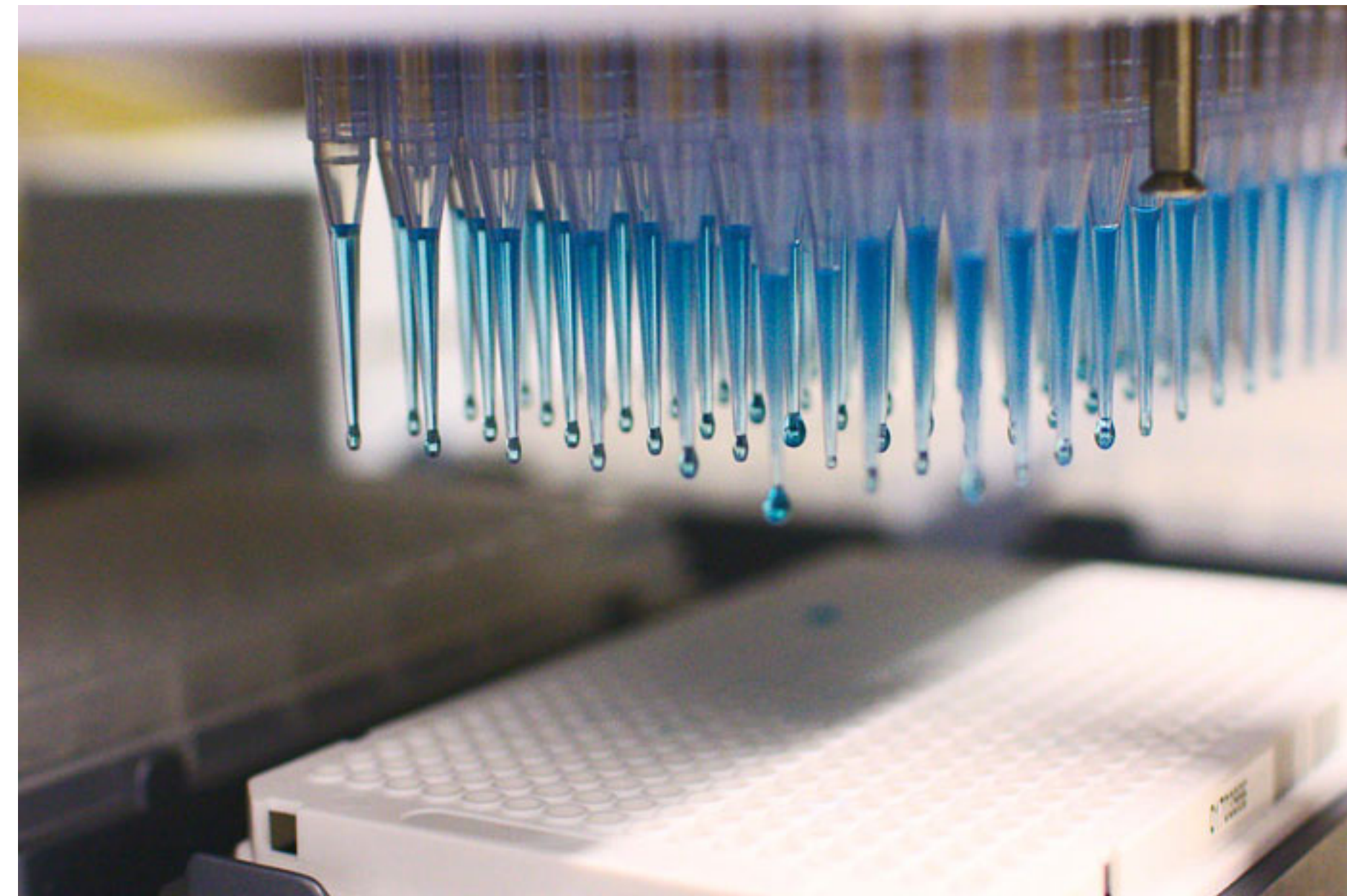


# *High throughput screening (HTS) strategies in drug discovery*



**Blair Dong**

MacMillan Research Group

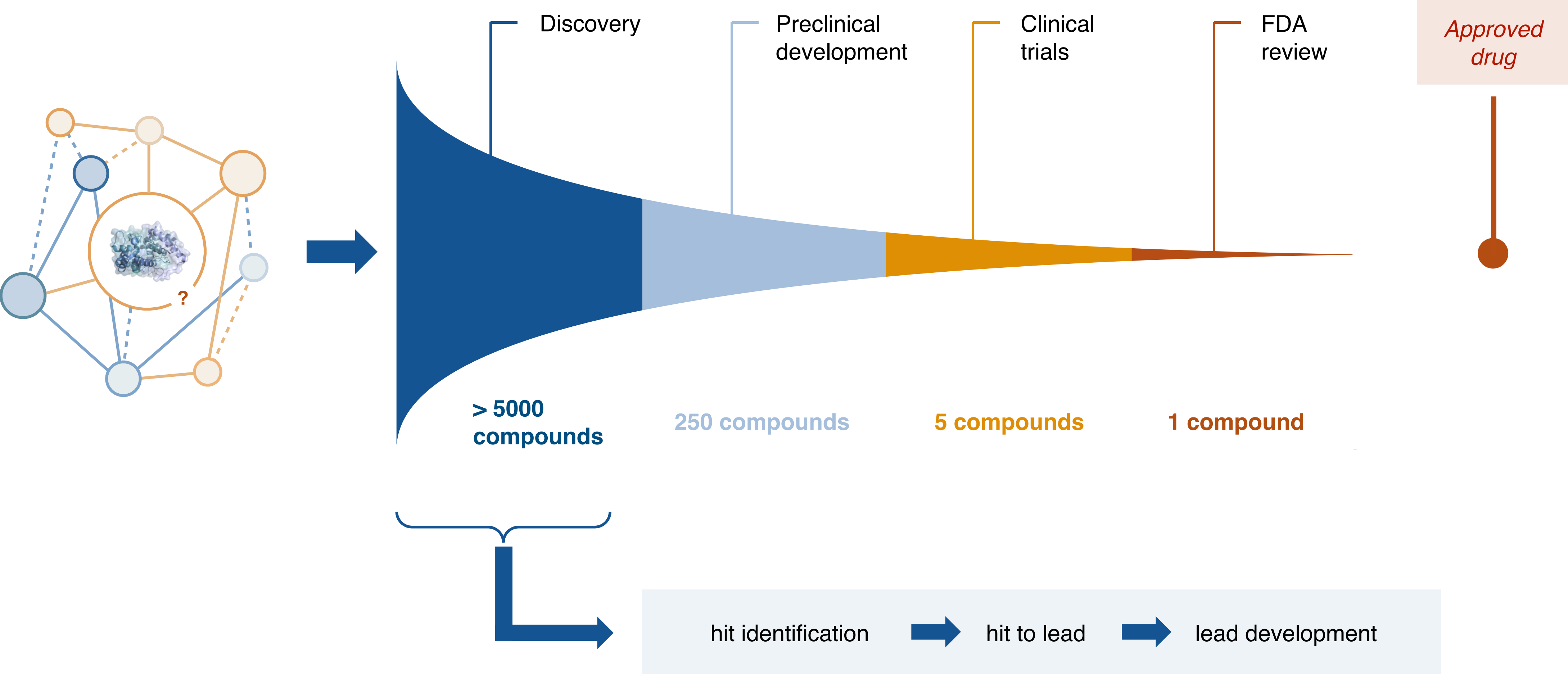
Literature Talk

Sep 13, 2024

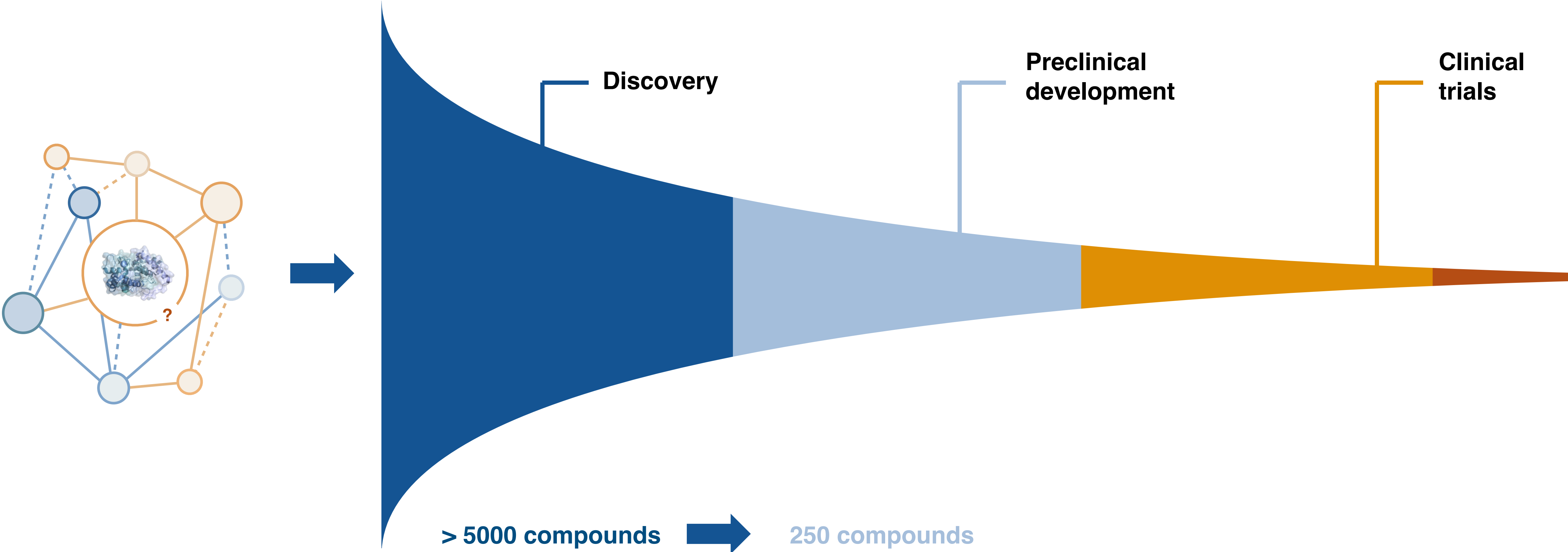
# *Outline*

- Origin and early evolution of HTS
- Strategies for small molecule HTS
  - Compound library types
  - Screening methods
  - Case study
- HTS for antibody and RNA based therapies

# The drug discovery pipeline



# The drug discovery pipeline



***How do we screen through so many compounds?***

# Origin of HTS

## Natural products screening



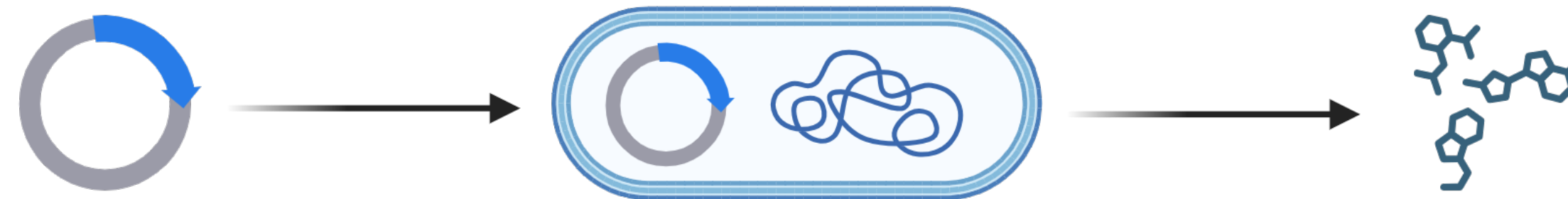
**Pfizer Nagoya, Japan  
1984**

### Streptomycete

*-source of a large number of natural products*



### Recombinant DNA technology for natural product discovery



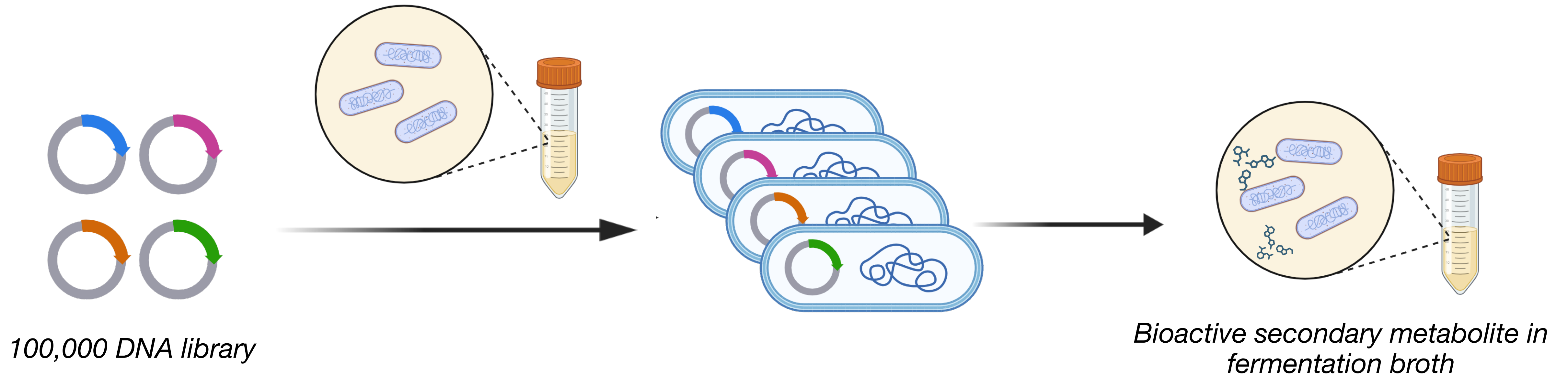
*Recombinant plasmid*

*Transformed bacteria*

*Novel natural products*

# Origin of HTS

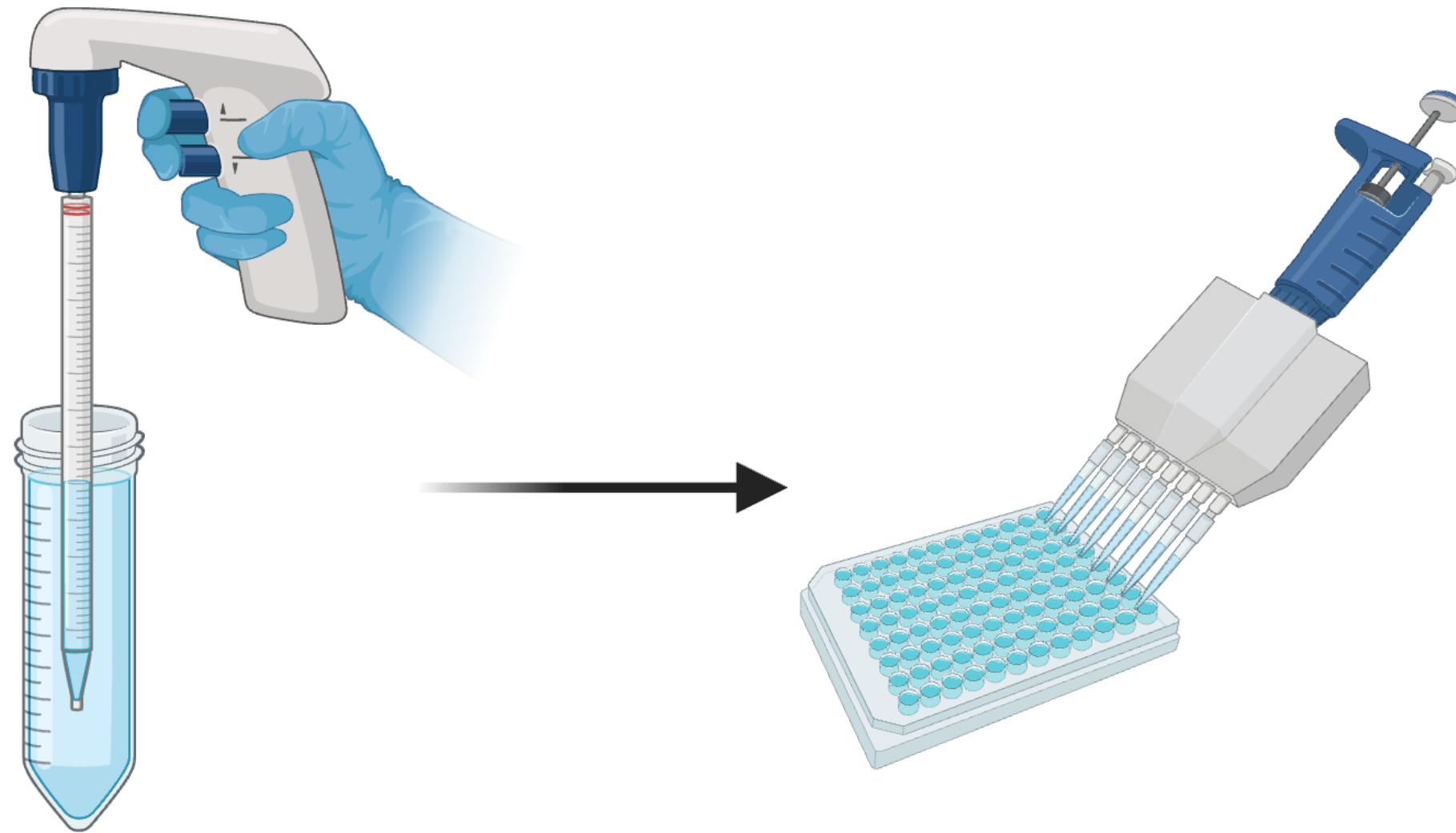
## Natural products screening



**Problem: manual fermentation process will take too long to screen for their DNA library**

# Origin of HTS

*Natural products screening*



*10 mL fermentation tube*

*96 well plate*

## **Advantages**

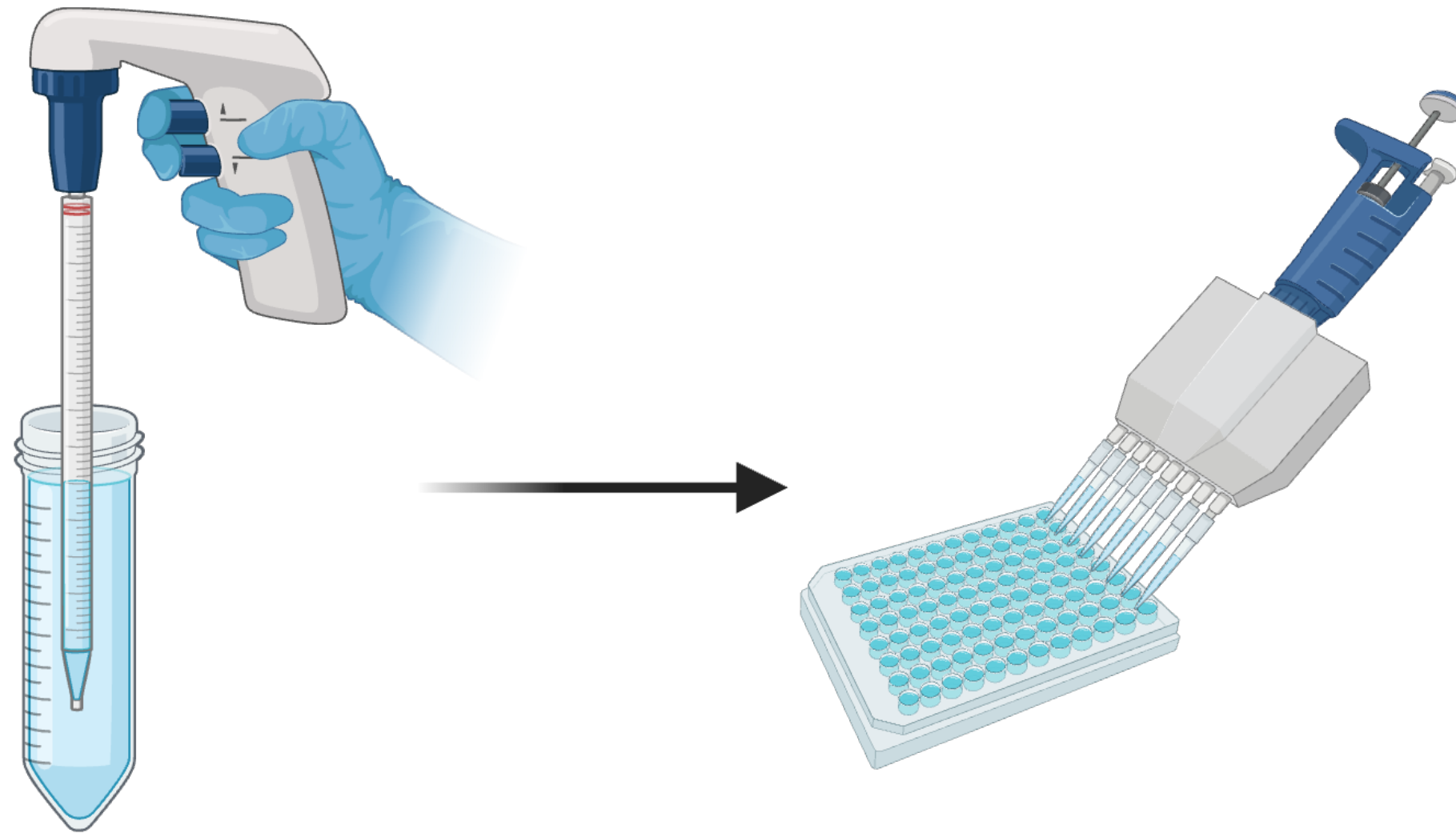
*Accessing 96 samples simultaneously*

*Compatible with multichannel pipettes*

*Increasing capacity by reducing incubation space*

## *Origin of HTS*

*Natural products screening*



**200 samples / week**

**10,000 samples / week**

***Started the automation project in 1984 and fully implemented in 1990 in the Nagoya site***

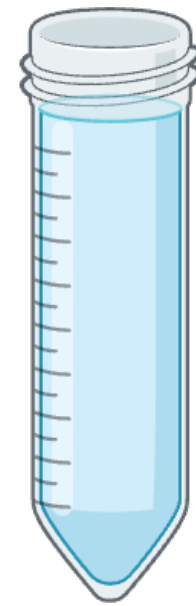
***Can we adapt it to screen synthetic compounds?***



# Origin of HTS

## Screening synthetic compounds

### Natural products screening

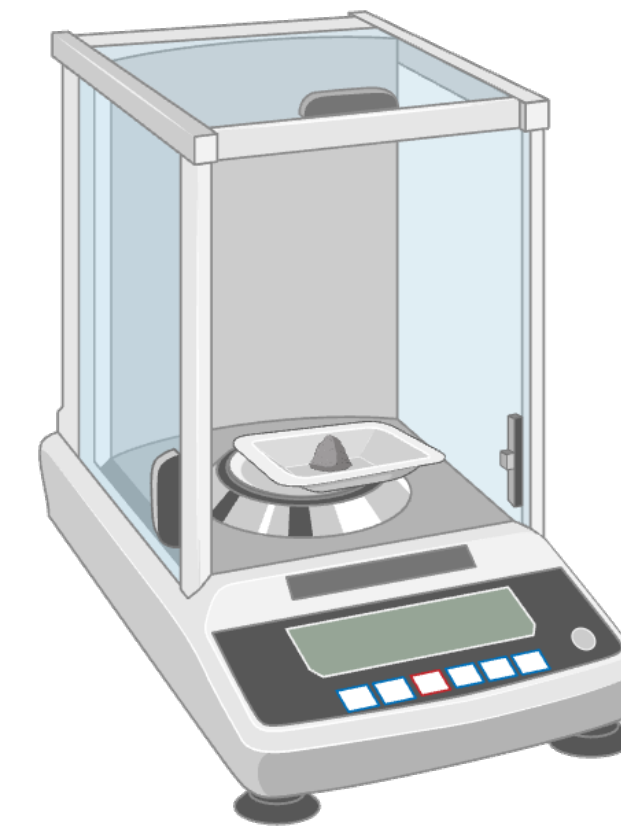


Compounds in  
fermentation broth

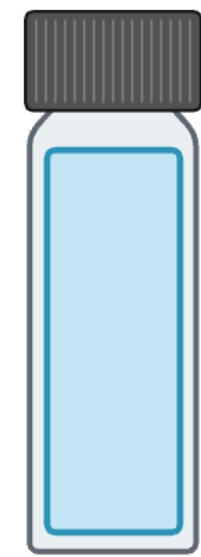
### Synthetic compounds screening



Compounds stored  
as solids



Weigh out 5-10 mg



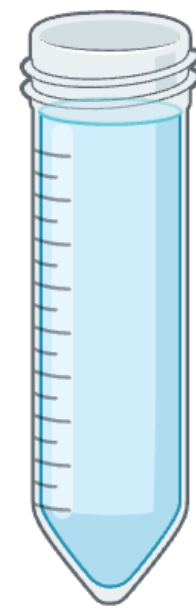
Dissolve in  
solvent cocktail

**Accessing compounds in solution would be the rate-limiting step**

# Origin of HTS

## Screening synthetic compounds

### Natural products screening

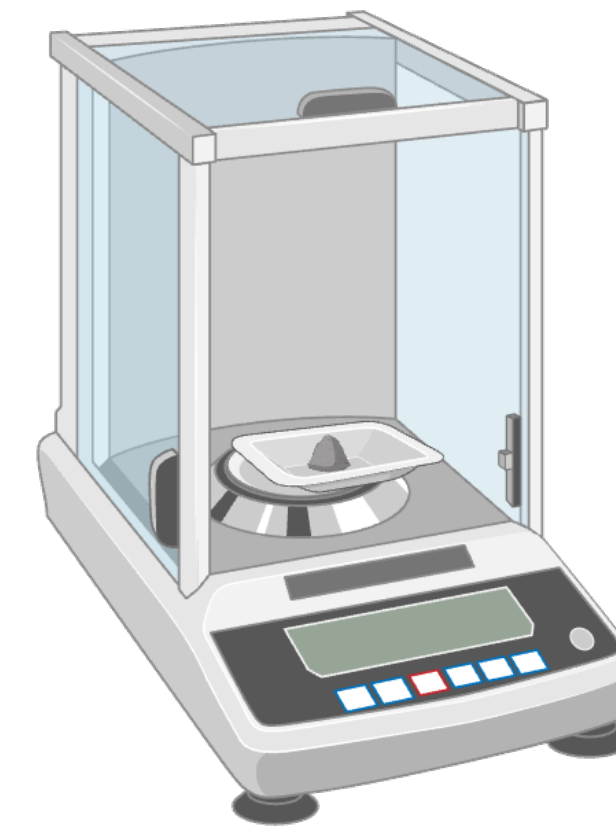


Compounds in  
fermentation broth

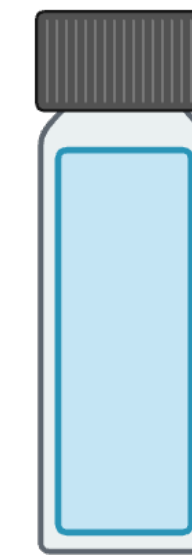
### Synthetic compounds screening



Compounds stored  
as solids



Weigh out 5-10 mg



Dissolve in  
solvent cocktail

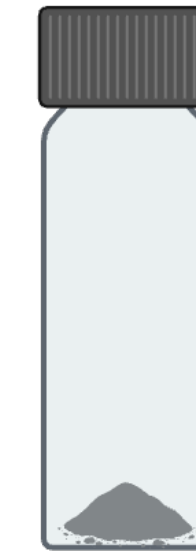
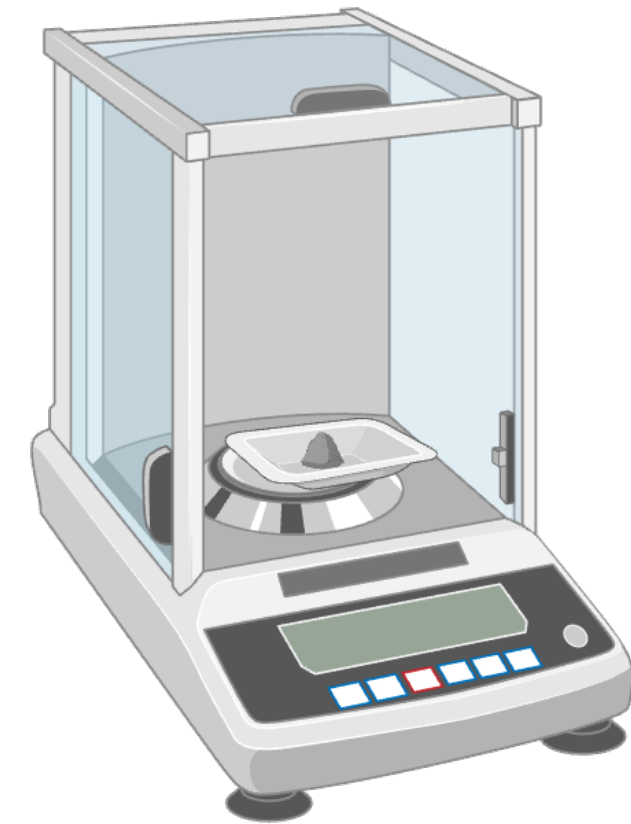
- variable concentrations

