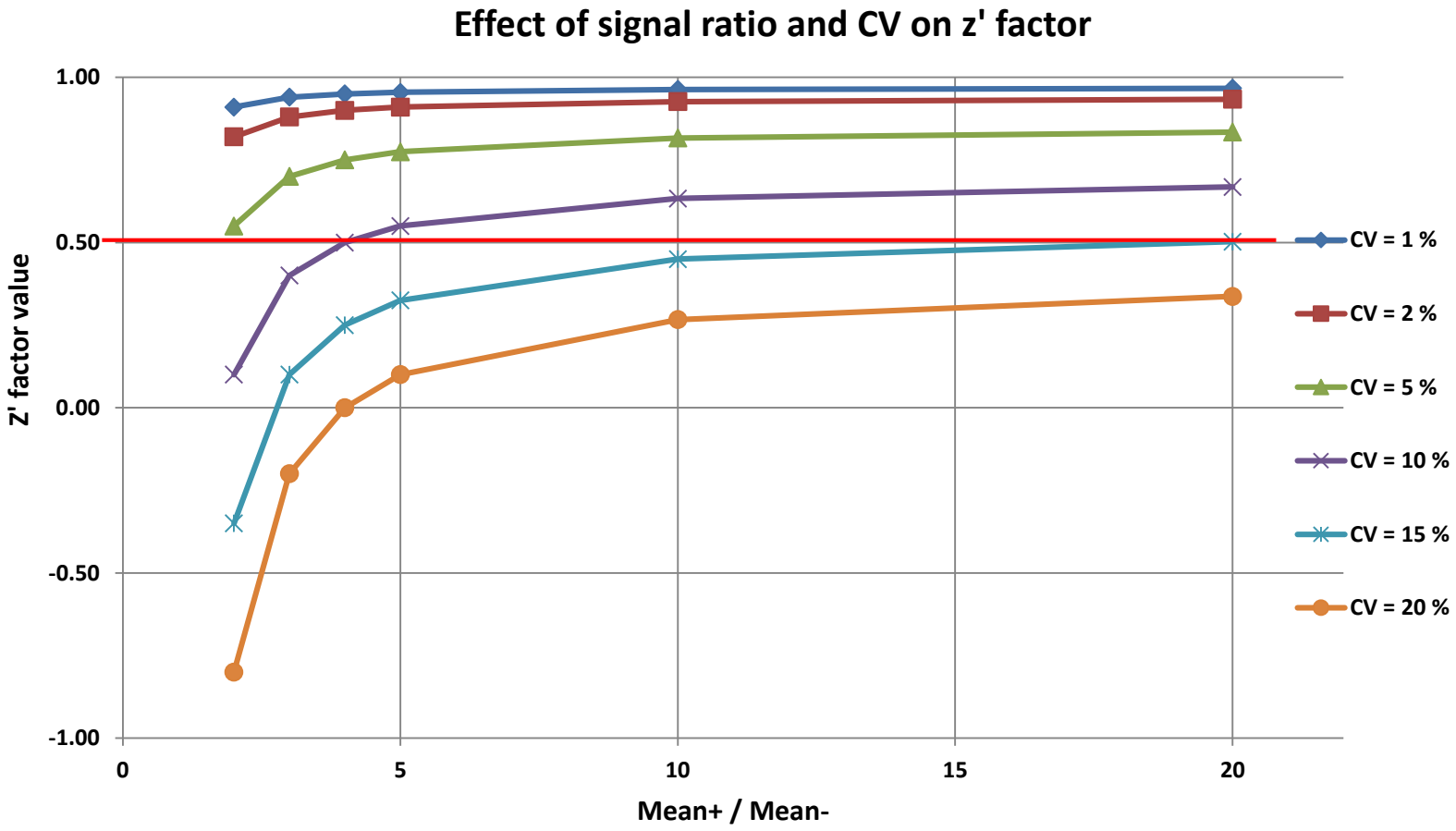


Z’ factor characterizes the quality of primary screening assay
Z’ factor allows to expect the quality of quantitative analysis like dose response curve

Parameters impacting z’ factor



Parameters impacting z’ factor



z’ factor as a key parameter for driving assay improvment

- What is driving the value of my z’ factor?
  -> variability and/or mean difference?

