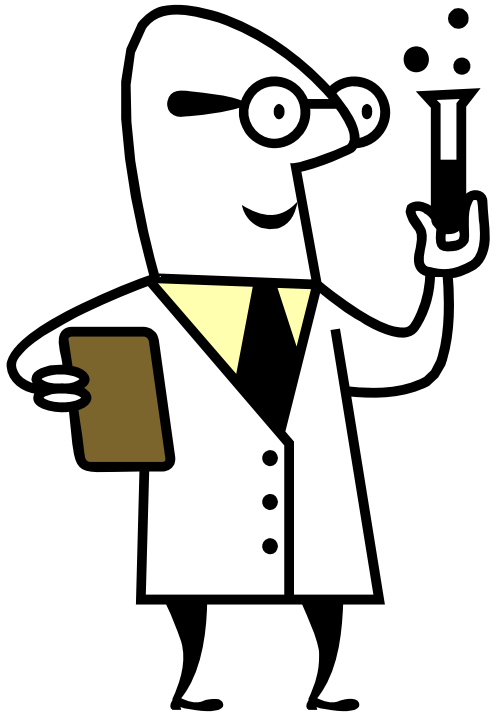
Screening in Drug Discovery



Göteborgs Universitet

Läkemedelsutveckling

November 2007

Fanyi Jiang, PhD

Lead Discovery Science

Lead Generation

AstraZeneca, Mölndal

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Outline

- Drug Discovery
- What is screening? Background & Context
- The biology of screening
- The chemistry of HTS
- The automation of HTS
- The computing & data of HTS
- The future

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Drug Discovery

Find a new drug (small molecule) for therapeutic targets

Therapeutic Target:

An enzyme, receptor or other protein in disease-relevant biological systems is regulatable

Therapeutic Area (TA):

Central Nervous System (CNS), Cancer, Cardiovascular (CV), etc

Disease Area (DA):

Obesity, Depression, Diabetes, etc

Drug discovery – an expensive & risky business

However beneficial for human life & life quality

- Average drug costs >$500-1000m and 15 years
- Prices are under pressure
- Regulations very demanding & complex
- Patent situation is very sensitive
- Generics have 43% of market
- Economics are tough
  - Only 3 in 10 drugs recover costs
  - Only 2 in 10 drugs are profitable
  - Only 1 in 50 drugs are block-busters

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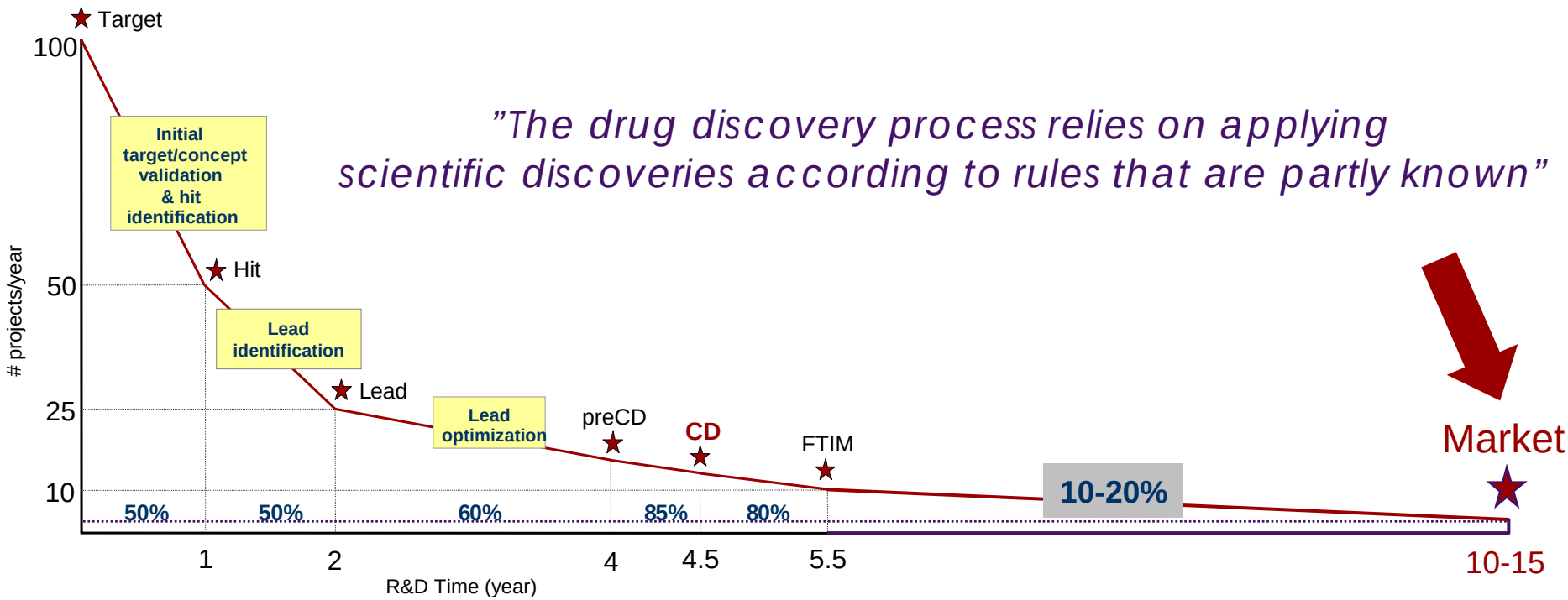
Failure rates are high in drug discovery and development



"The drug discovery process relies on applying scientific discoveries according to rules that are partly known"

"Imagine trying to design a modern aircraft with the knowledge that there might be rules of aerodynamics that are yet undiscovered"

Carl M. Cohen, Nature Reviews 2003; 2: 751-753

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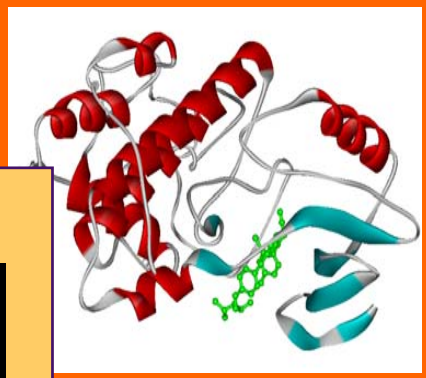
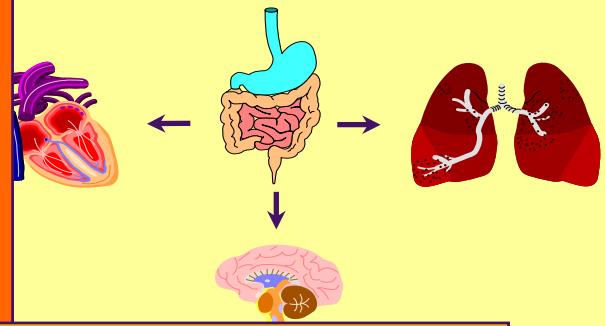
Requirements & Challenges

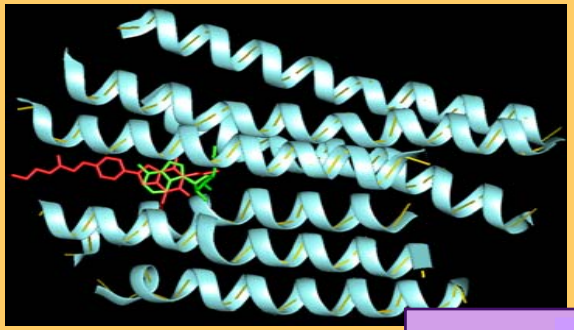
Potency & Selectivity



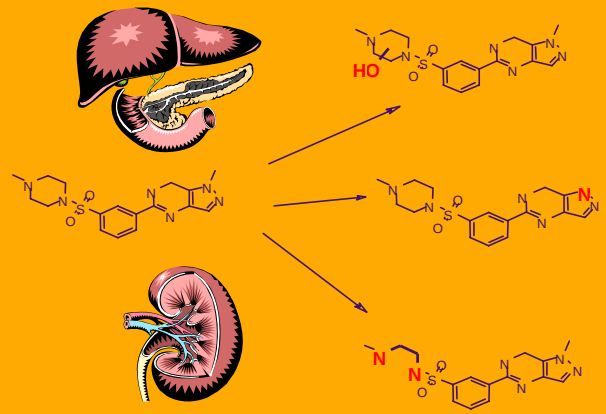
Absorption & Distribution



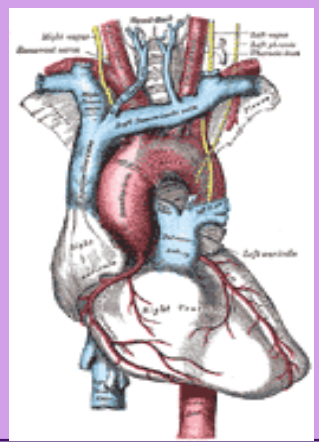
Safe & effective target



Metabolism & Excretion



Toxicology



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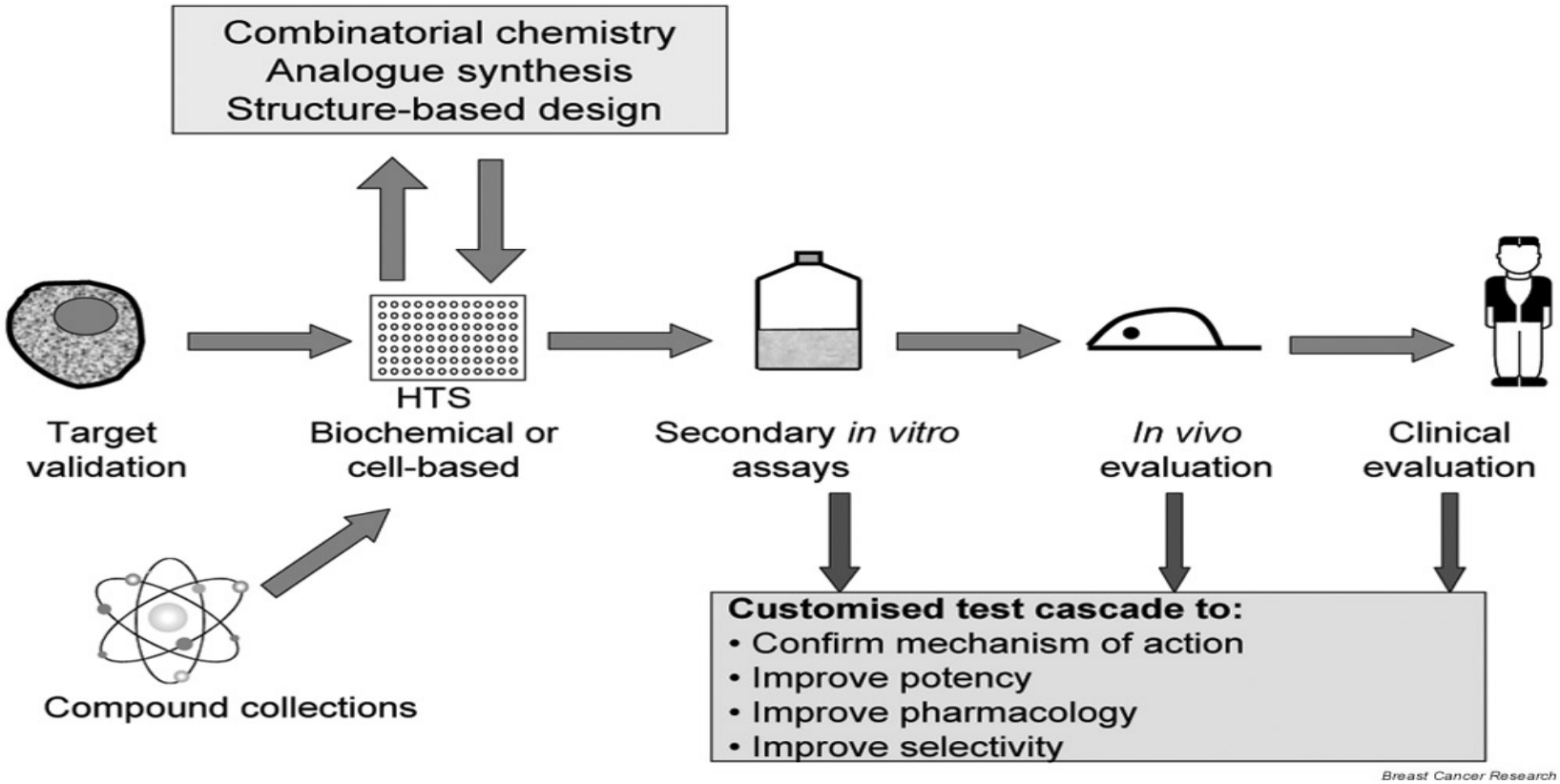
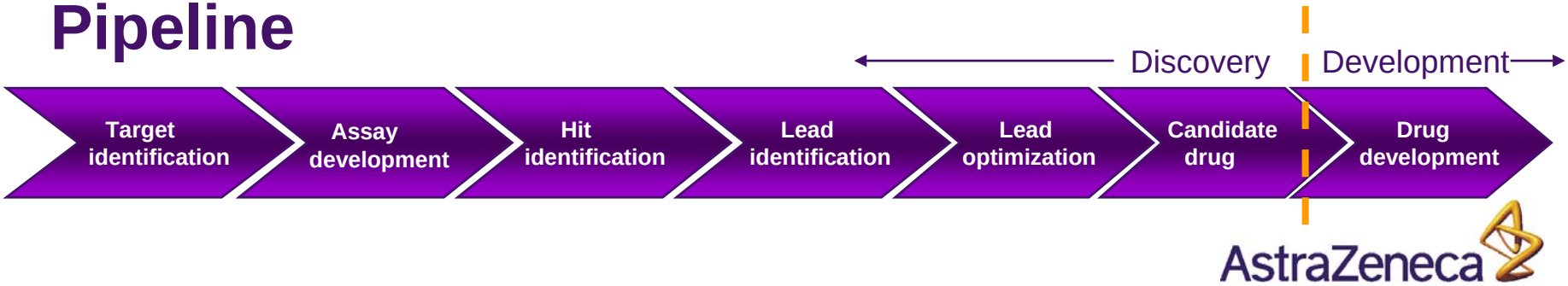
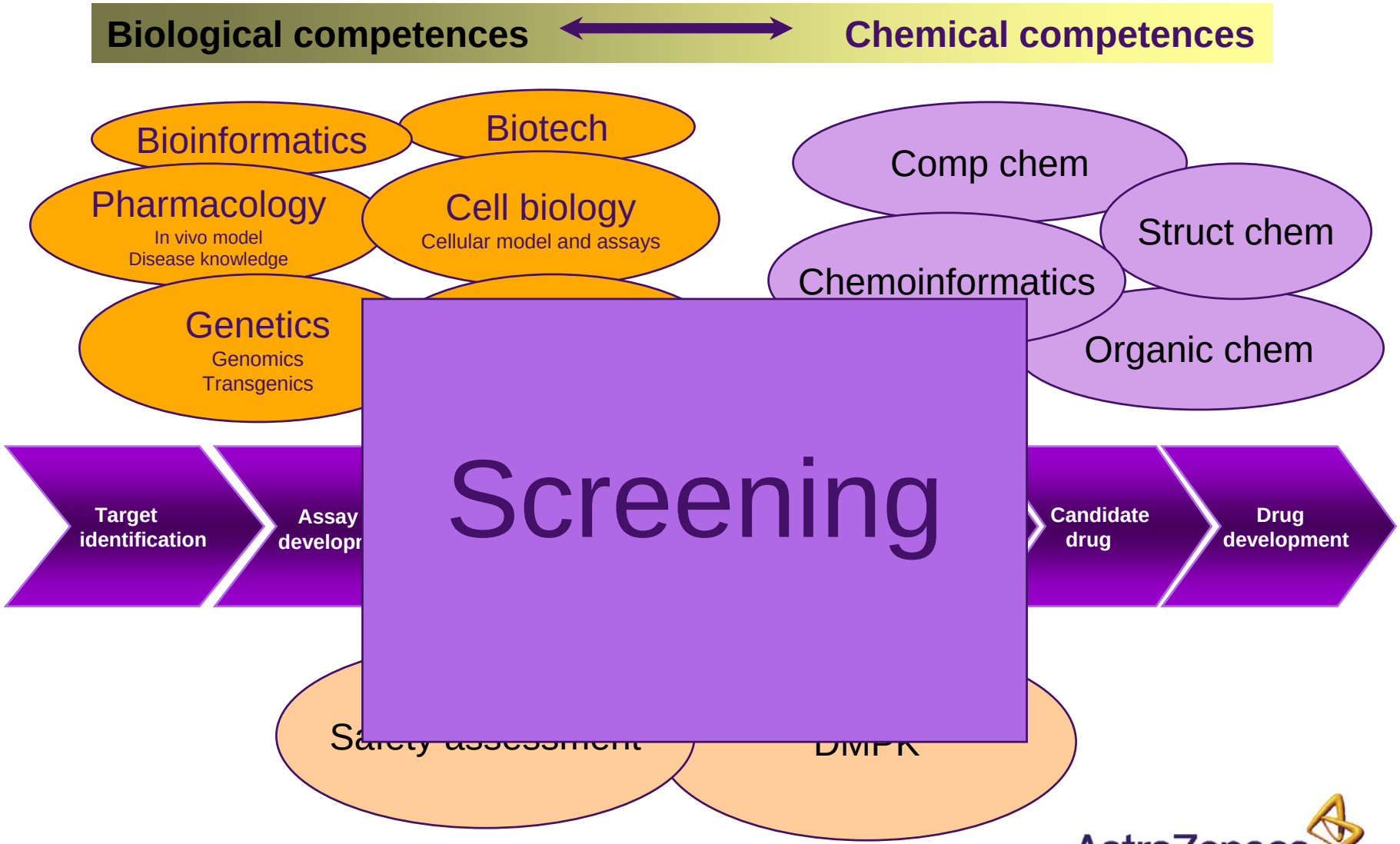
Drug discovery process