**Exact compound concentrations don't matter, as long as they pass the minimum threshold**

# Origin of HTS

## Screening synthetic compounds

**Solution: flicking**



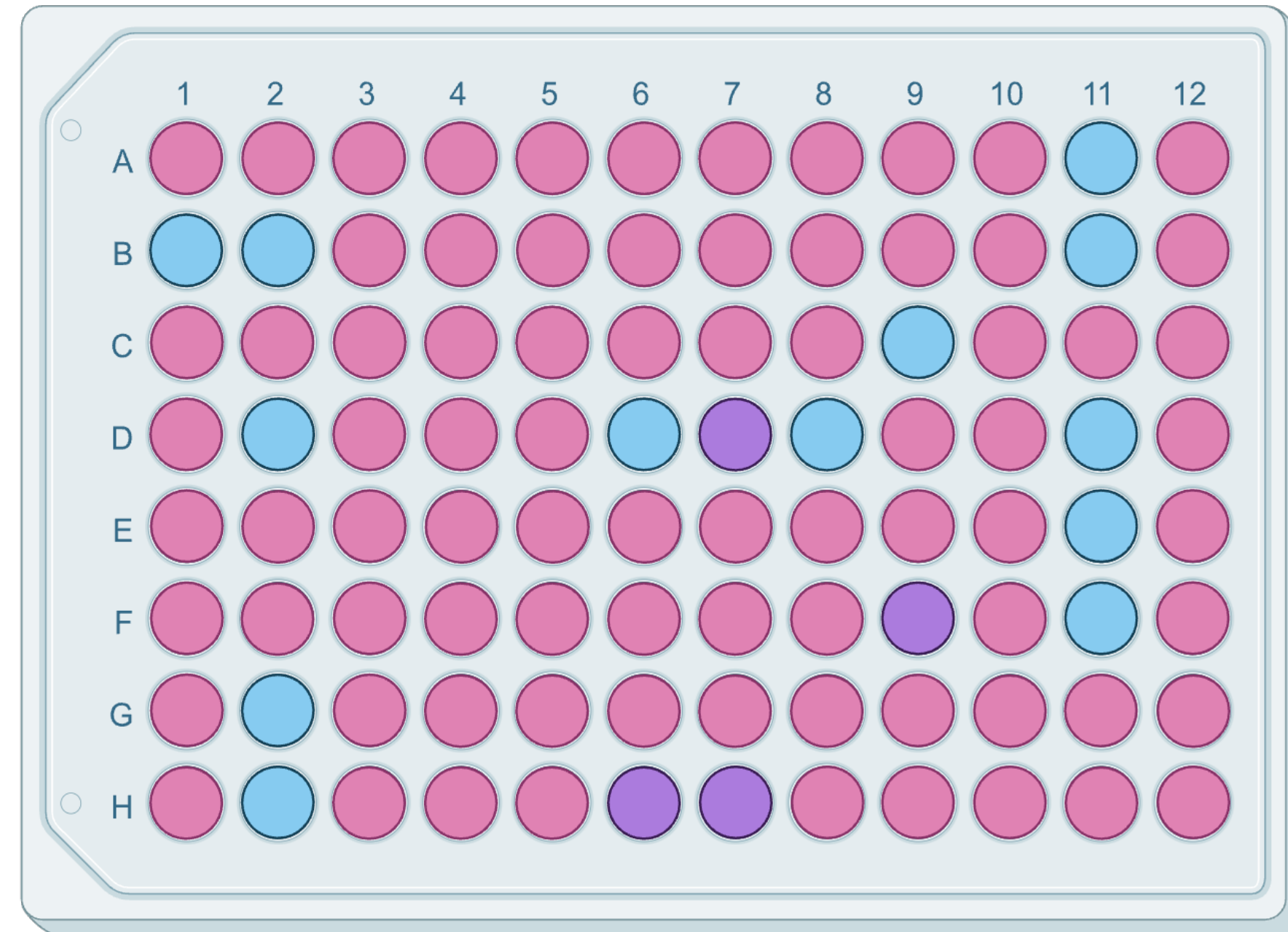
*Compounds stored  
as solids*

*Weigh out 5-10 mg*

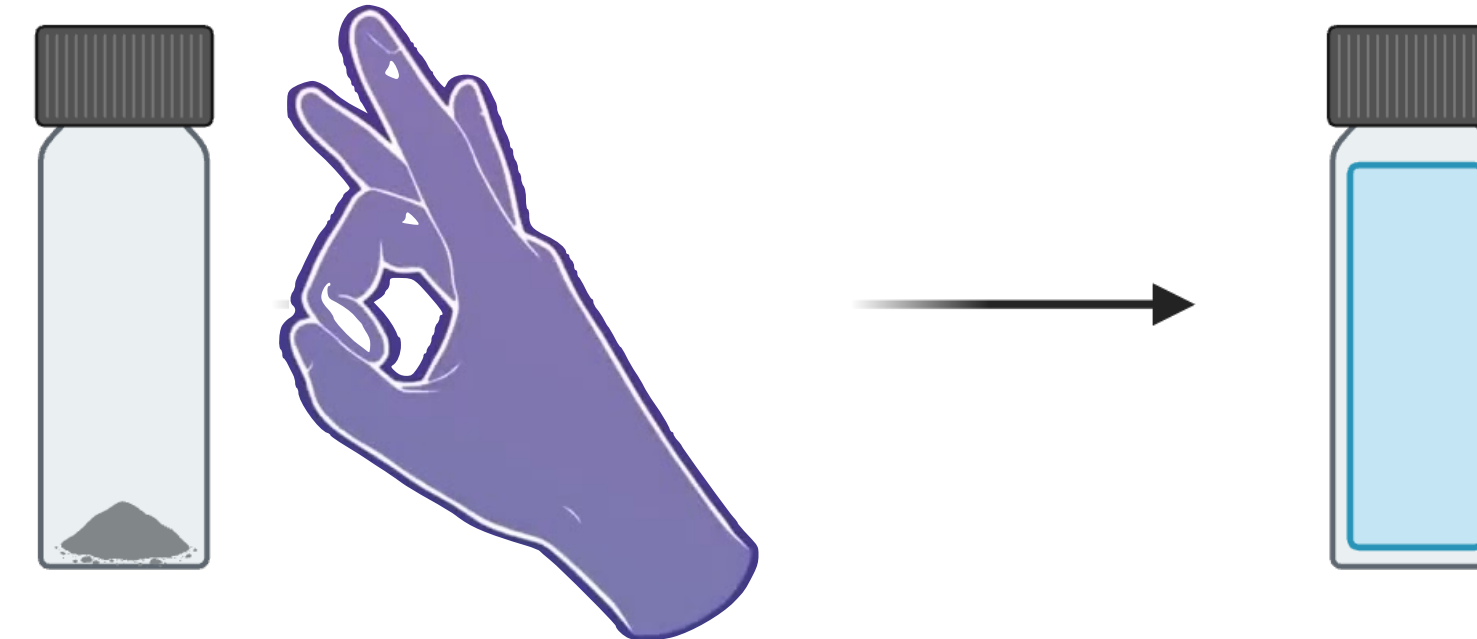
*Dispenses ~1 mg, with 5x variation*

# Origin of HTS

## Screening synthetic compounds - example



● **Hits: active at 1  $\mu\text{M}$**



**Target concentration: 30  $\mu\text{M}$**

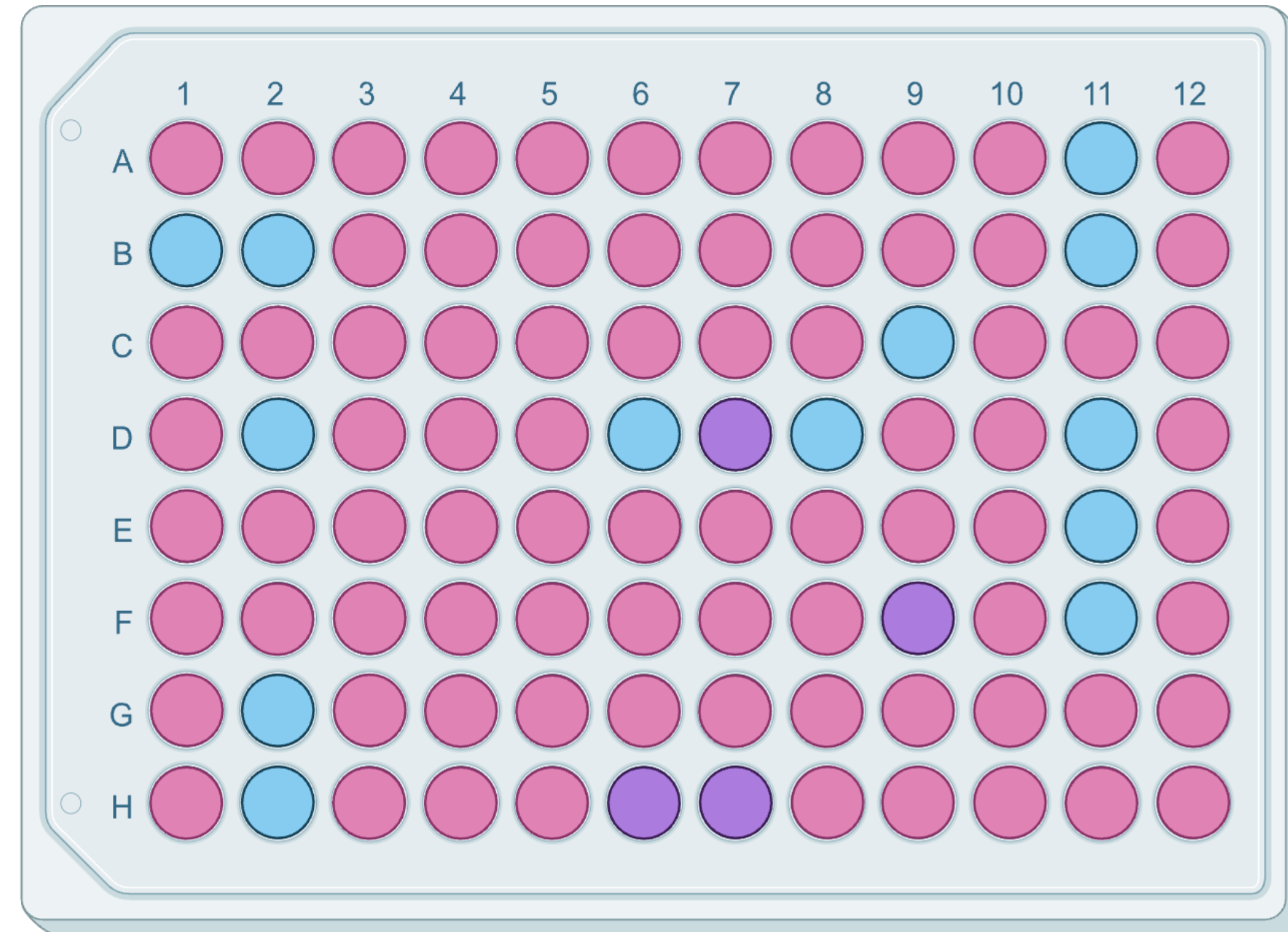
**Reality: varies from 6-150  $\mu\text{M}$**

**Exceed target concentration ✓**

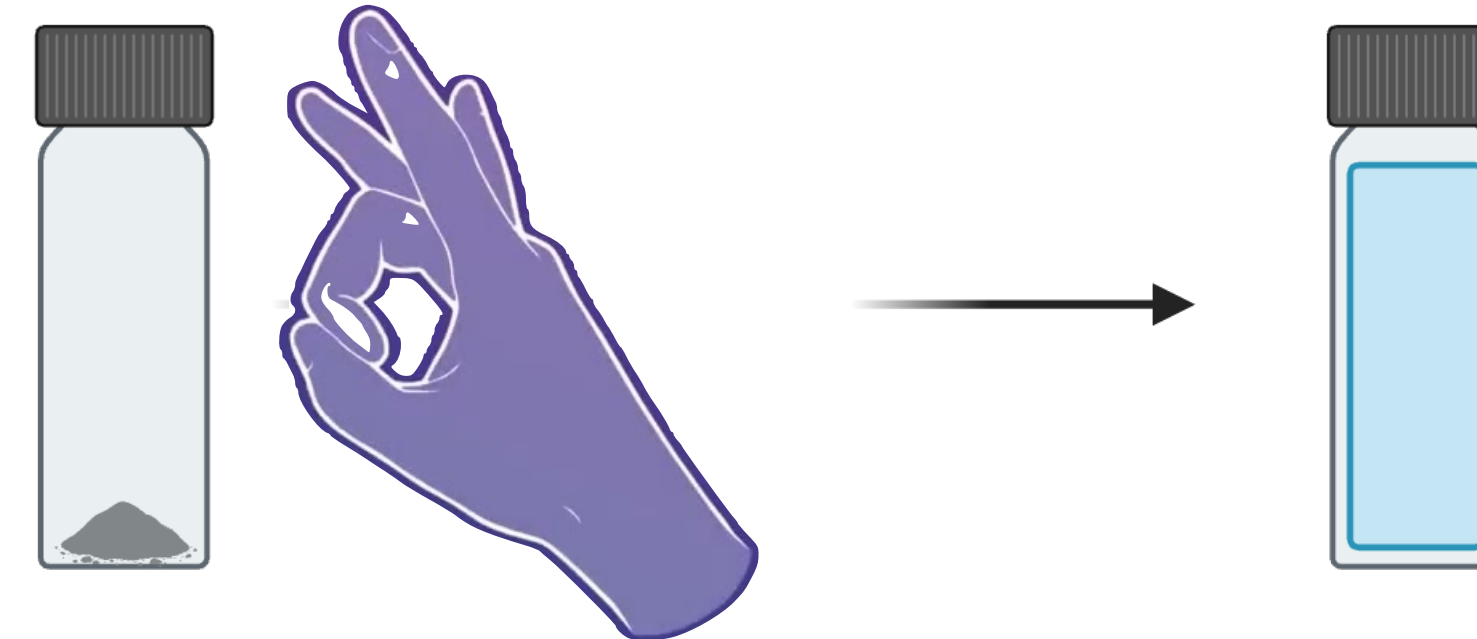
**Nowadays weighing is much more accurate with automation**

# Origin of HTS

## Screening synthetic compounds - example



● **Hits: active at 1  $\mu\text{M}$**



**Target concentration: 30  $\mu\text{M}$**

**Reality: varies from 6-150  $\mu\text{M}$**

**Exceed target concentration ✓**

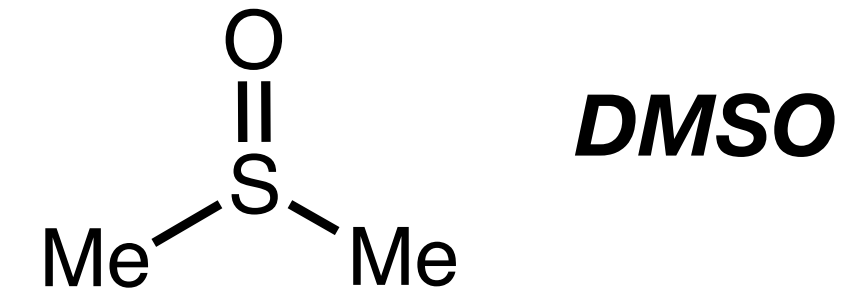
**The problem of weighing is solved, but what about dissolving the compounds?**

# Origin of HTS

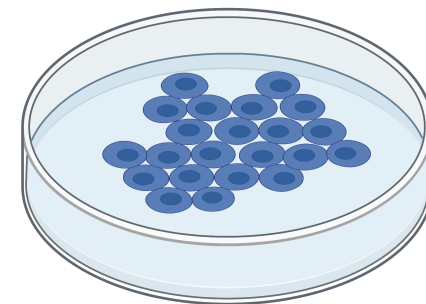
Screening synthetic compounds - solvent consideration

## Solvents used in the past

DMSO  
DMF  
Methanol  
Ethanol  
Mixtures with detergents  
...



**Compatible with cell based assays**



Up to 0.1% DMSO

**Compatible with biochemical assays**



1-5% DMSO

**Good solubility for most compounds**



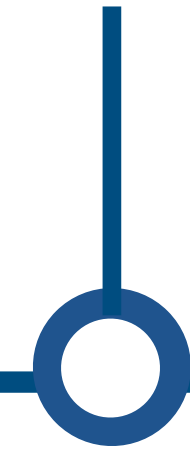
>30 mM solution for 95% compound

**HTS concept was successfully implemented**

# Origin of HTS

## Early evolution of HTS

**Natural product  
screening automation**



1984

1986



**Therapeutic Target HTS**

1989

**Centralized screening**  
Reporter genes  
Multiplex assays with RT-qPCR  
72,000 compounds / week

**HTS concept**

96 well plates, 50-100  $\mu$ L assays  
Manual pattern recognition and autoradiography  
800-1,440 compounds / week

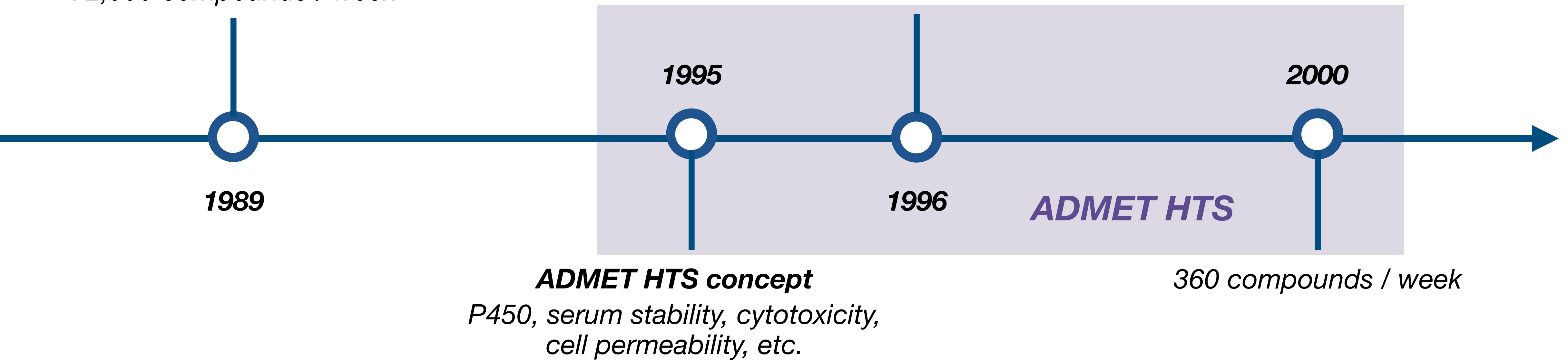
# Origin of HTS

Early evolution of HTS

**Centralized screening**  
Reporter genes  
Multiplex assays with RT-qPCR  
72,000 compounds / week

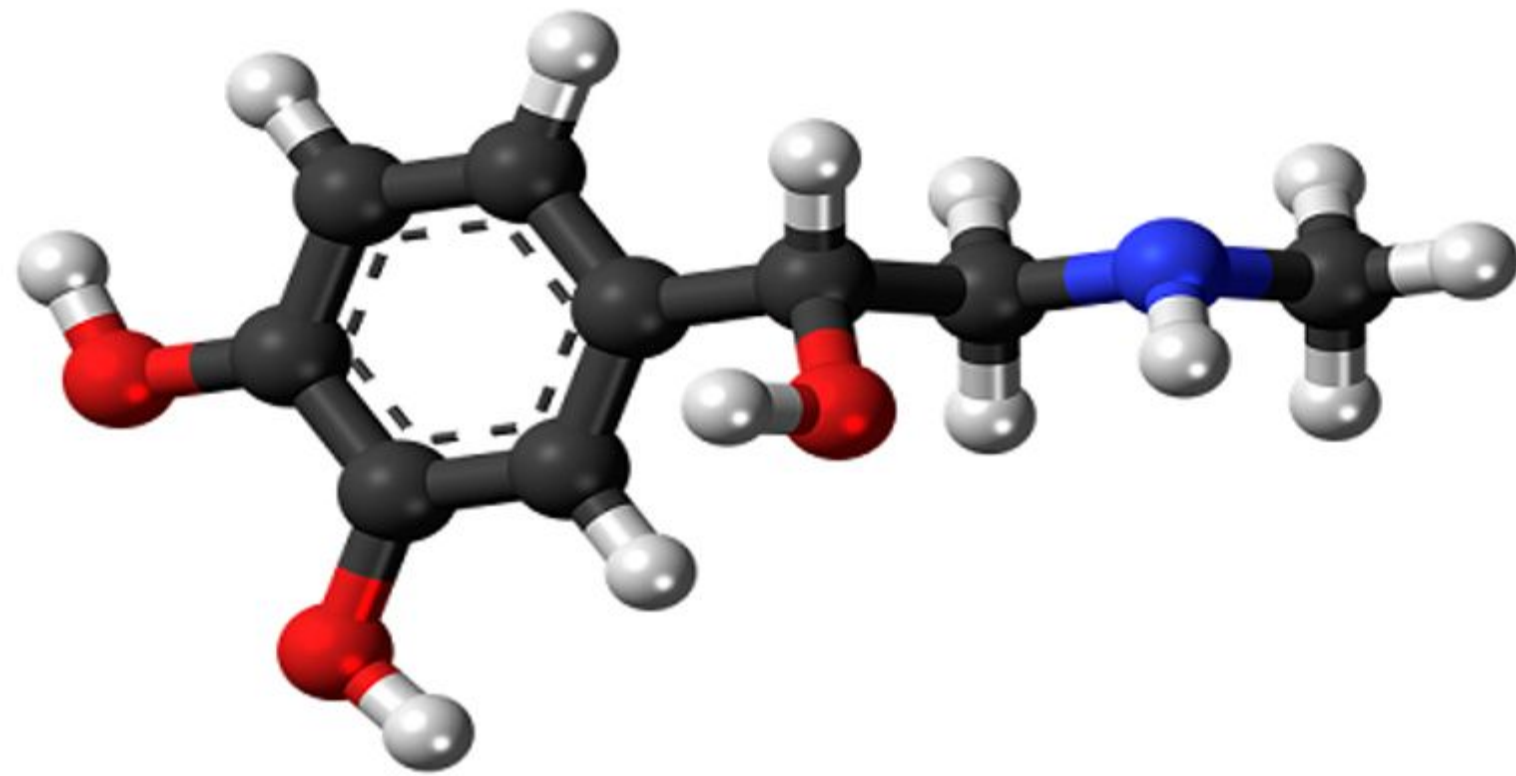
## PreCandidate Technology Group

90 compounds / week  
High throughput HPLC

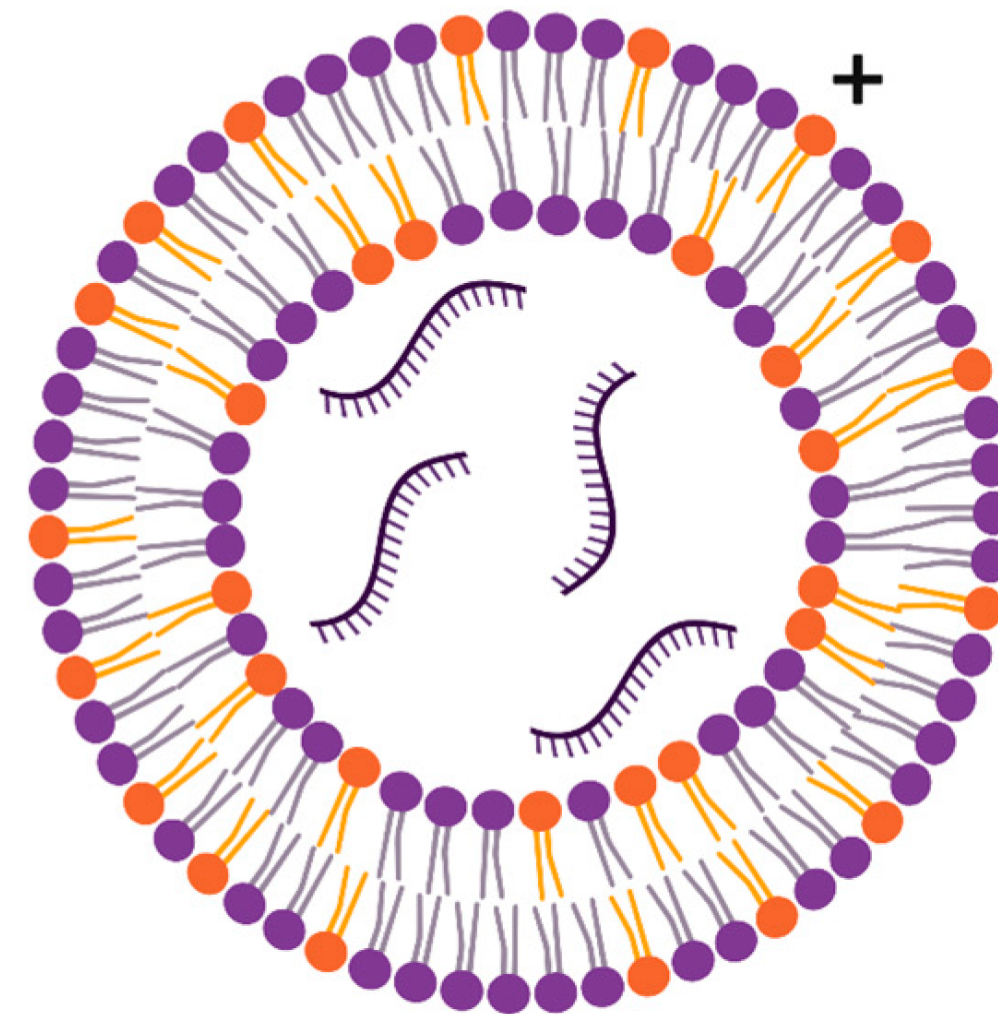




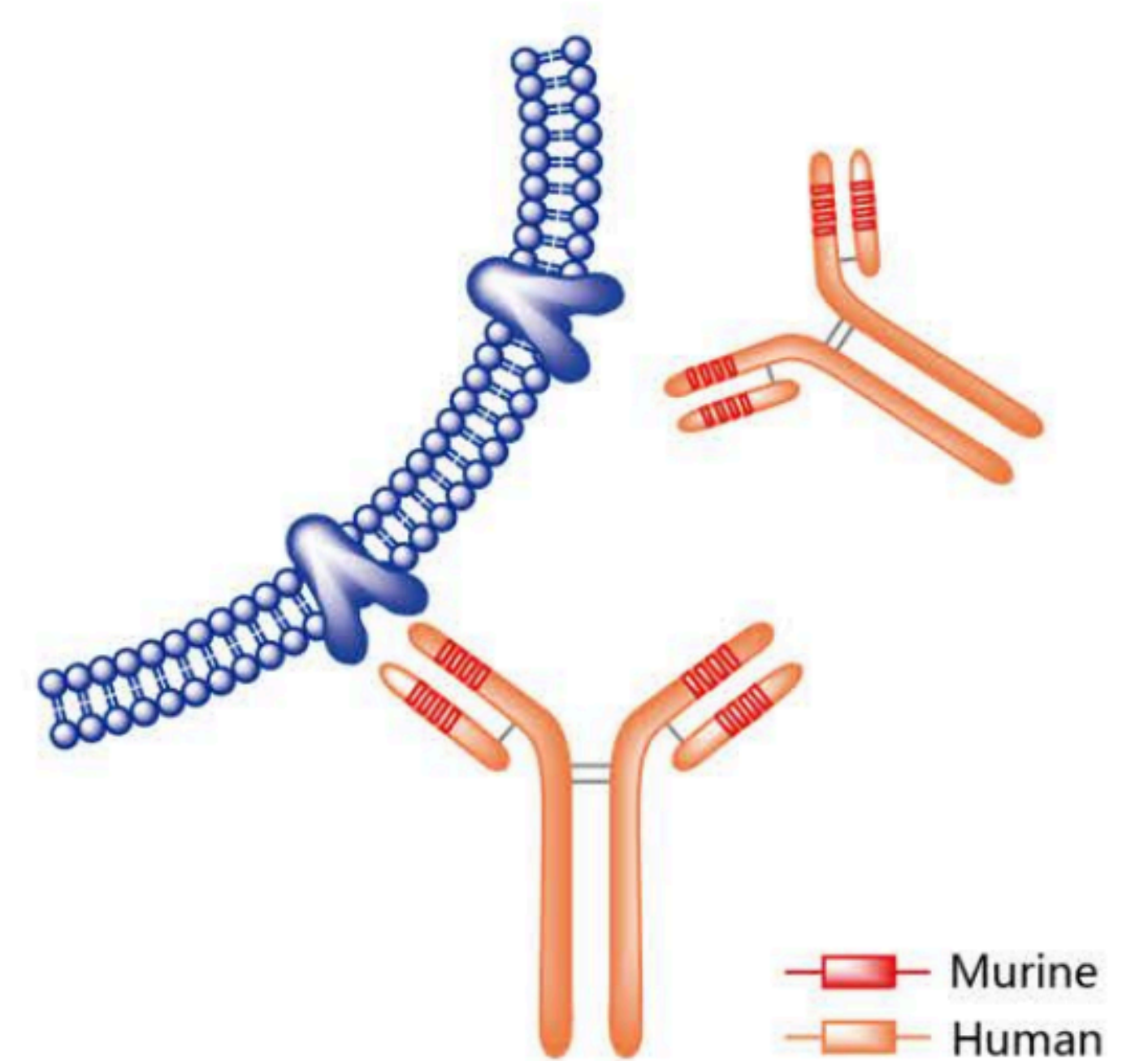
# *HTS strategies in drug discovery*



***Small molecule drugs***

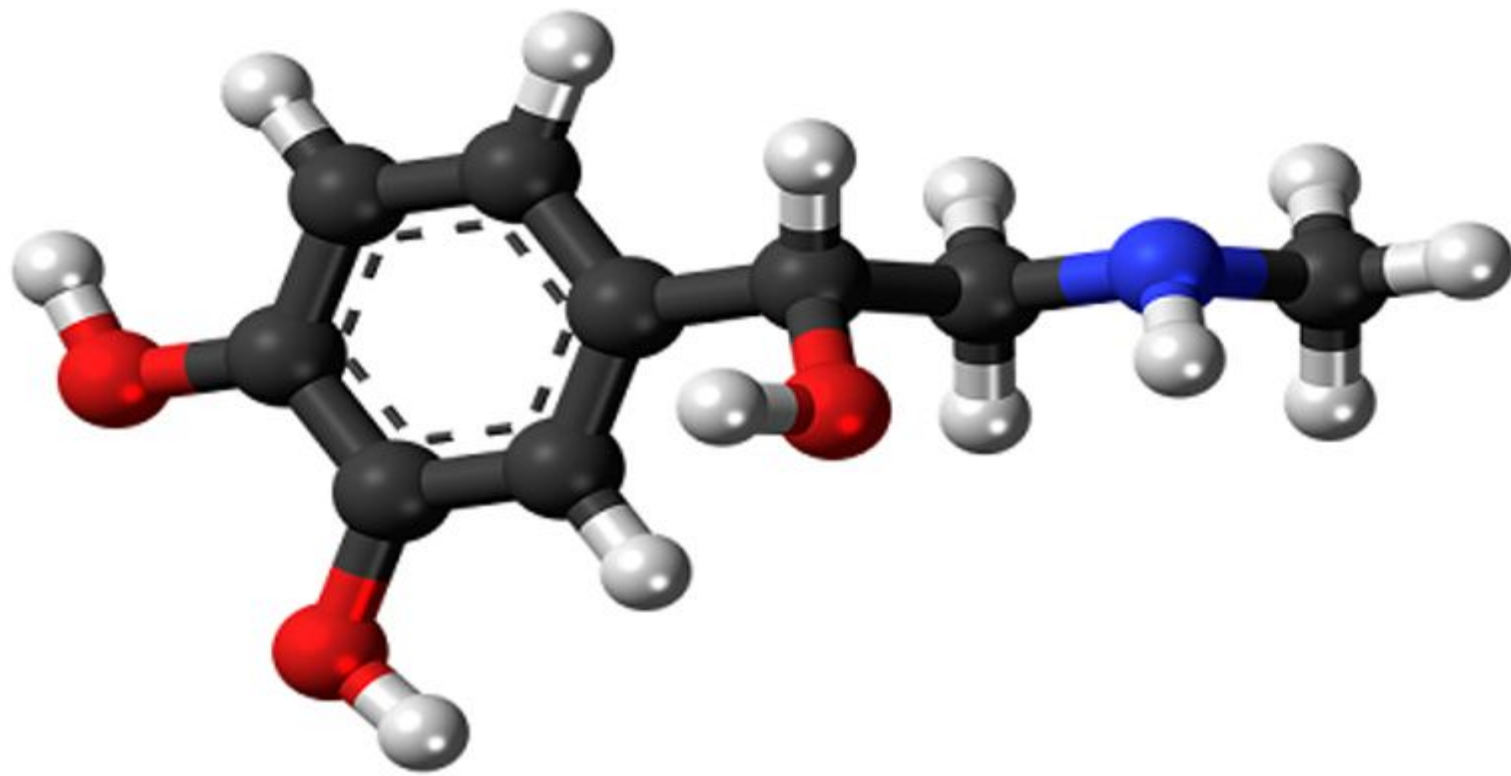


***RNA based therapies***

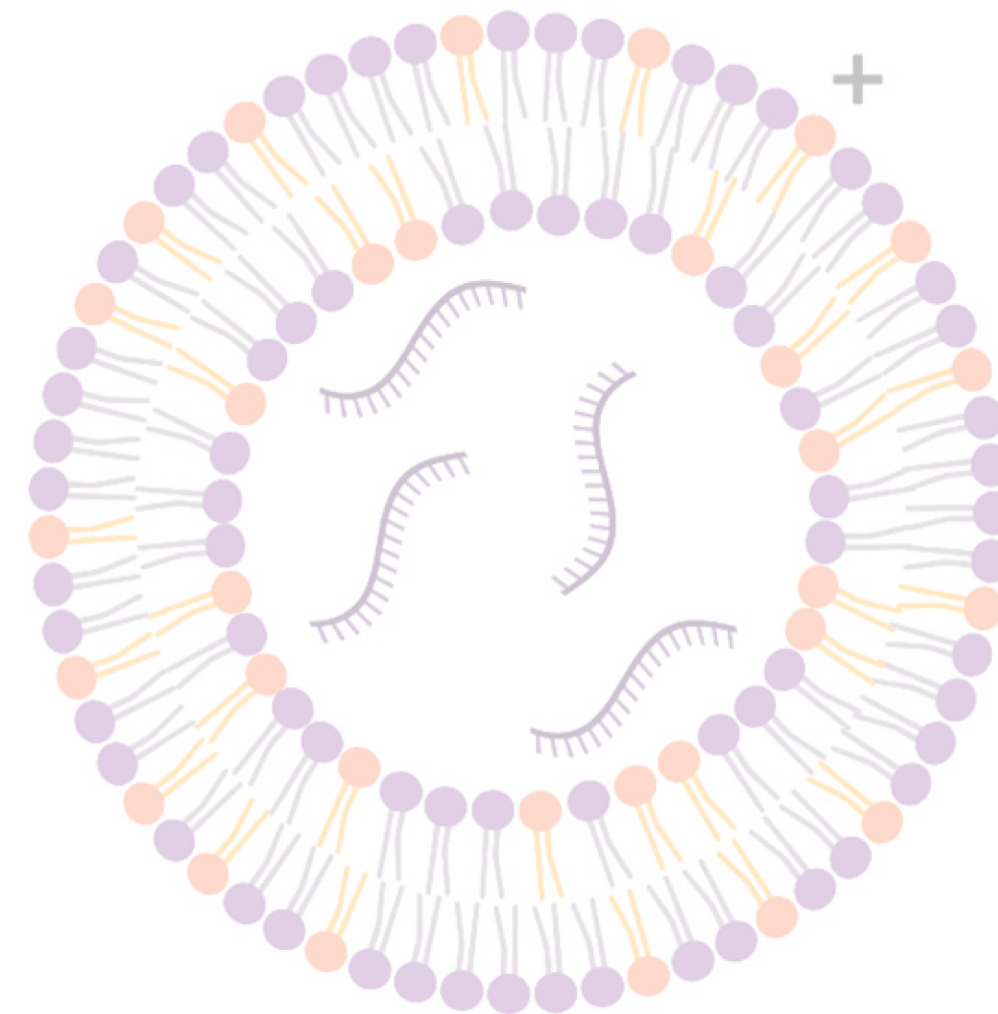


***Antibody based therapies***

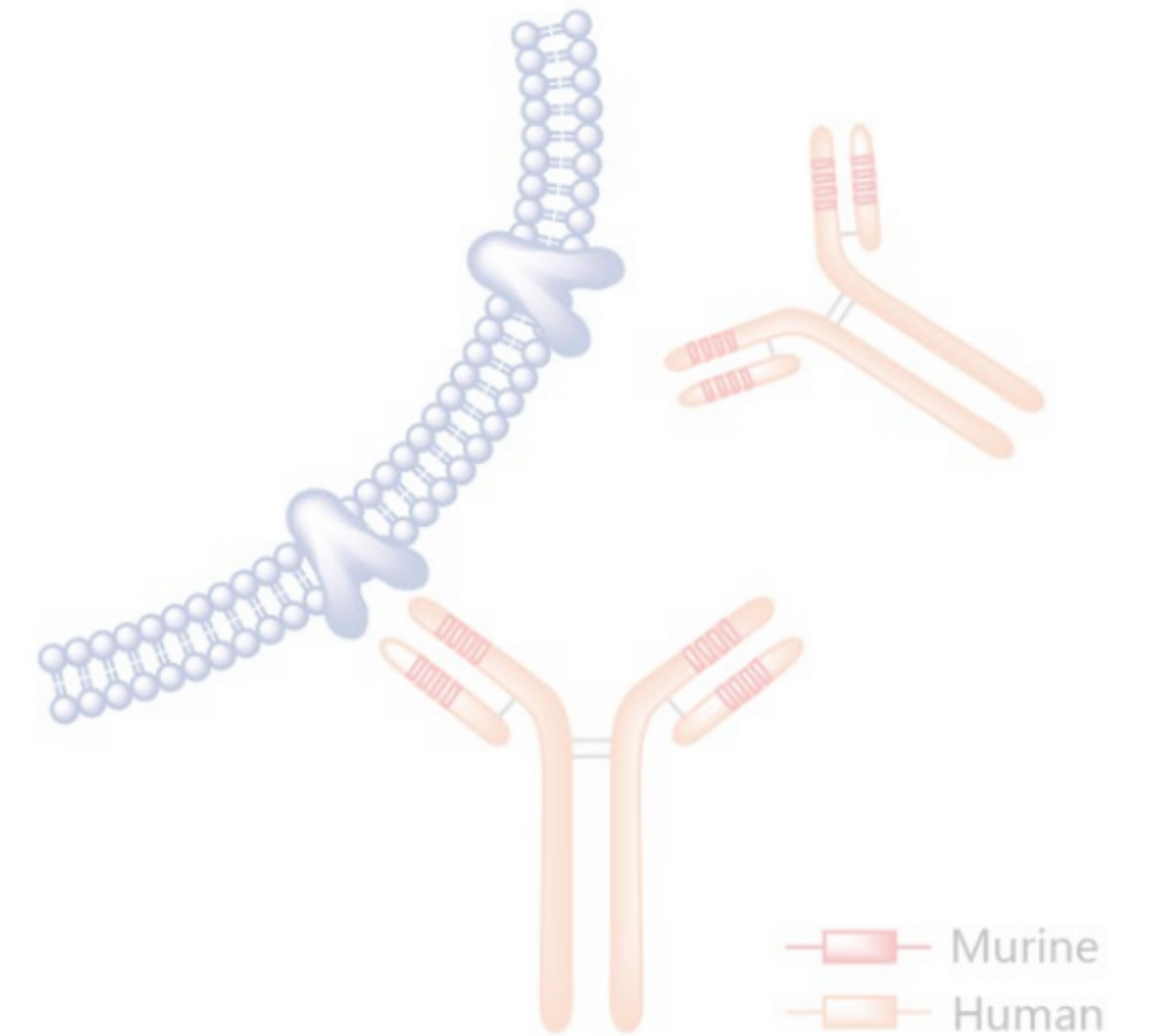
# *HTS strategies in drug discovery*



***Small molecule drugs***



***RNA based therapies***



***Antibody based therapies***

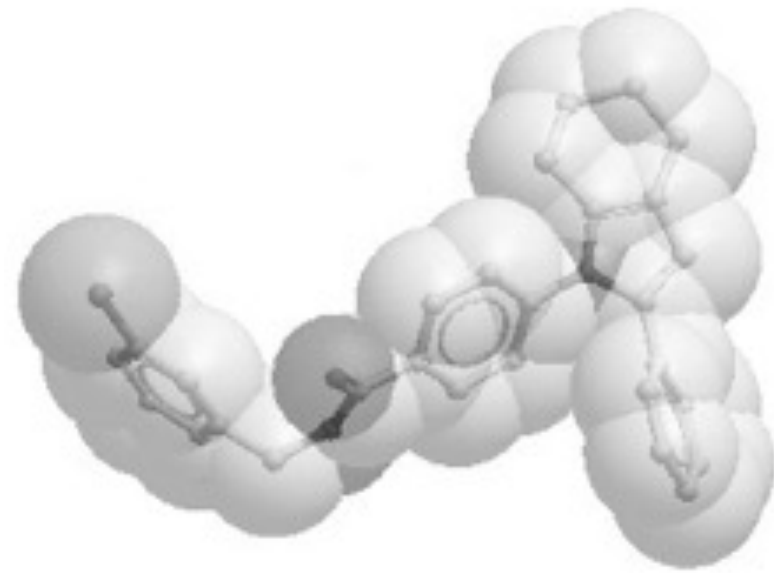
# *HTS in small molecule drug discovery*