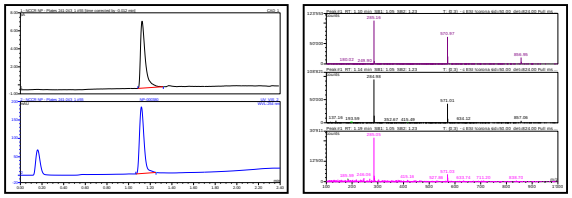
- Why is the difference between negative and positive means so small?
  - need of technology shift, e.g from absorbance to fluorescence
  - stable cell line vs transcient transfection…

- From what is arising the variability?
  - single step as the main variability source or cumulative effect
  - Variability analysis of each step (through CV)

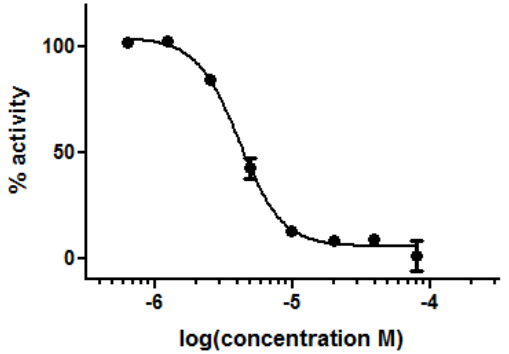
Defining primary screening follow-up

This process should address any potential limitation of primary screening

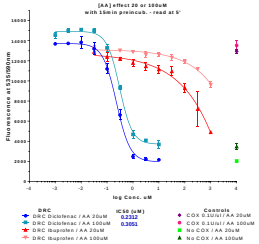
- Confirmation step(s) : repeat, post-reaction testing, interference detection (like autofluorescence), filtering/counterscreen assay *(mainly linked to the screening assay)*
- Compounds QC



- Dose response curve (quantitative analysis)



- Secondary assays (cytotoxicity, additional cell line, other technology for readout…)
- Study of MOA (inhibition type)



- Direct interaction measurement (MST, SPR, ITC…)

NT.115: cox2 vs. diclofenac:

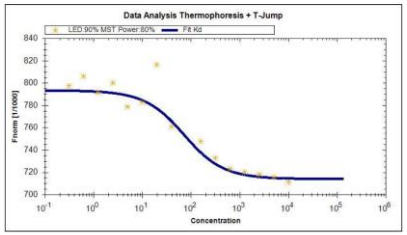


Figure 2: For performing experiments with the cox2 protein, a fluorescent label (NT-647) was covalently attached to the protein (NHS coupling). In the MST experiment we have kept the concentration of NT-647 labeled cox2 constant, while the concentration of the non-labeled diclofenac was varied between 10 µM – 0.3 nM. The assay was performed in buffer provided by the experimenter containing Tween20 (0.05%) and centrifuged before loading. After a short incubation the samples were loaded into MST NT.115 standard glass capillaries and the MST analysis was performed using the Monolith NT.115. Concentrations on the x-axis are plotted in nM. A K_d of **60.9 nM +/- 4.9 nM** was determined for this interaction.

Medicinal chemistry / Hit to lead

- 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

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

Presentation of the practical course at BSF

- *Measurement of chemical cytotoxicity in myoblast cells (H9C2) with different readouts (Digital Holographic Microscopy versus automated fluorescence microscopy versus metabolic fluorescent reporter)*
- *Introduction to automated compound management and demo of using an acoustic dispenser (Echo, Labcyte) for plating chemical compounds (single dose and dose response curves) in a 96W plate*

Selected books & general bibliography

Handbook of drug screening
Seethala & Fernandes, Ed. Marcel Dekker

Handbook of assay development in drug discovery
Minor, Ed. CRC

High throughput screening, Methods and protocols
Janzen, Ed. Humana Press

Structure and mechanism in protein science
Fersht, Ed. Freeman

Evaluation of enzyme inhibitors in drug discovery
Copeland, Ed. Wiley

High content screening
Taylor et al., Ed. Humana Press

Imaging cellular and molecular biological functions
Shorte & Frischknecht, Ed. Springer

Cancer cell culture
Cree, Ed. Humana Press

The practice of Medicinal Chemistry
Wermuth, Aldous, Raboisson & Rognan, Ed. Academic Press

A simple statistic parameter for use in evaluation and validation of high throughput screening assays
Zhang et al., Journal of Biomolecular Screening, 4 (2), 1999

Early probe and drug discovery in academia: a minireview
Roy, High-Throughput, 7, 2018

Principles of early drug discovery
Hughes et al., British Journal of Pharmacology, 162, 2011

Cyclooxygenase assays
Gierse and Koboldt, Current Protocols in Pharmacology, 1998

High throughput screening assays for the identification of chemical probes
Inglese et al., Nature Chemical Biology, 8 (3), 2007

CellProfiler: image analysis software for identifying and quantifying cell phenotypes
Carpenter et al., Genome Biology, 7, 2006

Integrating high-content screening and ligand-target prediction to identify mechanism of action
Young et al., Nature Chemical Biology, 4 (1), 2008

High content screening: seeing is believing
Zanella et al., Trends in Biotechnology, 28 (5), 2008

Genomic screening with RNAi: results and challenges
Mohr et al., Annual Review Biochemistry, 79, 2010

Optimization procedure for small interfering RNA transfection in a 384-well format
Borawski et al., Journal of Biomolecular Screening, 12 (4), 2007