Pipeline



Competences in drug discovery



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Screening – an important tool box in drug discovery

Therapeutic Target:

An enzyme, receptor or other protein in disease-relevant biological systems is regulatable

Assay:

Analytical method to monitor an activity or a biological process involving of the desired or a potential therapeutic target

Screen:

A process to identify small molecules from a large collection pool interacting with the desired target in an assay that is reproducible, scale-able & robust

HTS:

High through-put screening = HTS (>100,000 cmp/day)

- test as many sample as possible, as quick as possible
- chemical starting point in drug discovery

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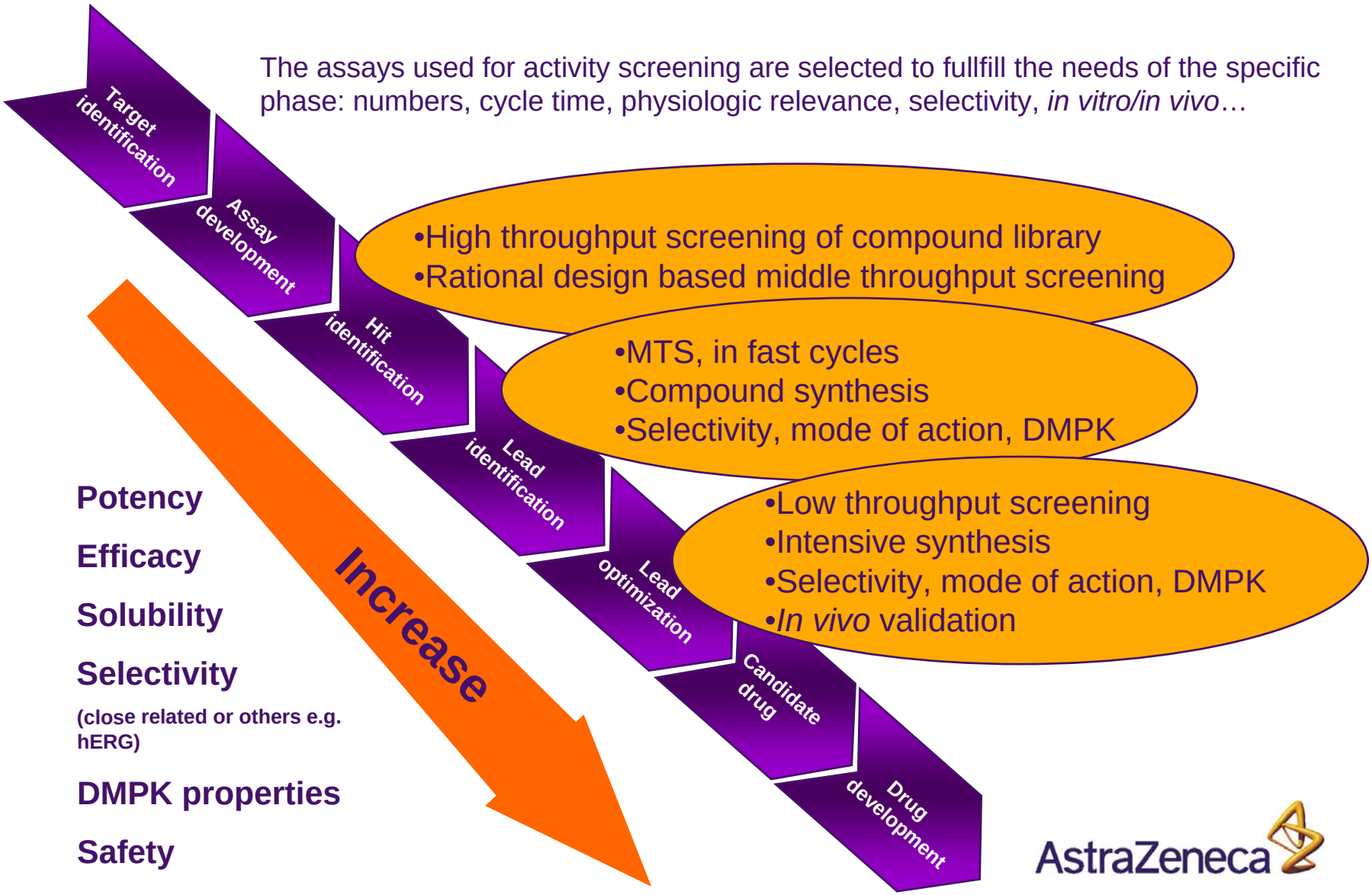
Screening in drug discovery

The assays used for activity screening are selected to fullfill the needs of the specific phase: numbers, cycle time, physiologic relevance, selectivity, *in vitro/in vivo*…



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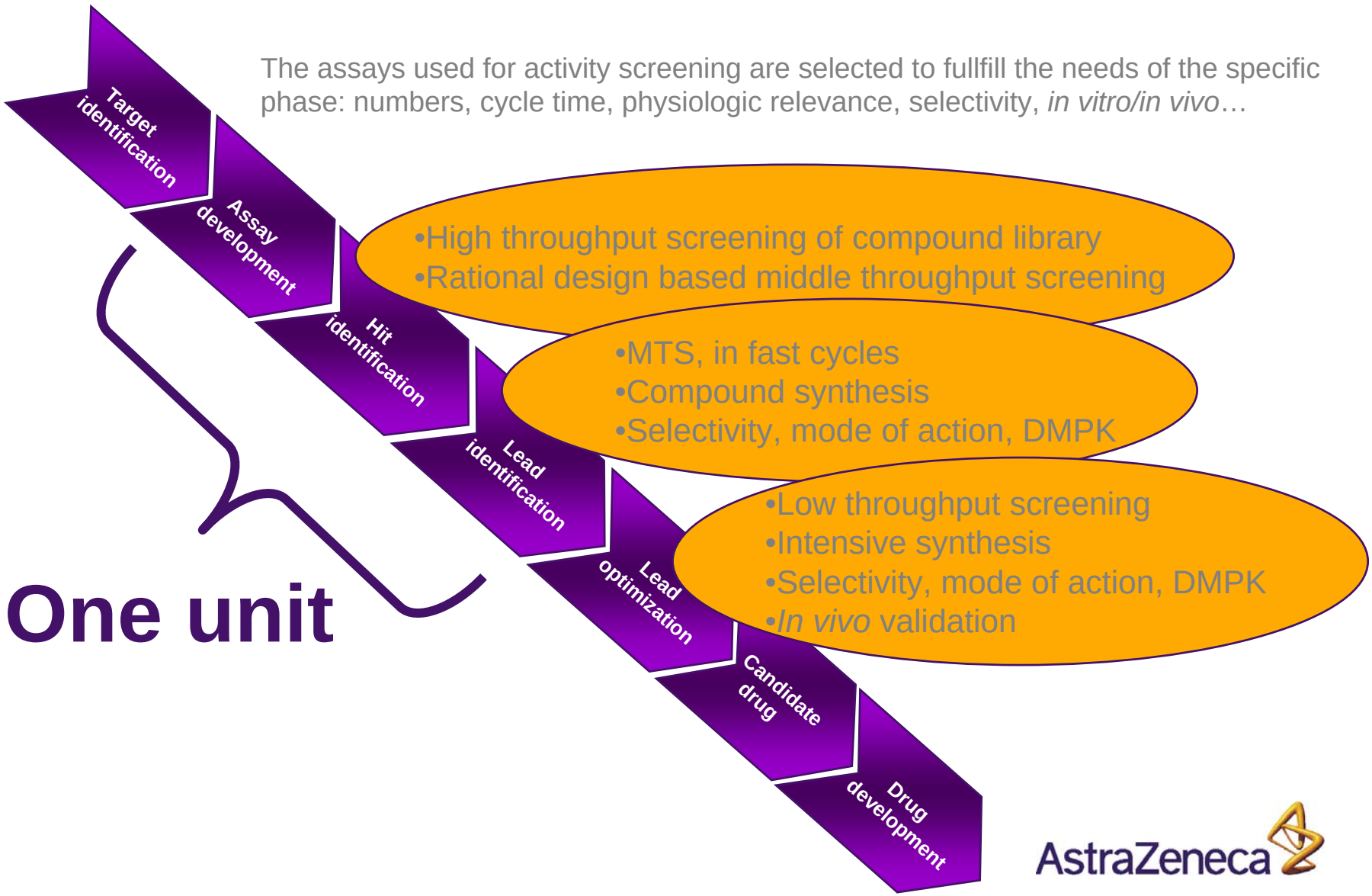
Effective early drug discovery

The assays used for activity screening are selected to fullfill the needs of the specific phase: numbers, cycle time, physiologic relevance, selectivity, *in vitro/in vivo*…



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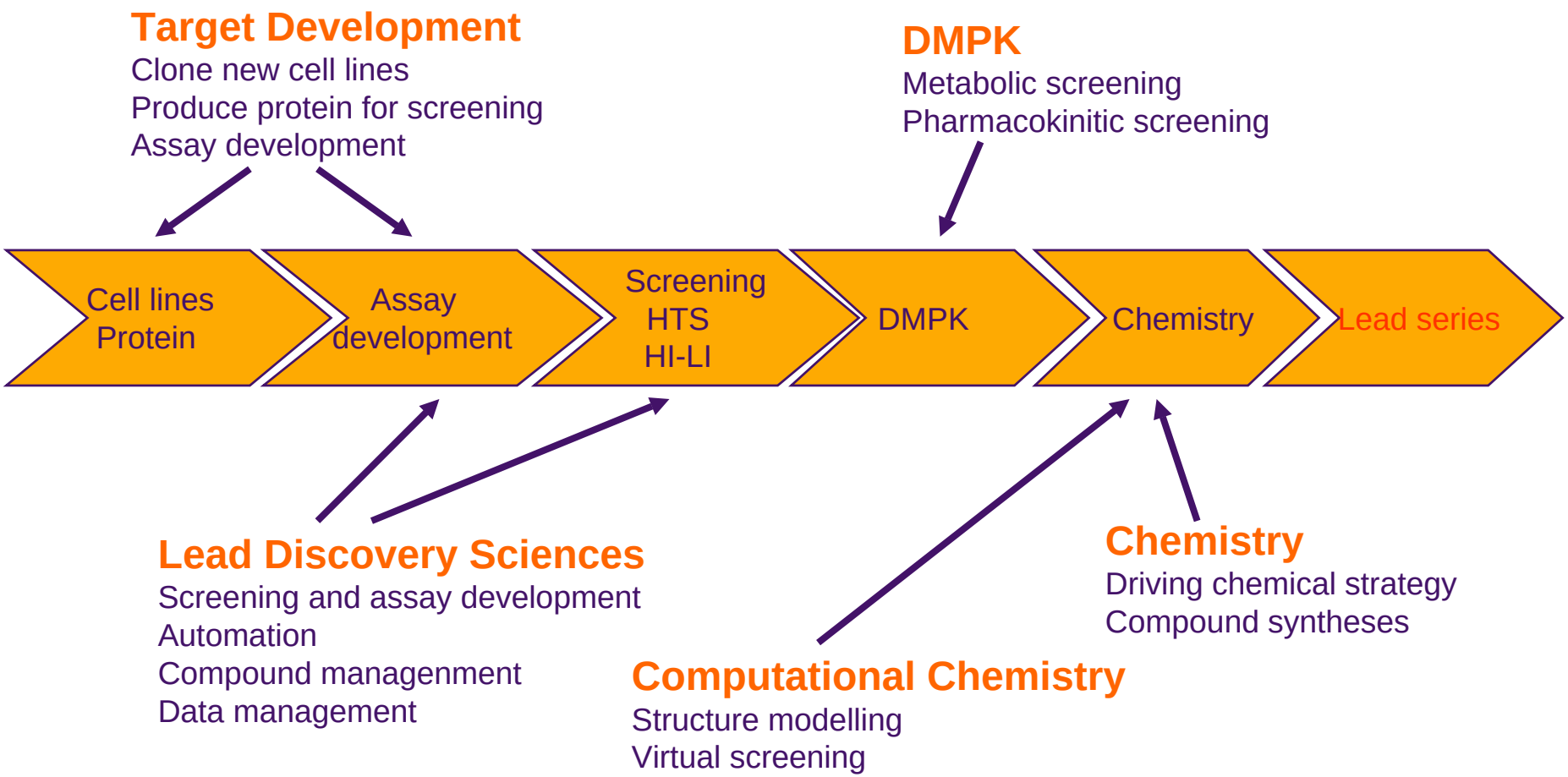
Lead Generation –
Combining Technologies within one department



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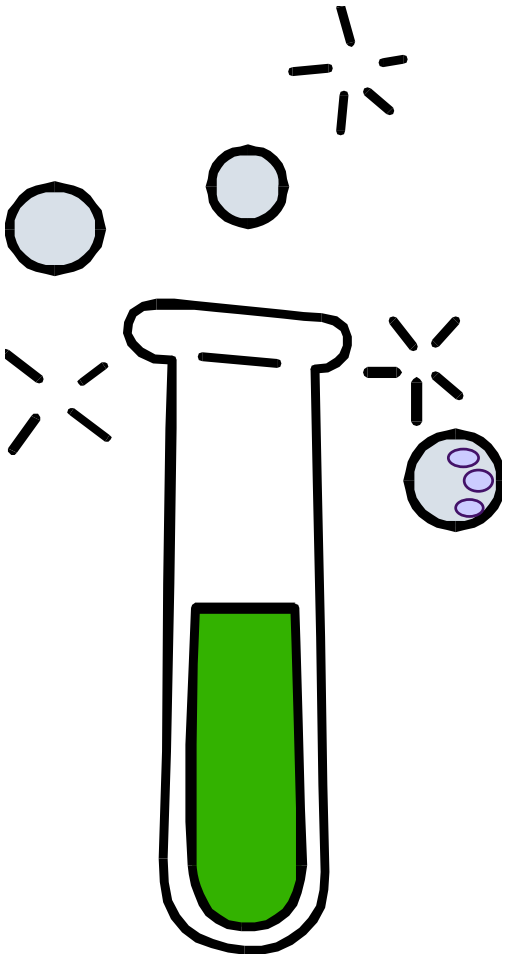
5 powerful ingredients of Lead Discovery Scienses …



Biology:
Big, experienced & versatile screening capacity

Automation:
Robots & sophisticated instruments

Chemistry:
Big & advanced compound collection & management

Computing:
Powerful data management systems

Teamwork!

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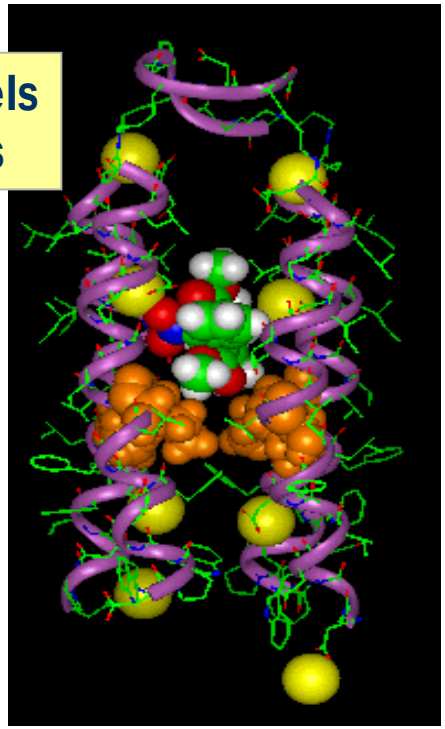
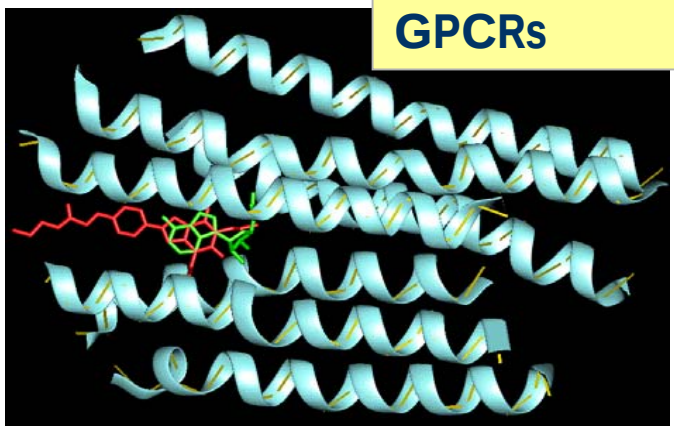
Major targets of successful drugs