## *Compound library types*

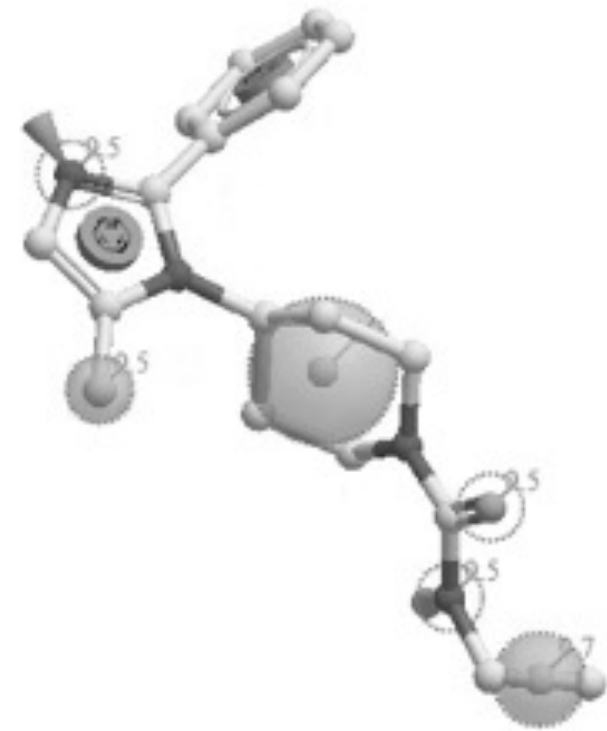
# HTS in small molecule drug discovery

## Compound library types

### 3D Shape Diversity



### Pharmacophore Diversity



### Diversity screening library

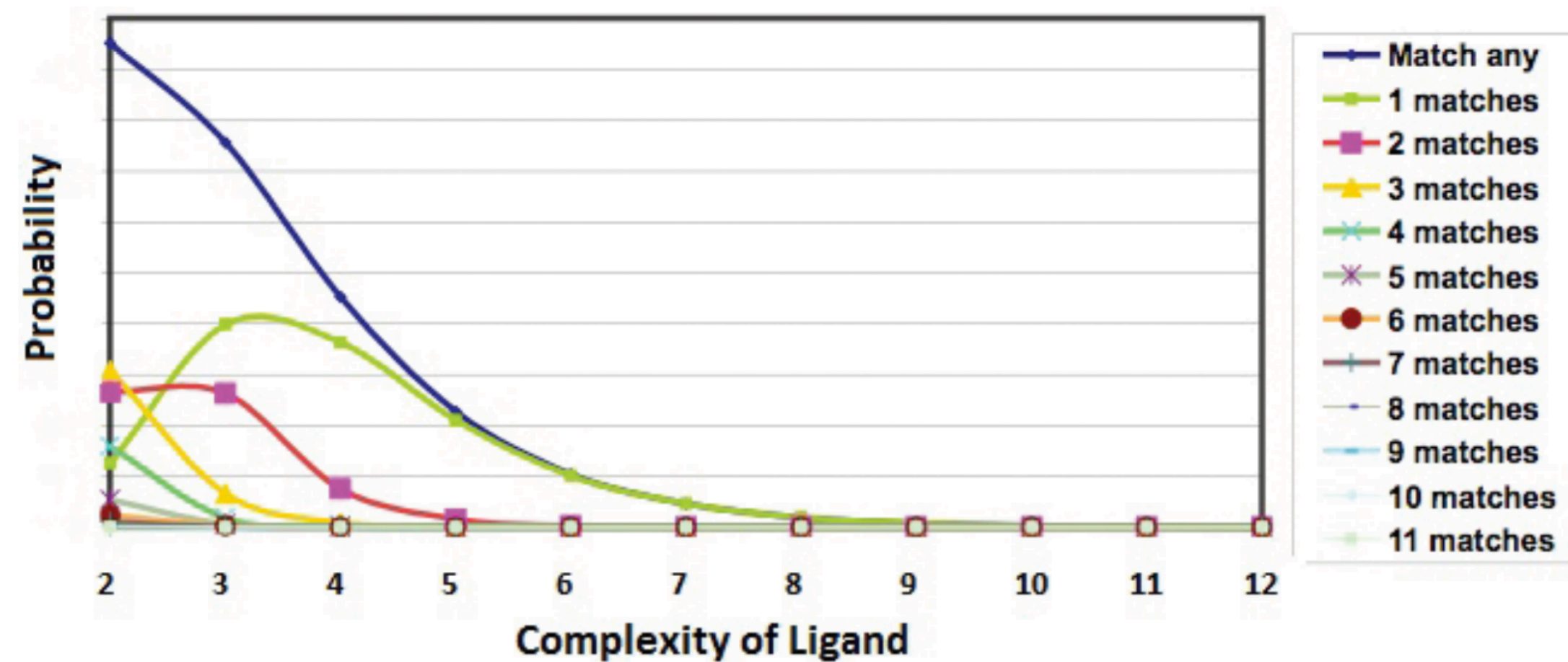
Key metrics: dissimilarity between molecules  
e.g. Tanimoto Coefficient, unique chemical scaffolds

### Compound fragment library

Used in fragment based drug discovery (FBDD)

Low molecular weight ligands (<300 Da)

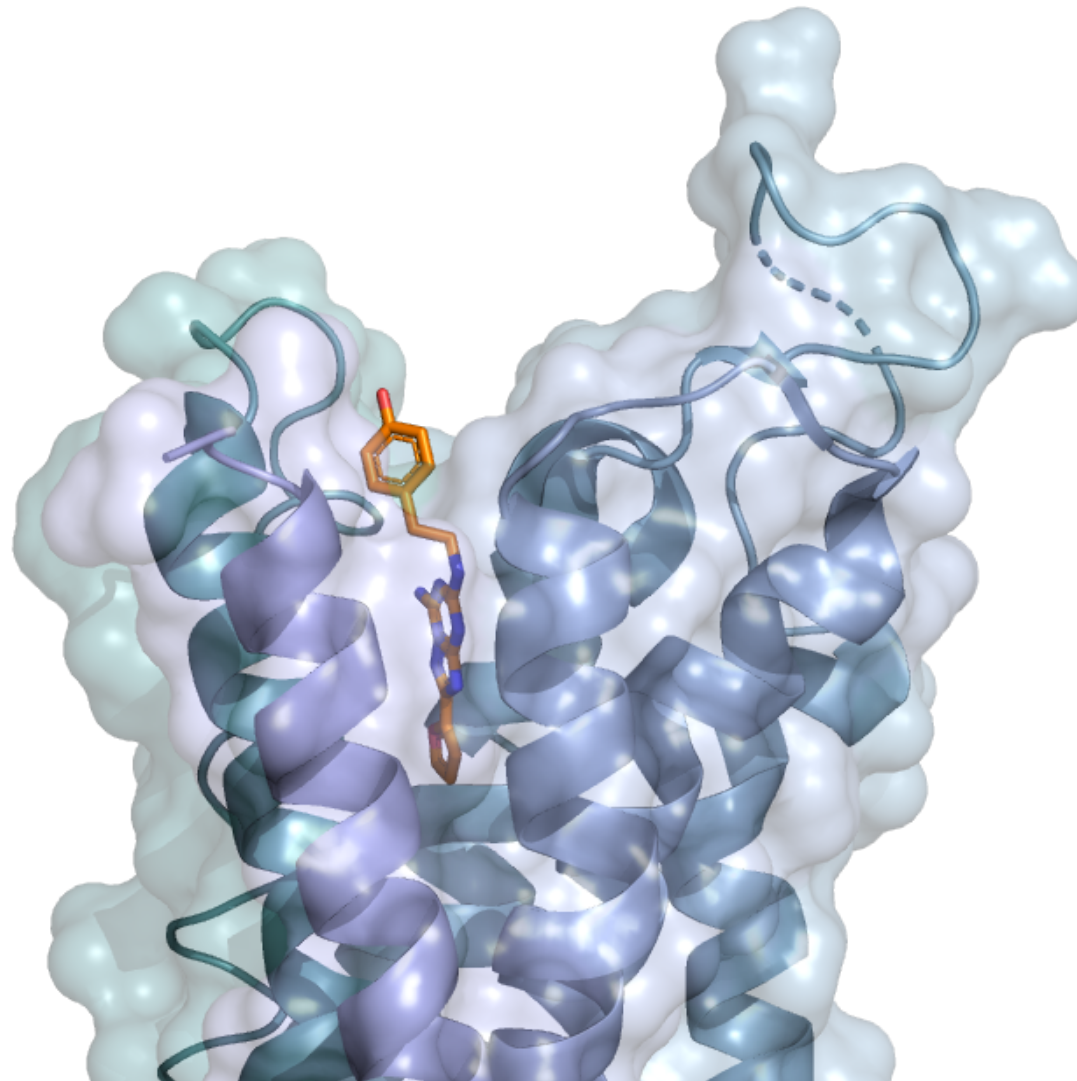
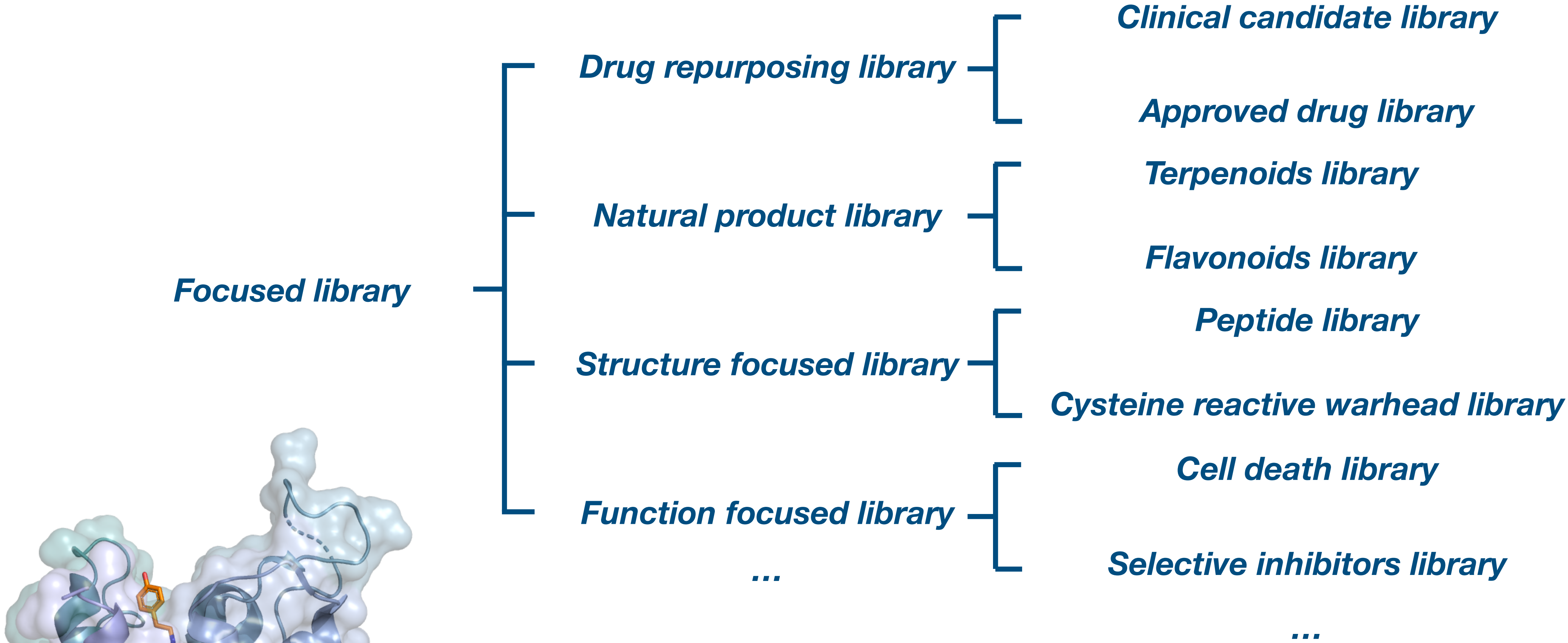
Usually used for in vitro assays



**Some drug discovery campaigns would use a more focused library**

# HTS in small molecule drug discovery

## Compound library types



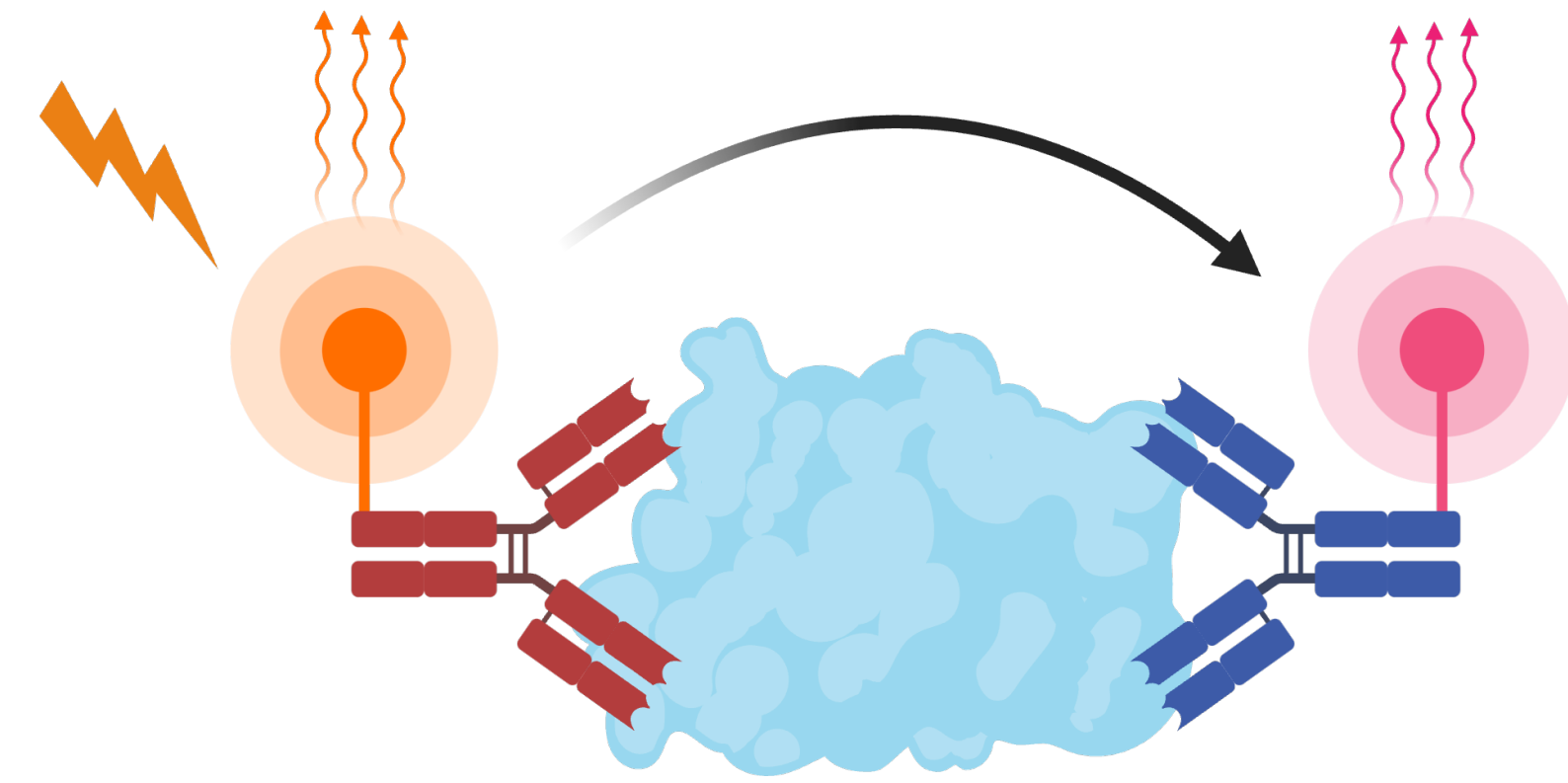
# *HTS in small molecule drug discovery*

## *Screening methods*

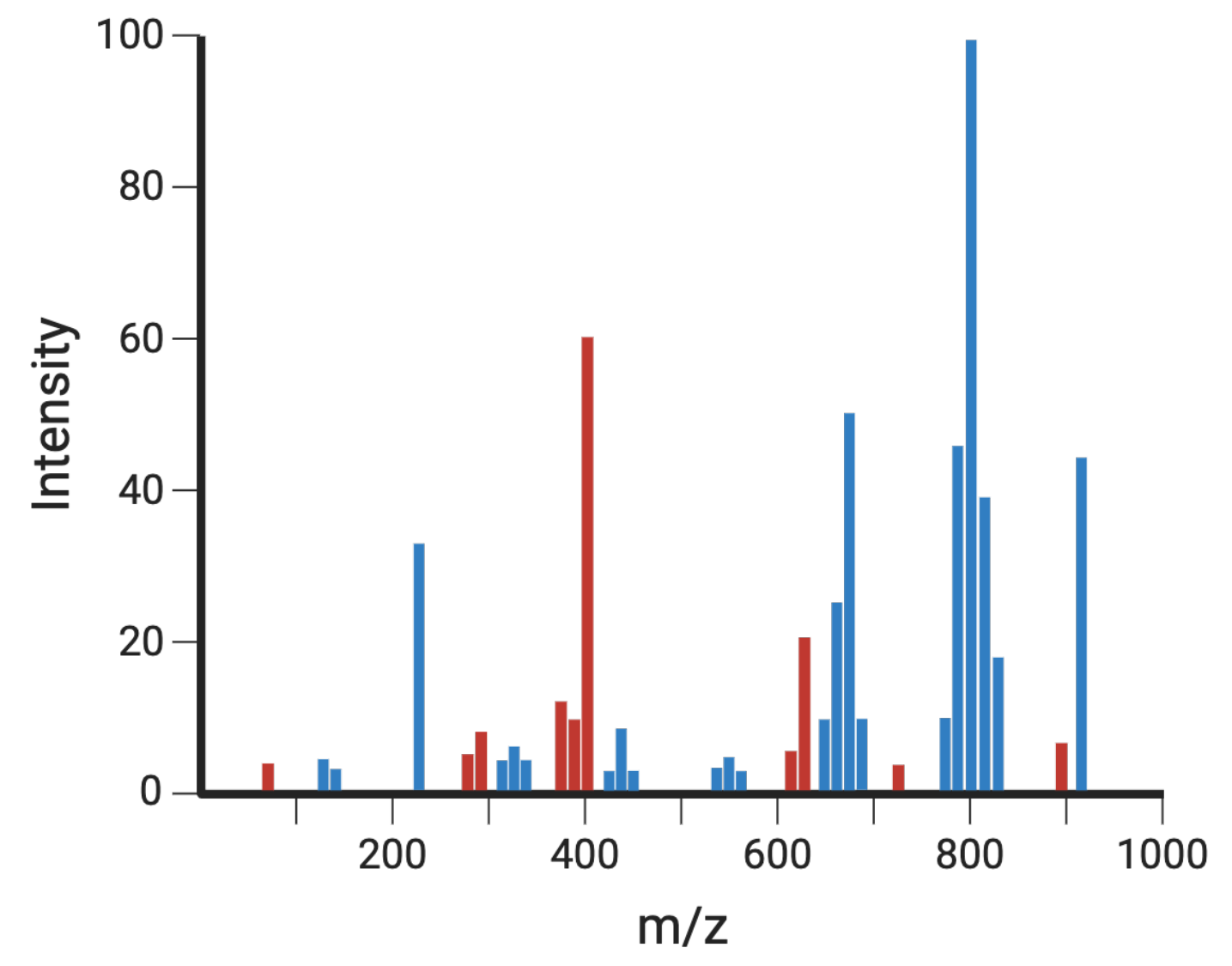
# HTS in small molecule drug discovery

## Screening methods

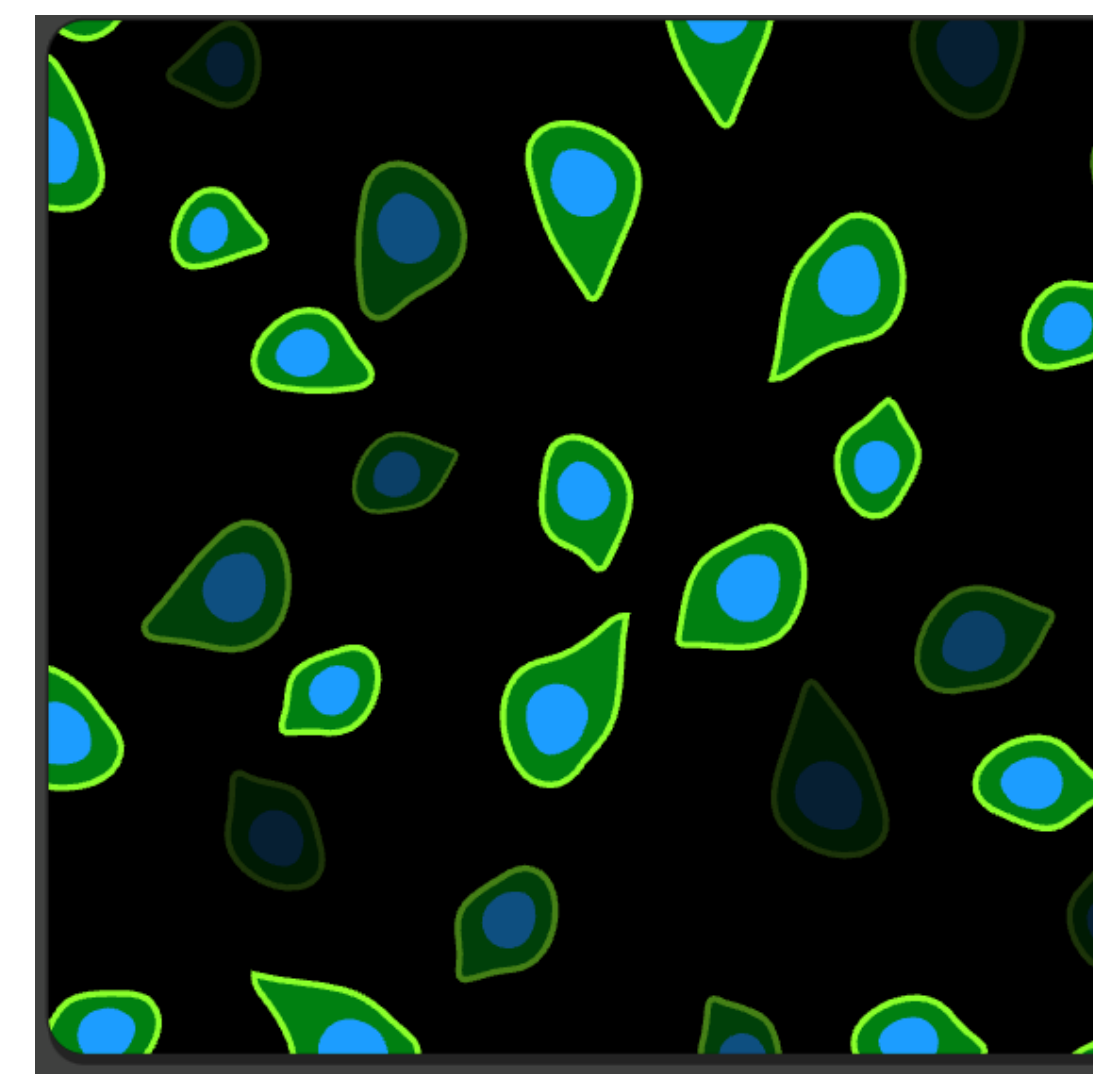
- Requirements:**
- Biologically relevant
  - Sensitive
  - Robust
  - Economic