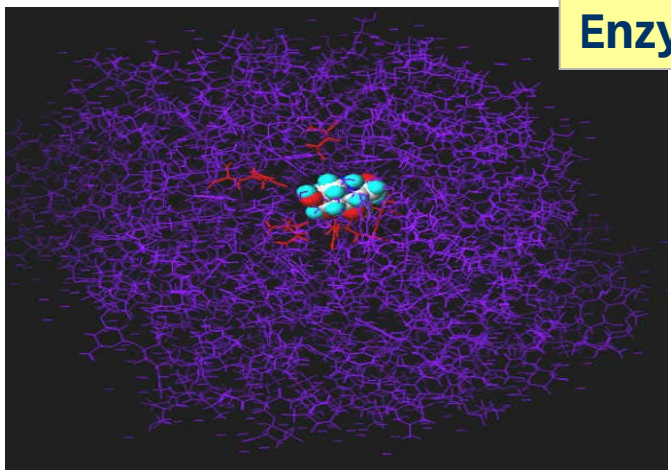
Ion channels and pumps



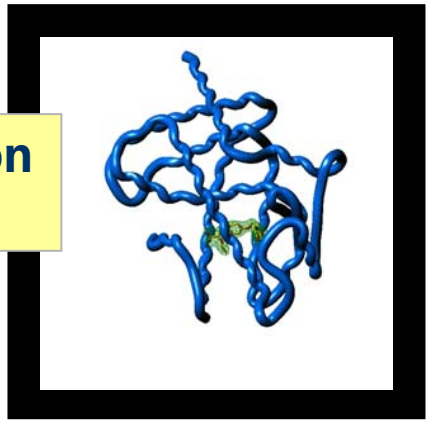
7-TM receptors; GPCRs



Enzymes



Transcription factors



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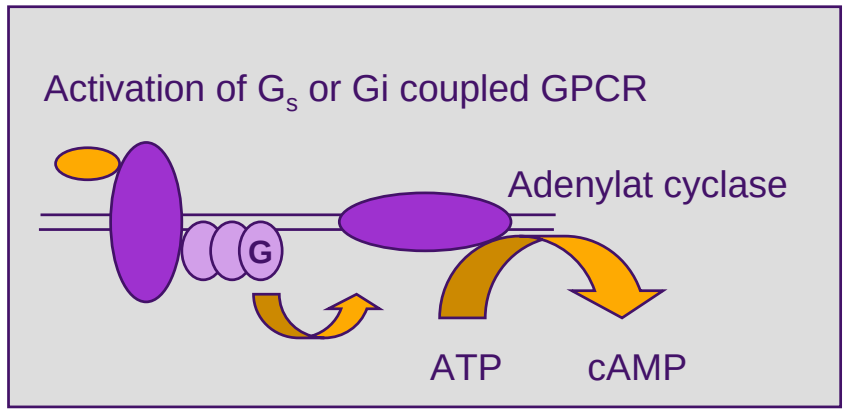
Targets - receptors

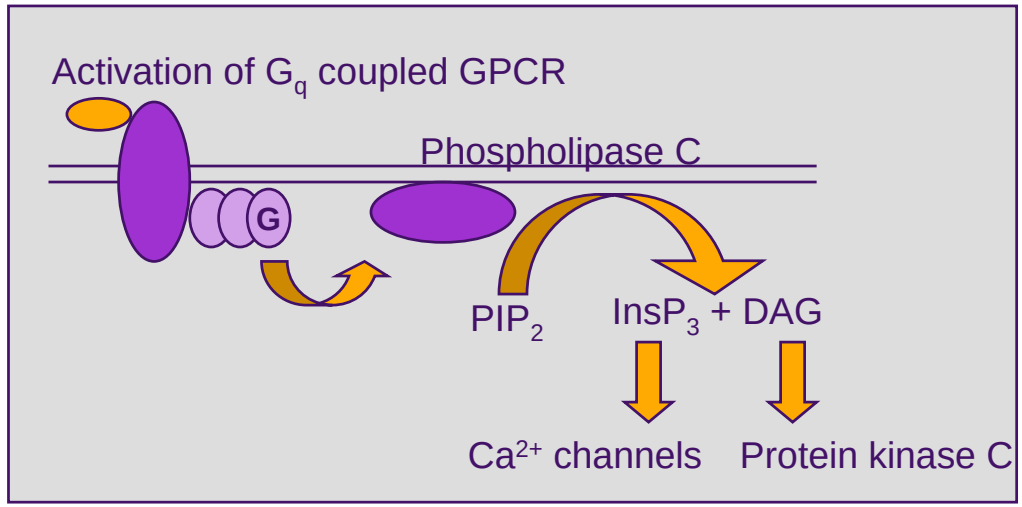
Receptors:

- G-protein coupled receptors
  = GPCRs
- Ion-channel coupled receptors
  = ligand-gated ion-channels
- Enzyme-linked receptors



GPCRs

- Seven TM alpha helixes
- Desensitized after activation through internalisation
- G-protein have three subunits
- Type of alpha subunit determines signaling pathway



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Targets – ion-channels

Ion-channels show ion selectivity and act very fast:

- Ligand-gated channels (intra- and extracellular ligands), ex neurotransmittor

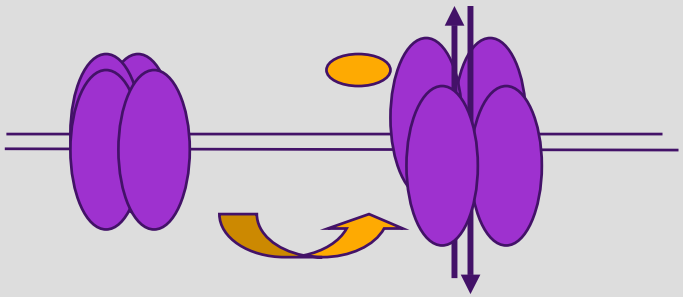
- Voltage-gated channels

  Closed in polarized cell, opened when the membrane is depolarized

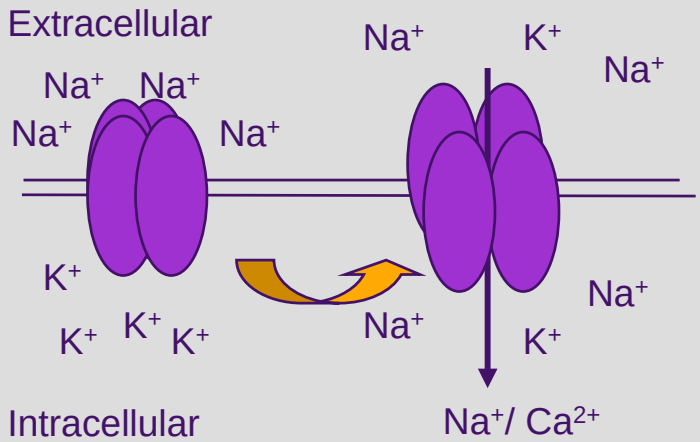
  In neurons, voltage gated Na^+ channels

- Mechanically gated channels

Ligand-gated channels:



Votage-gated channels:



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Targets – enzymes

- Proteases
- Kinases
- Phosphatases
- Metabolic (ex ATP or NADPH consumption)
- .....

Targets – transcription factors

- Ligand activited transcriptinal regulation
- Nuclear receptors (cytoplasma to nucleus translocation)

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Screen flow in HTS

Assay development

↓

Robot test (liquid handling and reagent stability)

↓

Primary screen (single test of compounds in core collection)

↓

Retest (confirmation in triplicates)

↓

Concentration response (3 or 7 concentrations in triplicates)

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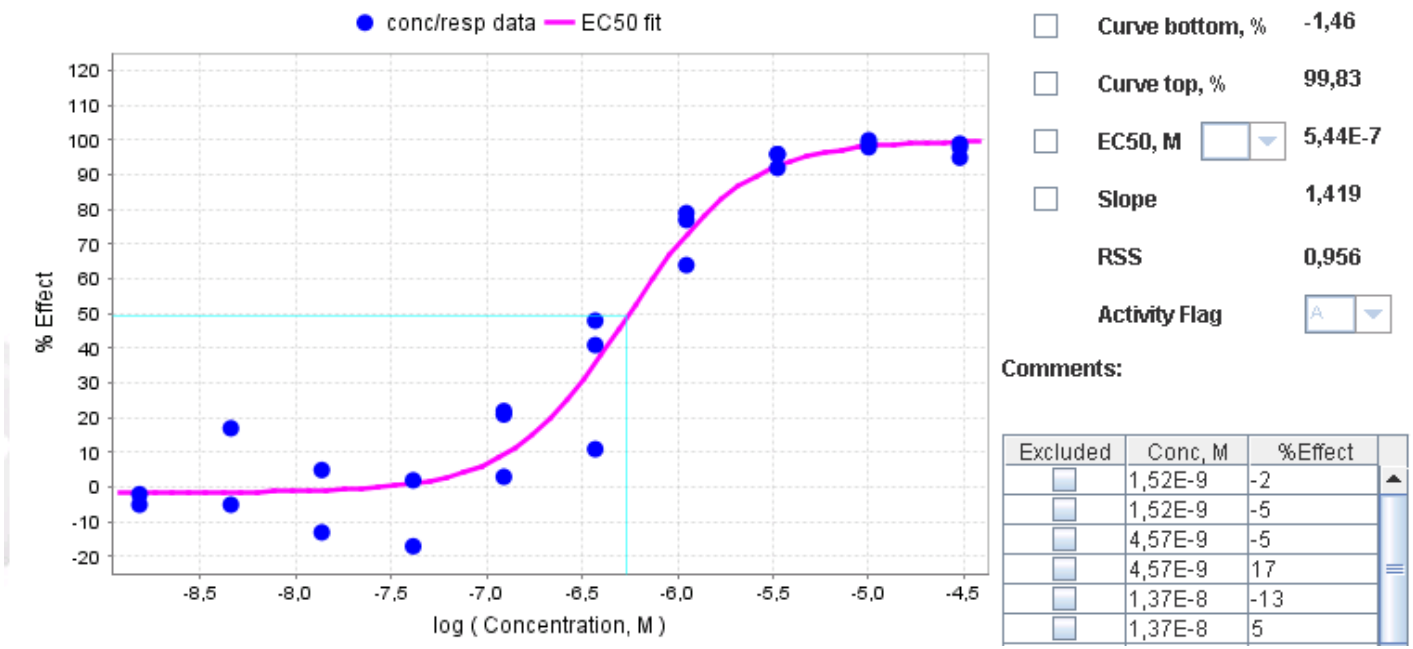
What is a dose response curve?



- The effect of different concentrations of compound is tested in an activity assay.
- The % effect of a reference compound is plotted against the compound concentration.
- An EC50 (activation) or IC50 (inhibition) value is calculated from the plot,that is the half maximal effect

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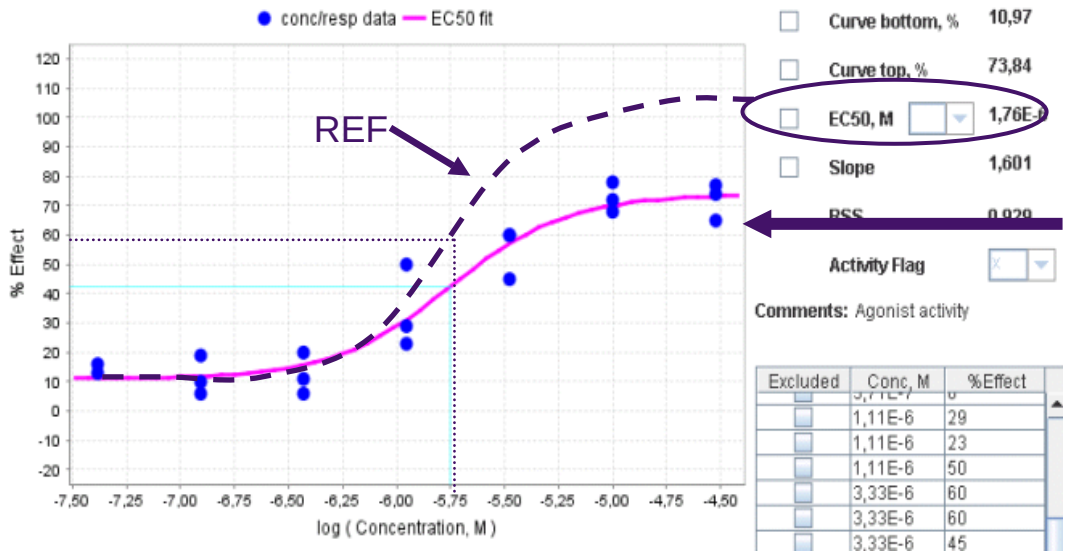
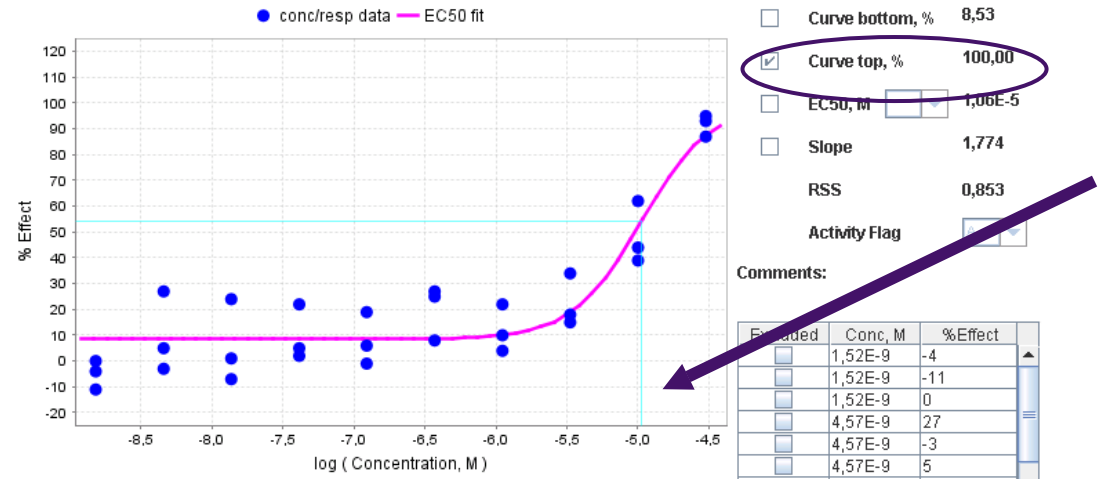
Why dose response curves?



To determine efficacy:
Is the maximal effect of a compound as efficient as a reference compound?



To determine potency:
How much of the compound do you need for half maximal effect?

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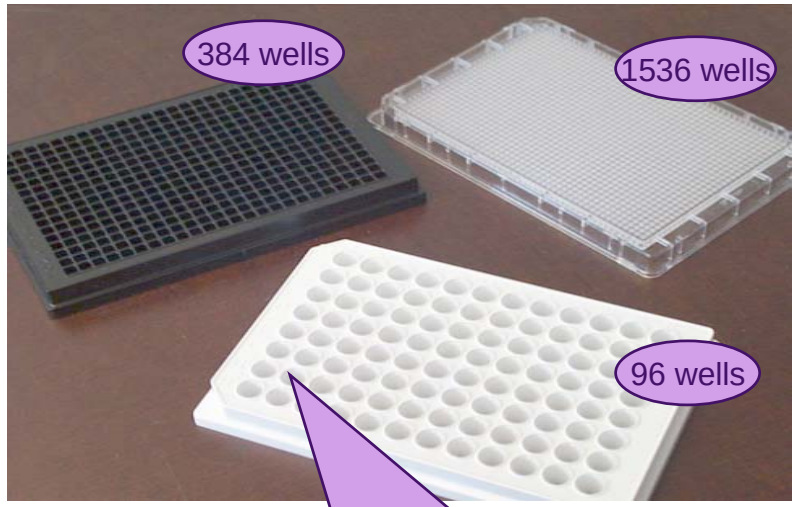
Assay development for HTS

- HTS assays must be…
  - Simple!
  - Reliable!
  - Robust
  - Cheap!
- We consider…
  - Assay type (binding, enzymatic, functional)
  - Cell, membrane or protein based
  - Signal type (fluorescence, absorbance, luminescence…)
  - Solvent tolerance (DMSO)
  - Compound interference
  - Reference compounds
  - Volume (10-80 µl in 384)



One compound and one assay per well!

And they must have physiological (disease) relevance!

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HTS likes “homogeneous” assay technologies

- “Mix and Measure”
- Ideal for HTS
- No separation steps
- Simple!

Radioactive assays:

- SPA (Scintillation Proximity Assay)
- Filtation assays (includes filtration step, lower through-put)

Non-radioactive assays:

- Time Resolved Fluorescence
- Fluorescence resonance energy transfer (FRET)
- FLIPR
- Fluorescence Polarisation
- AlphaScreen
- Aequorin
- Cellomics – ArrayScan etc

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Assay Technologies

- Binding Assays
  - Scintillation proximity assay (SPA)
  - Fluorescence polarisation (FP)
  - Biacore …
- Enzyme assays
  - Enzyme as targets (Catalytic substrates or products with changes in colour, fluorescence & luminescence)
  - Enzyme mediated measurement
- Functional assays

  Non cell-based
  - Fluorescence resonance energy transfer (FRET)
  - GTPgS for GPCR

  Cell-based
  - Reporter (gene transcription)
  - Secondary messager: cellular Ca^{++} and cAMP content etc
  - Cell viability and proliferation
  - Cell Image

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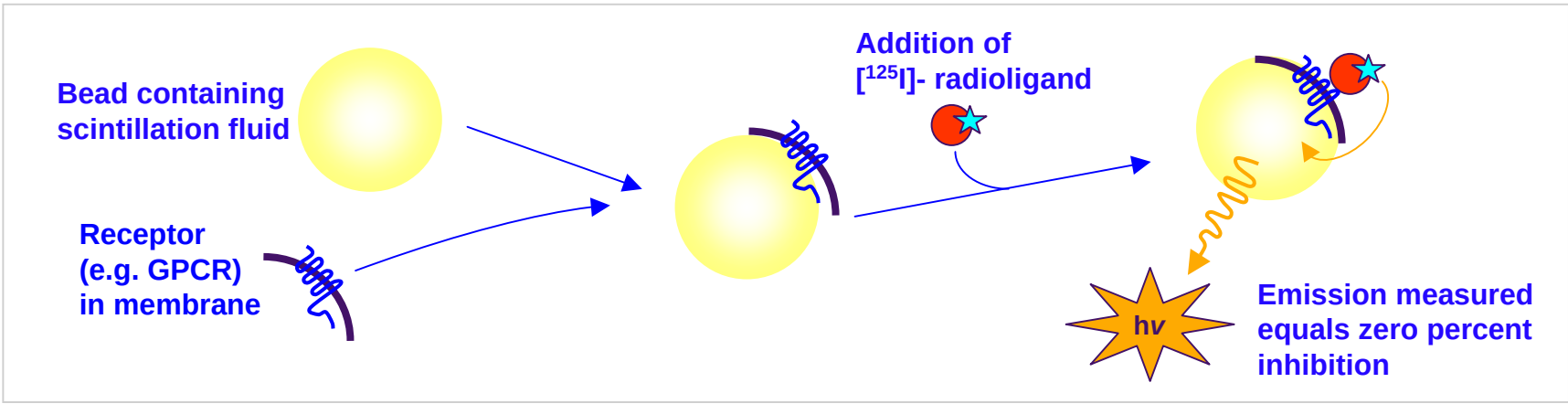
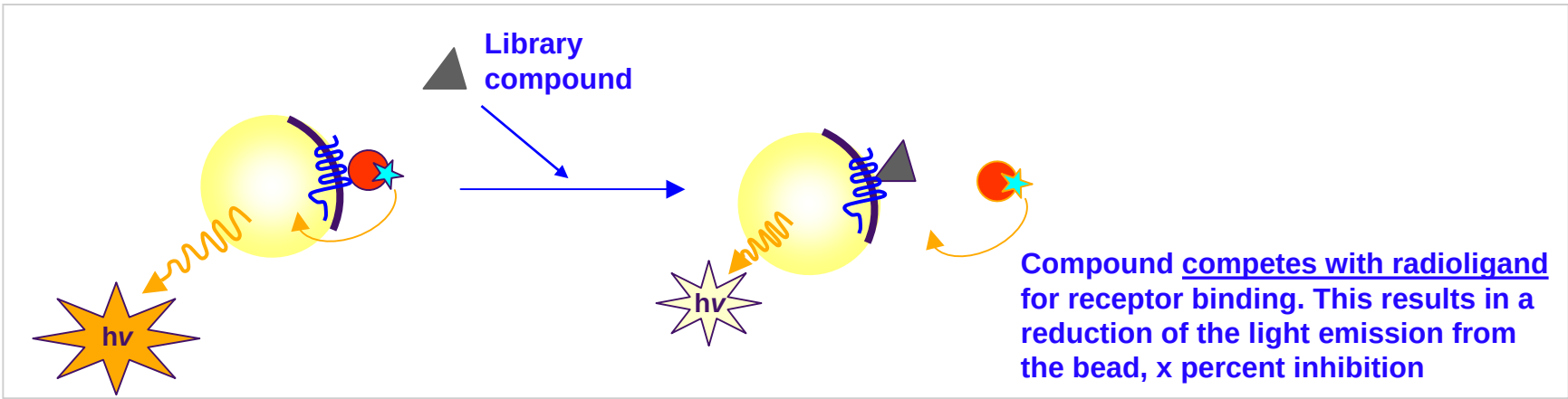
Scintillation Proximity Assay (SPA)

Rationale of the SPA binding assay:



Binding of an antagonist from compound library:



Disadvantage: radioactive

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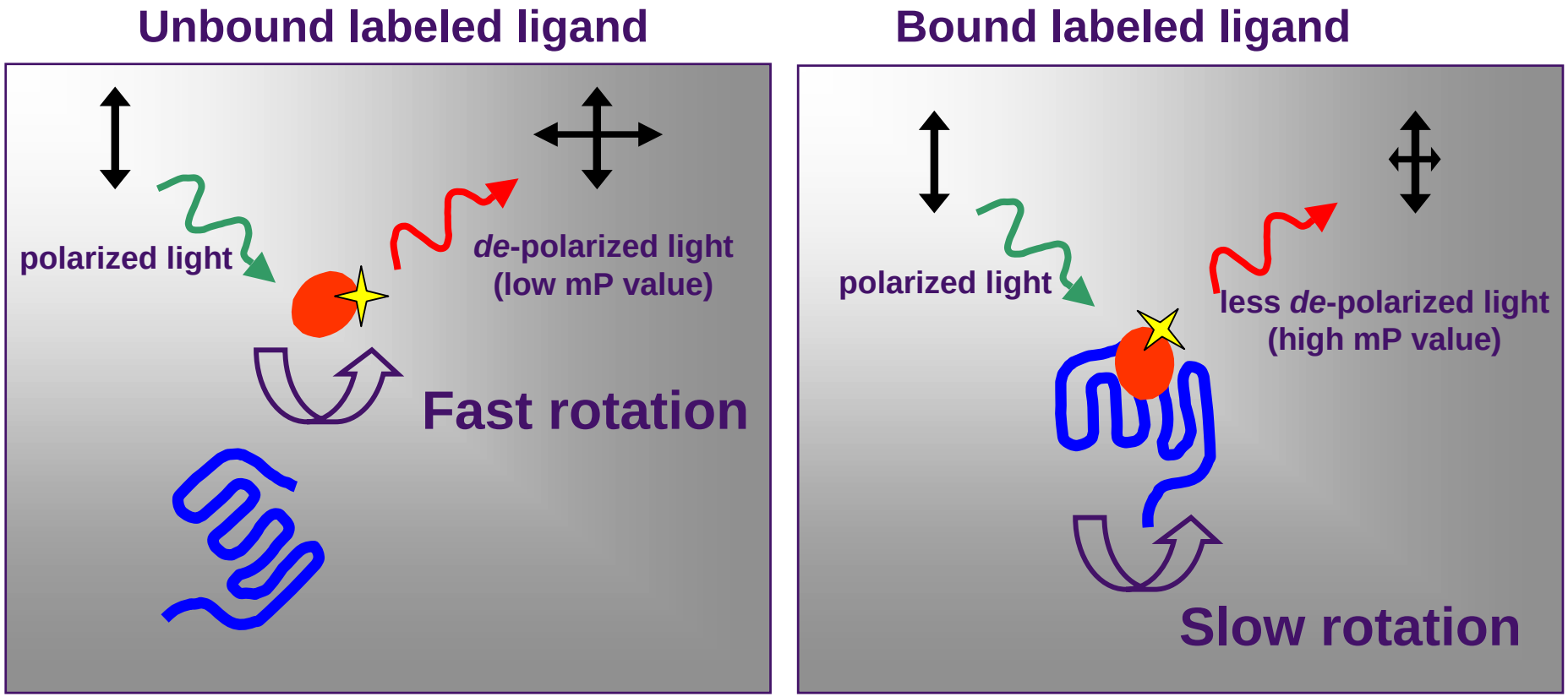
Fluorescence polarisation (FP)



- A non-radioactive binding assay, not very sensitive comparing to SPA
- A library compound compete out the ligand with a fluorescent label => faster rotation
- mP = 1000 * (II - ⊥) / (II + ⊥); (II: fluorescence in parallel plane, ⊥ : fluorescence in perpendicular plane)

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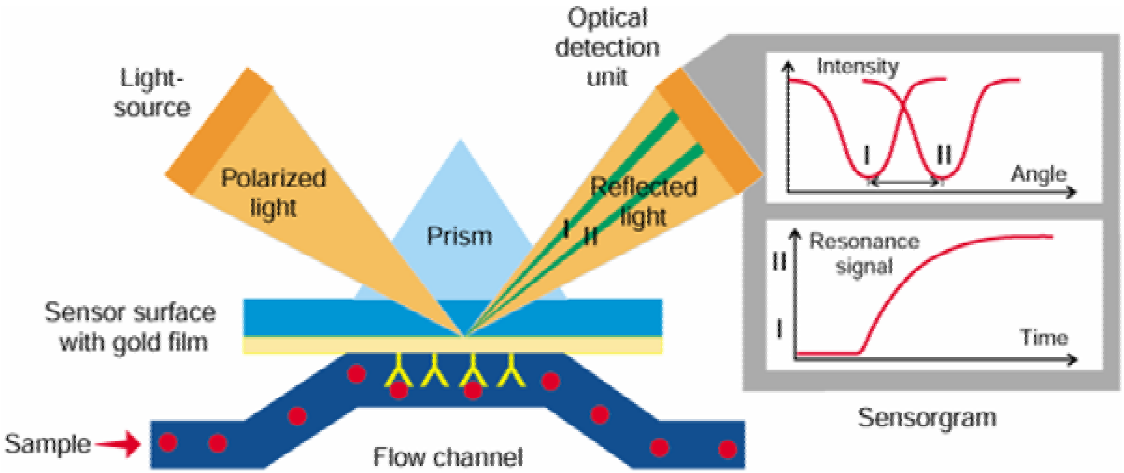
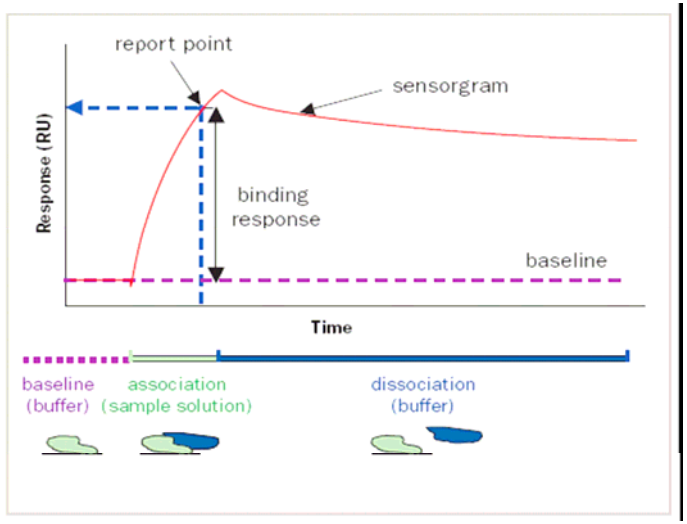
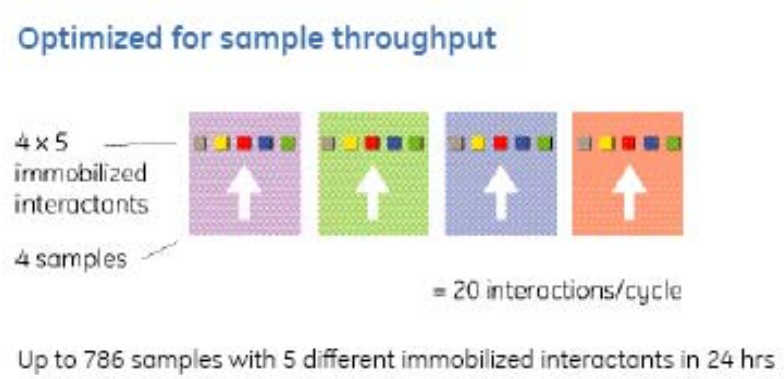
BiaCore

- Principle - Surface Plasmon Resonance





- Biacore A100 - a high throughput Biacore



- Binding kinetics
- Search for binding when no ref available
- Not for HTS purpose
- Immoblization can be difficult

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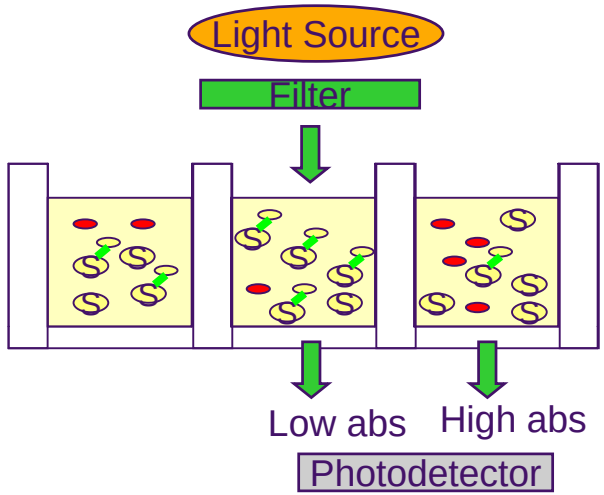
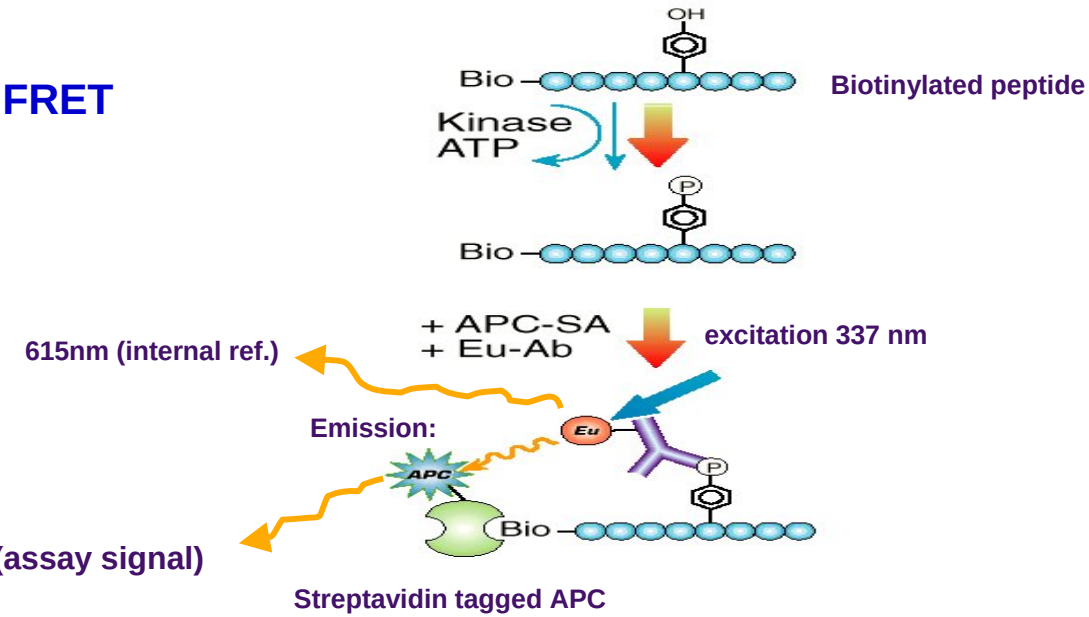
Enzyme assays - enzyme as targets

- **Substrate measurement**



- **Indirect Substrate measurement**
  - FRET, AlphaScreen, Enzyme fragment complemetation *etc*

Kinase FRET



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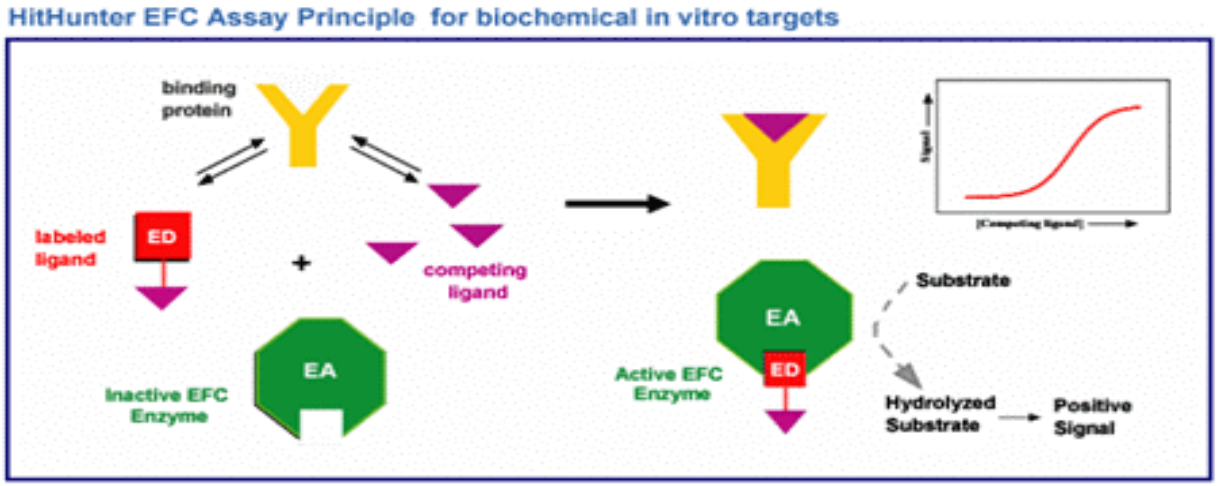
Enzyme assays - enzyme mediated measurement

- Enzyme mediated measurement
  - Enzyme Fragment Complementation Tech - DiscoveRx



  - ✓ GPCR – cAMP (cAMP-doner): very sensitive, 3456 well assay worked very well
  - ✓ Translocation assay: cell lines with EA β-gal fragment restricted to the cell nucleus
  - ✓ Kinase
  - ✓ Protase
  - ✓ etc
  - Luciferase, Galactosidase etc

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Assay Technologies

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  - Scintillation proximity assay (SPA)
  - Fluorescence polarisation (FP)
  - Biacore …
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  - Cell viability and proliferation
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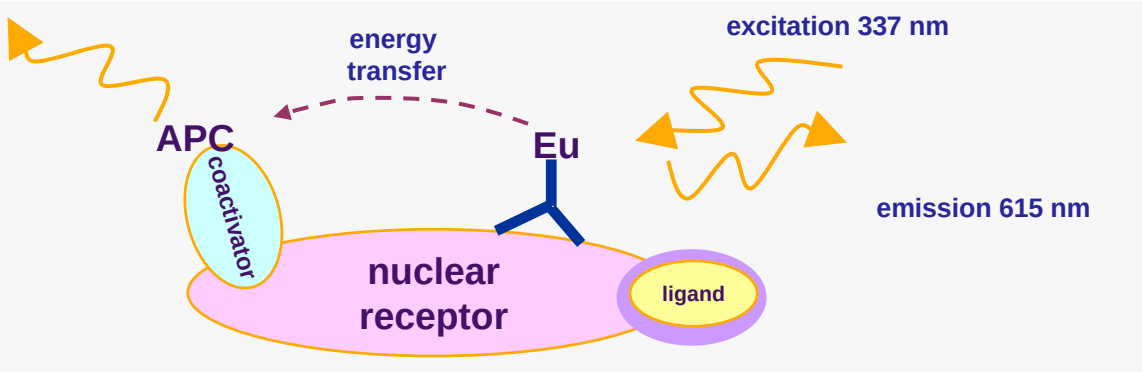
Homogenous Time Resolved Fluorescence Energy Transfer (HTRF, FRET)

Background:

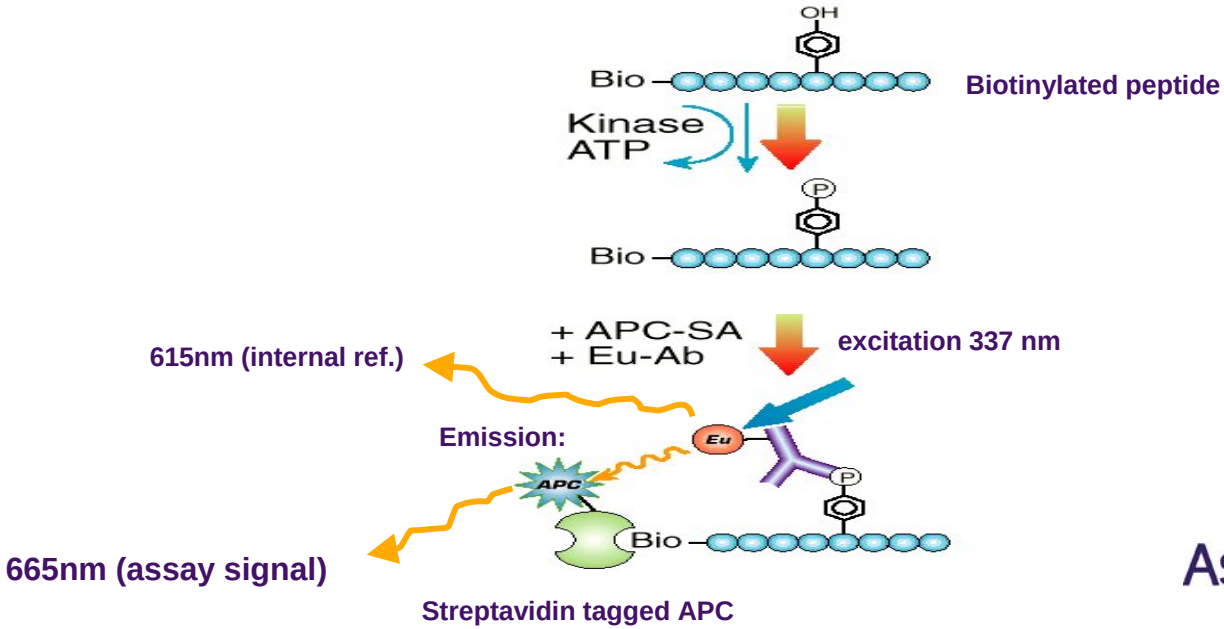
- Streptavidin and biotin bind to stable complex
- Energy transfer can take place between Europium and APC if close enough

Nuclear Receptor

- Biotin-cofactor
- Streptavidin – APC
- His tagged NR
- Anti his tagged Ab – Eu



Kinase



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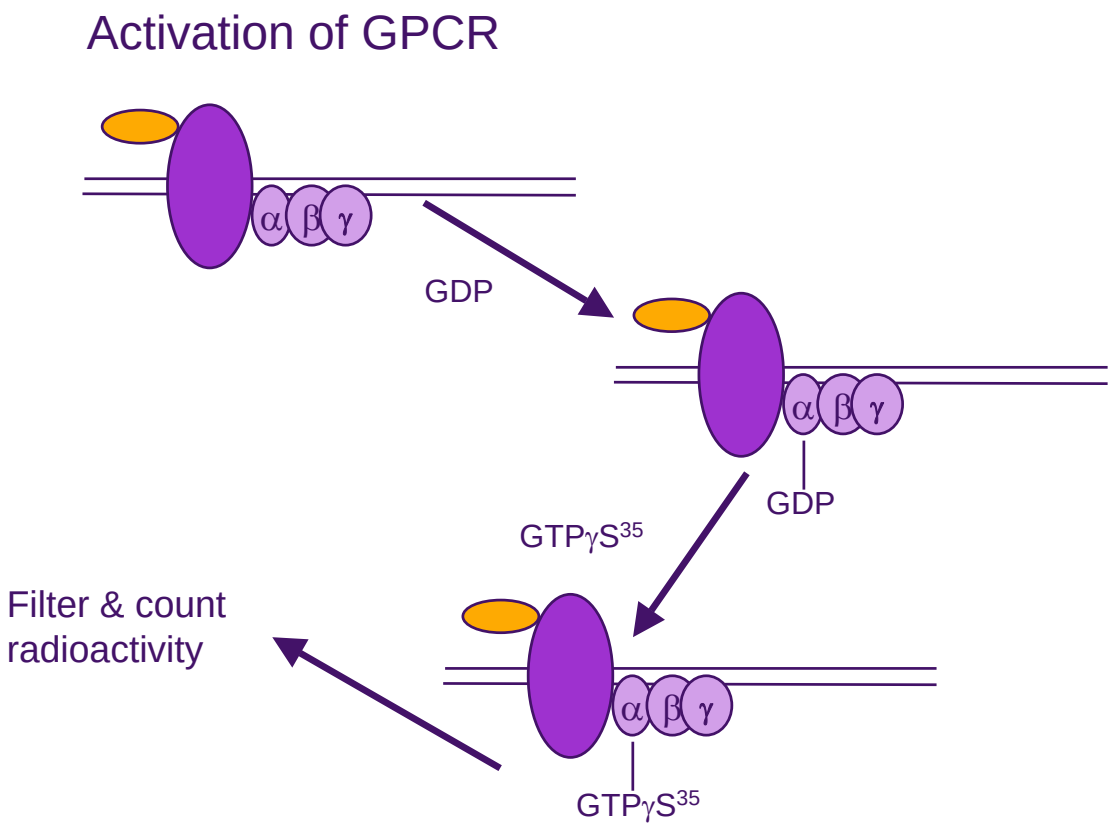
GTPγS functional binding assay



- GTPγS^{35} is a GTP analog
- In principle, the assay can be applied to all GPCRs (Gs, Gi, Gq …)

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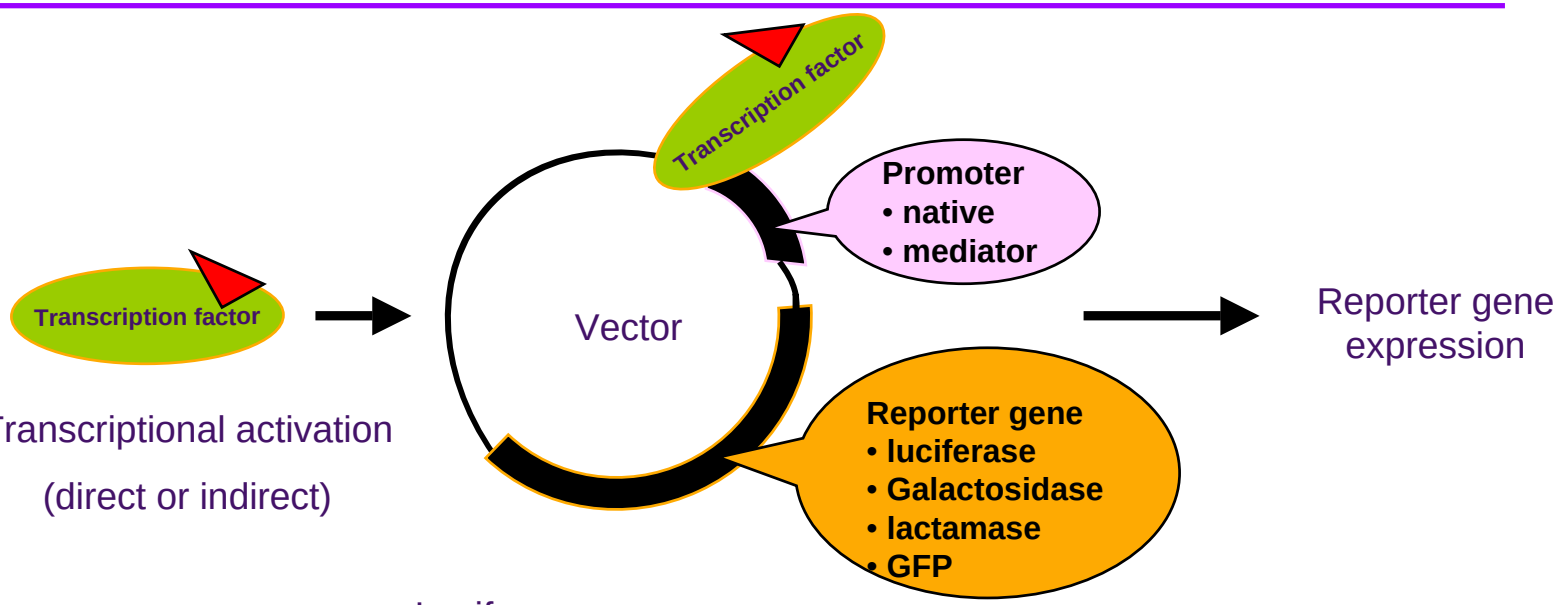
Reporter assays



- luciferin + ATP + O_2 $\xrightarrow[Mg^{2+}]{\text{Luciferase}}$ oxyluciferin + PP_i + AMP + CO_2 + light

- X-Gal (colorless) $\xrightarrow{\beta\text{-gal}}$ galactose (colorless) + 4-Cl-3-Br-indigo (deep blue)

Application

- Transcription factor as target
- Up-stream receptors
- “Black box” assay…

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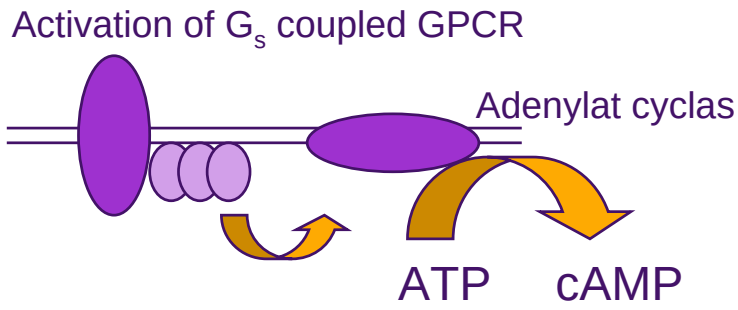
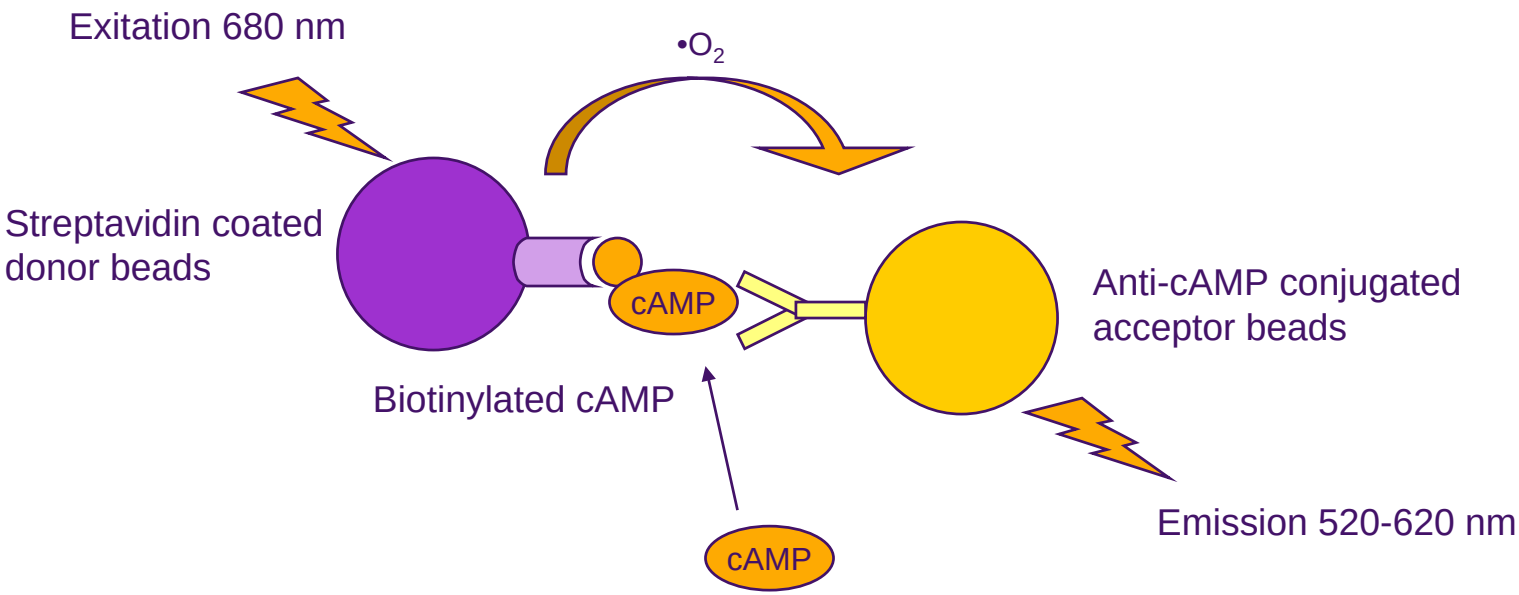
AlphaScreen techonlogy



Applications:

GPCR, cAMP, IP_3 , kinases, proteases….



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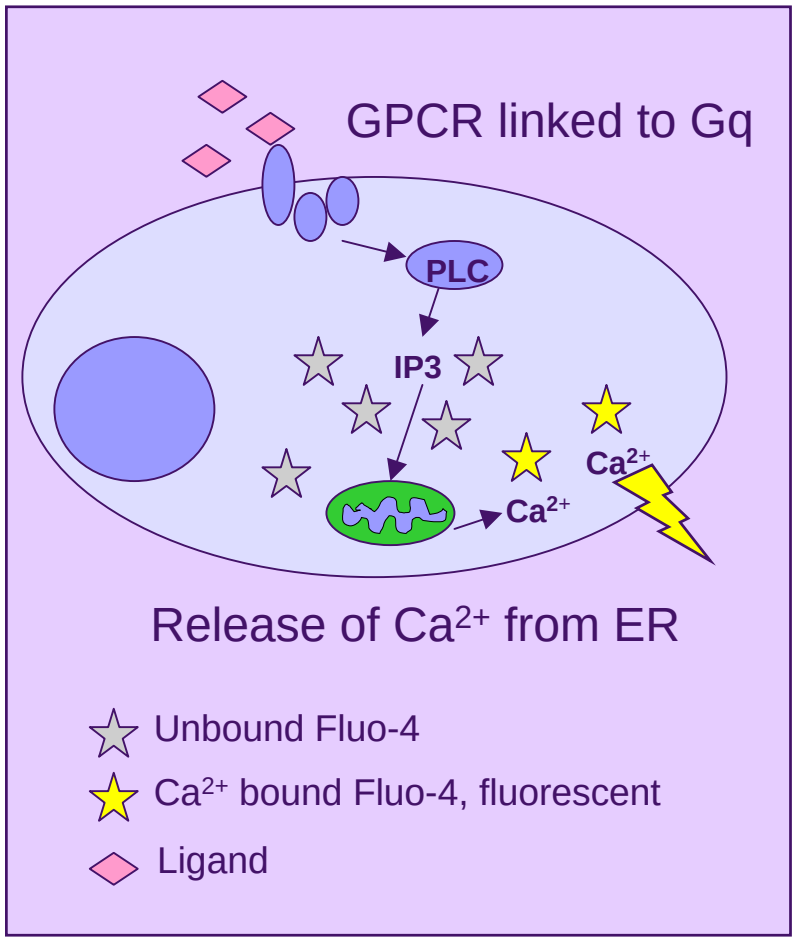
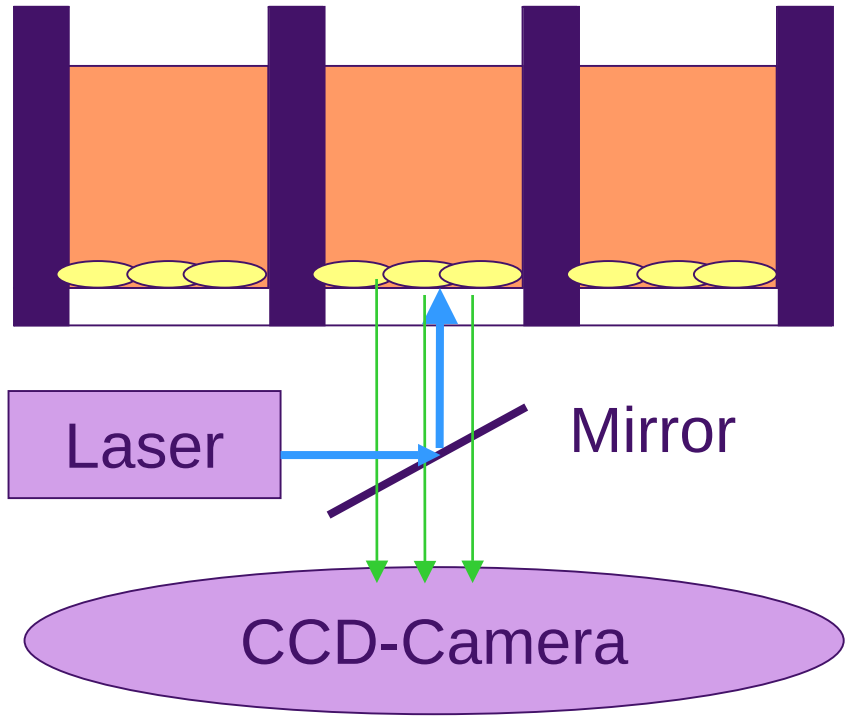
Fluorometric Imaging Plate Reader (FLIPR)