**Biochemical assays**



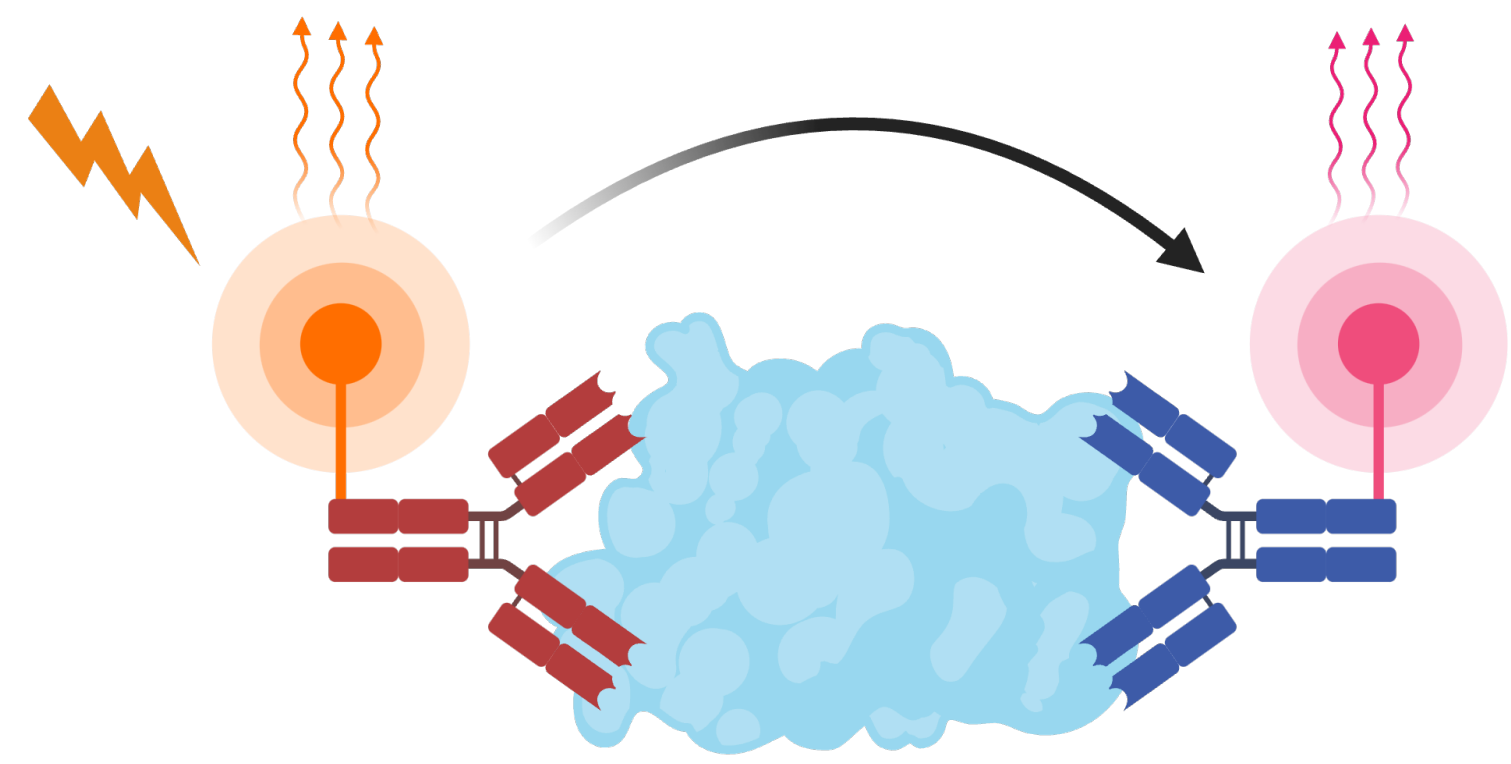
**Spectroscopic assays**



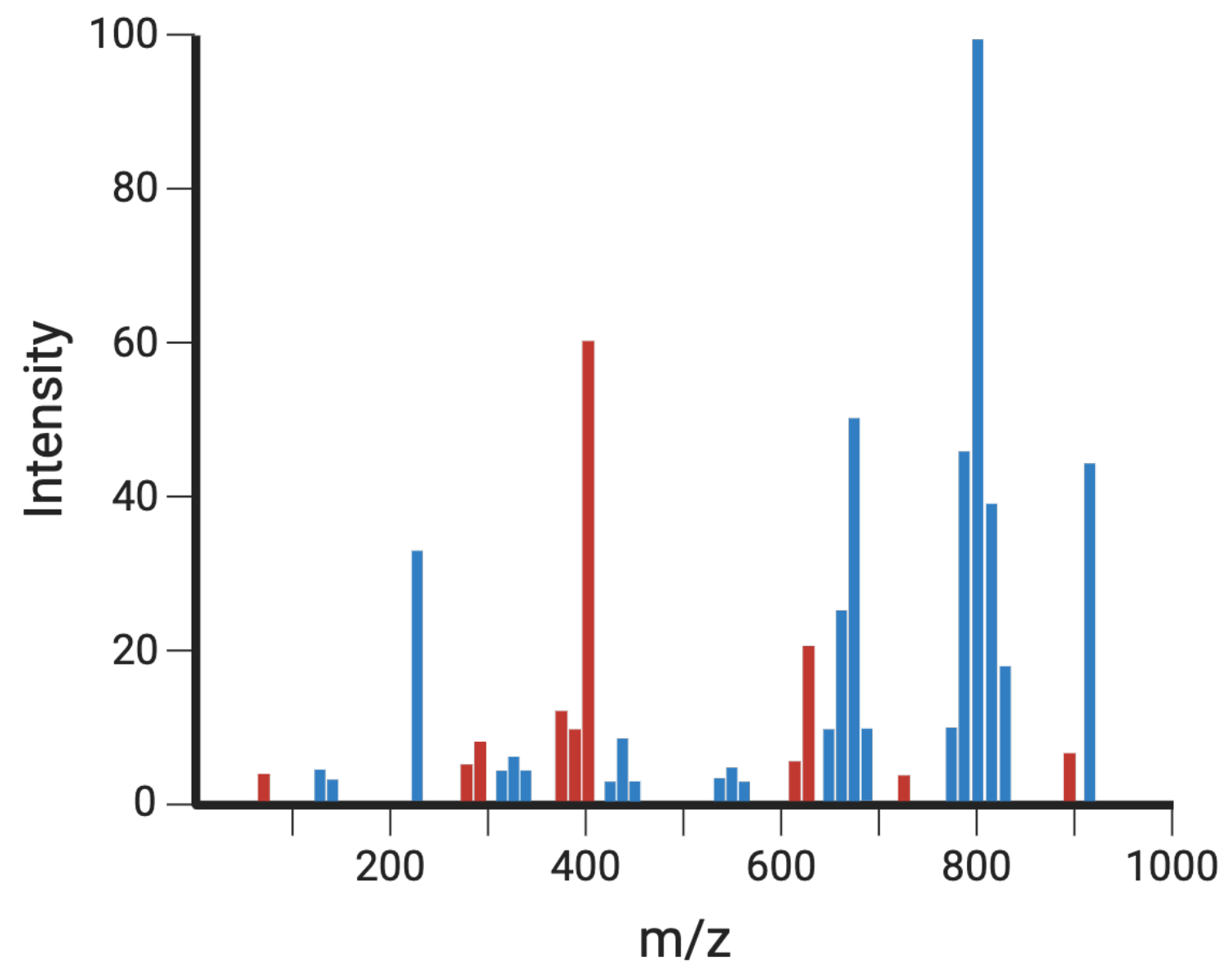
**Cell based assays**

# HTS in small molecule drug discovery

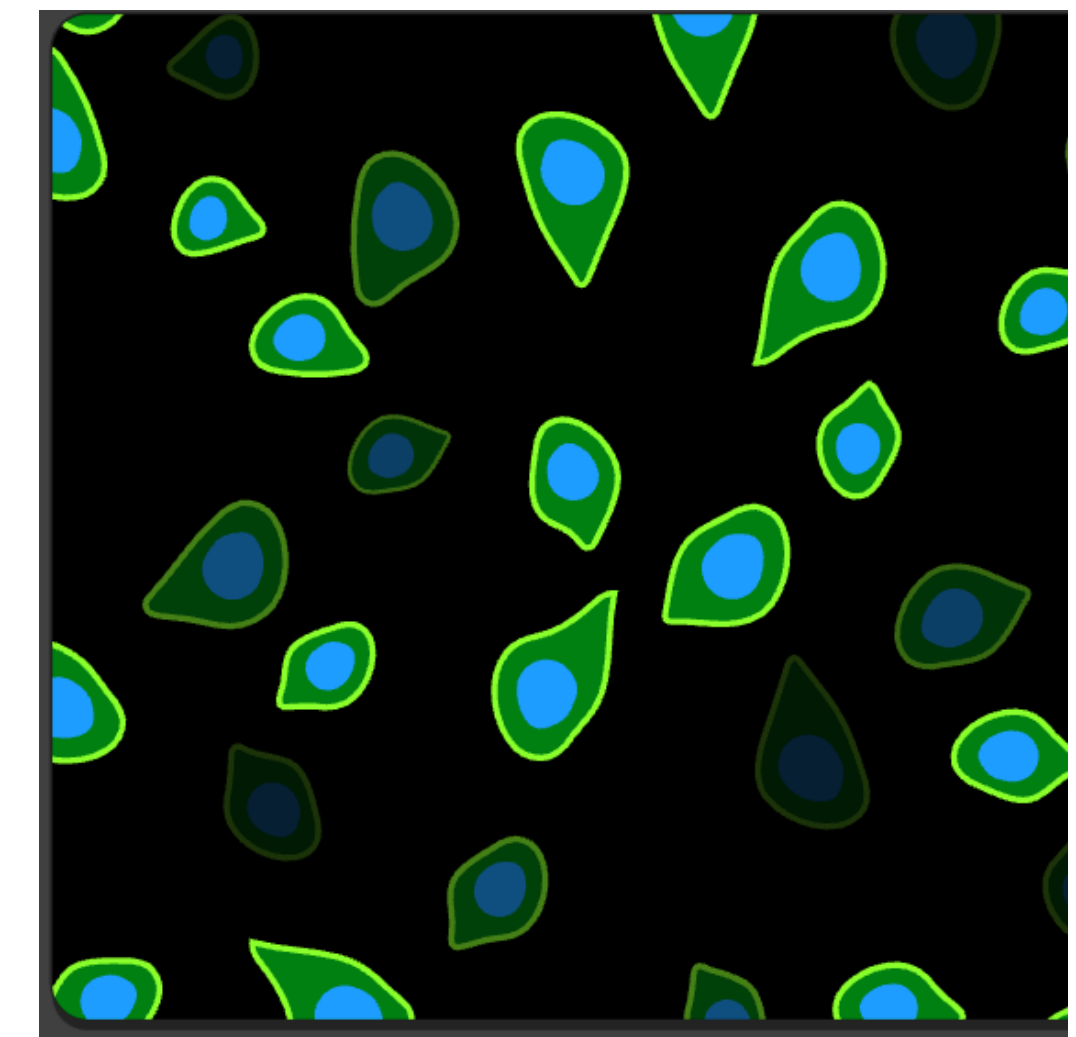
## Screening methods



**Biochemical assays**



**Spectroscopic assays**



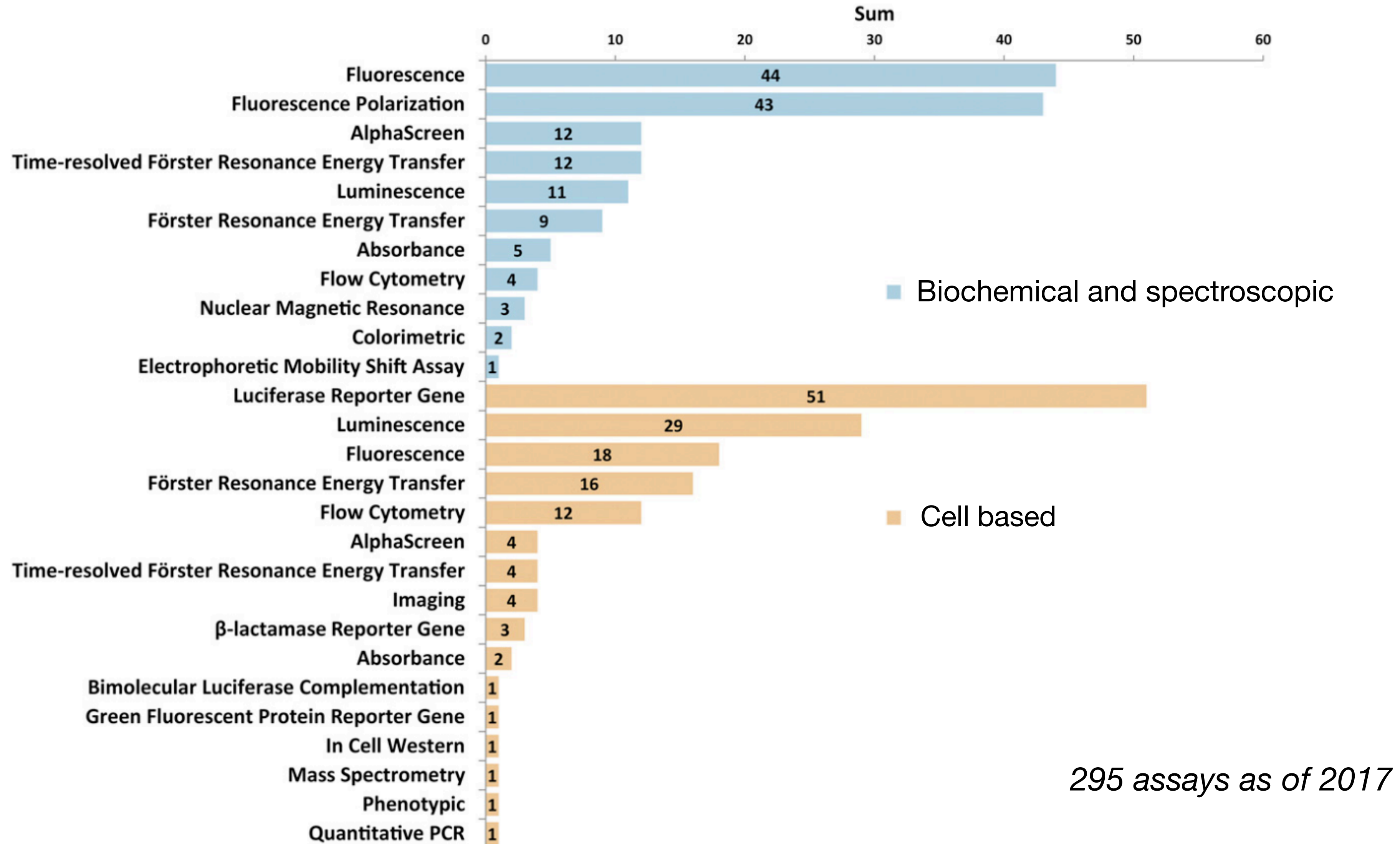
**Cell based assays**

***The choice of HTS assay is important because each compound is typically only assayed once in a primary screen***



# HTS in small molecule drug discovery

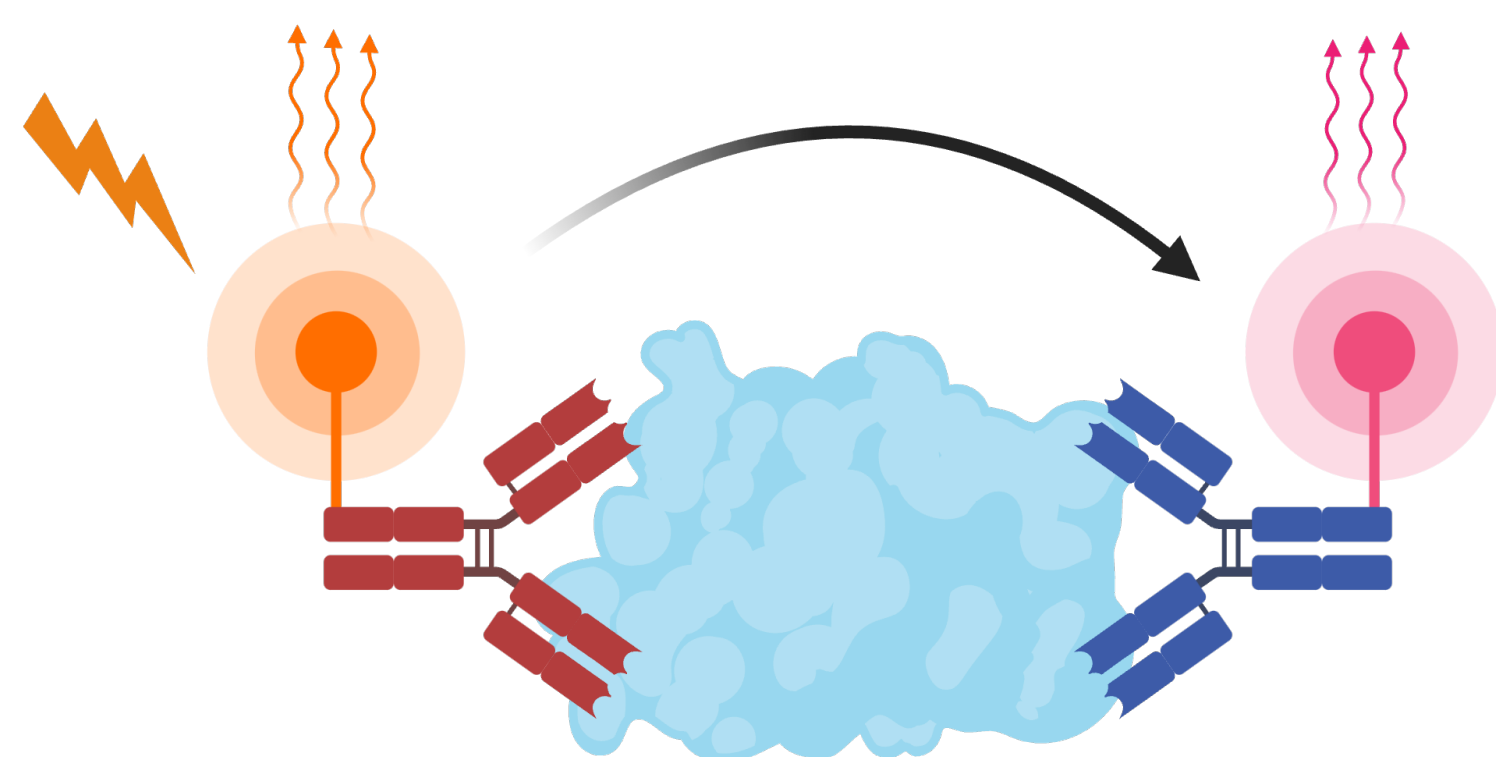
## Cancer-relevant HTS assays reported in the PubChem database



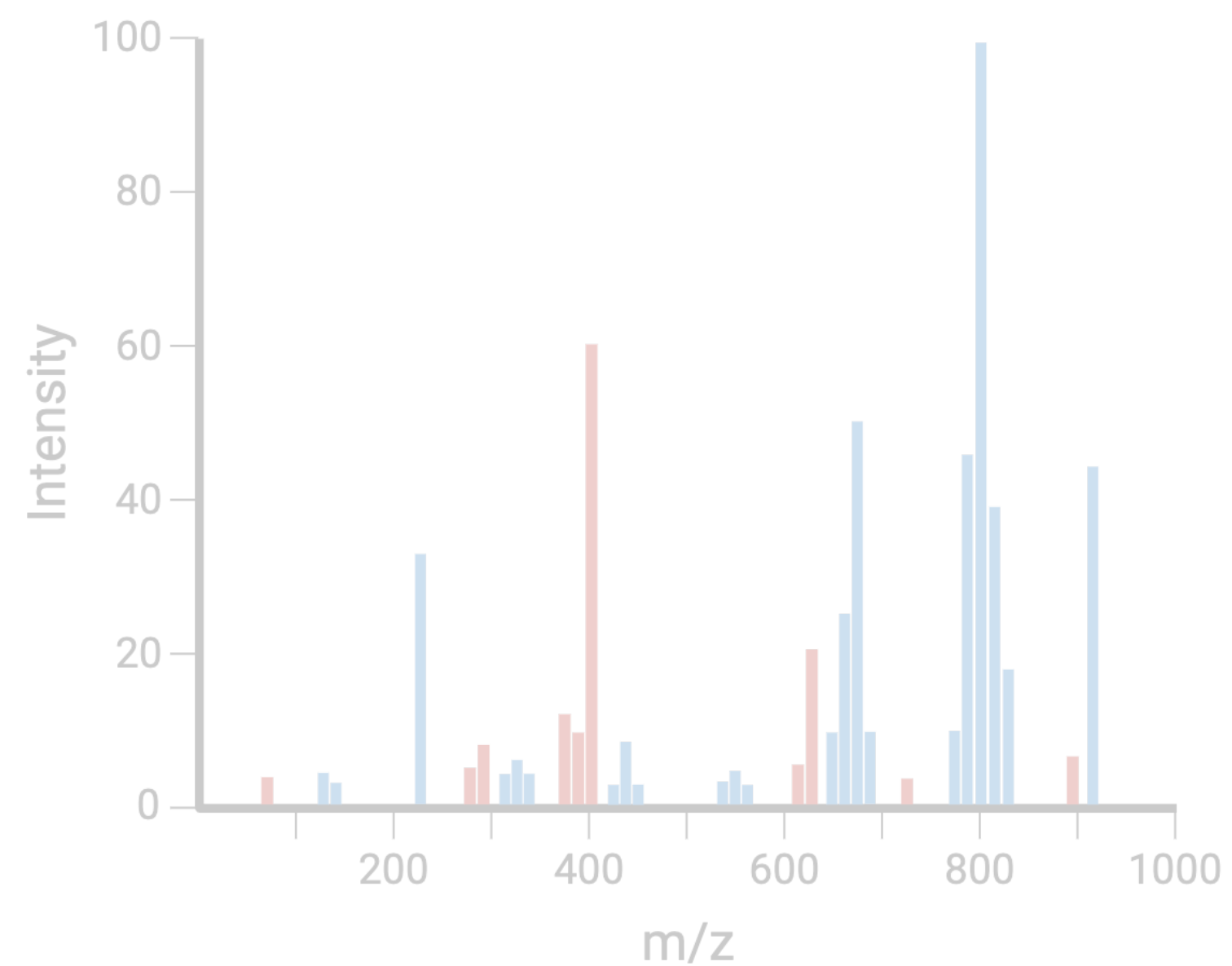
295 assays as of 2017

# HTS in small molecule drug discovery

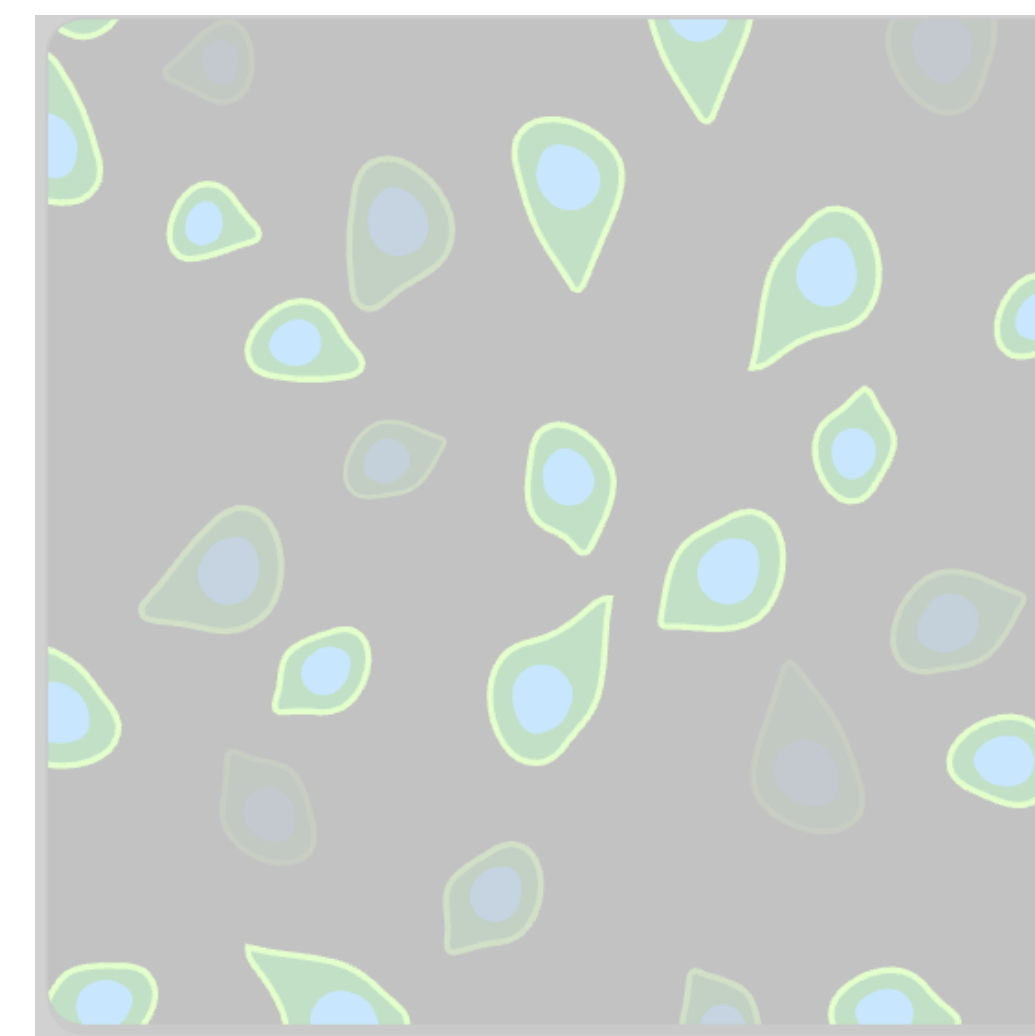
## Screening methods



**Biochemical assays**



**Spectroscopic assays**

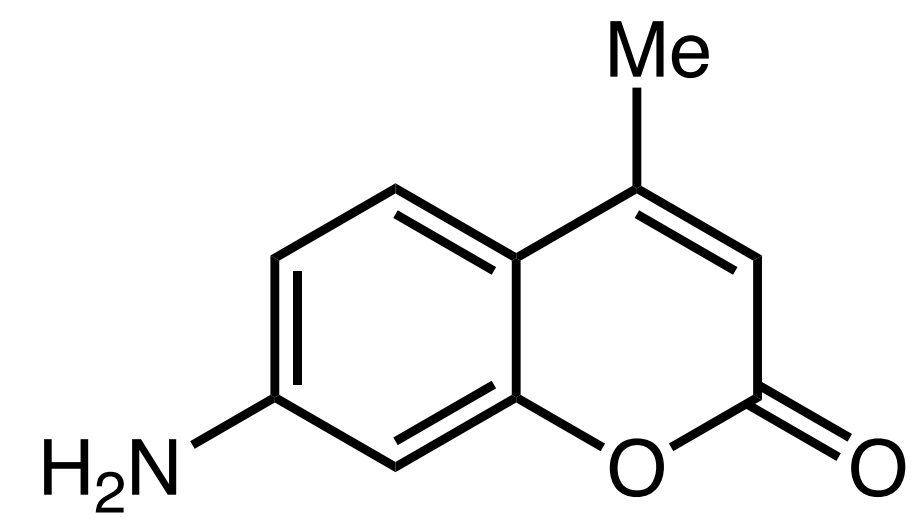
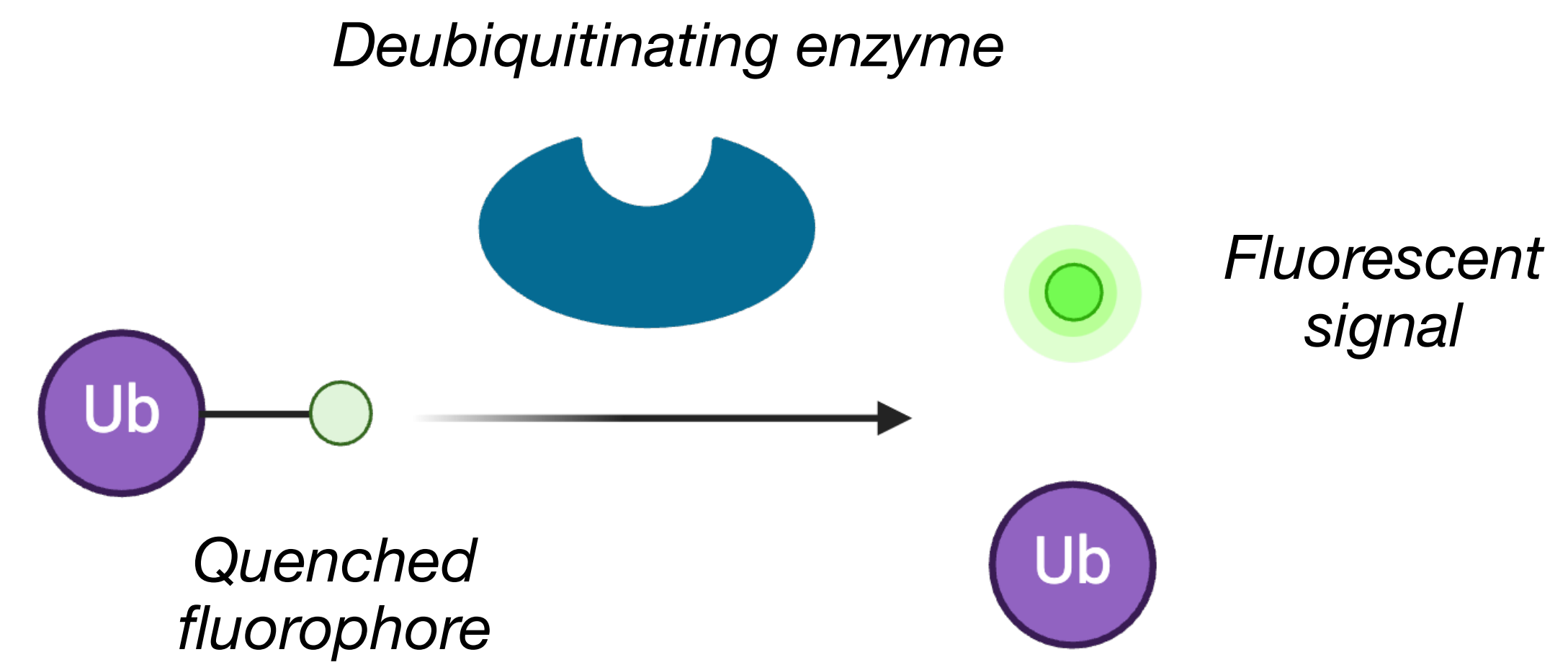


**Cell based assays**

# HTS in small molecule drug discovery

## Screening methods - biochemical assays

### Fluorescent intensity assay (FLINT)



**7-Amino-4-methylcoumarin (AMC)**

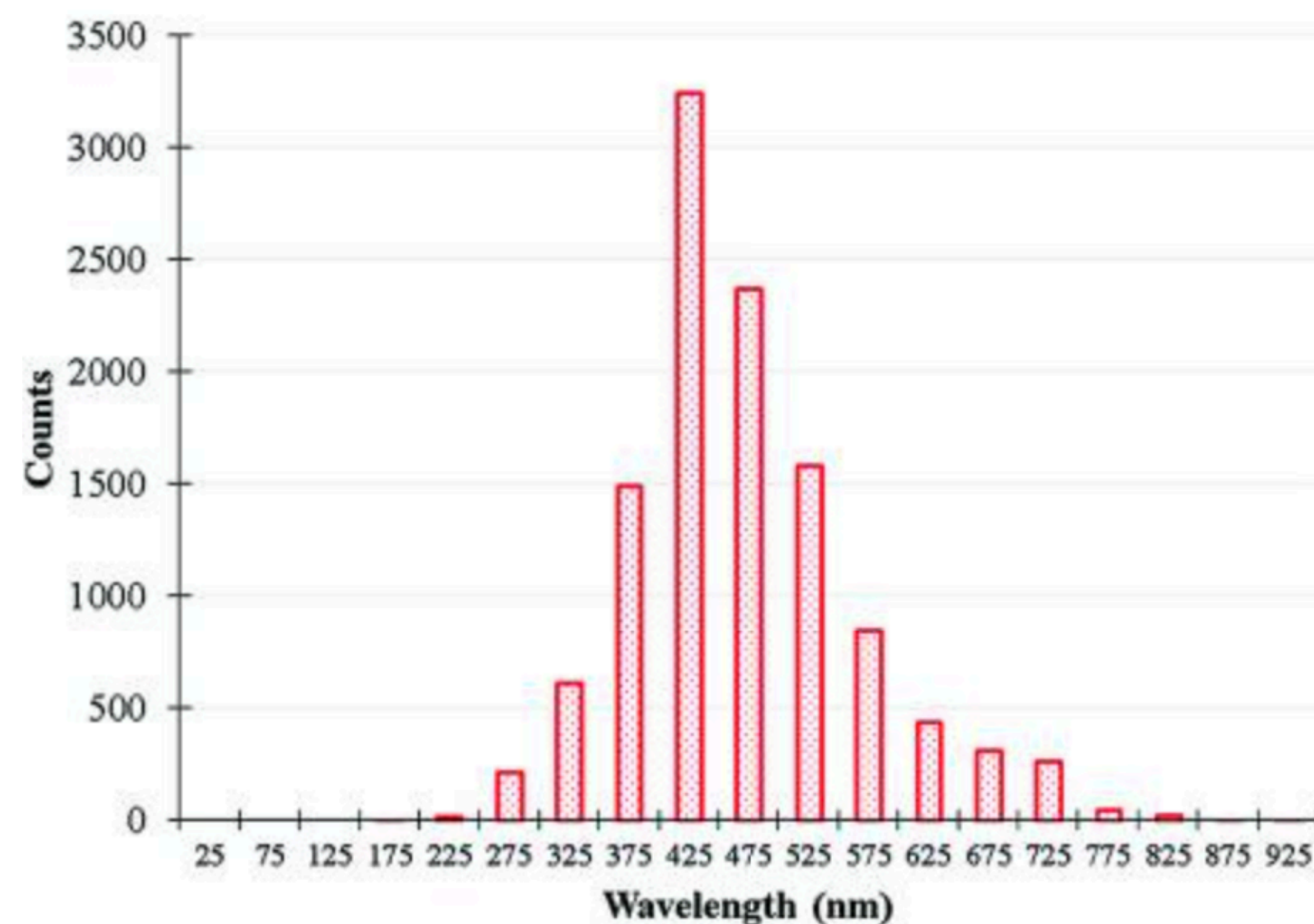
445 nm emission



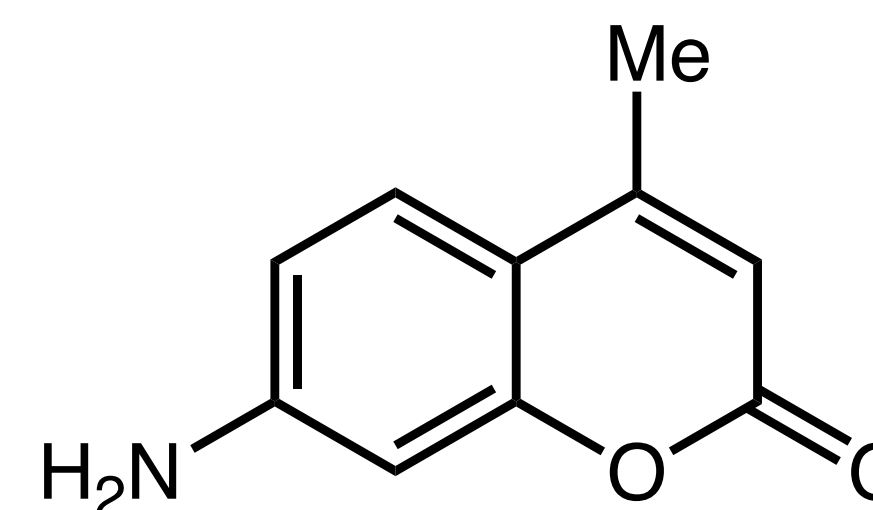
# HTS in small molecule drug discovery

## Screening methods - biochemical assays

### Predicted emission wavelength of organic molecules



11,460 organic molecules from Reaxys database



**7-Amino-4-methylcoumarin (AMC)**

445 nm emission

**Autofluorescent of testing molecules can be a problem**

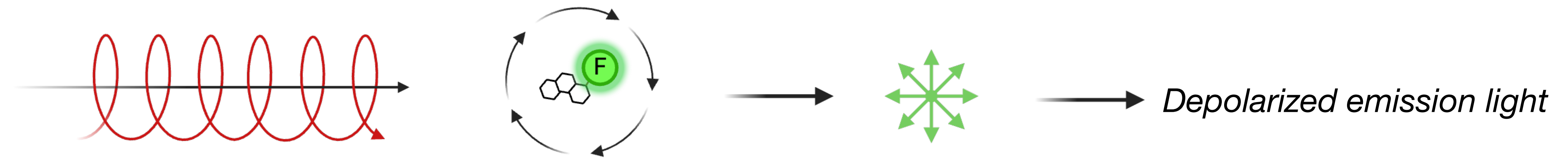
**One solution is to measure the test compounds before adding detection reagents**