Black plate with clear bottom, monolayer of cells



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FLIPR

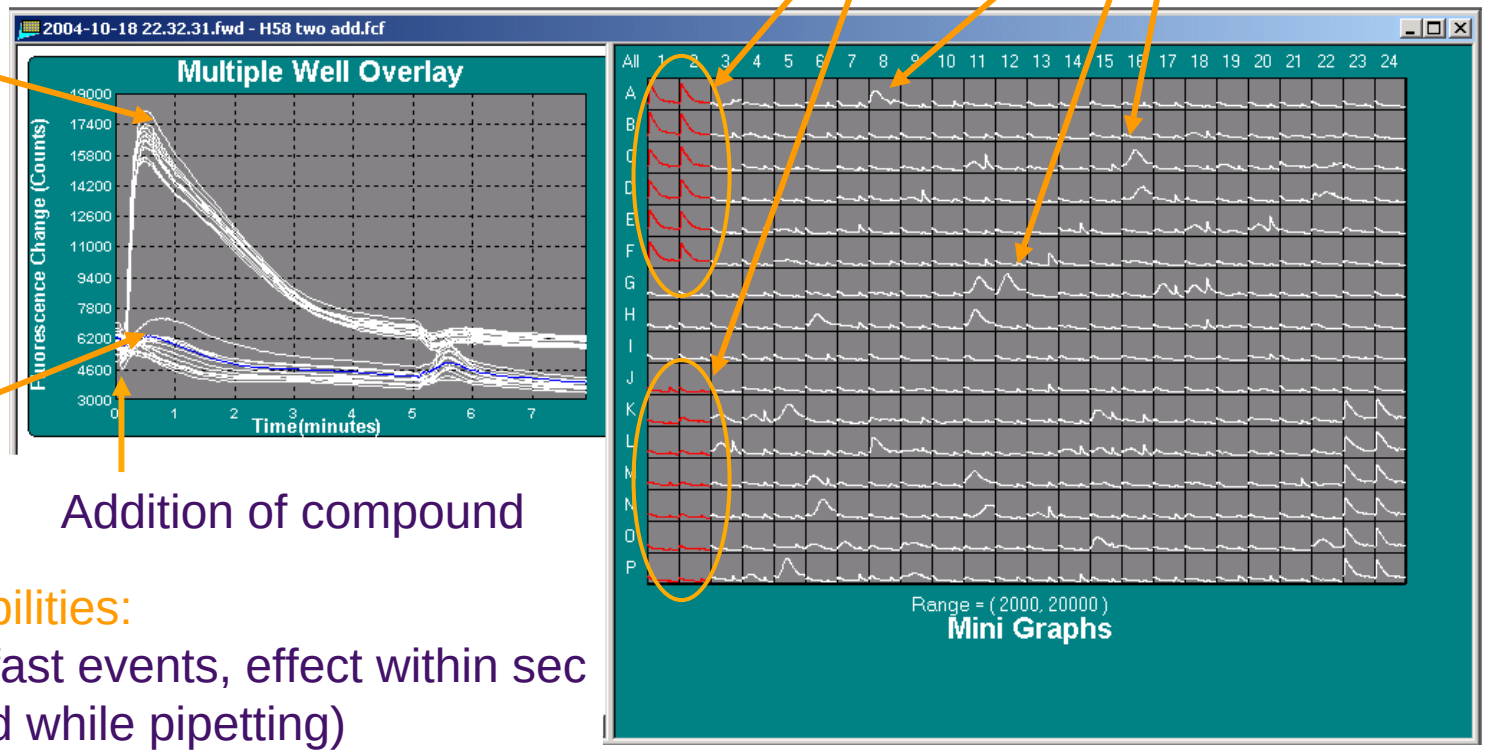
Example from agonist screen:

Wells with active compound

Control wells

100% effect

0% effect



Addition of compound

Screen possibilities:

- Assay very fast events, effect within sec (read while pipetting)
- Assay GPCR and ion channel activity

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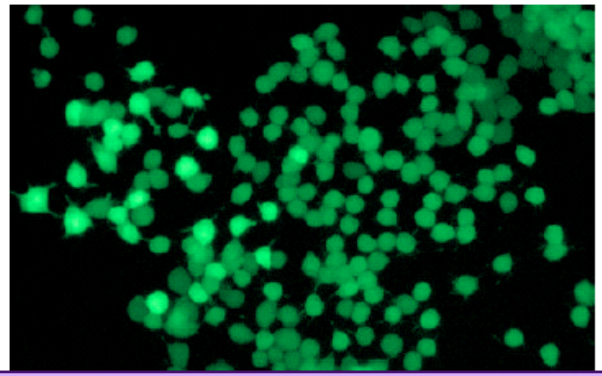
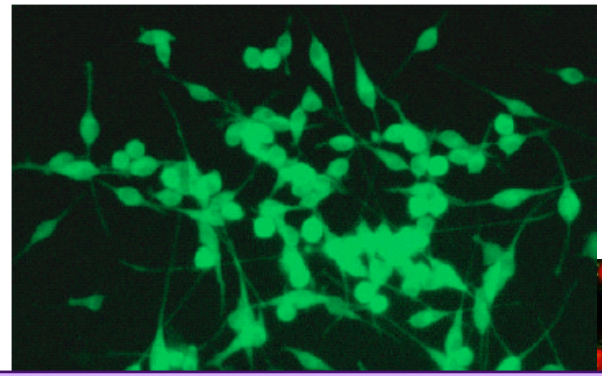
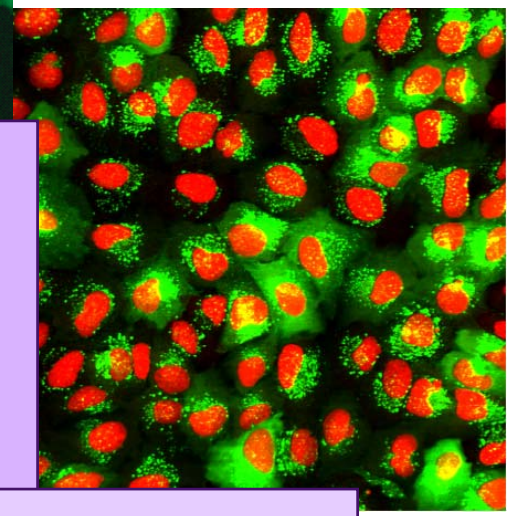
Cell imaging methods







High-content, high-throughput cell biology
- High-resolution microscopic imaging
- Microplate format & speed
- Real time
- Multi-parameter

- Transcription
- Trafficking
- Translocation
- Morphology

Exempel: Opera from Evotec
- Confocal microscope
- Laser exitation
- CCD cameras as detectors
- High speed data acquisition

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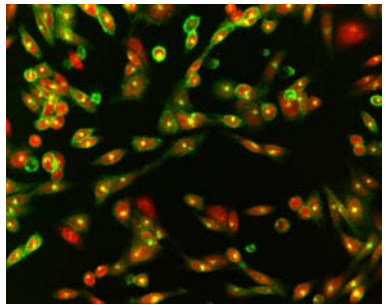
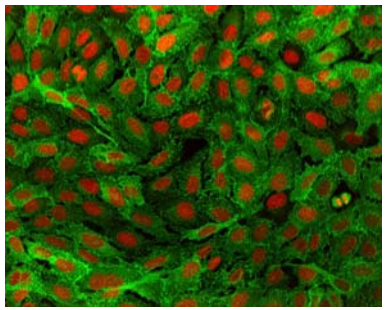
Image-based HTS: Opera Assays: Fluorescence Redistribution Assays

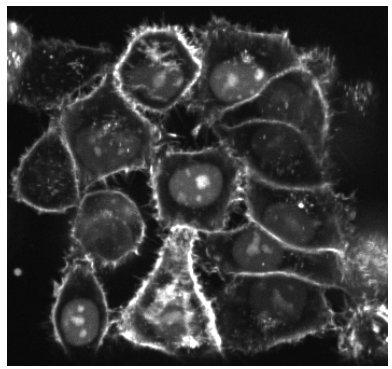
- GPCR Internalisation Assay



- ß-Arrestin Translocation Assay



- Signaling Molecule Recruitment Assay



From Evotec

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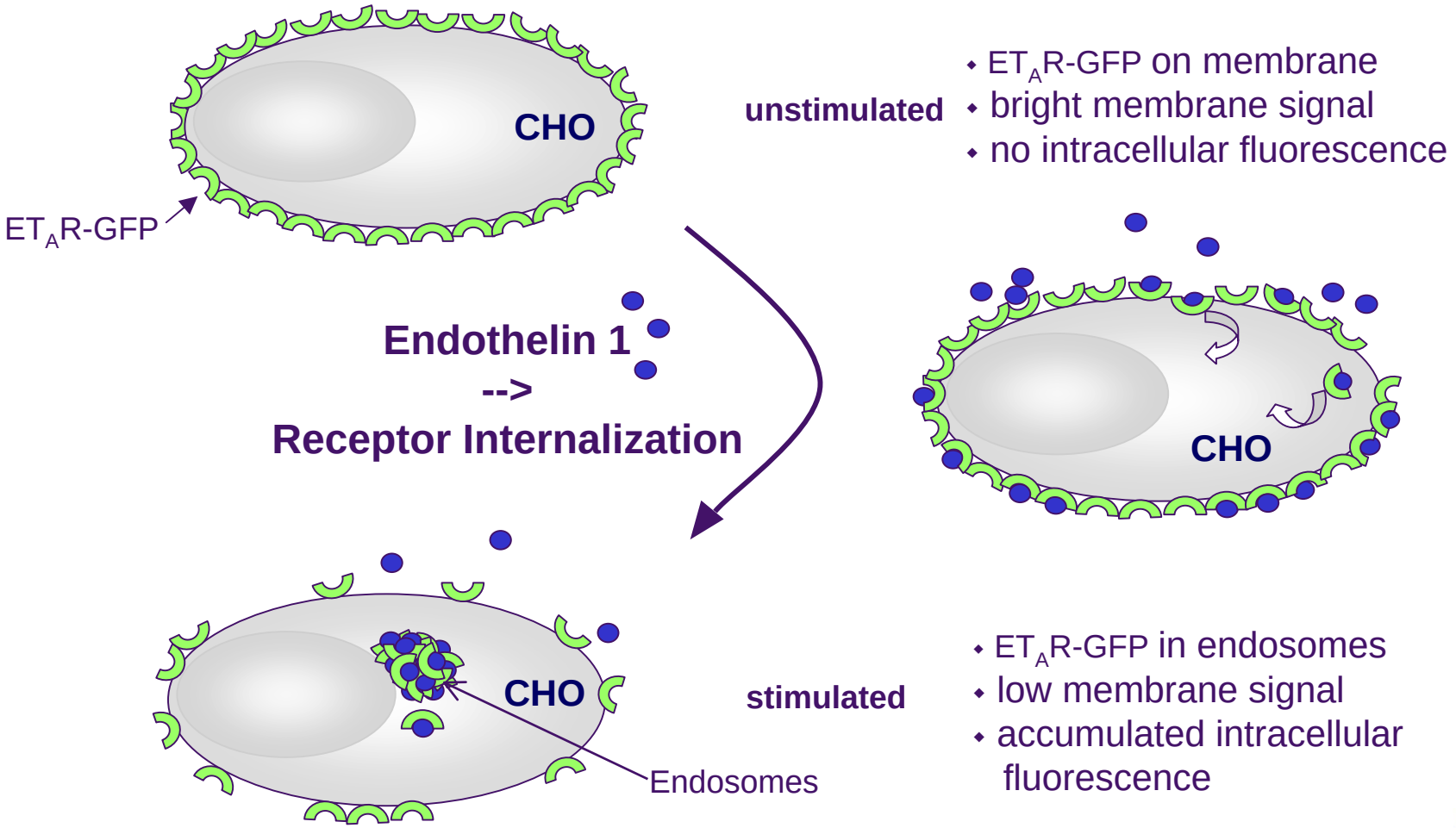
GPCR Internalization Assay:

Assay Principle using Endothelin A Receptor



From Evotec

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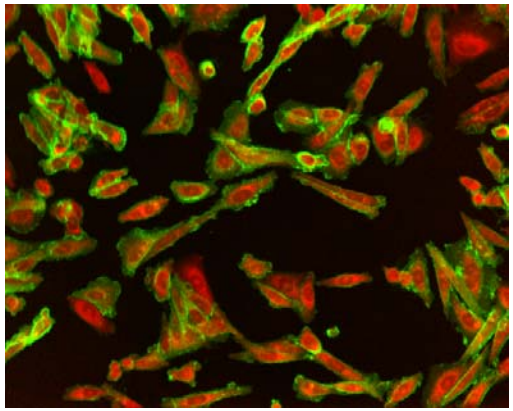
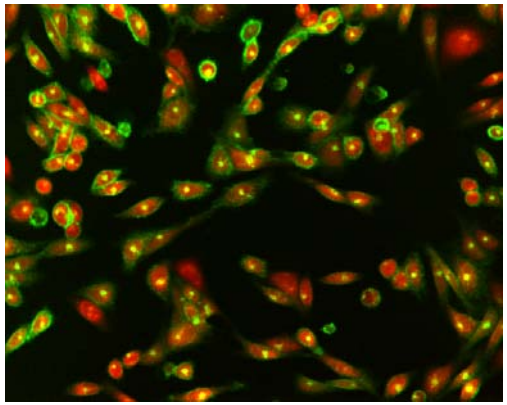
GPCR Internalization Assay:

ET_A-Receptor Internalisation Assay



Control Image: - Endothelin

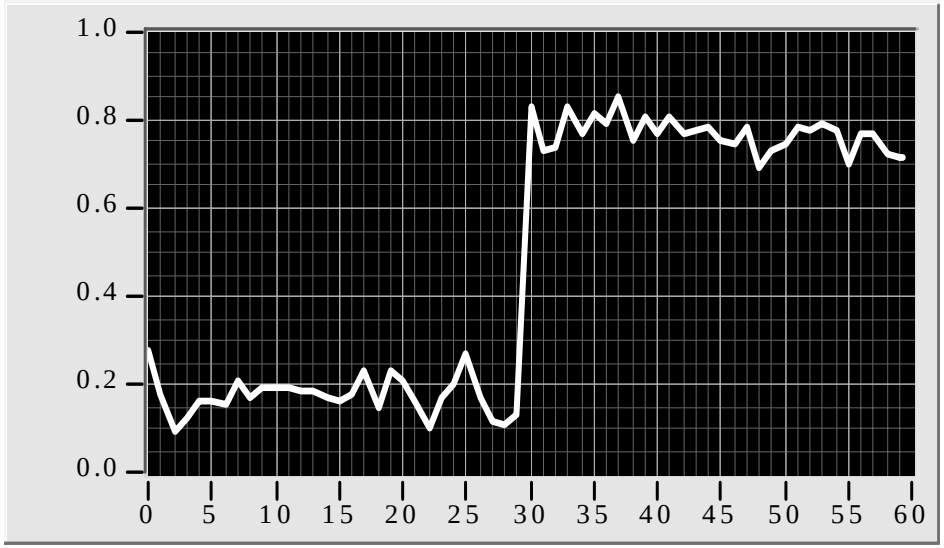


Activated Image: + Endothelin

From Evotec

Relative number of cells showing translocation

Z' = 0.58



- Endothelin control situation

+ Endothelin activated situation

measurements of individual wells

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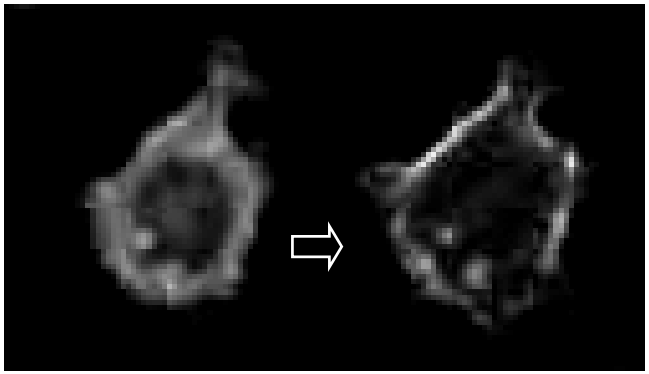
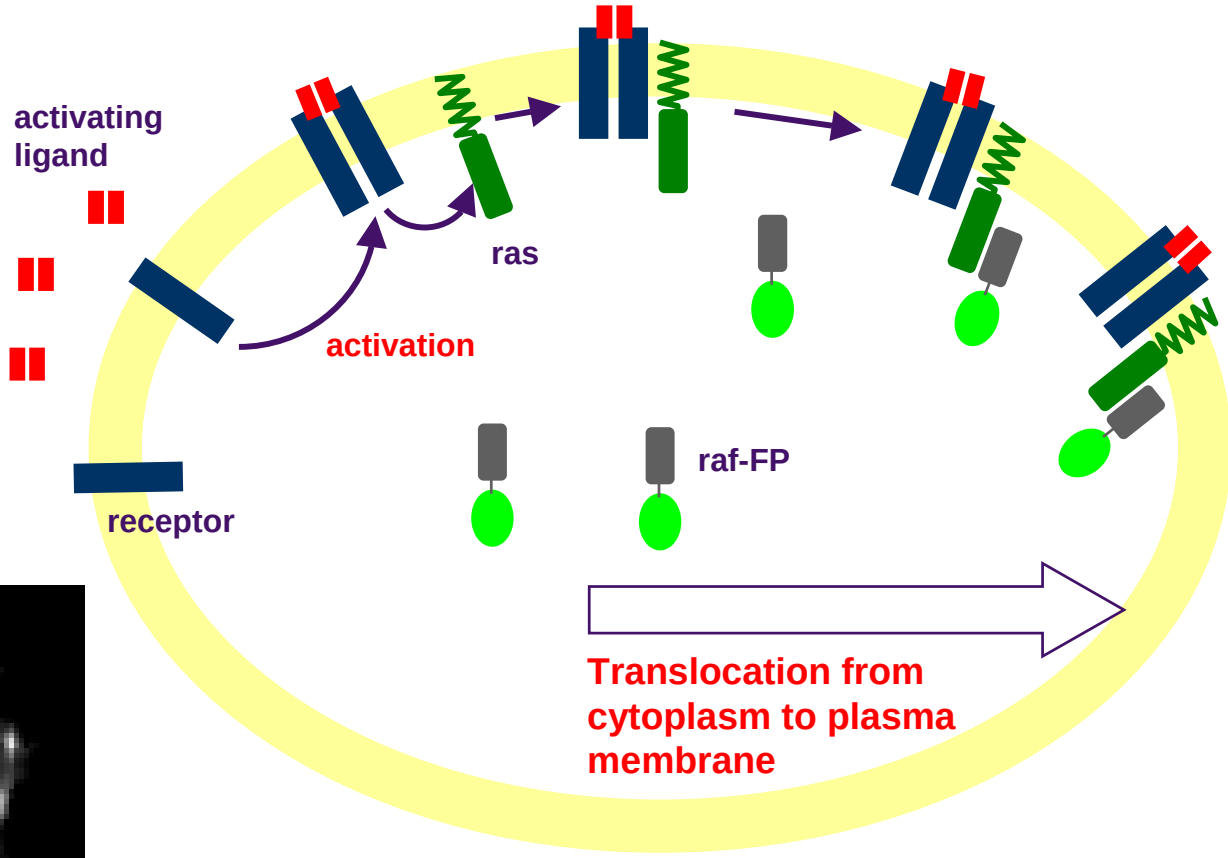
Signal Molecule Recruitment Assay:

Protein - Protein Interaction: Ras/Raf Assay

- MCF7 double transfectants
- Activation results in raf translocation to plasma membrane
- Stimulation with EGF





From Evotec

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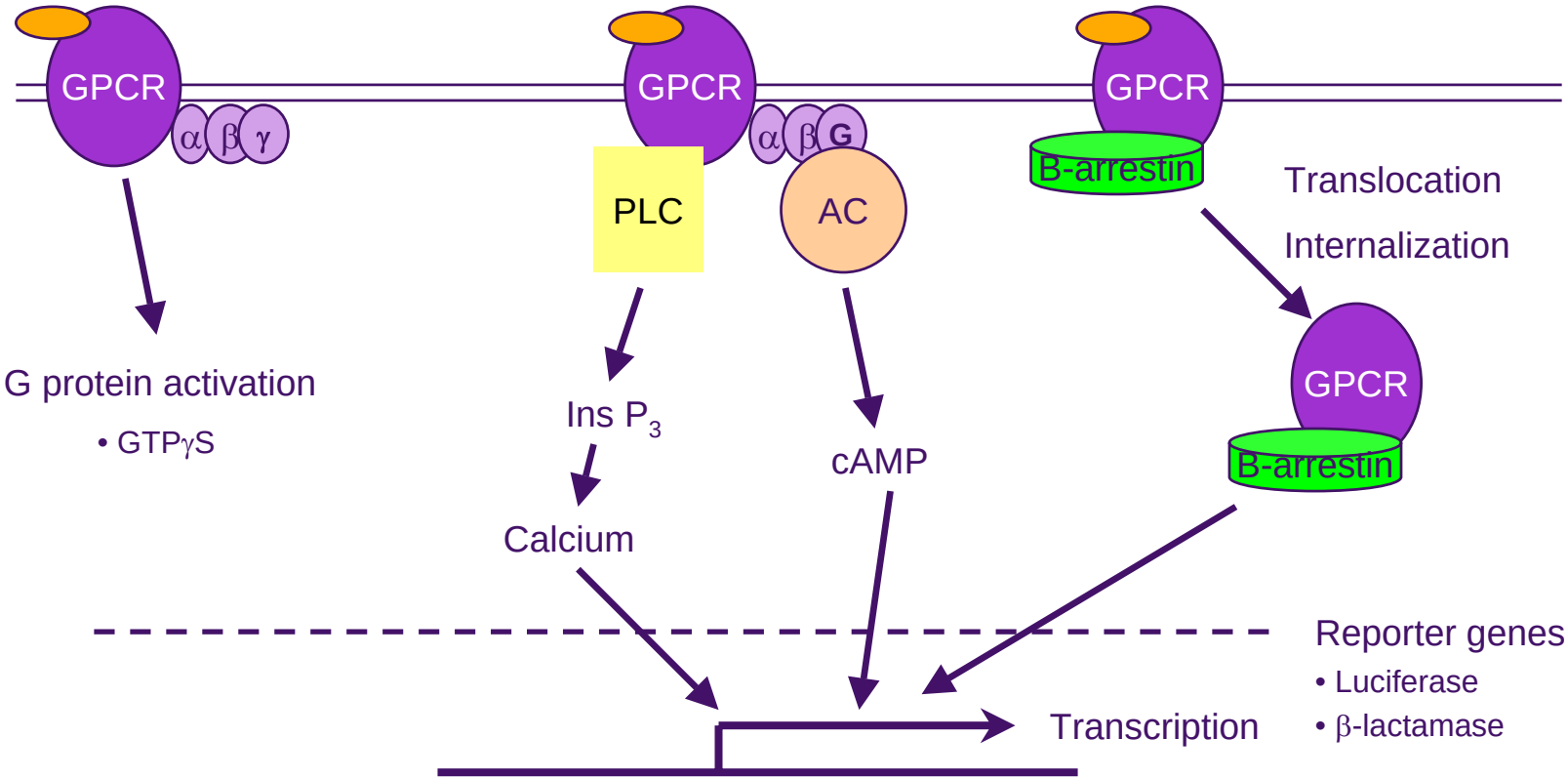
Current GPCR Screening Approaches

Receptor binding
- Radioligand filtration
- Radiolabeled SPA
- FP

Second messengers
- cAMP
- Ca++
- IP3
- MAPK

Protein redistribution
- Translocation
- Receptor Internalization



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Outline

- Drug Discovery
- What is screening? Background & Context
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- The automation of HTS
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- The future

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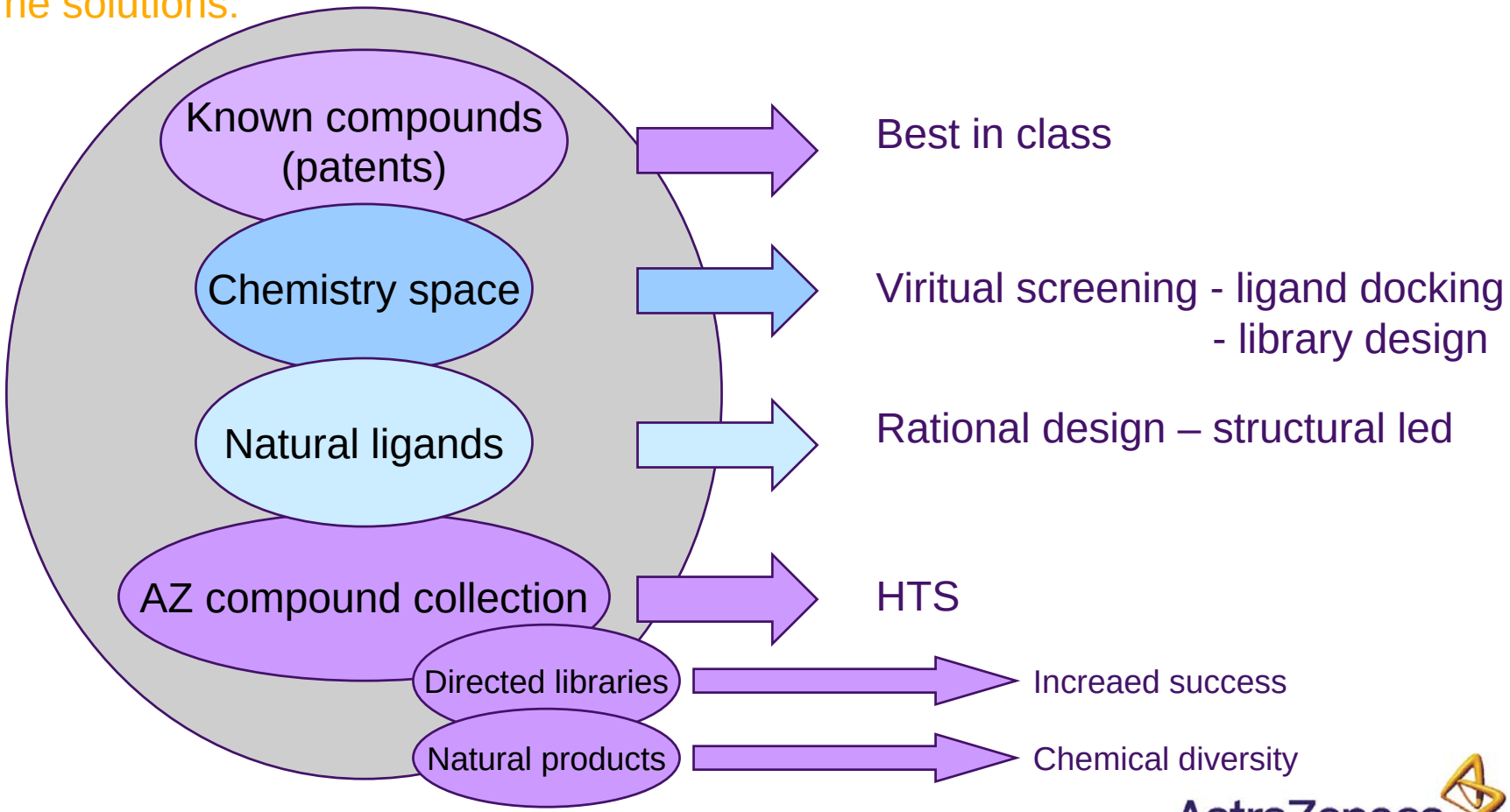
Approaches to finding small molecule drugs

The challange: an interesting target is identified, but how do we find compounds that interact with the target

The solutions:



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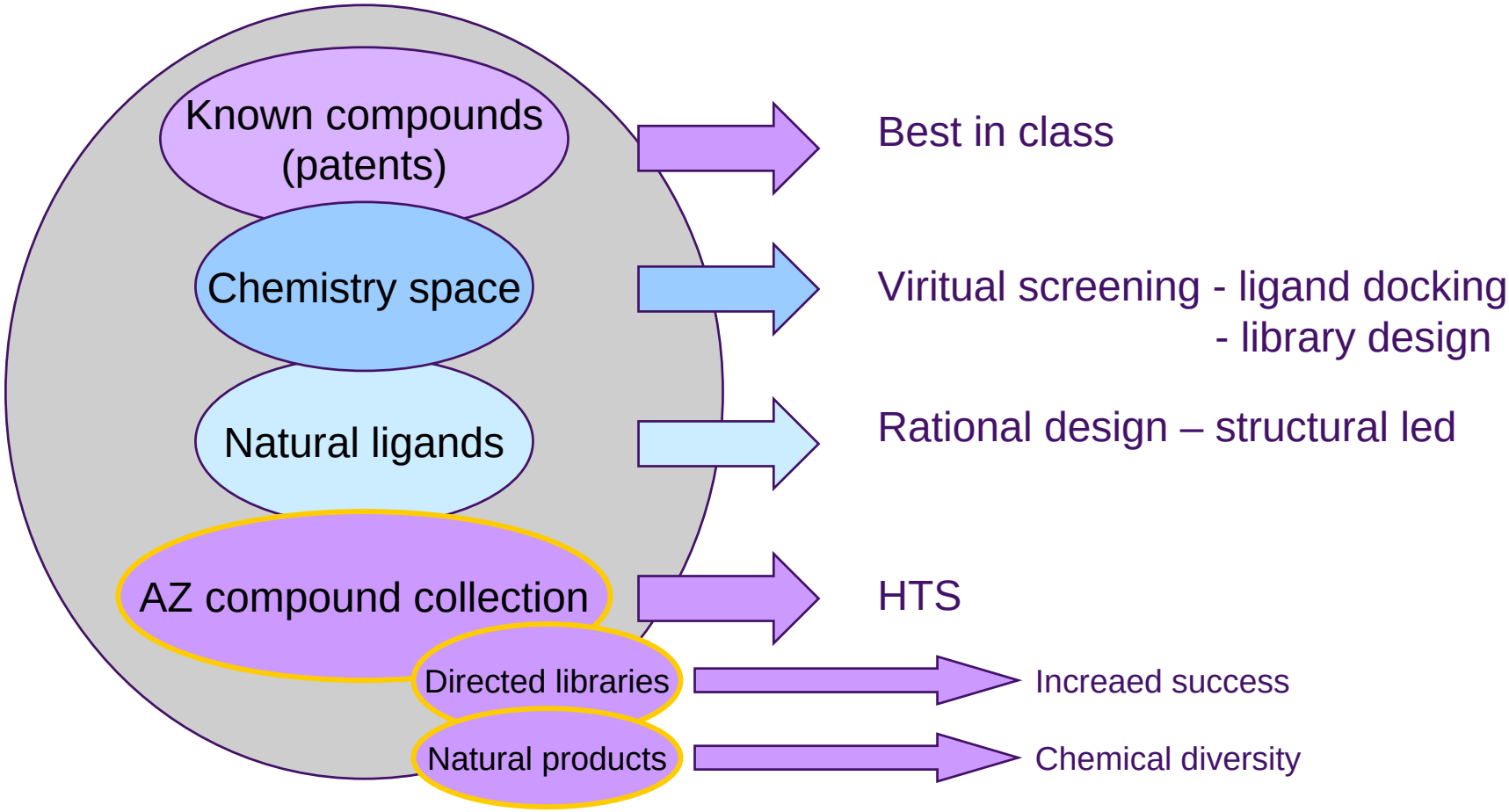
The compound library



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Compound library…

- **Logistics**
  - storage conditions
  - high throughput, high precision handling
  - tight integration with screening
- **Chemical quality**
  - library “richness”
- **Physical quality**
  - purity, stability
- **Data**
  - mapping quality parameters
  - Using data on today’s library to improve tomorrows

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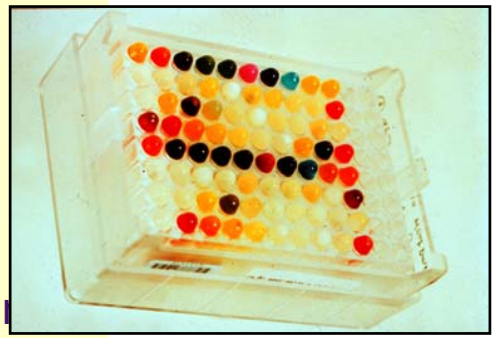
An evolution of chemical thinking in drug discovery

- Size
  - Smash & grab philosophy of early HTS
- Diversity
- Clean out the trash
  - Toxics, reactives, non-drugs

- Drug-like
- Lead-like
- Hit-like
- Quality, Quality, Quality
- Logistics
  - A must for global companies and cross site worki



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Medicinal Chemical Quality:

- **Drug-like properties**
  - Lipinski's rules
- **Lead-like properties**
  - allow for lead optimisation
  - smaller and more hydrophilic
- **Undesirable properties**
  - reactives
  - toxics

Lipinski's "rule of 5"

H-donor <5
Molecular weight <500
LogP (hydrophobicity) <5
Sum of N + O <10

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Physical quality

- Routine chemical QC of hits revealed using LCMS
  - Degradation (DMSO thirst for water)
  - Precipitation ($\rightarrow$ wrong concentration)
  - Data errors (structure & id)
- We have now a NEW compound collection
  - Analysis of incoming compounds
  - Better automation
  - Better storage
  - Better software

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Stores for compound solutions in tubes and plates

- High density, automated storage
  - RTS Thurnall
- Controlled humidity and temperature
  - 10% RH, N2, Cooled
- 3M tubes, 16K plates
- Powerful software
  - Robot control
  - Inventory tracking
  - Structure database
  - Registration & ordering

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Compound handling is critical with big HTS collections: ”Turbo” sample picking…

- ”Pick-&-place” automation
  - Flex-picker robots (ABB)
- Highest ever sample picking throughput
  - Ca. 35K / day
- Reformating from storage tubes to desired lab-ware (plates)
  - 96-384-1536
- Nano-litre liquid-handling

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Natural product screening…
… history or still relevant?

… Chemistry too complex or over-active?

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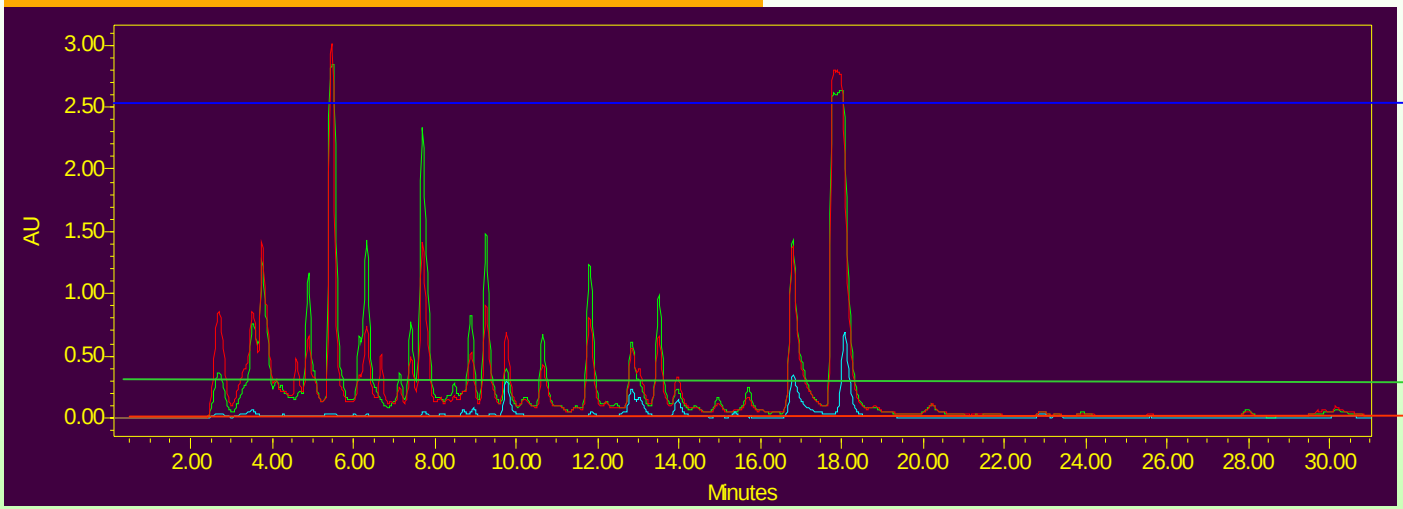
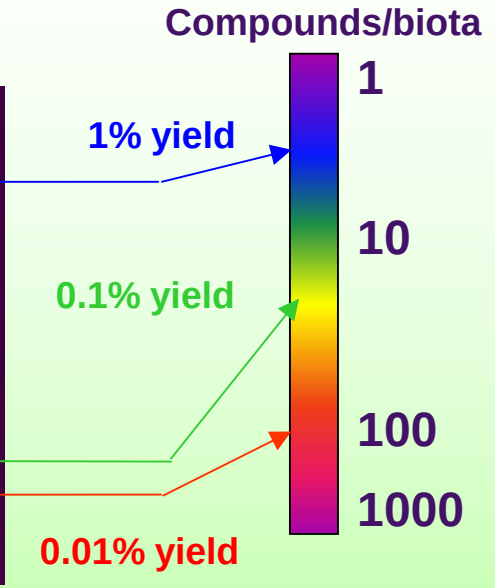
There are certainly many compounds in nature…

Typical Extract Chromatogram





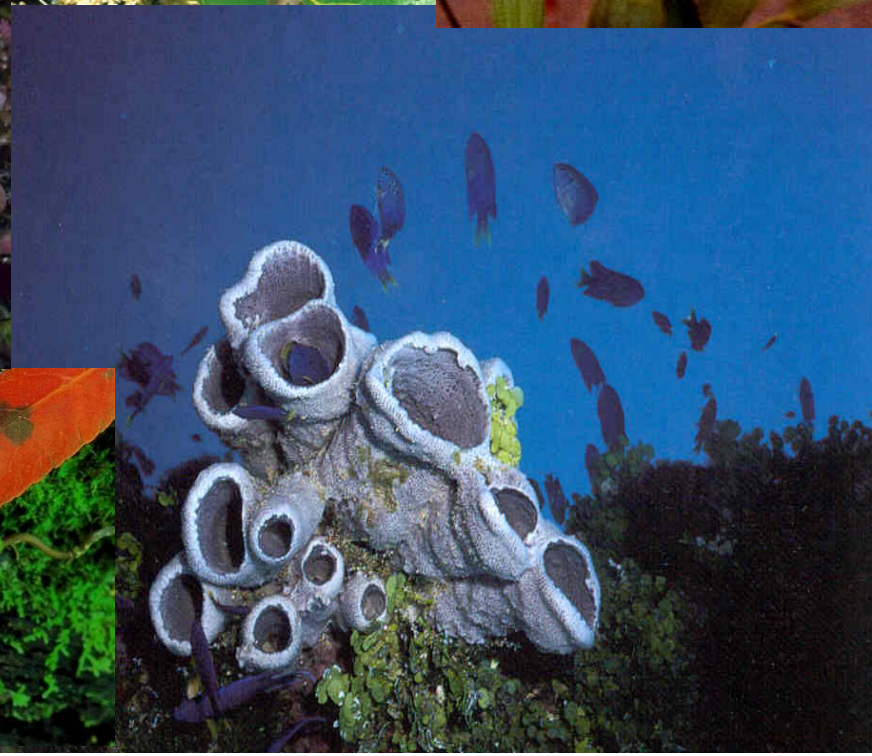
Potential accessible chemical diversity (compounds in yield >0.01%):
5 - 7 million compounds in a biota library of 30,000 extracts

Enormous diversity but…
- many cpds non-drug-like & chemistry-unfriendly
- interfering cpds common
- isolated cpds often inactive in secondary assays
- lack of recent successes?

And natural products have delivered drugs…

- Anti-microbial
  - Penicillin, tetracycline…
- Anti-viral
  - Vincristine
- Anti-cancer
  - Taxol
- Immuno-supressant
  - Cyclosporin
- Lipid-lowering
  - Statin

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How “High” is HTS?

- Plate standard is 384 or 1536 well
- Assay controls are included to track screen quality
- Each robot can process >100 plates/ 24 h
- Multiply by the number of robots

>150,000 data points per day!

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Bob

- Acquired 1999
- Orca arm (3m)
- Carousel
- Multimek
- Microbeta (2)
- Multidrop (3)
- Lid handler
- Plate sealer
- Shaker
- CO2 Incubator

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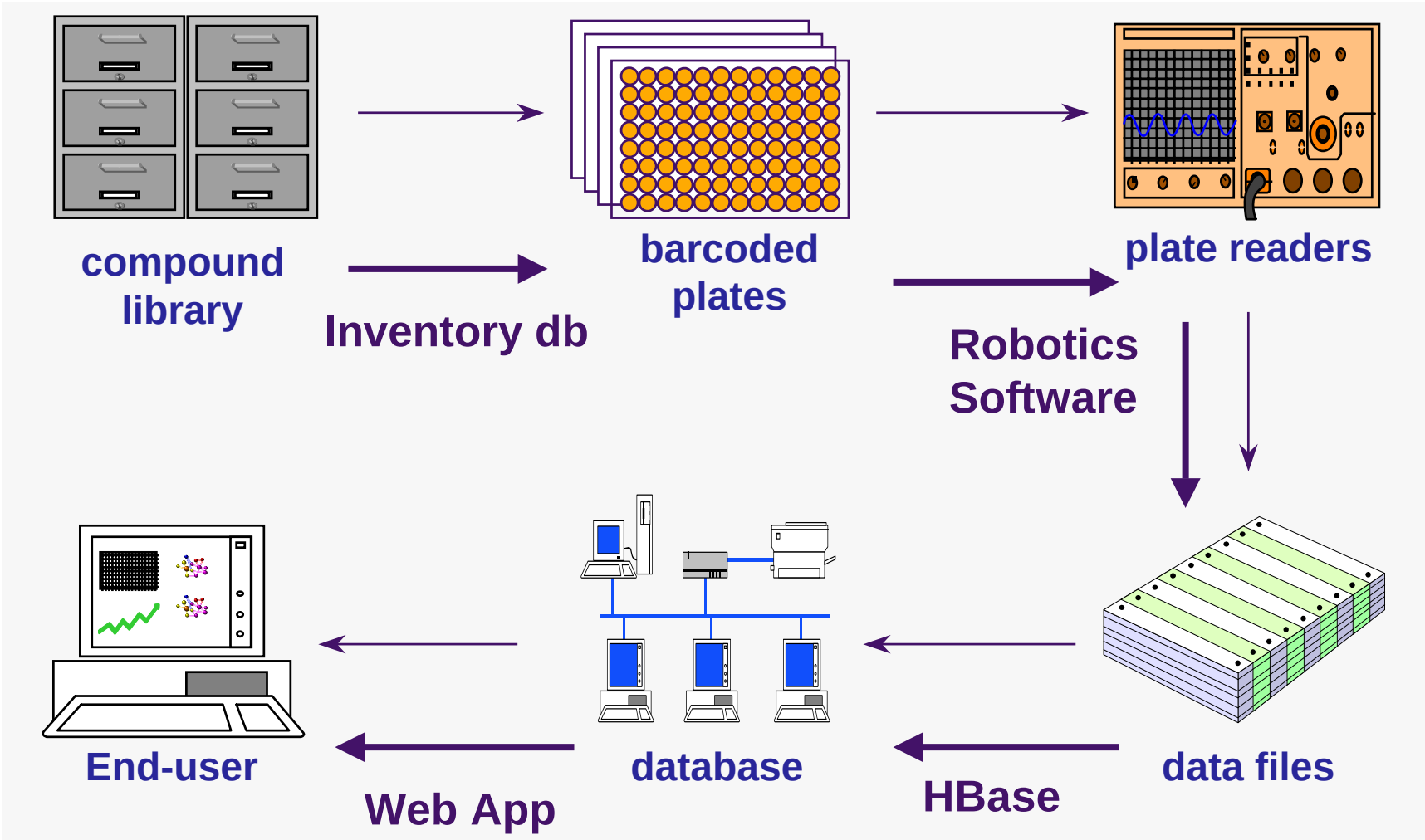
HTS Data Flow



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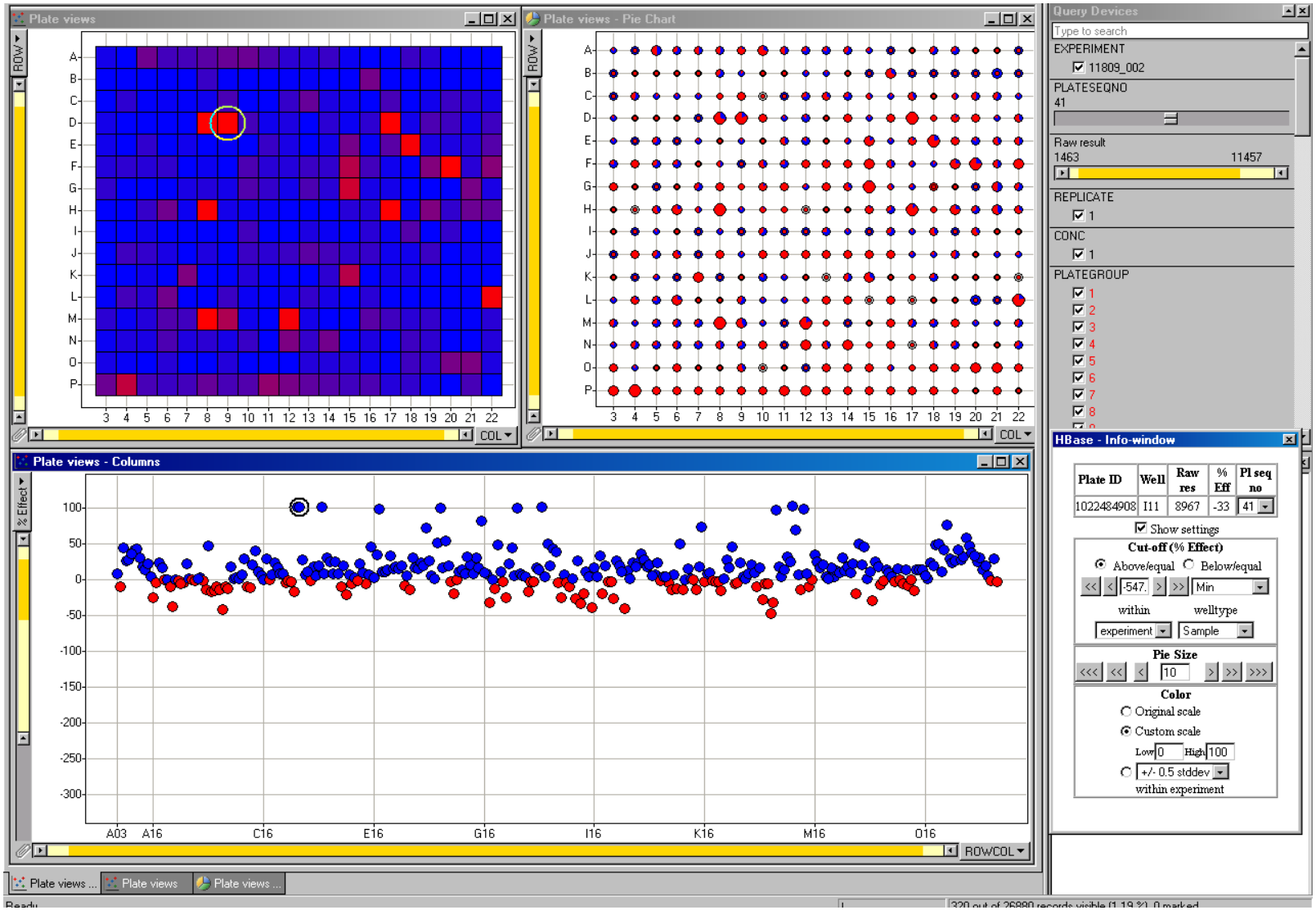
Powerful research data systems



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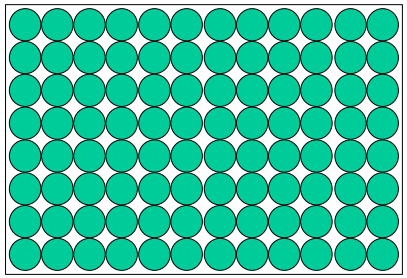
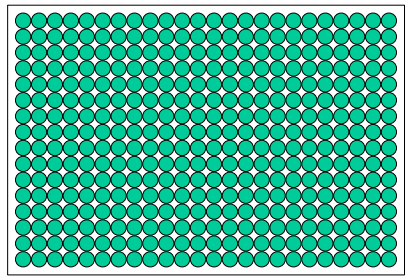
THE FUTURE:
new screening technology

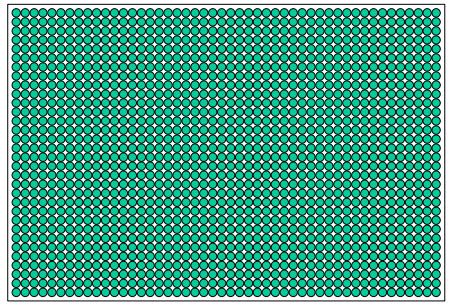


96 wells
(multi-μl)



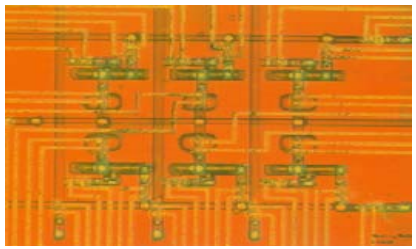
384
(μl)

evolutionary



1536
(nl)

revolutionary



Microfluidic chips (*pl*)
10^6 assays/day?

High density plates
10^5 assays/day?

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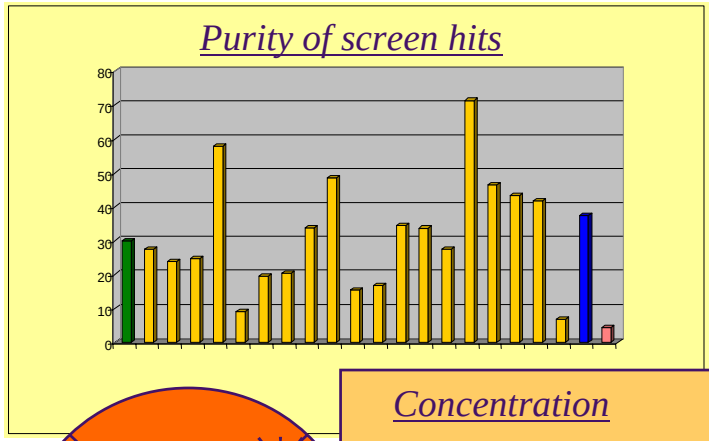
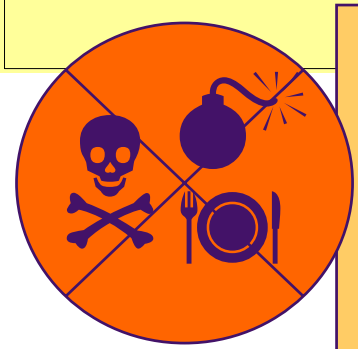
“Chemical failure” of HTS

- Poor physical quality
  - Structure integrity
  - Impurity
  - Solubility
  - Metabolic stability
- Poor med chem quality
  - Bad chemistry
  - Difficult chemistry
  - Wrong pharmacology





Concentration

		“10mM” stock real conc (mM)
AR-XXXXXXXX clogP 3.5	HTS soln	1.5
	Fresh	10
AR-YYYYYYYY clogP 4.1	HTS soln	0.6
	Fresh	10
AR-ZZZZZZZZ clog P 2.9	HTS soln	5
	Fresh	10

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“Biological failure” of HTS

Target failure

- Disease relevance
  - = target validation
- Target complexity
  - Multi-target interactions
  - Downstream consequences
  - Metabolic ”big picture”

Assay failure

- Practicalities (capacity, speed, reliability)
- Physiological realism
- Disease relevance
- Cellular processes in real time
- Tissue & organ models

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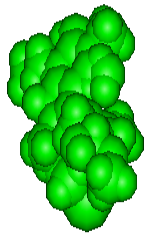
We can apply HTS in smarter ways…
…converging the empirical with the rational

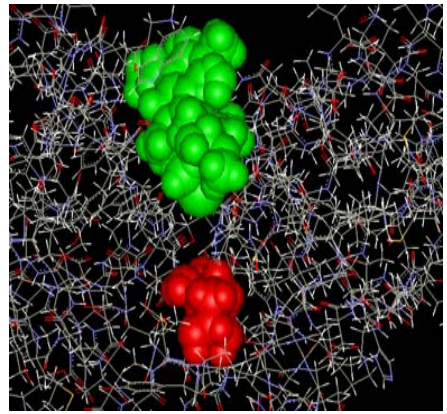
- Knowledge of protein structure gives enormous advantage
- Computational chemistry makes the journey easier

➔

- Rapid hit evaluation
- Hit rescue
- Hit expansion
- Virtual screening



HTS Hit



Protein structure

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…offers new ways to use HTS…

- Use capacity more imaginatively
  - selected compound subsets
  - target families in parallell
- Combine with rational platforms
  - Protein structure & computational chemistry
  - Virtual Screening + Real Screening
- Generate more systematic data
  - Target identification & validation opportunities
  - Compound sets to exploit clinical evidence?
  - Generate data for better predicitive models
  - Map biology space?

… and diversify discovery strategies

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Maybe there are yet many more targets than we see today…

- Our view of mono-molecular targets in isolation is not physiologically realistic
  - In healthy or diseased systems
- Target interactions, networks and metabolic flows must offer more permutations and opportunities for beneficial intervention
  - Assay construction is very significant

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