# HTS in small molecule drug discovery

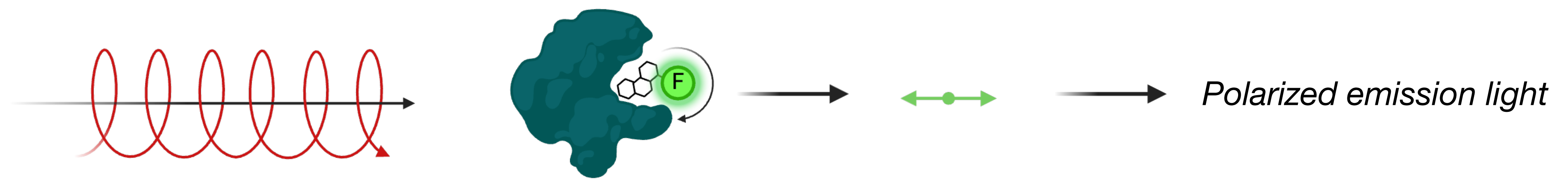
Screening methods - biochemical assays

## Fluorescence anisotropy/polarization (FA/FP)

Low affinity molecule



High affinity molecule

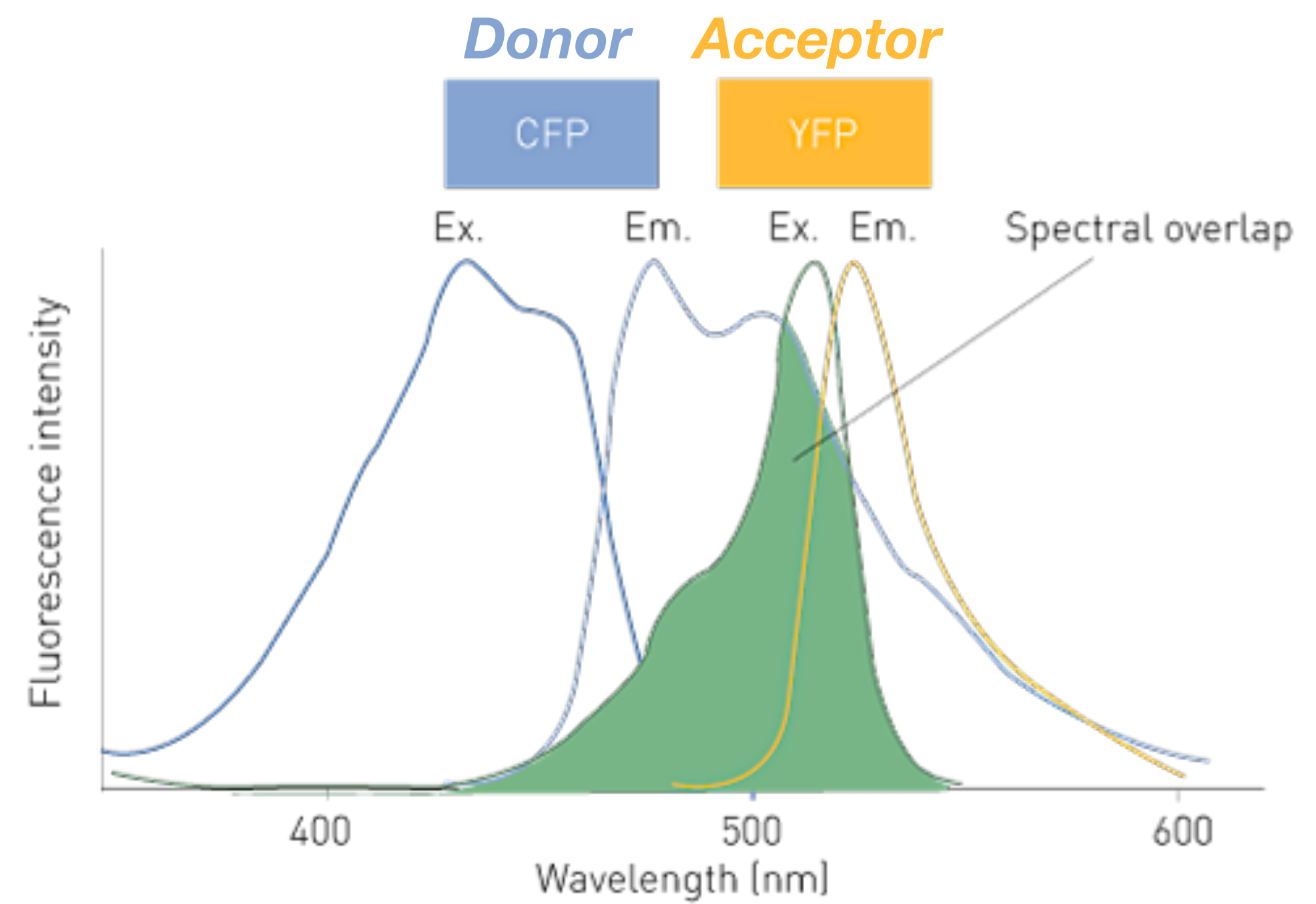


**Measuring fluorescent polarization is less sensitive to interference**

# HTS in small molecule drug discovery

## Screening methods - biochemical assays

### Fluorescent resonance energy transfer (FRET)



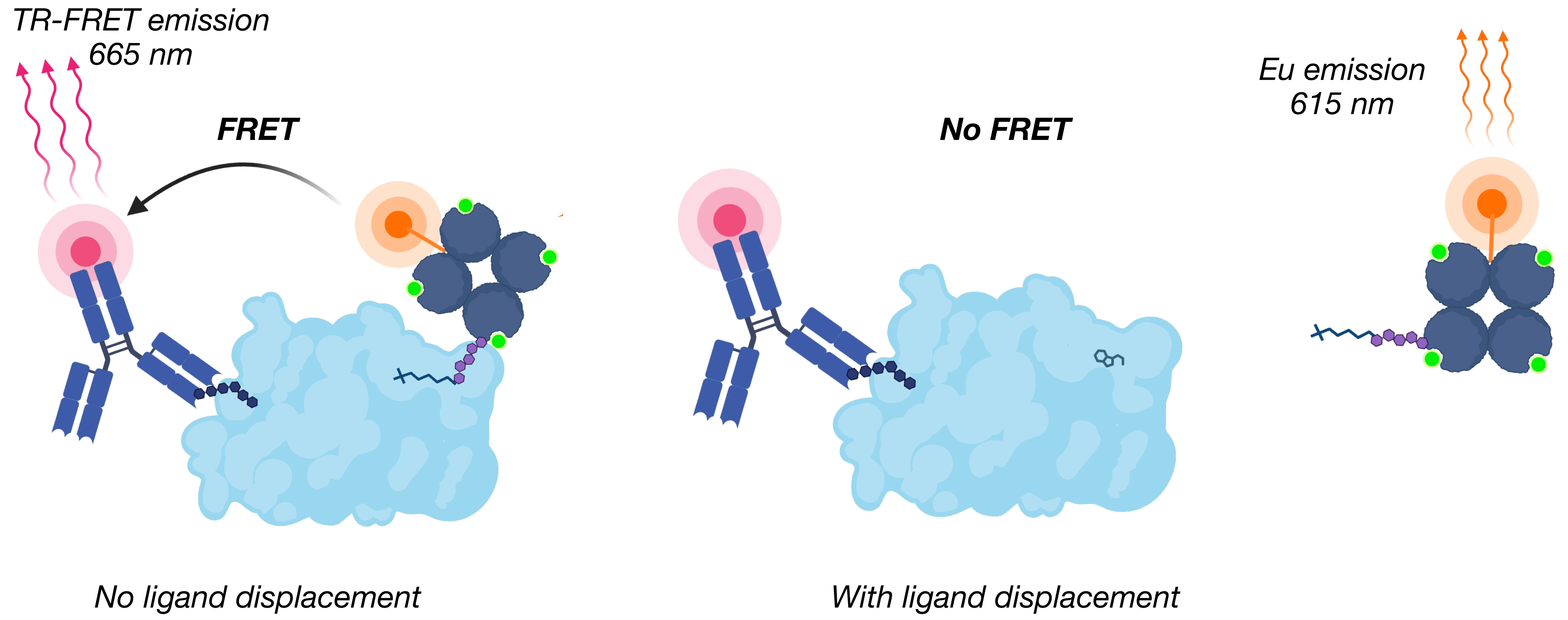
**With FRET: 528 nm emission**  
**No FRET: 485 nm emission**

**Similar to FLINT, FRET is sensitive to background signal**

# HTS in small molecule drug discovery

## Screening methods - biochemical assays

### Time-resolved fluorescent resonance energy transfer (TR-FRET)

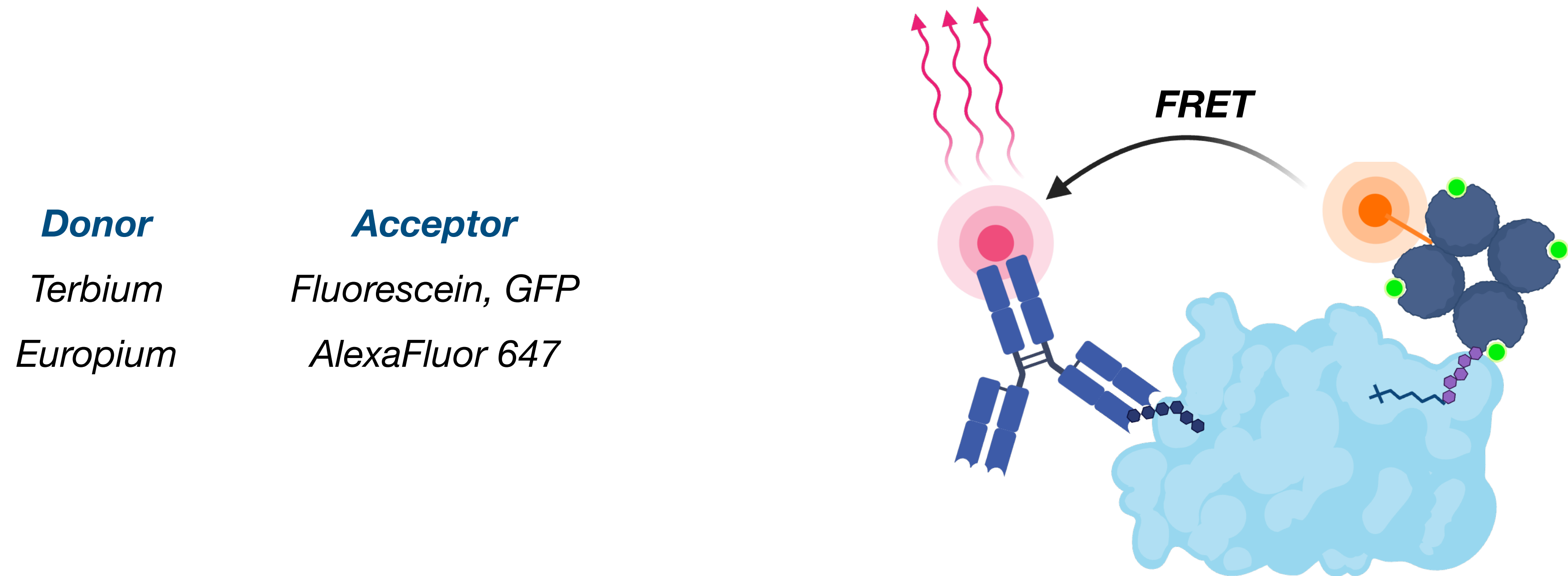


### Redshifted FRET emission reduces interference

# HTS in small molecule drug discovery

Screening methods - biochemical assays

## Time-resolved fluorescent resonance energy transfer (TR-FRET)



**Lanthanide complexes has fluorescent lifetimes of 100  $\mu$ s to ms, much longer than the ps range of organic library molecules**



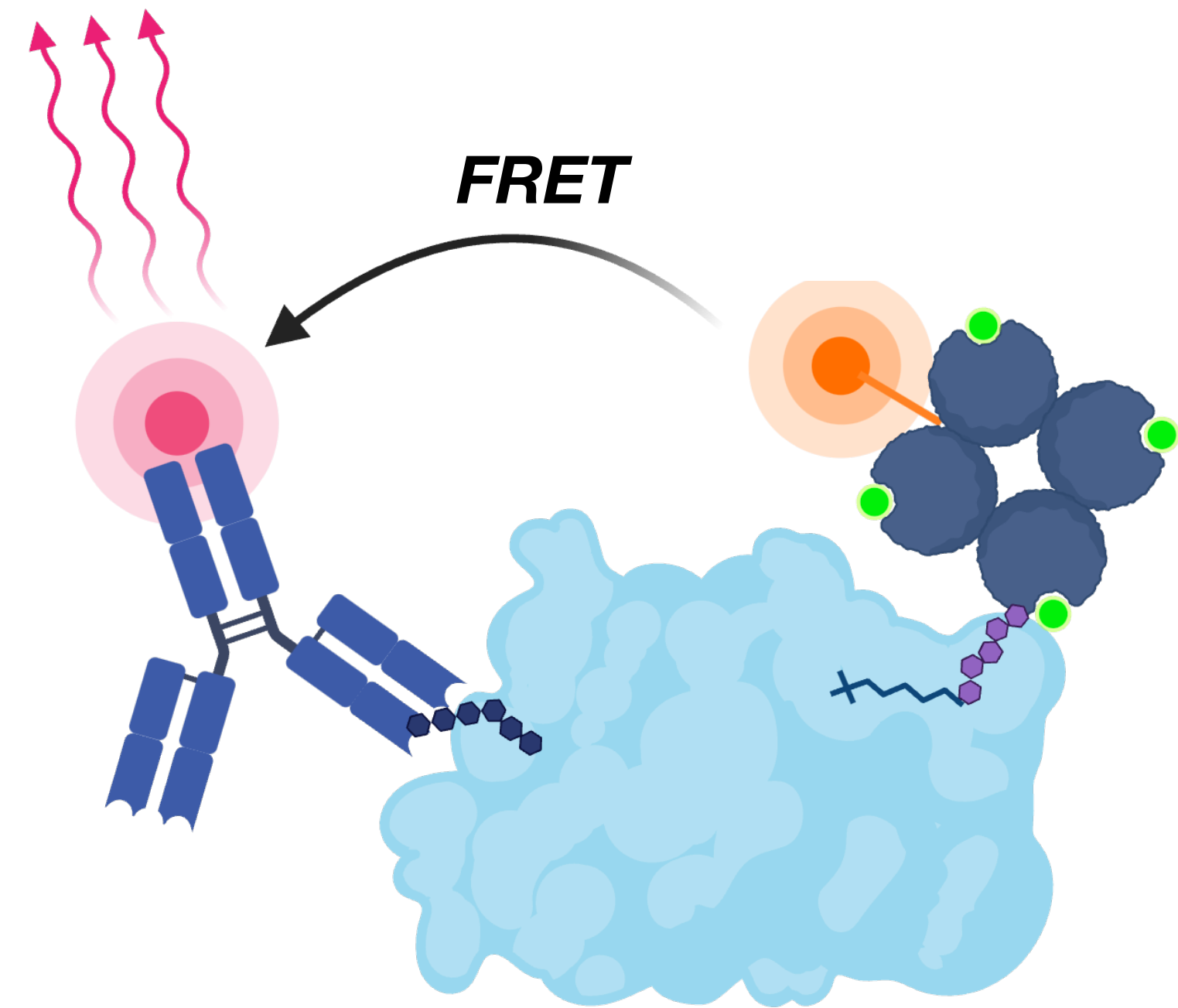
# HTS in small molecule drug discovery

Screening methods - biochemical assays

## Time-resolved fluorescent resonance energy transfer (TR-FRET)

**Donor**  
Terbium  
Europium

**Acceptor**  
Fluorescein, GFP  
AlexaFluor 647



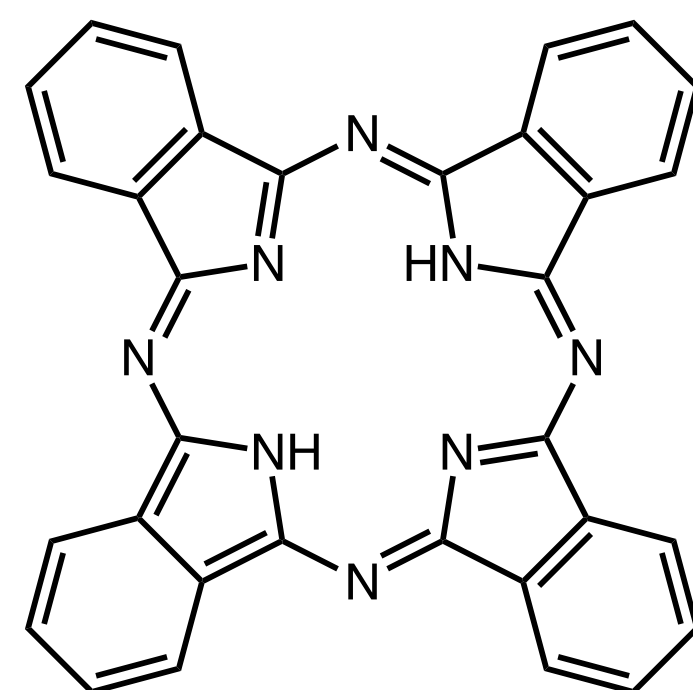
**Time-gated and red-shifted FRET to reduce interference**

# HTS in small molecule drug discovery

## Screening methods - biochemical assays

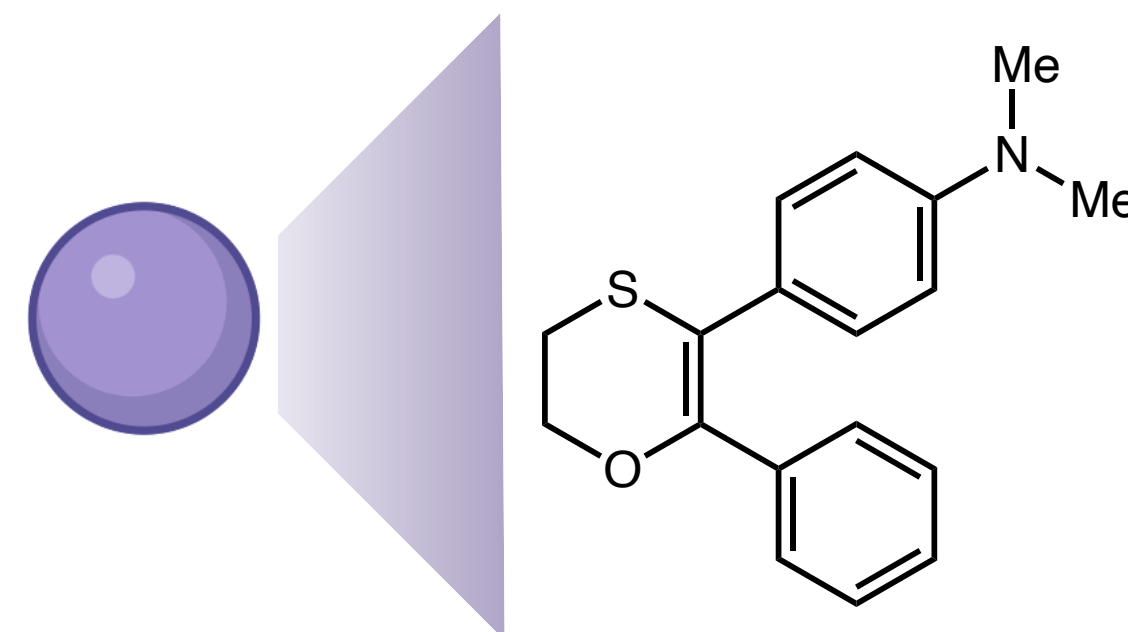
### Amplified Luminescent Proximity Homogeneous Assay (AlphaScreen)

Donor bead



Phthalocyanine  
(Photosensitizer)

Acceptor bead



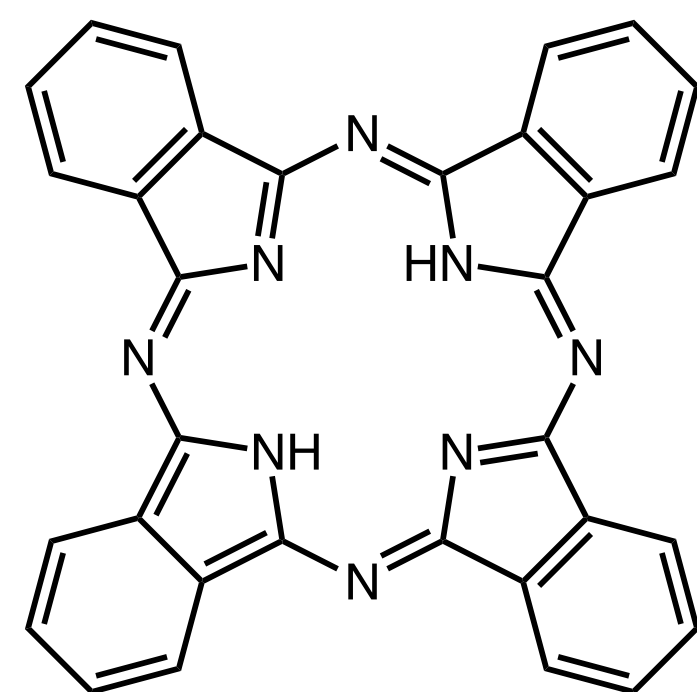
Thioxene

# HTS in small molecule drug discovery

## Screening methods - biochemical assays

### Amplified Luminescent Proximity Homogeneous Assay (AlphaScreen)

Donor bead

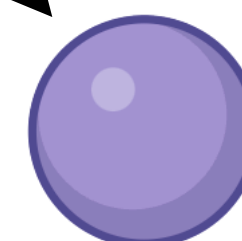
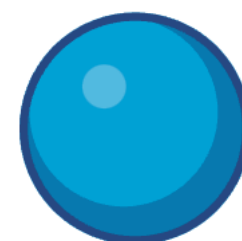


Phthalocyanine  
(Photosensitizer)

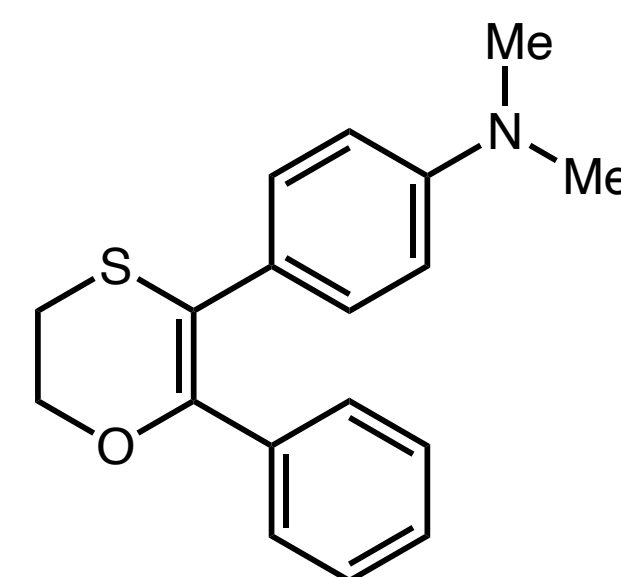
680 nm excitation



$^1O_2$



Acceptor bead

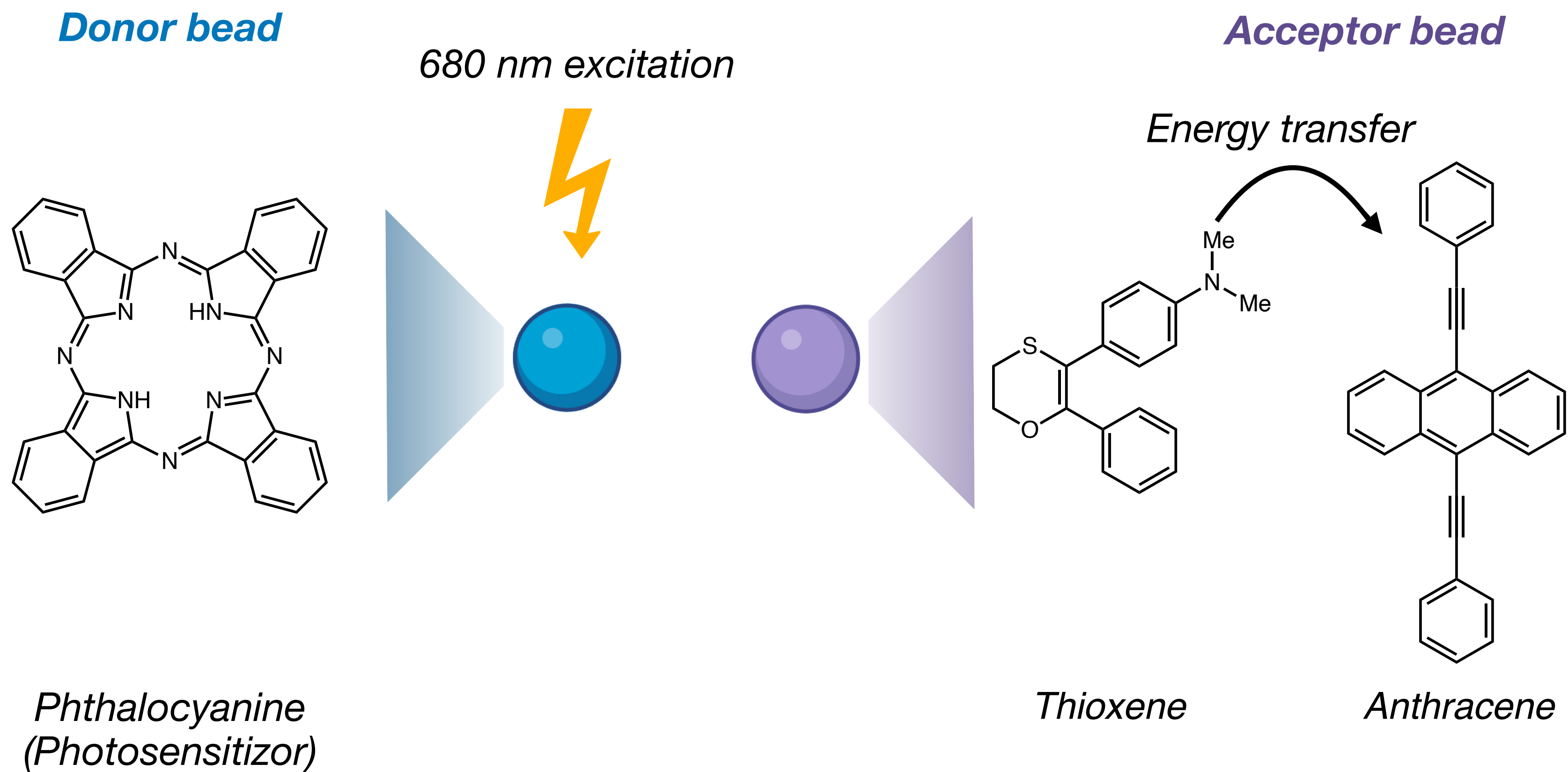


Thioxene

# HTS in small molecule drug discovery

## Screening methods - biochemical assays

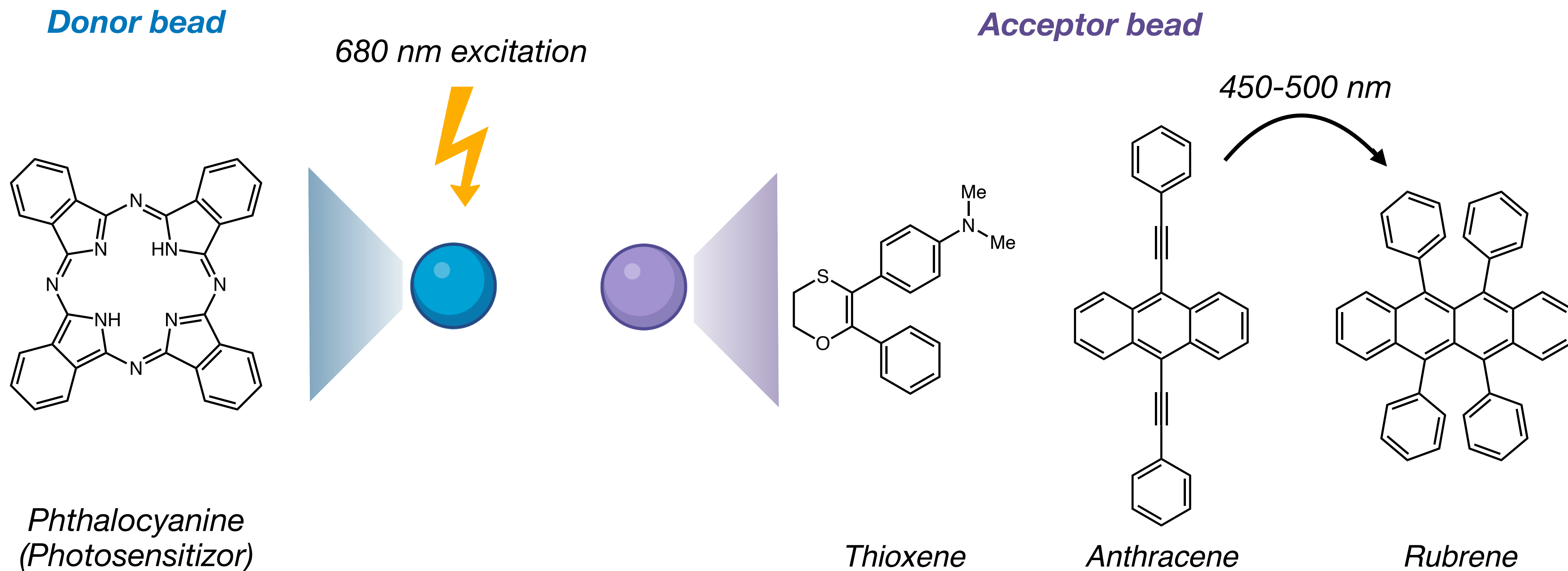
### Amplified Luminescent Proximity Homogeneous Assay (AlphaScreen)



# HTS in small molecule drug discovery

## Screening methods - biochemical assays

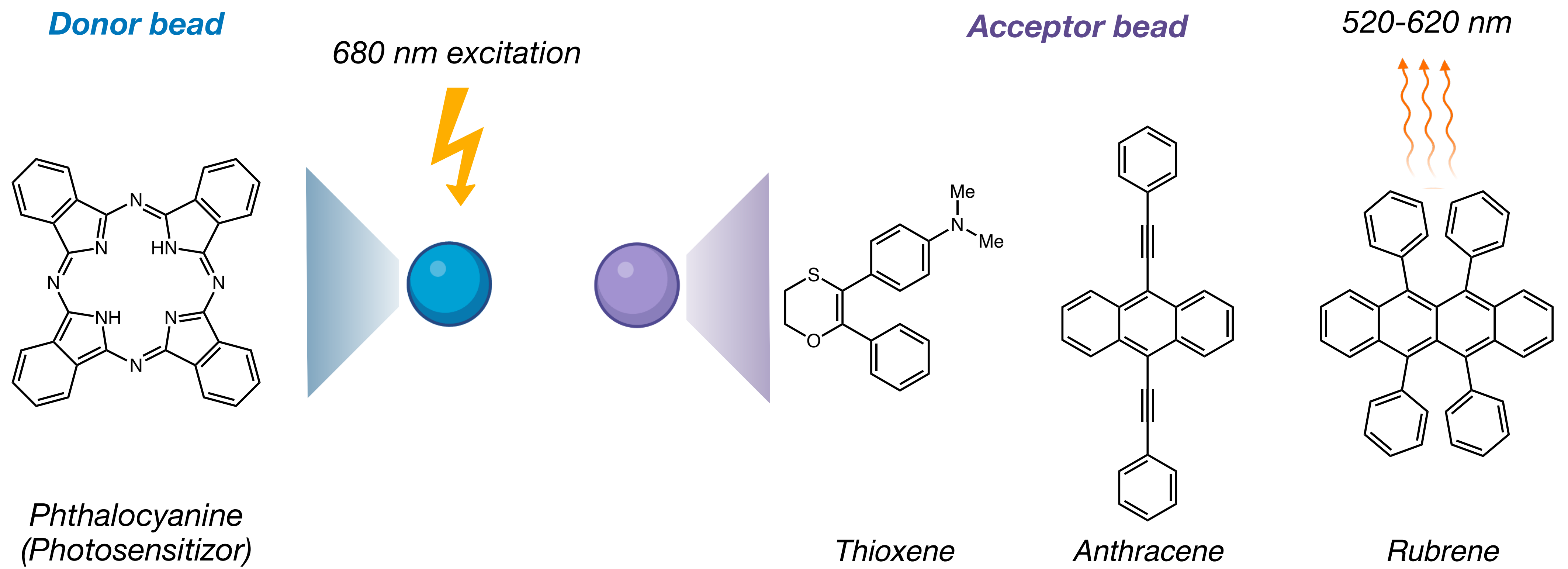
### Amplified Luminescent Proximity Homogeneous Assay (AlphaScreen)



# HTS in small molecule drug discovery

## Screening methods - biochemical assays

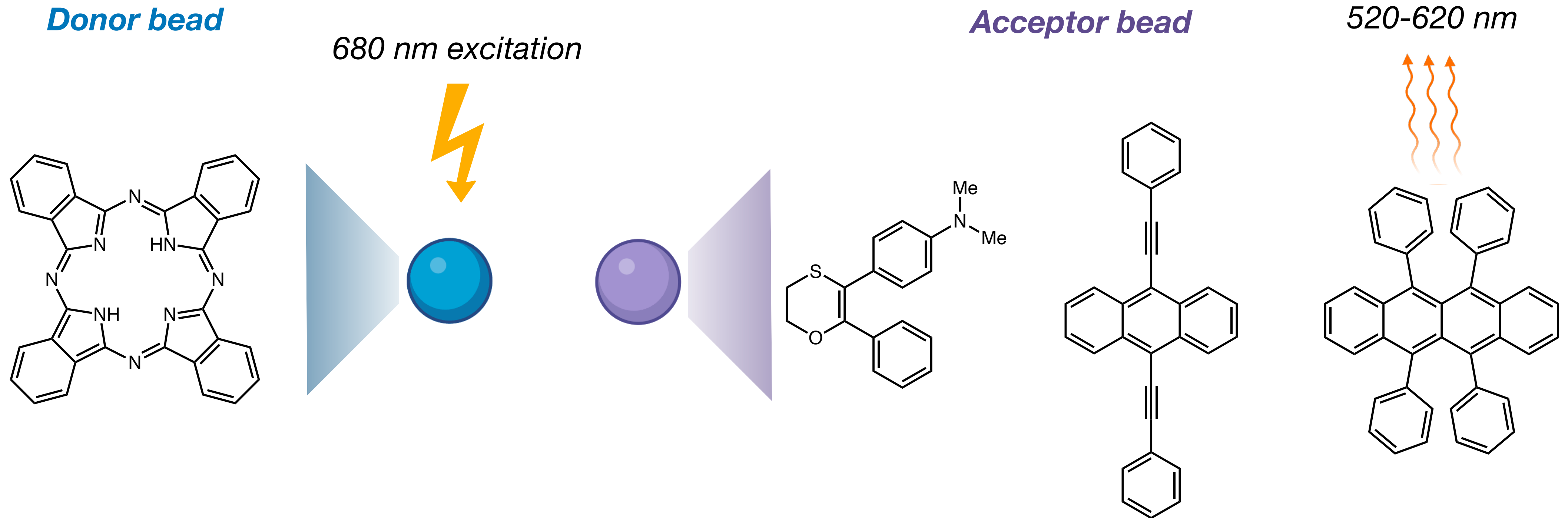
### Amplified Luminescent Proximity Homogeneous Assay (AlphaScreen)



# HTS in small molecule drug discovery

## Screening methods - biochemical assays

### Amplified Luminescent Proximity Homogeneous Assay (AlphaScreen)

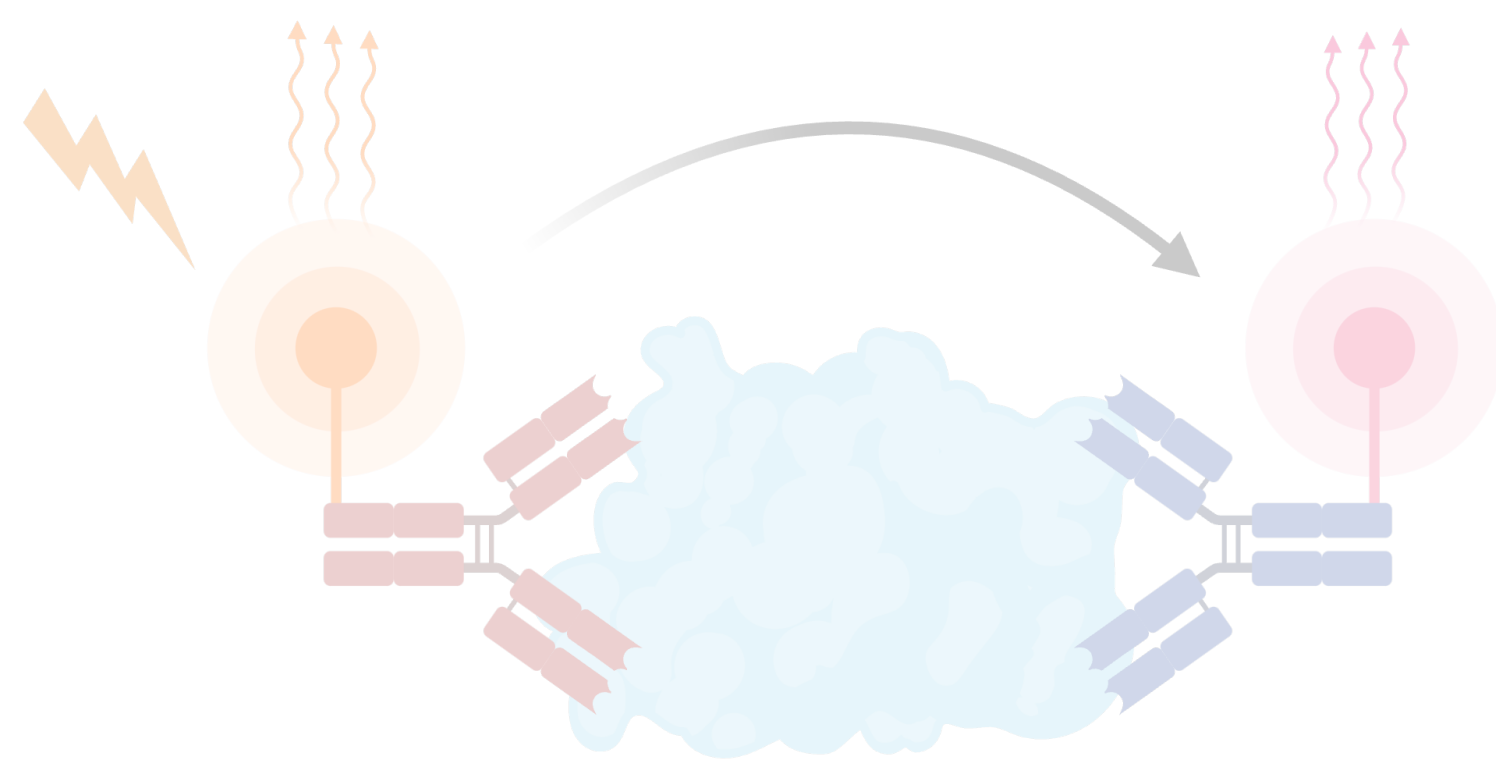


**Beads can be conjugated with peptides, proteins, or tracer ligands for assays**

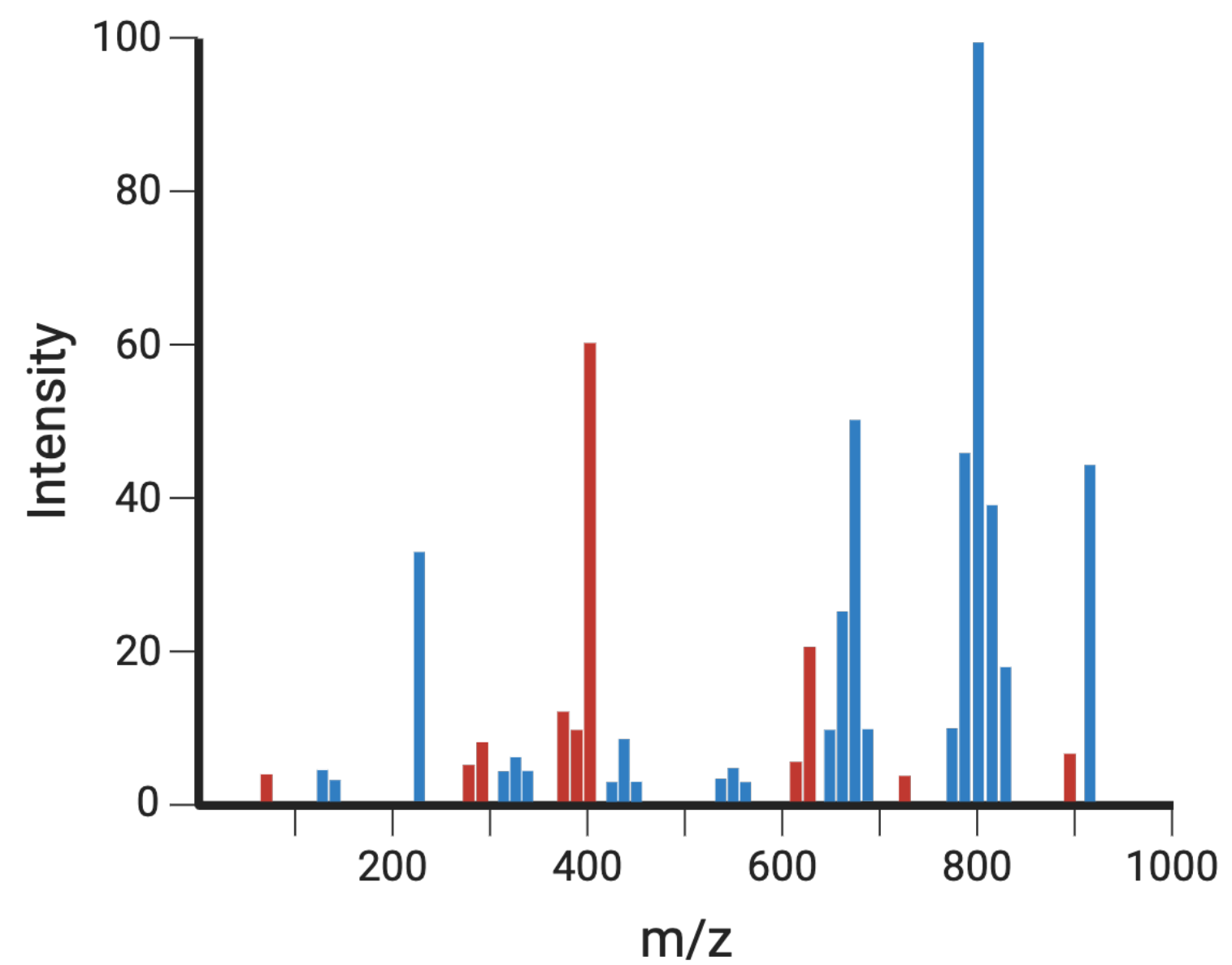
**AlphaLISA available as a faster alternative to ELISA**

# *HTS in small molecule drug discovery*

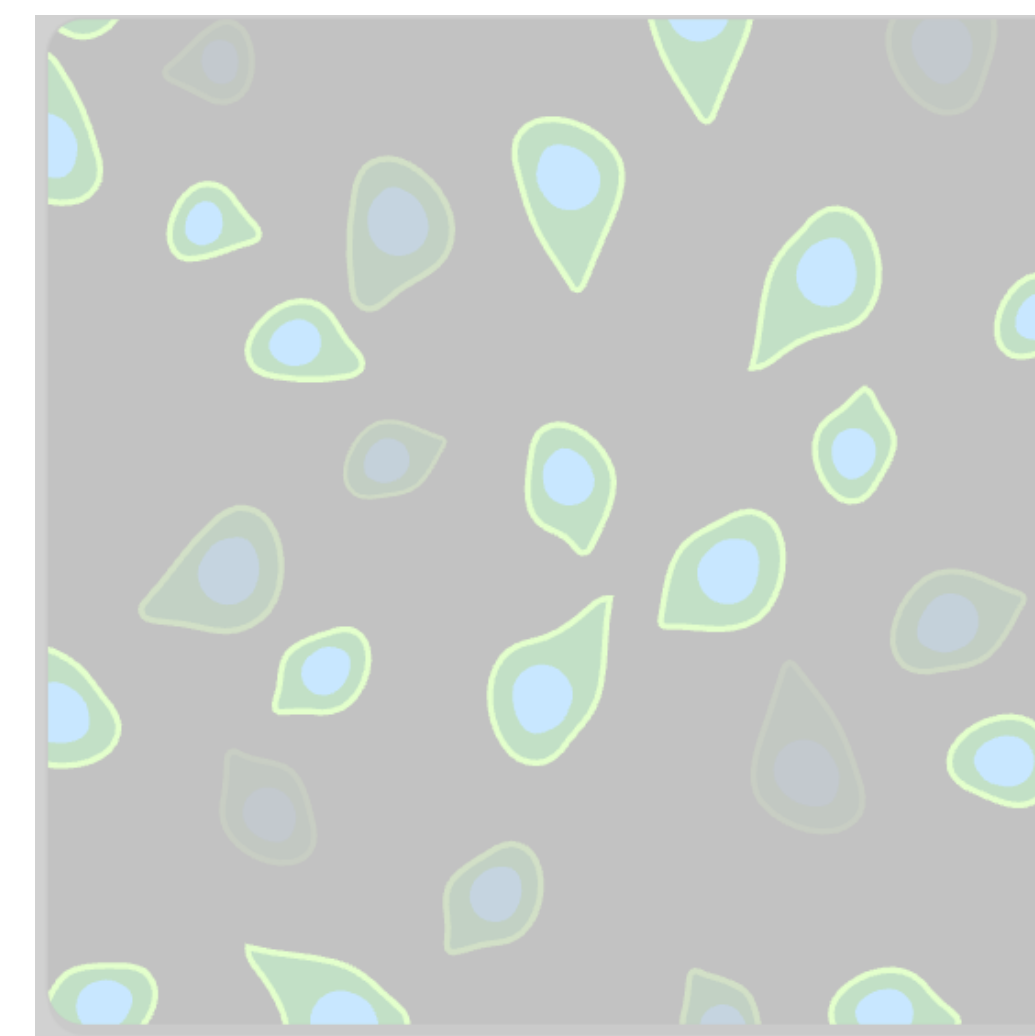
## *Screening methods*



*Biochemical assays*



*Spectroscopic assays*

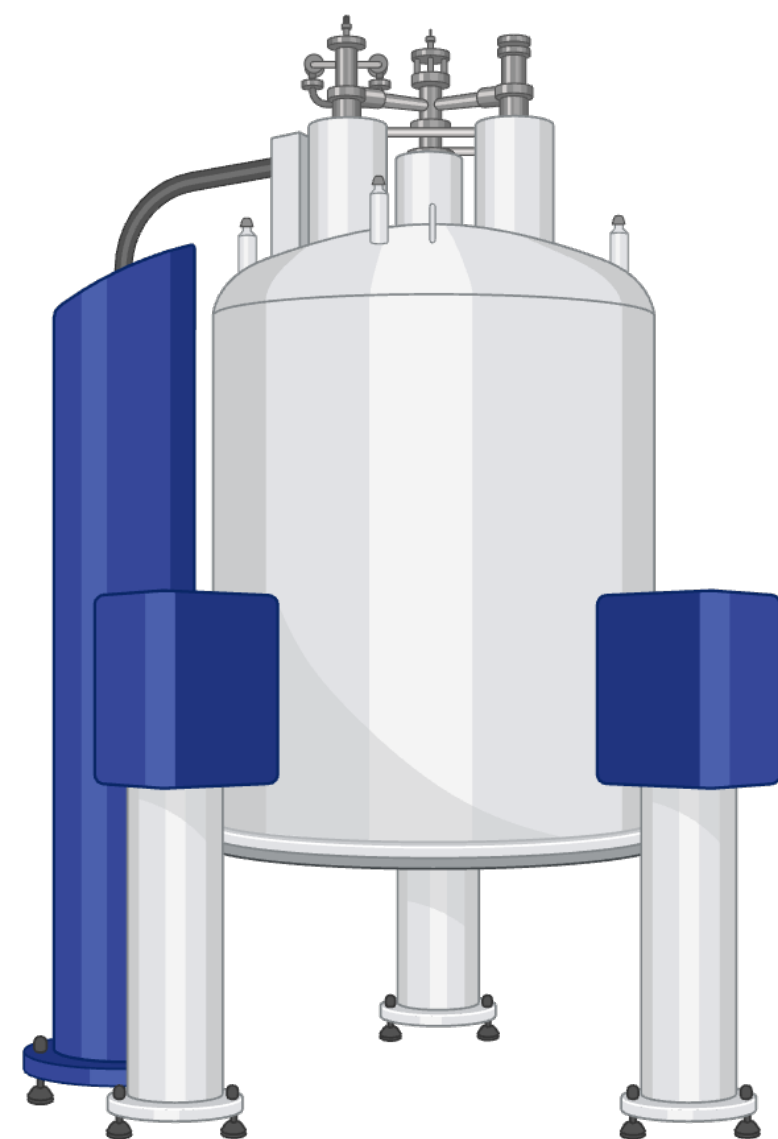


*Cell based assays*

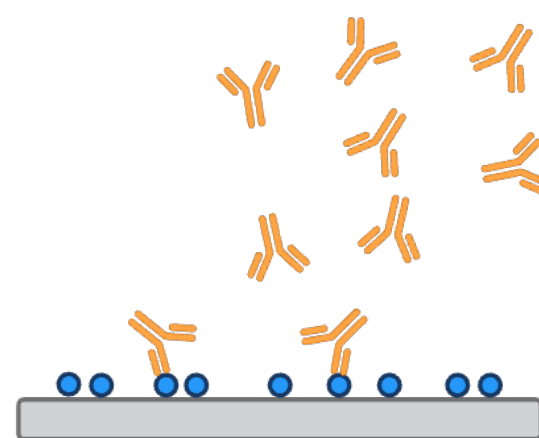


# *HTS in small molecule drug discovery*

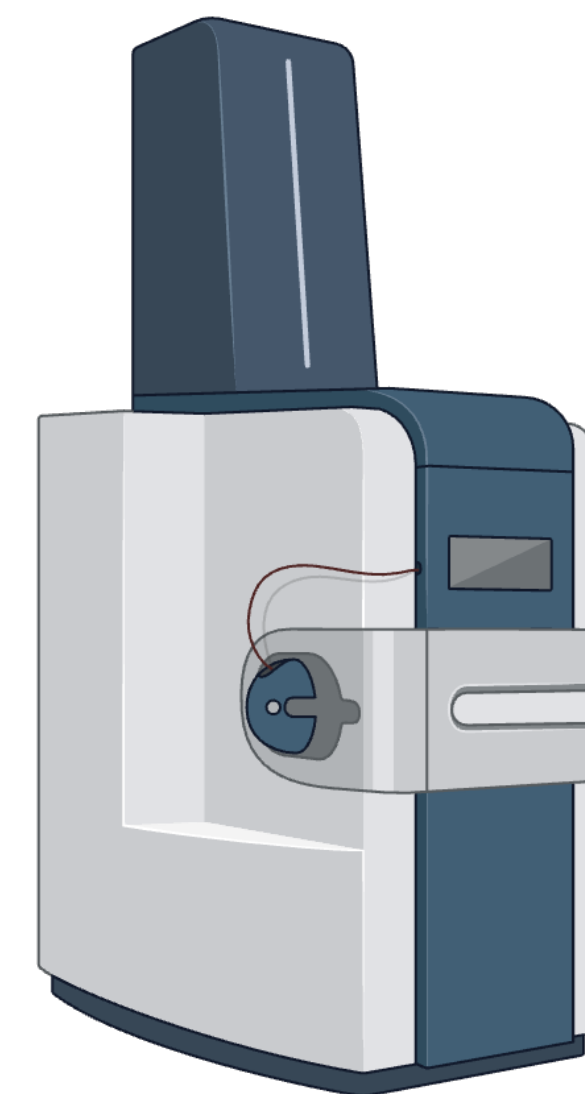
## *Screening methods - spectroscopic assays*



*NMR*



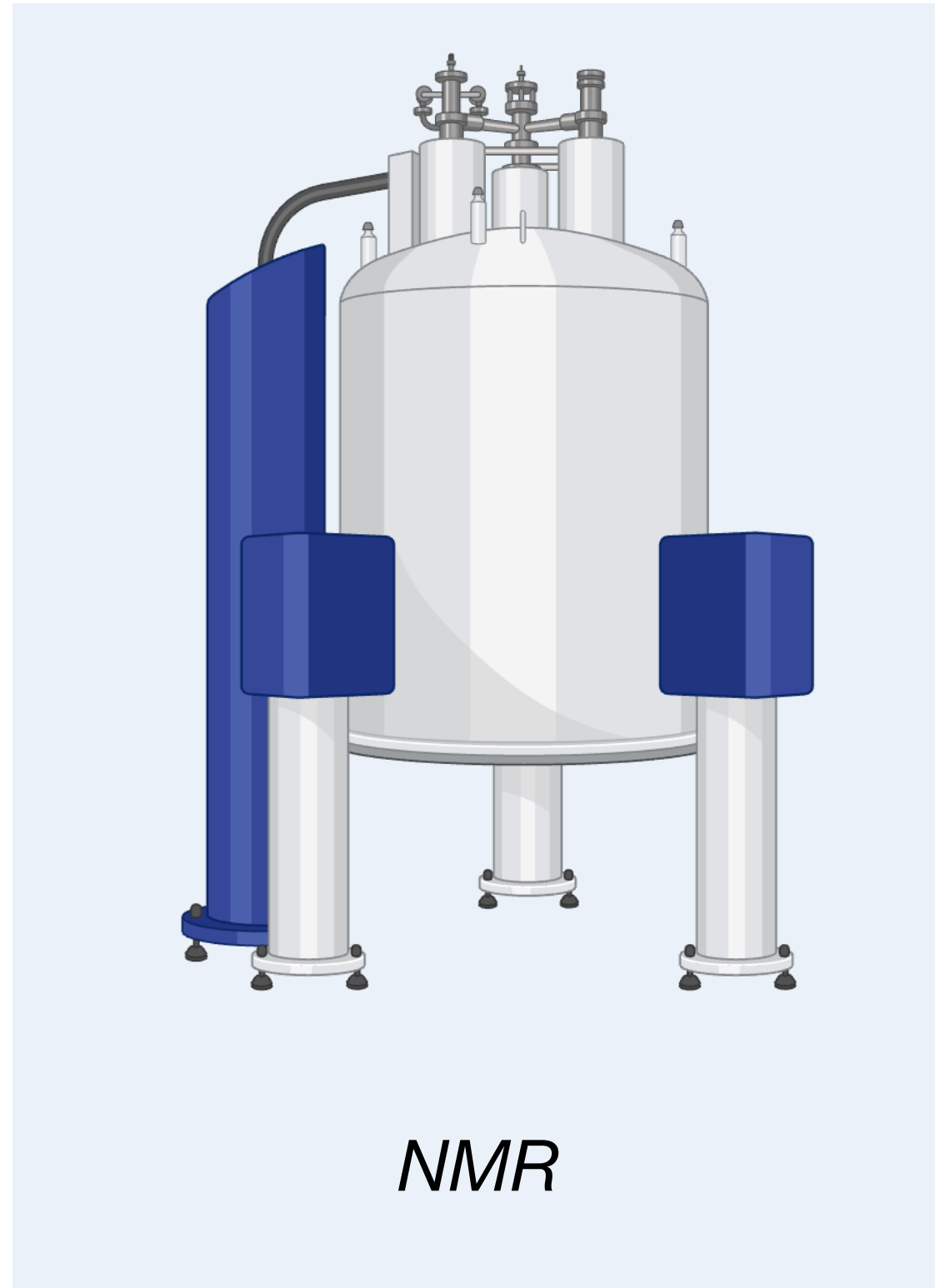
*Plate-based methods  
(e.g. SPR)*



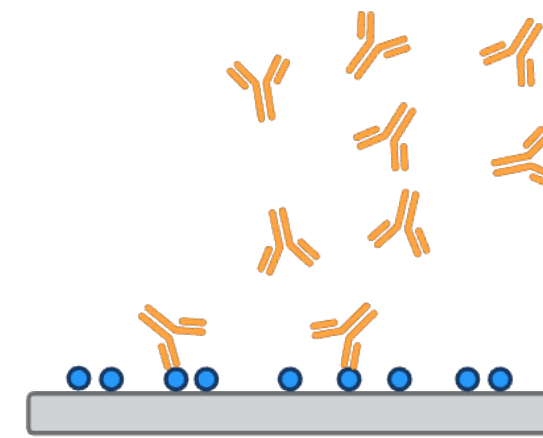
*Mass Spectrometry*

# *HTS in small molecule drug discovery*

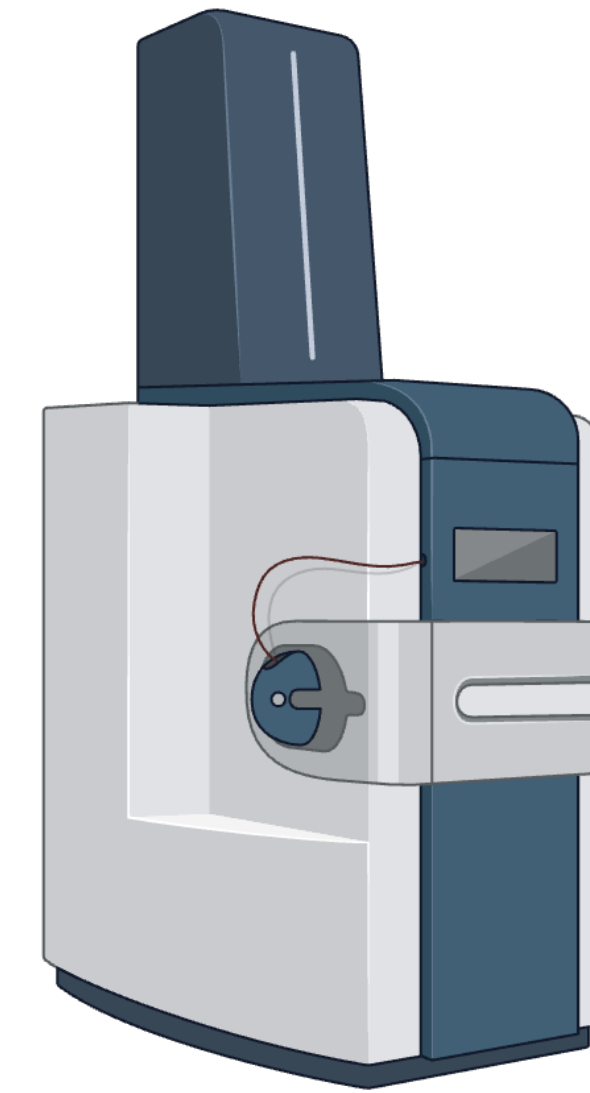
## *Screening methods - spectroscopic assays*



*NMR*



*Plate-based methods  
(e.g. SPR)*



*Mass Spectrometry*

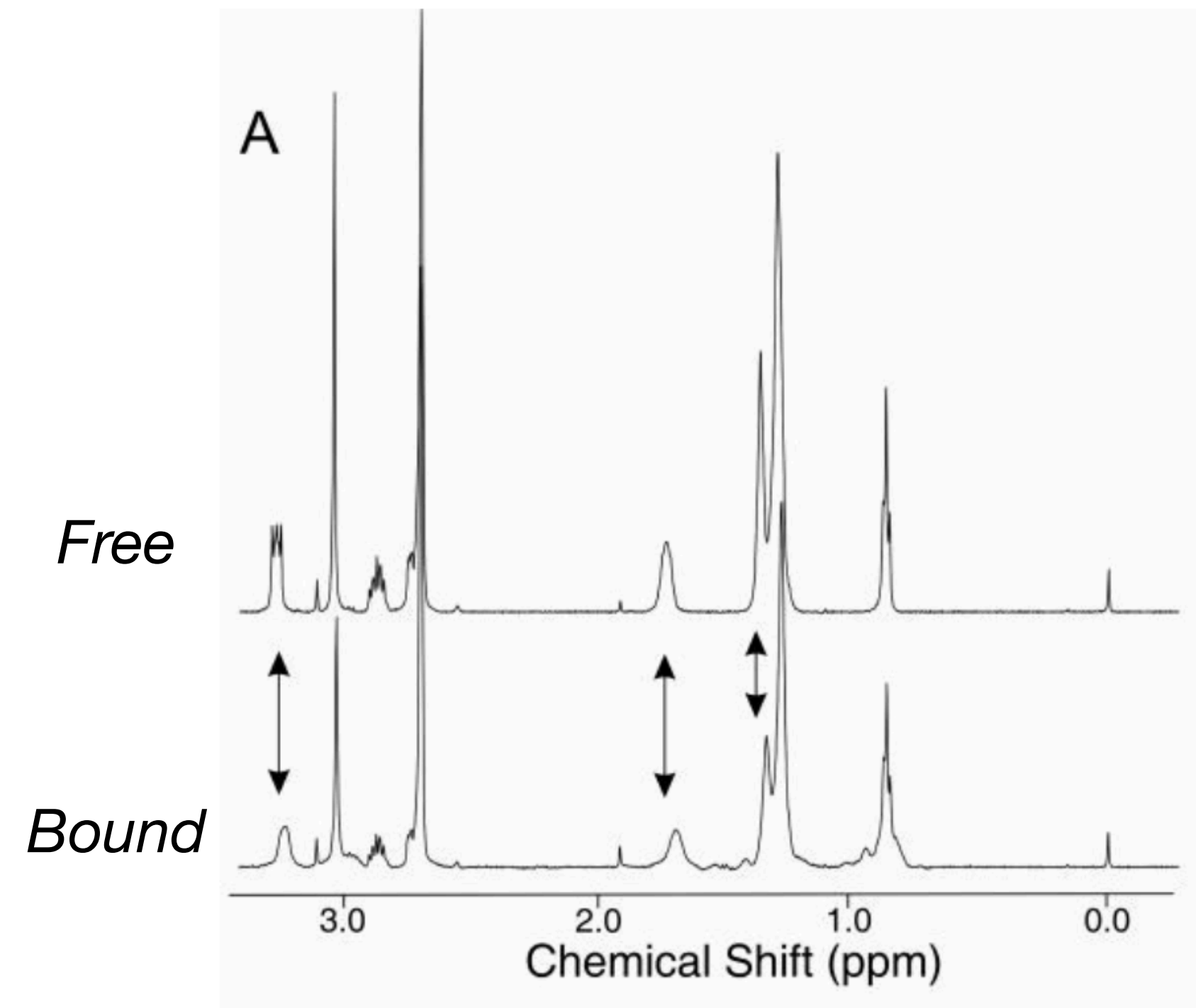
***More compatible with fragment based drug design***

***Less prone to artifacts but usually less high throughput***

# *HTS in small molecule drug discovery*

## *Screening methods - spectroscopic assays*

### ***Ligand detected NMR***

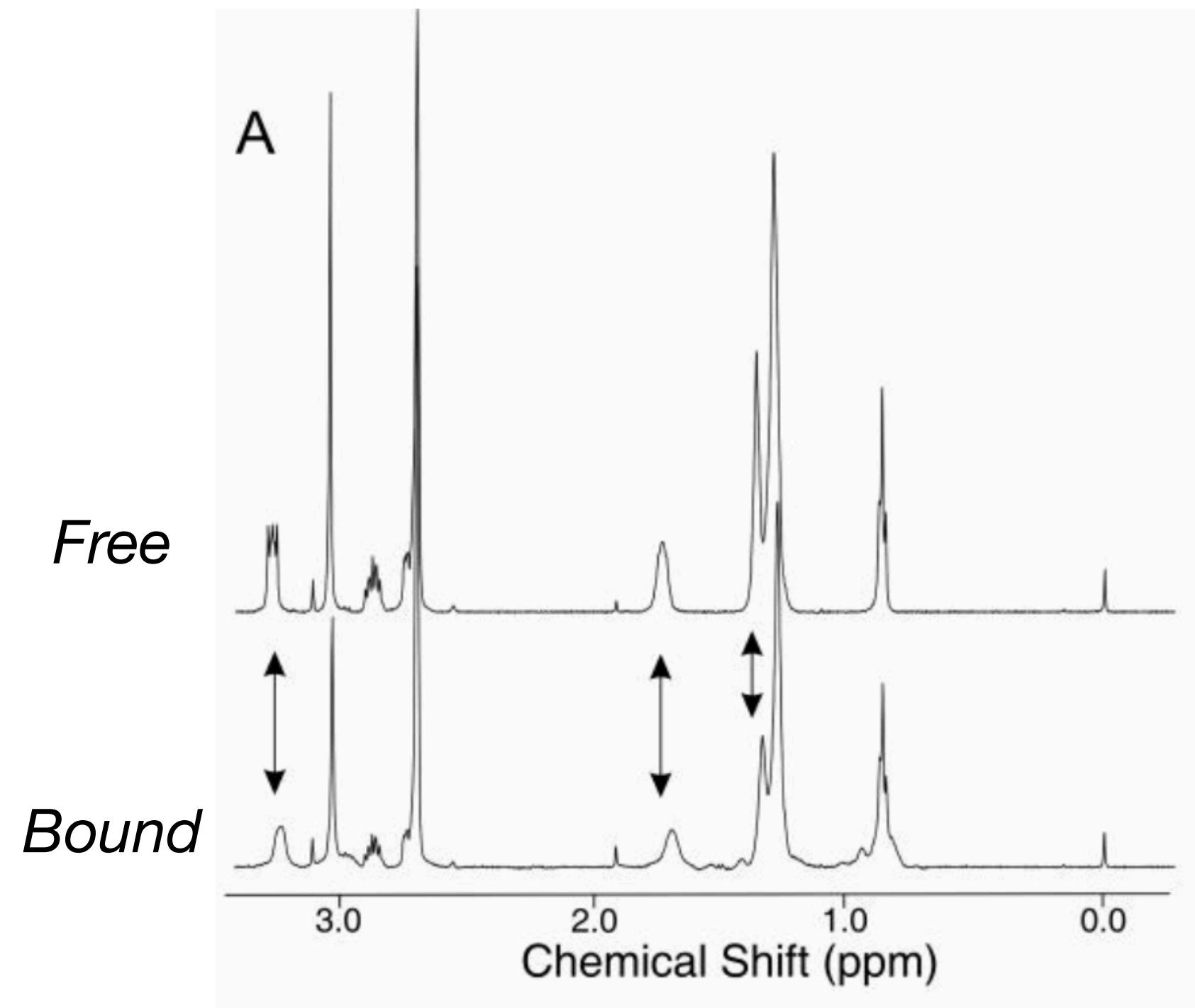


***Broadening of peaks and decreases in intensity***

# HTS in small molecule drug discovery

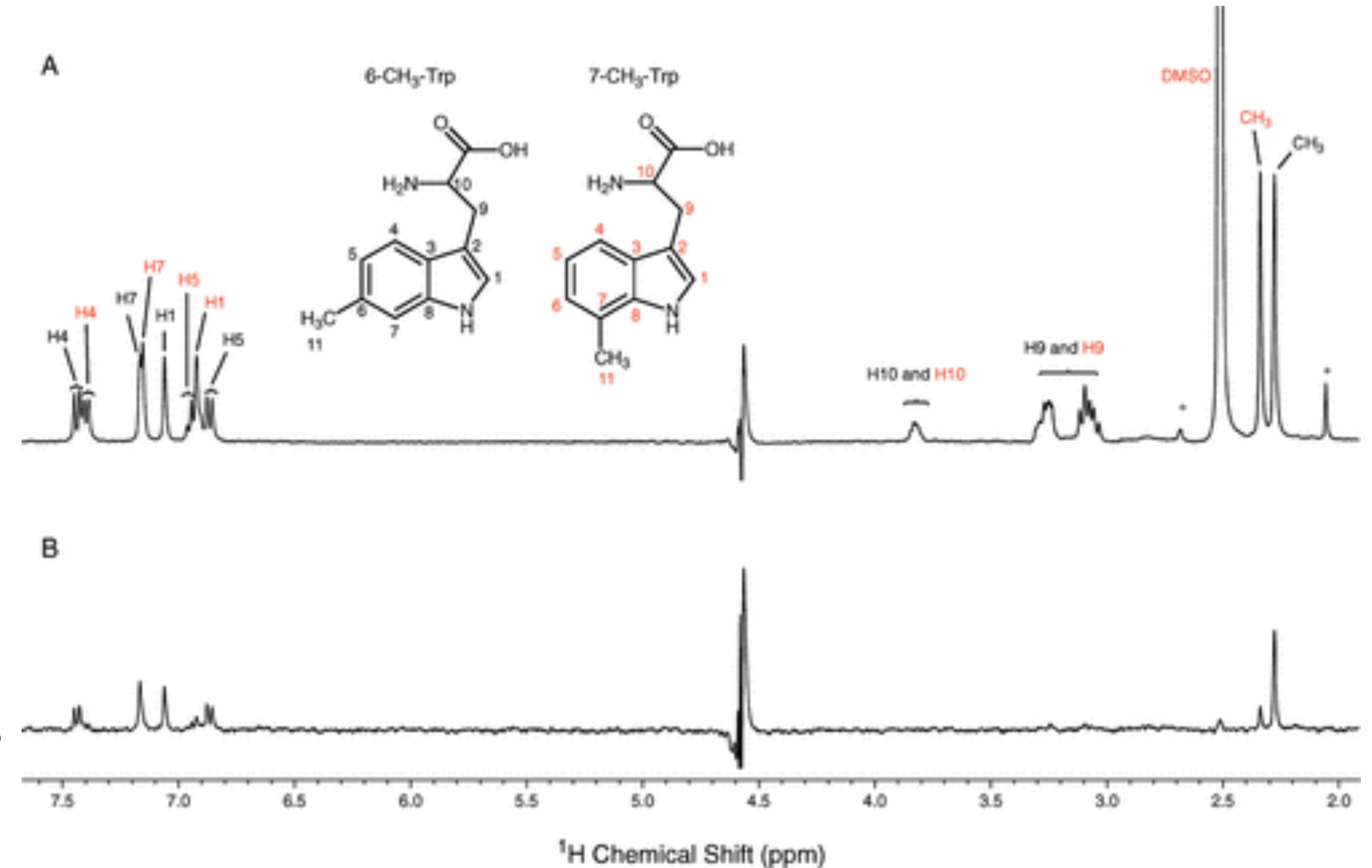
## Screening methods - spectroscopic assays

### Ligand detected NMR



$^1\text{H}$  NMR

STD NMR



Mixture of serum albumin,, 6-CH<sub>3</sub>-Trp, and 7-CH<sub>3</sub>-Trp

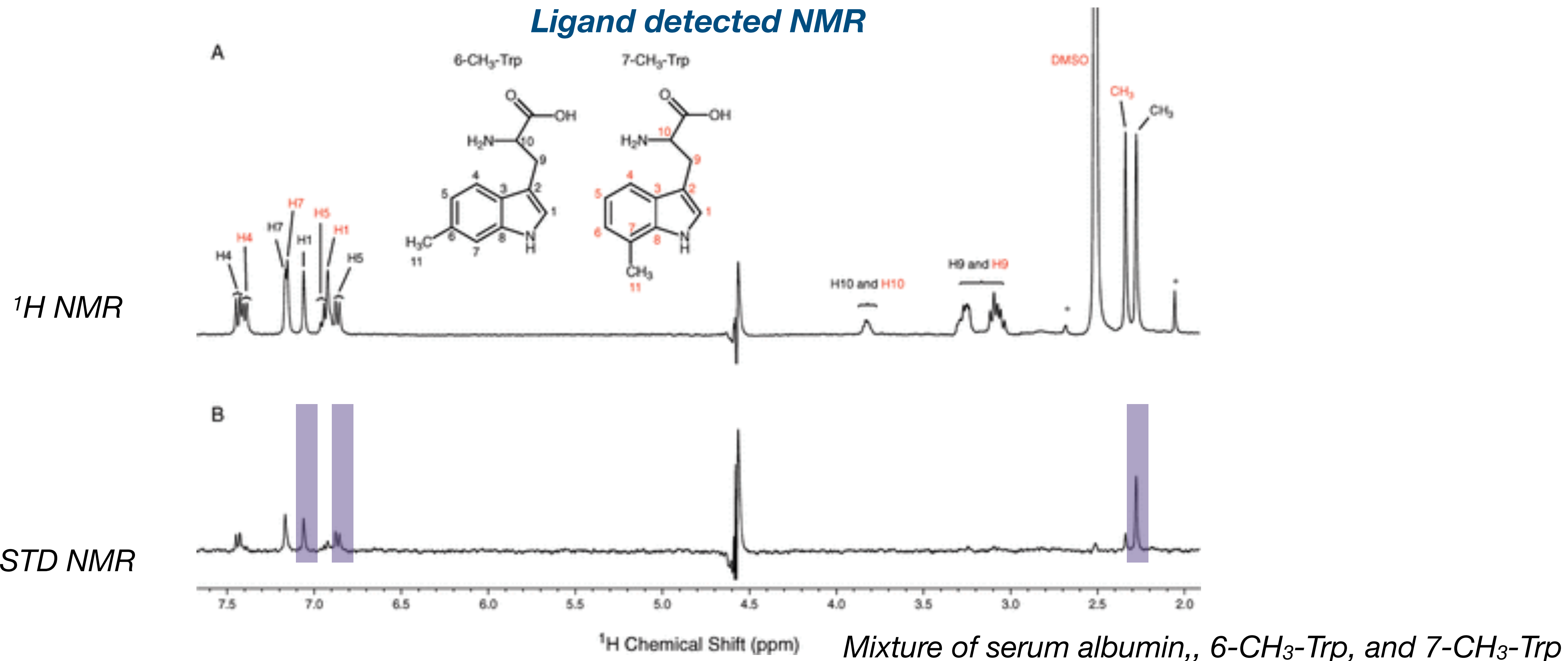
**Broadening of peaks and decreases in intensity**

**Only peaks of bound ligand are found in saturation transfer difference (STD) NMR**

# HTS in small molecule drug discovery

## Screening methods - spectroscopic assays

### Ligand detected NMR

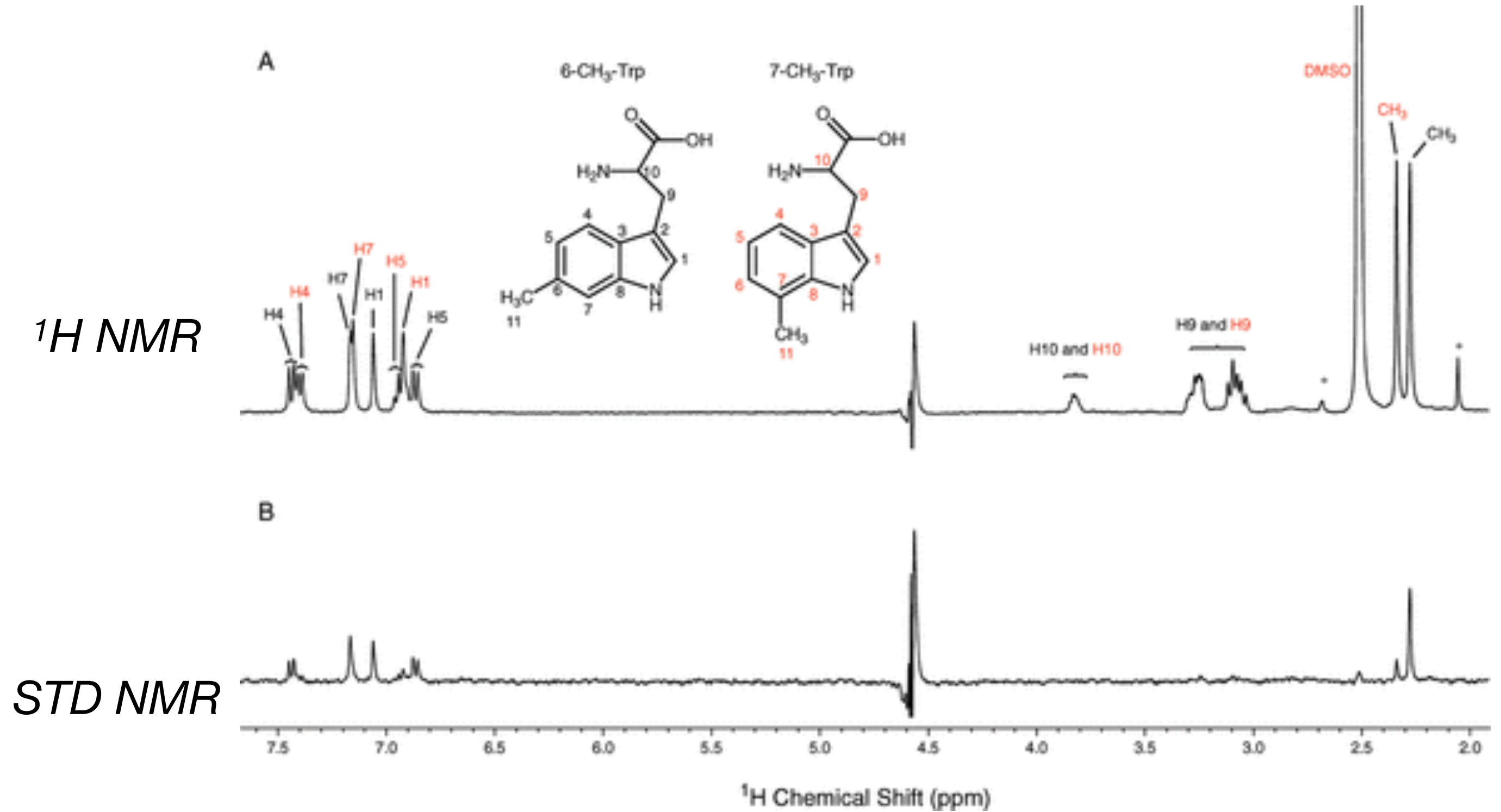
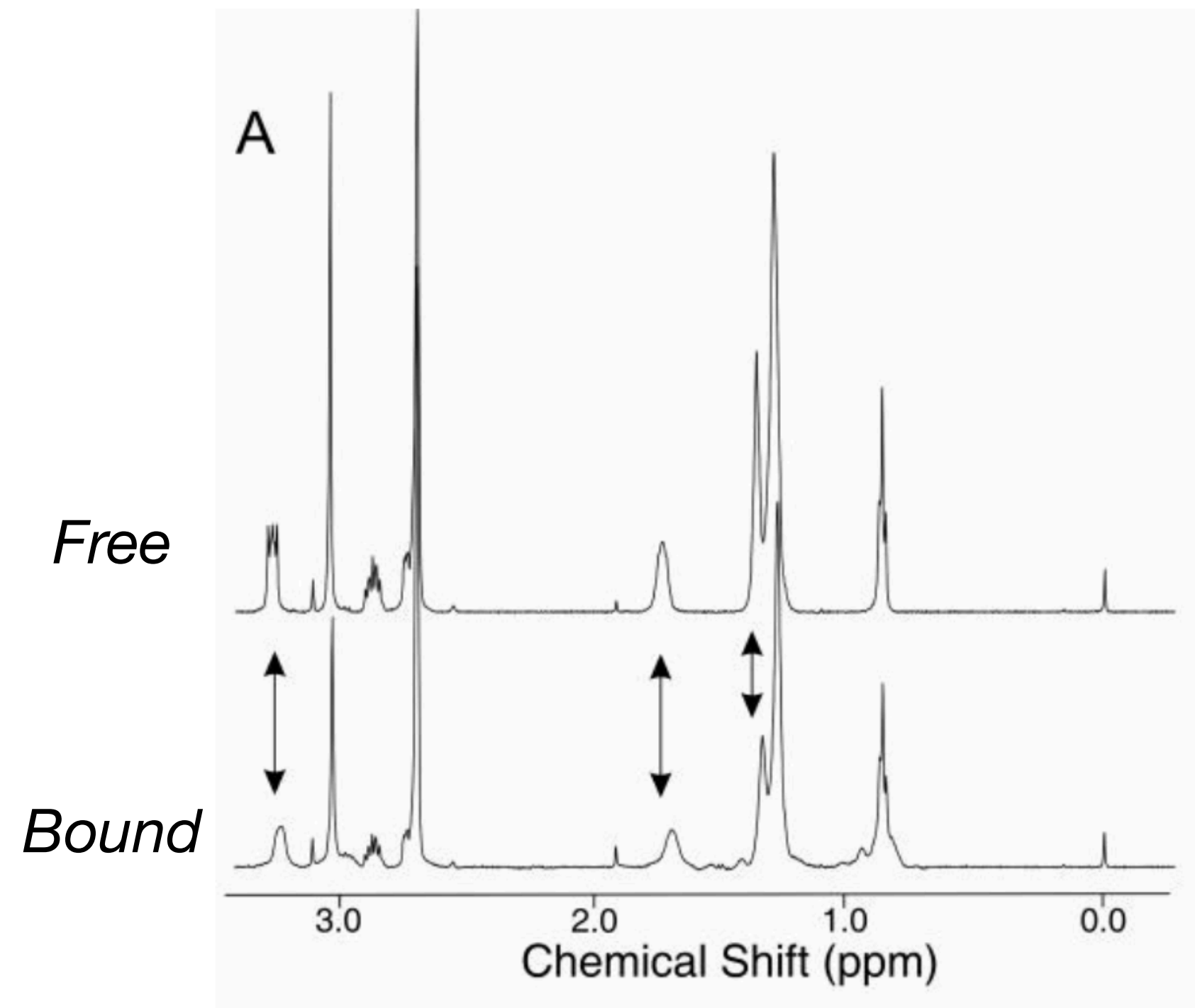


**Only 6-CH<sub>3</sub>-Trp peaks were found in STD NMR**

# HTS in small molecule drug discovery

## Screening methods - spectroscopic assays

### Ligand detected NMR

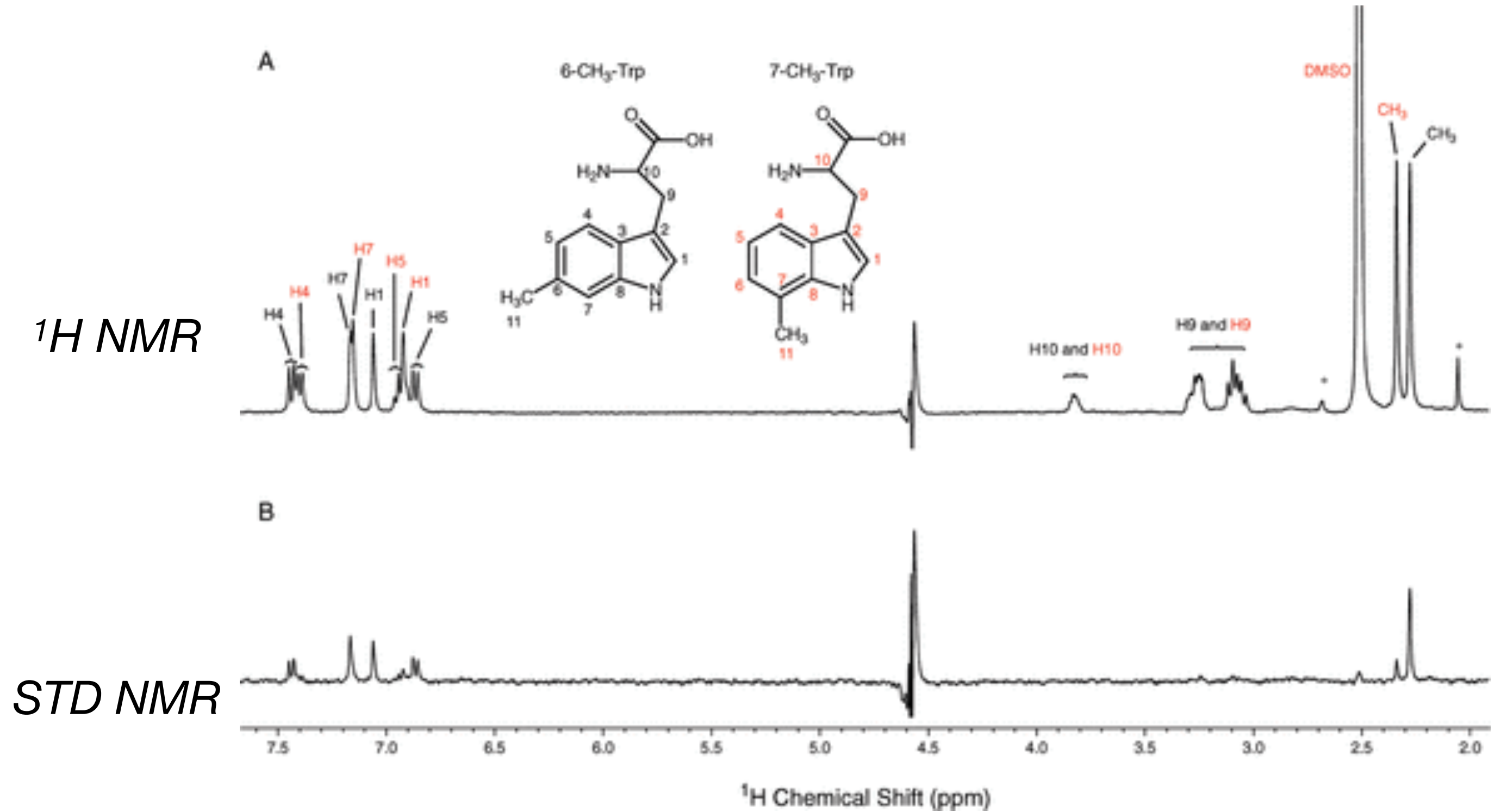
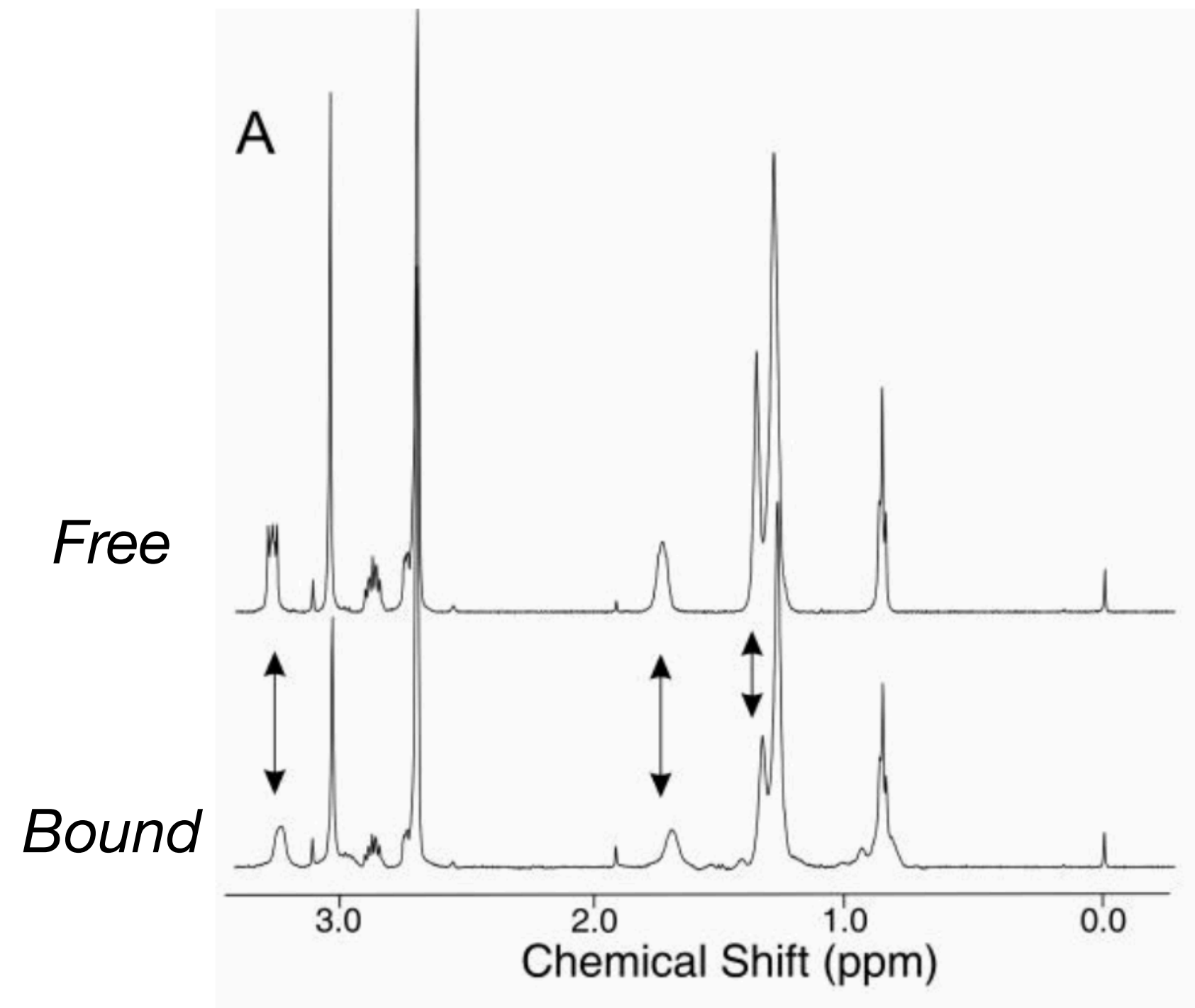


**Ligands bounded to protein exhibit negative Nuclear Overhauser Effect and longer molecular correlation times ( $\tau_c$ )**

# HTS in small molecule drug discovery

## Screening methods - spectroscopic assays

### Ligand detected NMR



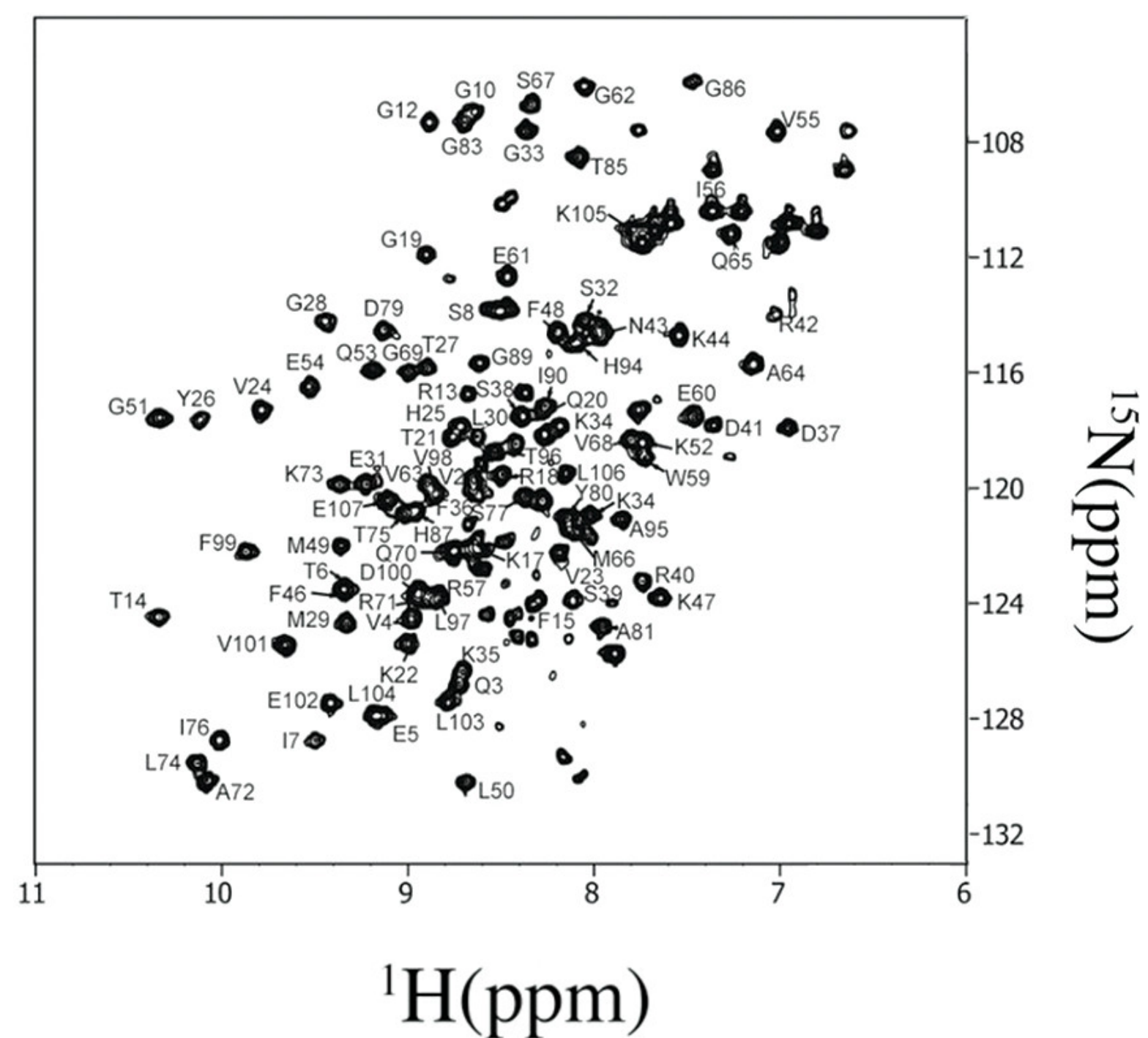
**No isotope labeling needed**

**Short experiment time and easy result interpretation**

# HTS in small molecule drug discovery

## Screening methods - spectroscopic assays

### Protein detected NMR



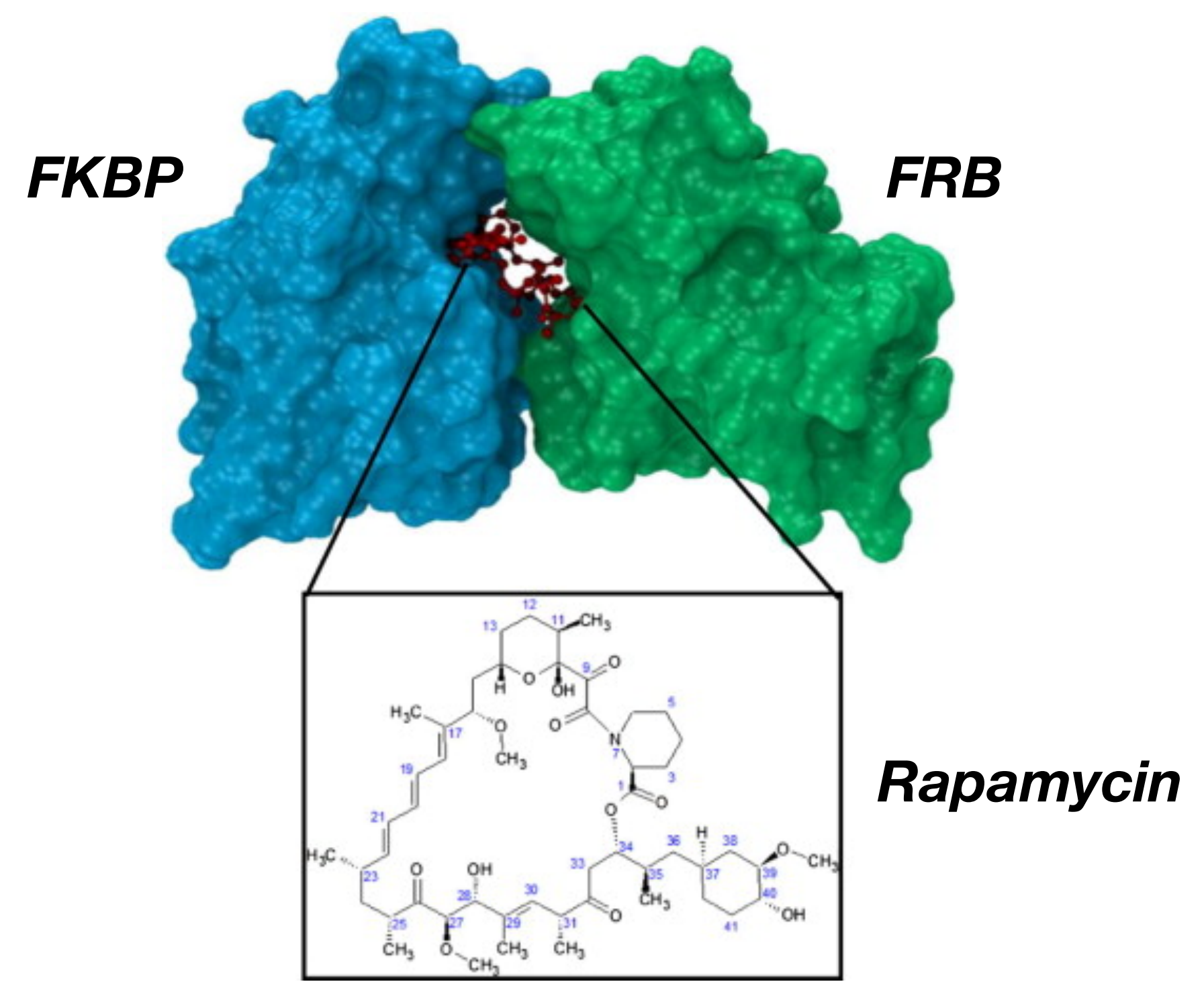
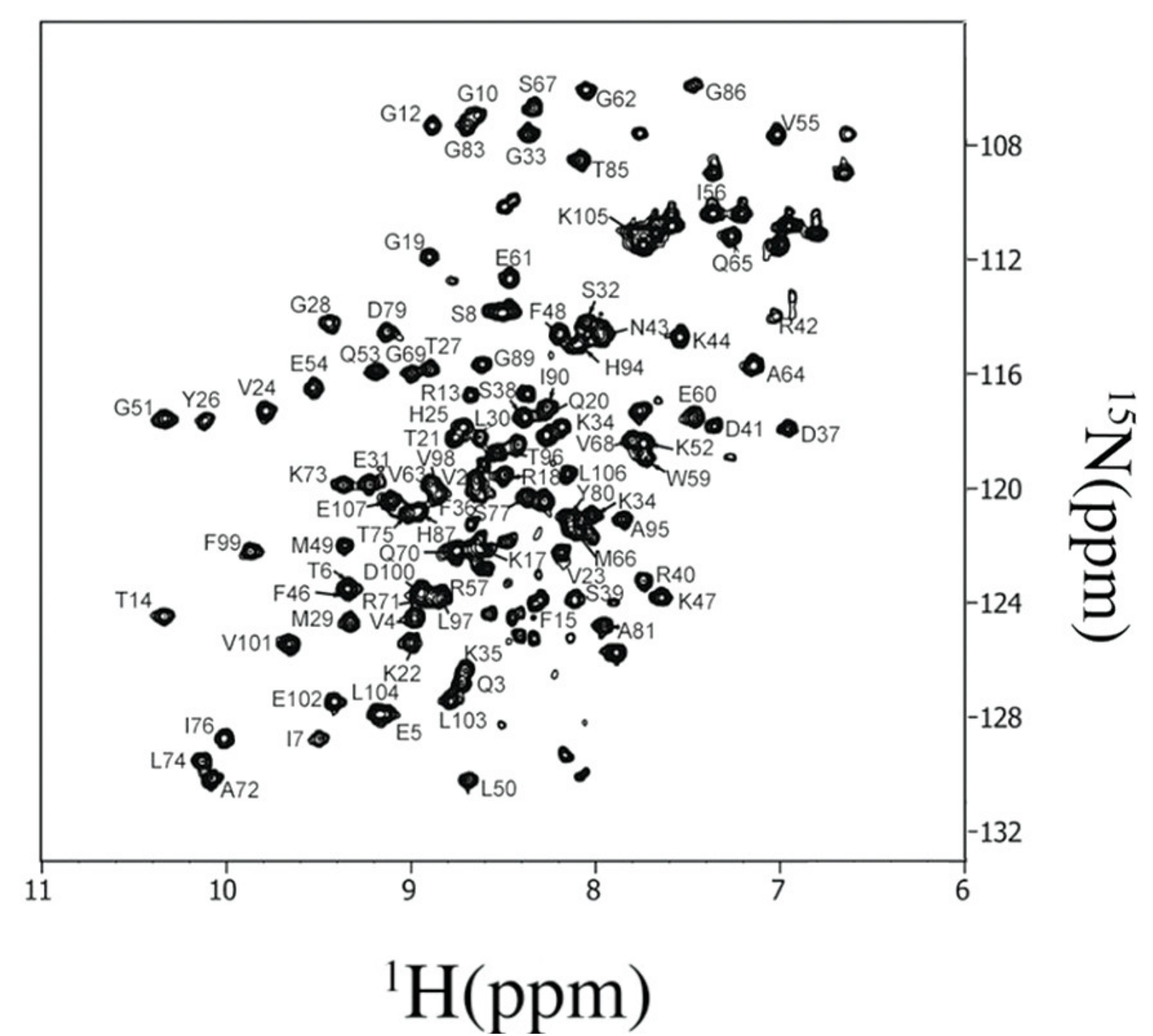
**$^1\text{H}$ - $^{15}\text{N}$  HSQC is commonly used to examine protein backbone environment**



# HTS in small molecule drug discovery

## Screening methods - spectroscopic assays

### Protein detected NMR



Formation of high affinity ternary complex

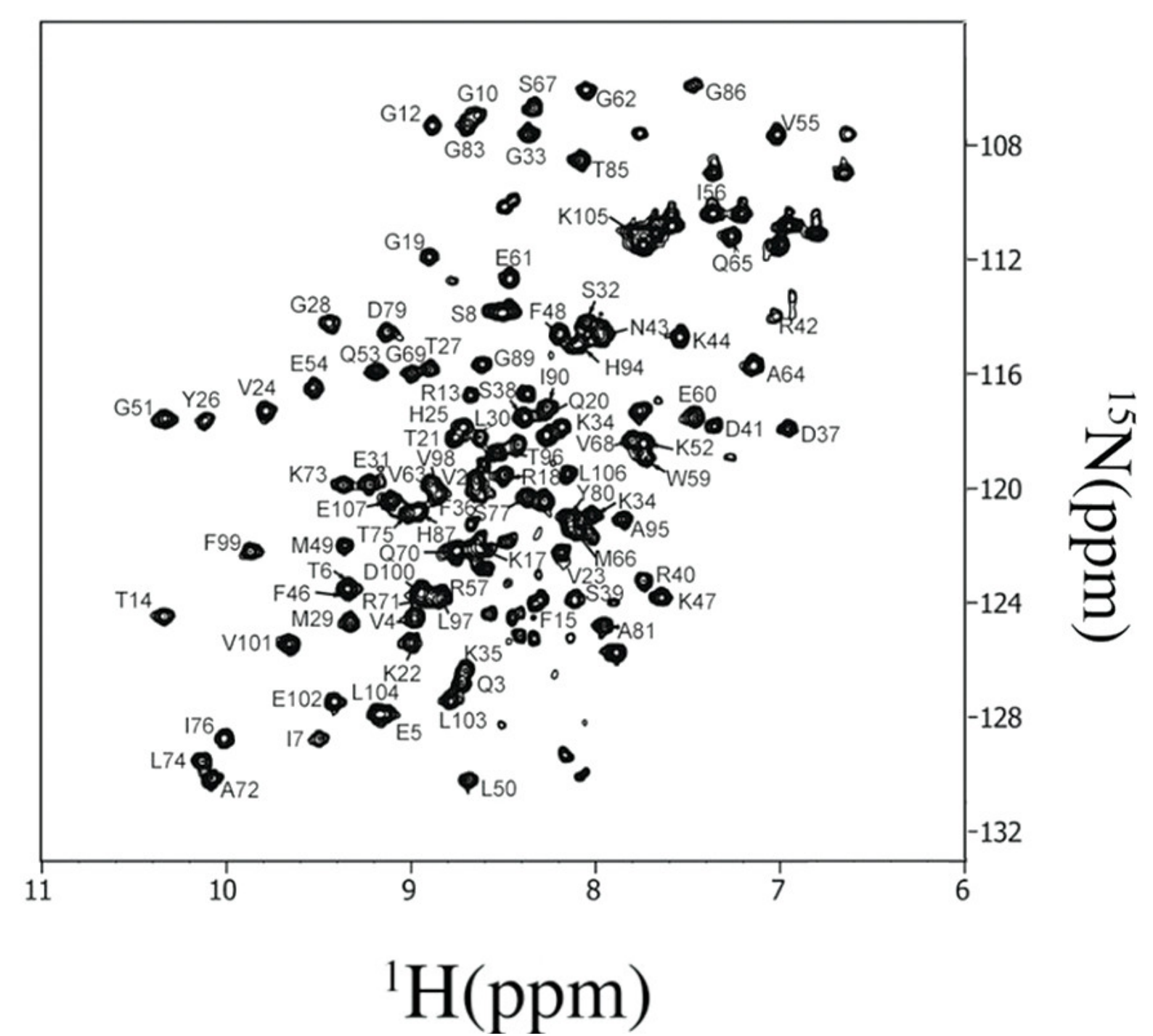
<sup>15</sup>N labeled FKBP and unlabeled FRB in *E. coli*

# HTS in small molecule drug discovery

## Screening methods - spectroscopic assays

### Protein detected NMR

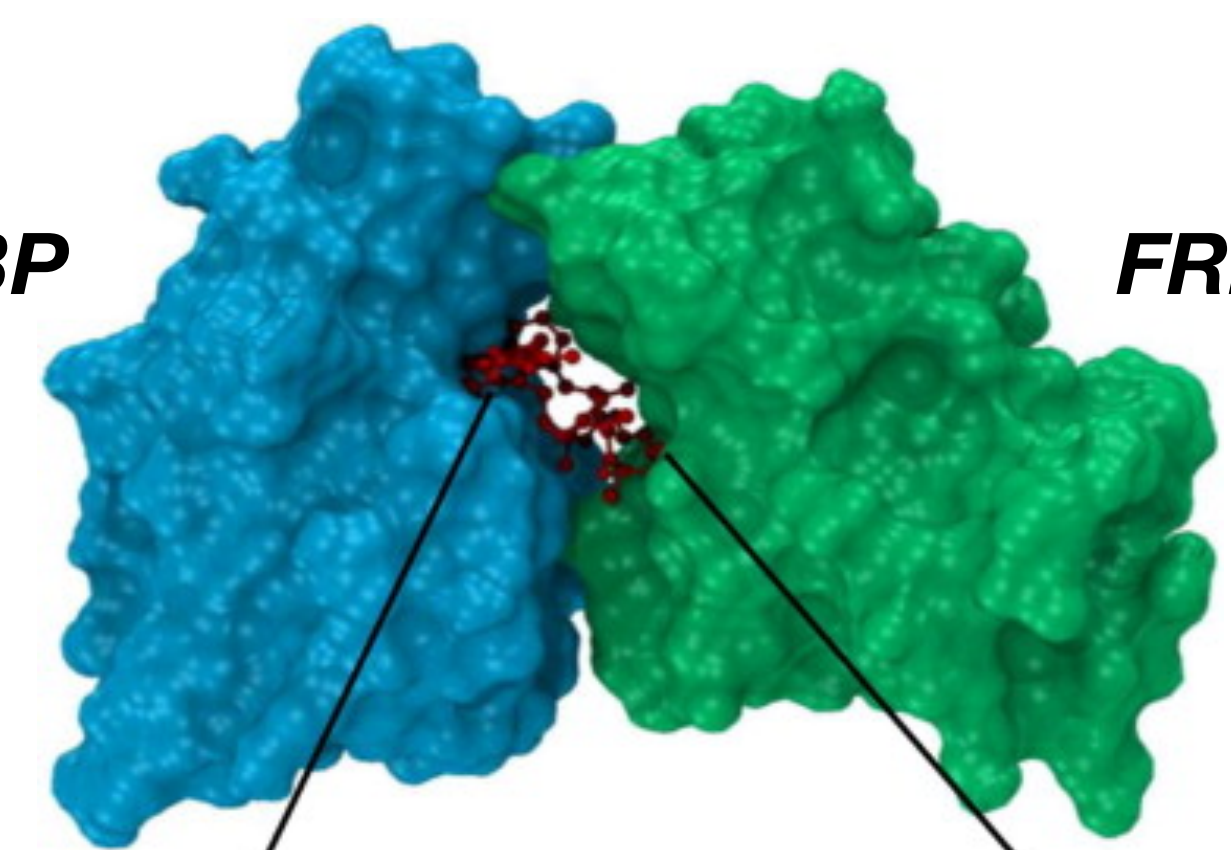
No rapamycin



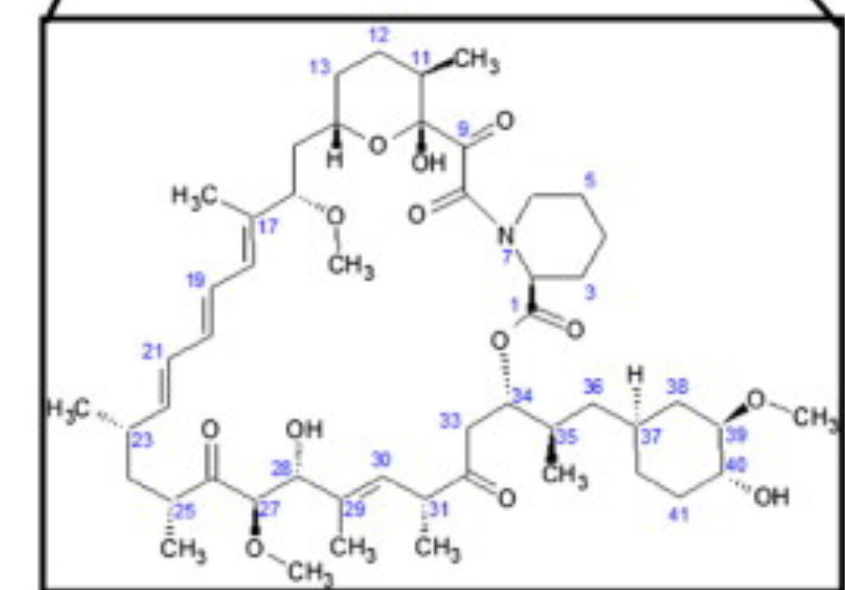
Only weak binding at FRB > 100µM

FKBP

FRB



Rapamycin



Formation of high affinity ternary complex

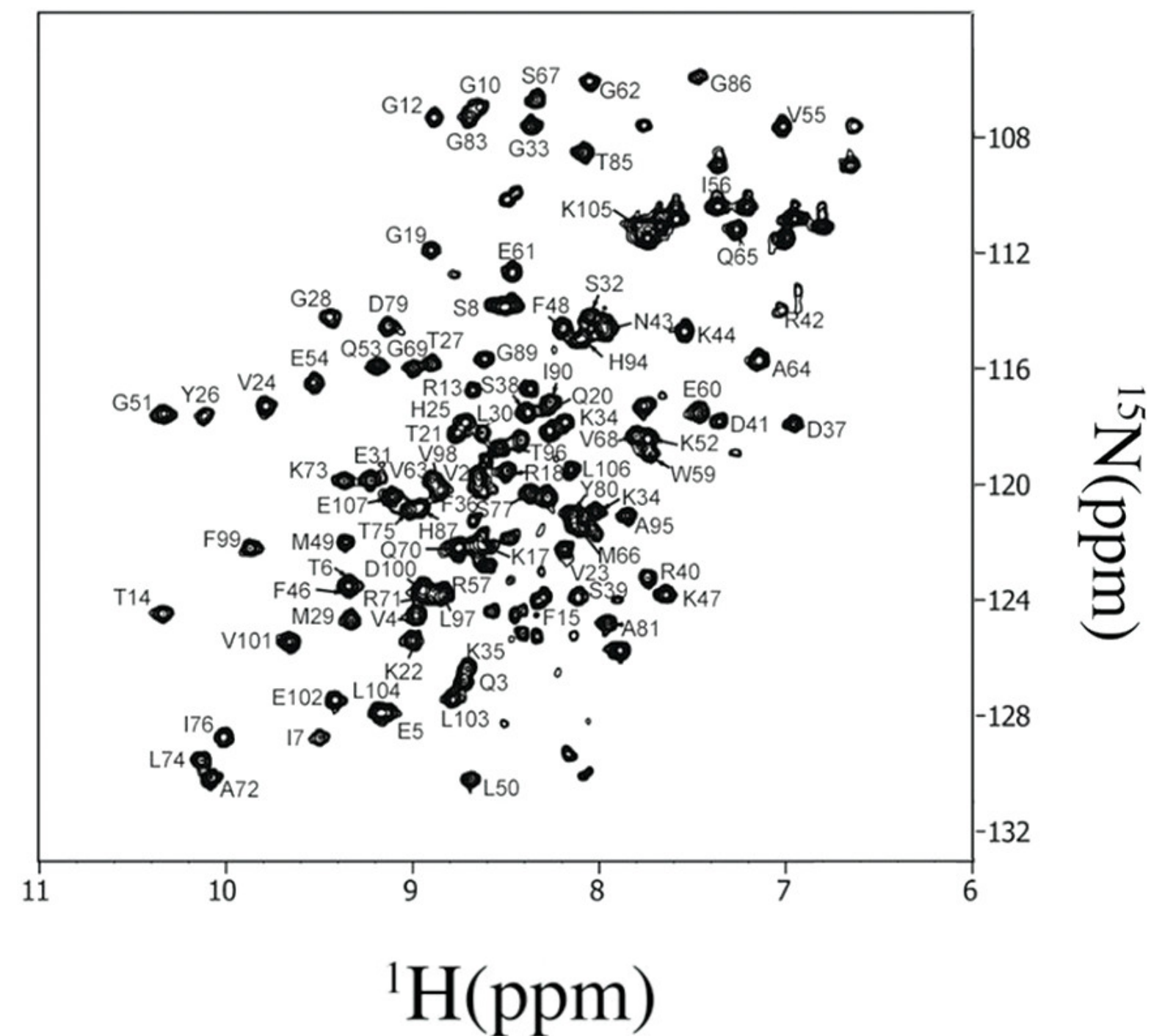
<sup>15</sup>N labeled FKBP and unlabeled FRB in *E. coli*

# HTS in small molecule drug discovery

## Screening methods - spectroscopic assays

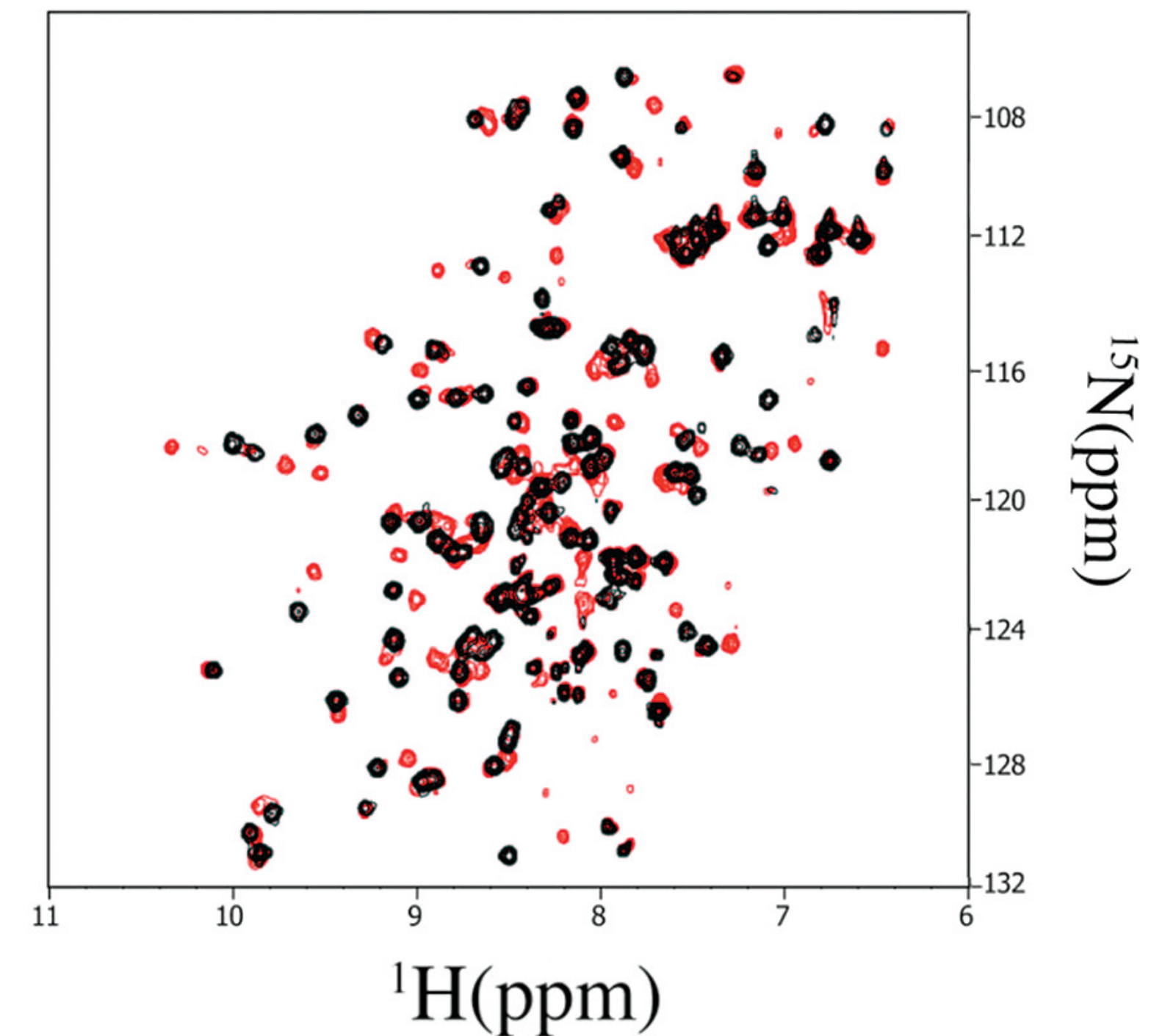
### Protein detected NMR

**No rapamycin**



*Only weak binding at FRB > 100 $\mu$ M*

**With rapamycin**



*Formation of high affinity ternary complex*

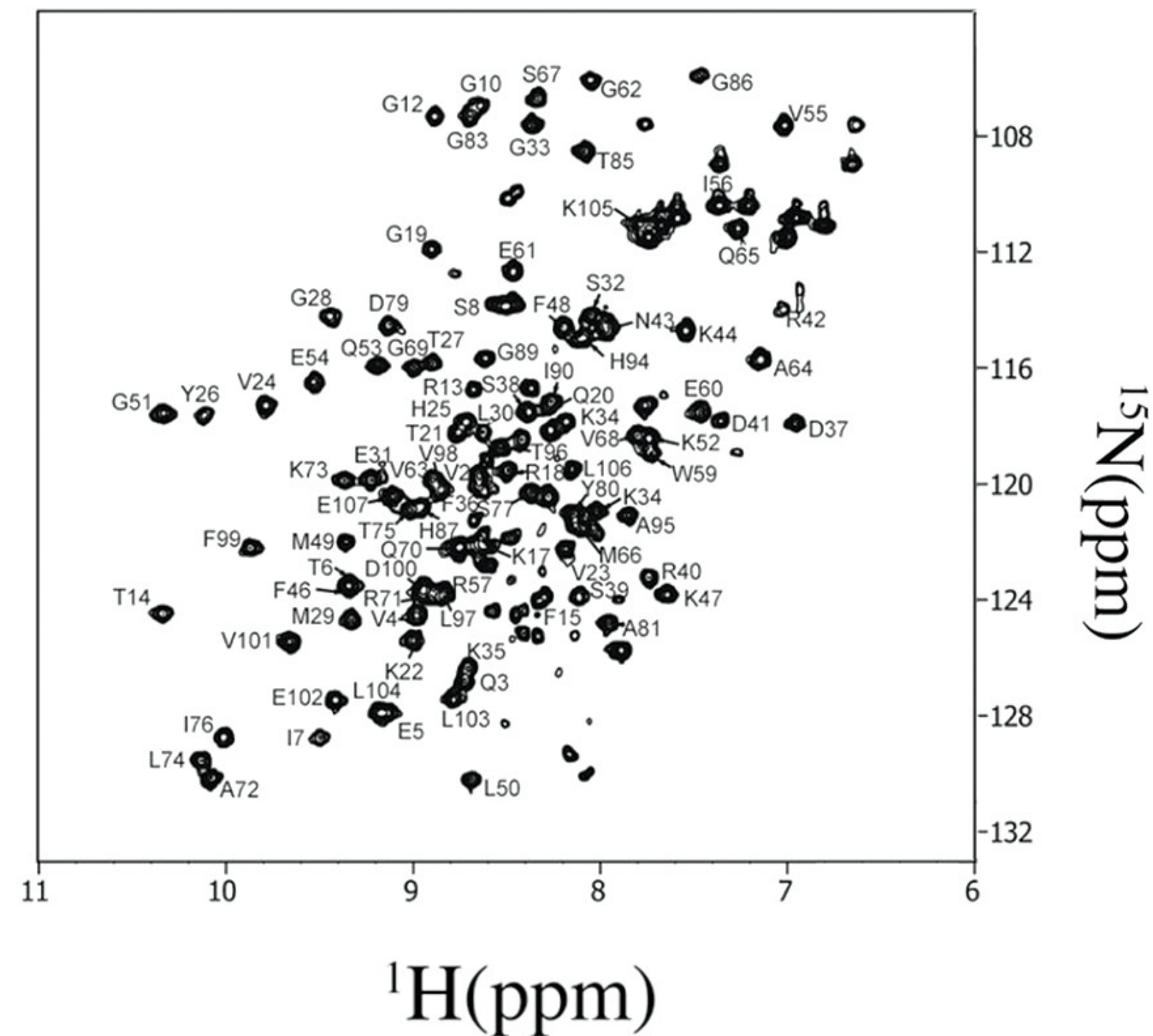
*<sup>15</sup>N labeled FKBP and unlabeled FRB in E. coli*

# HTS in small molecule drug discovery

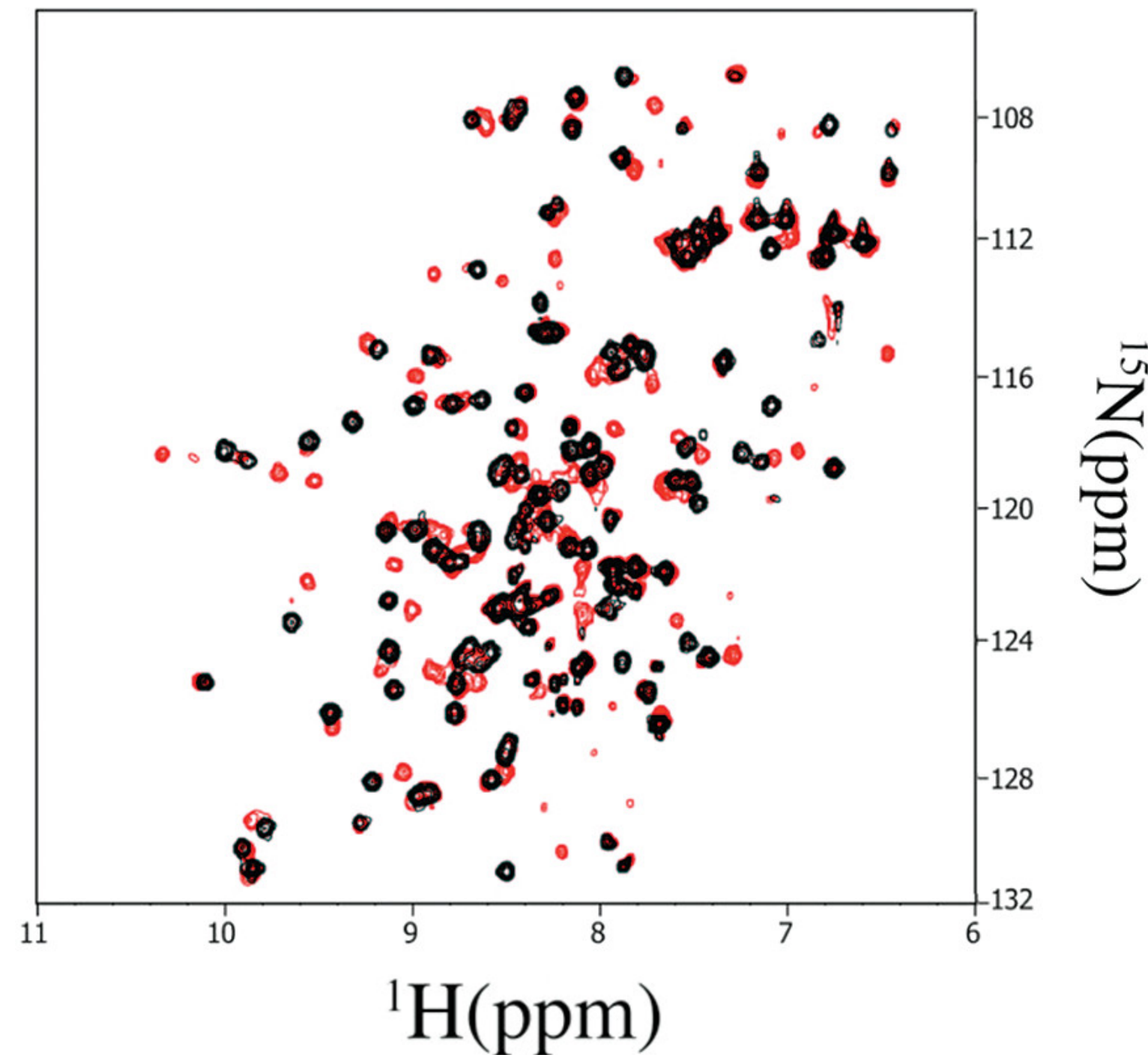
Screening methods - spectroscopic assays

## Protein detected NMR

No rapamycin



With rapamycin

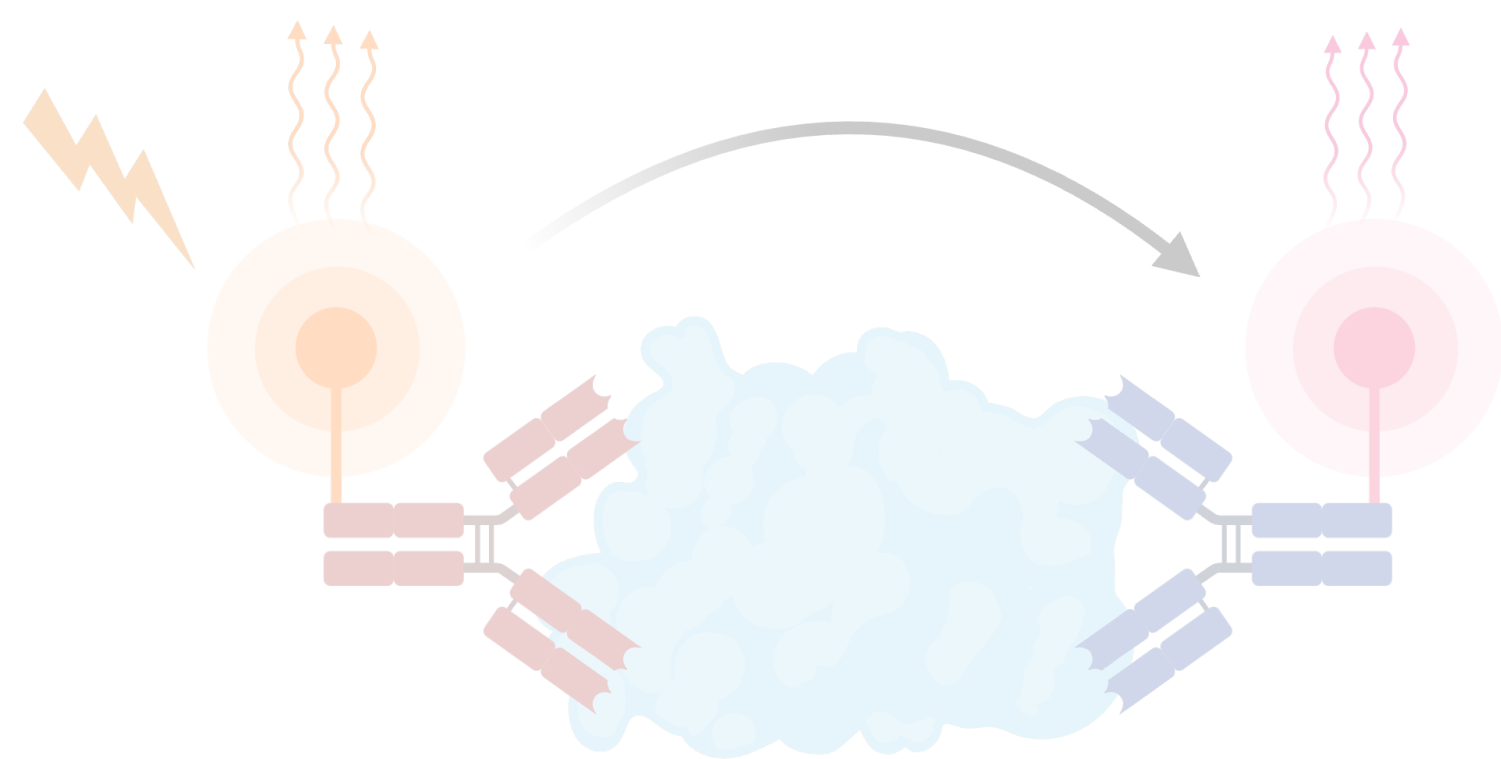


Screening of 289 dipeptides

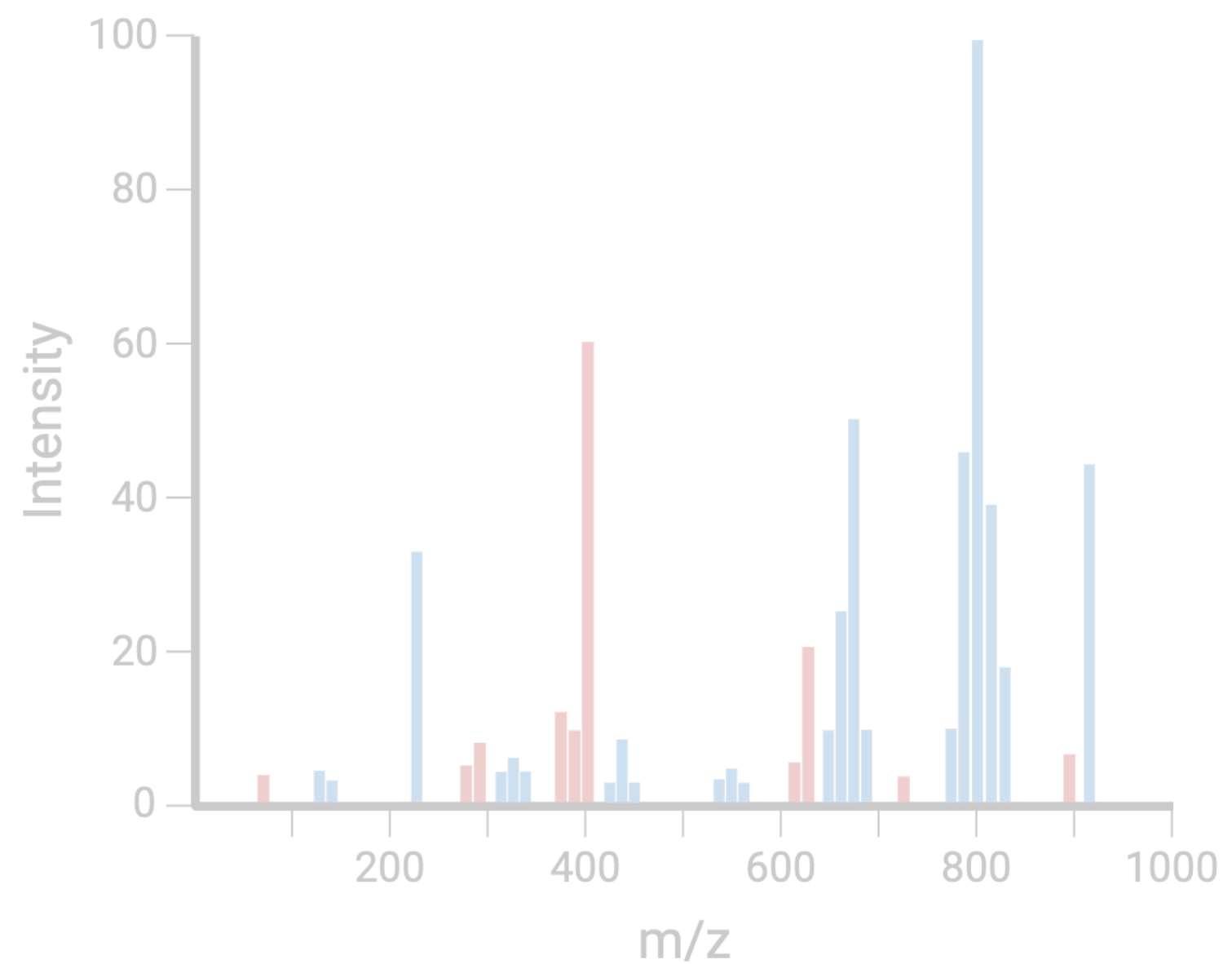
**More laborious and less high throughput, but provide information on binding sites**

# HTS in small molecule drug discovery

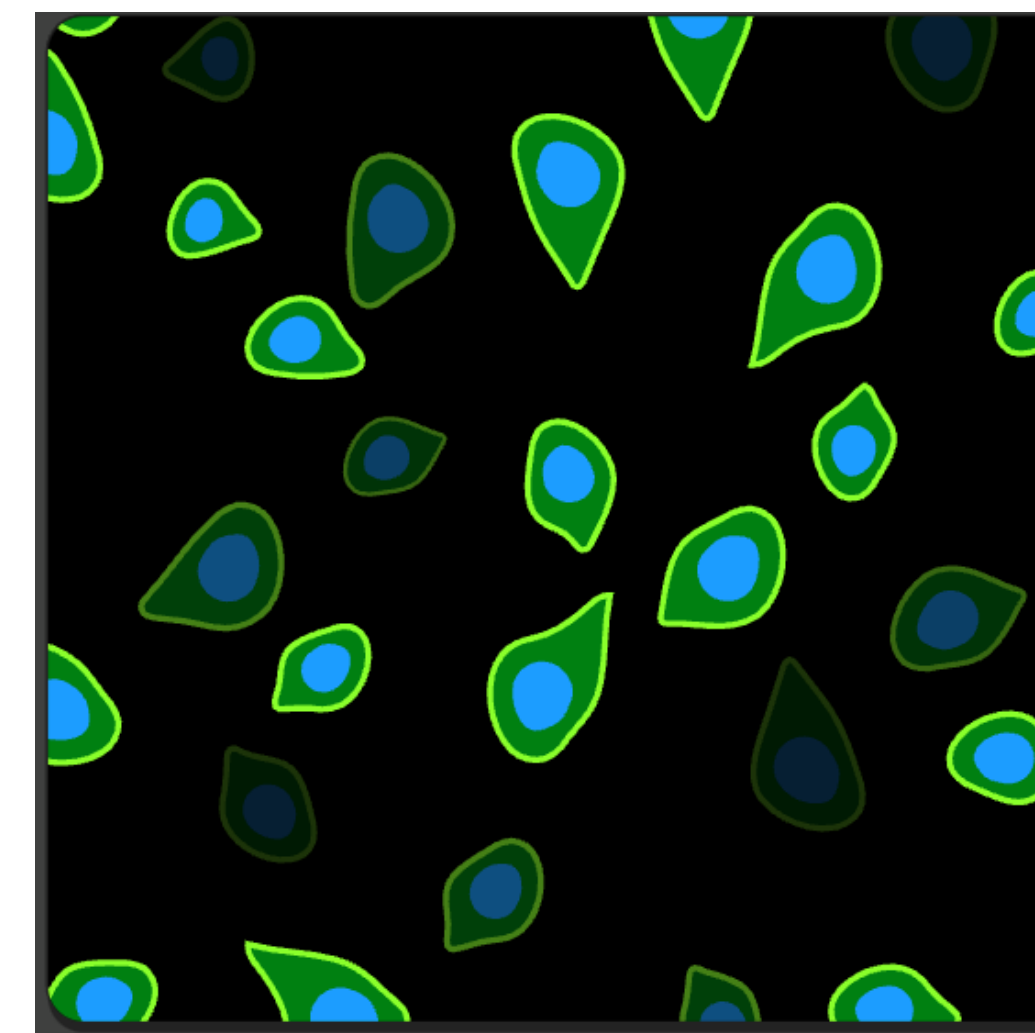
## Screening methods



*Biochemical assays*



*Spectroscopic assays*



*Cell based assays*

# HTS in small molecule drug discovery

## Screening methods - cell based assays

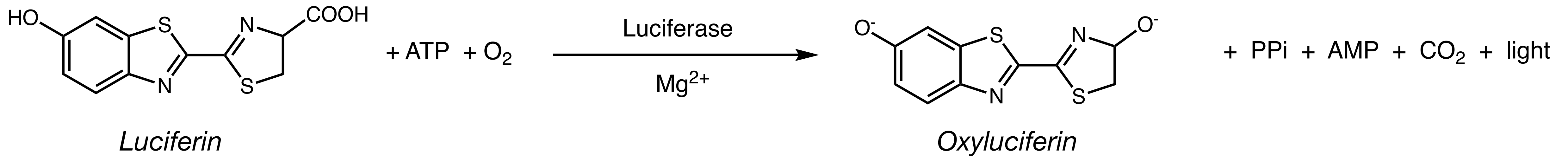
### **Bioluminescence**



# HTS in small molecule drug discovery

Screening methods - cell based assays

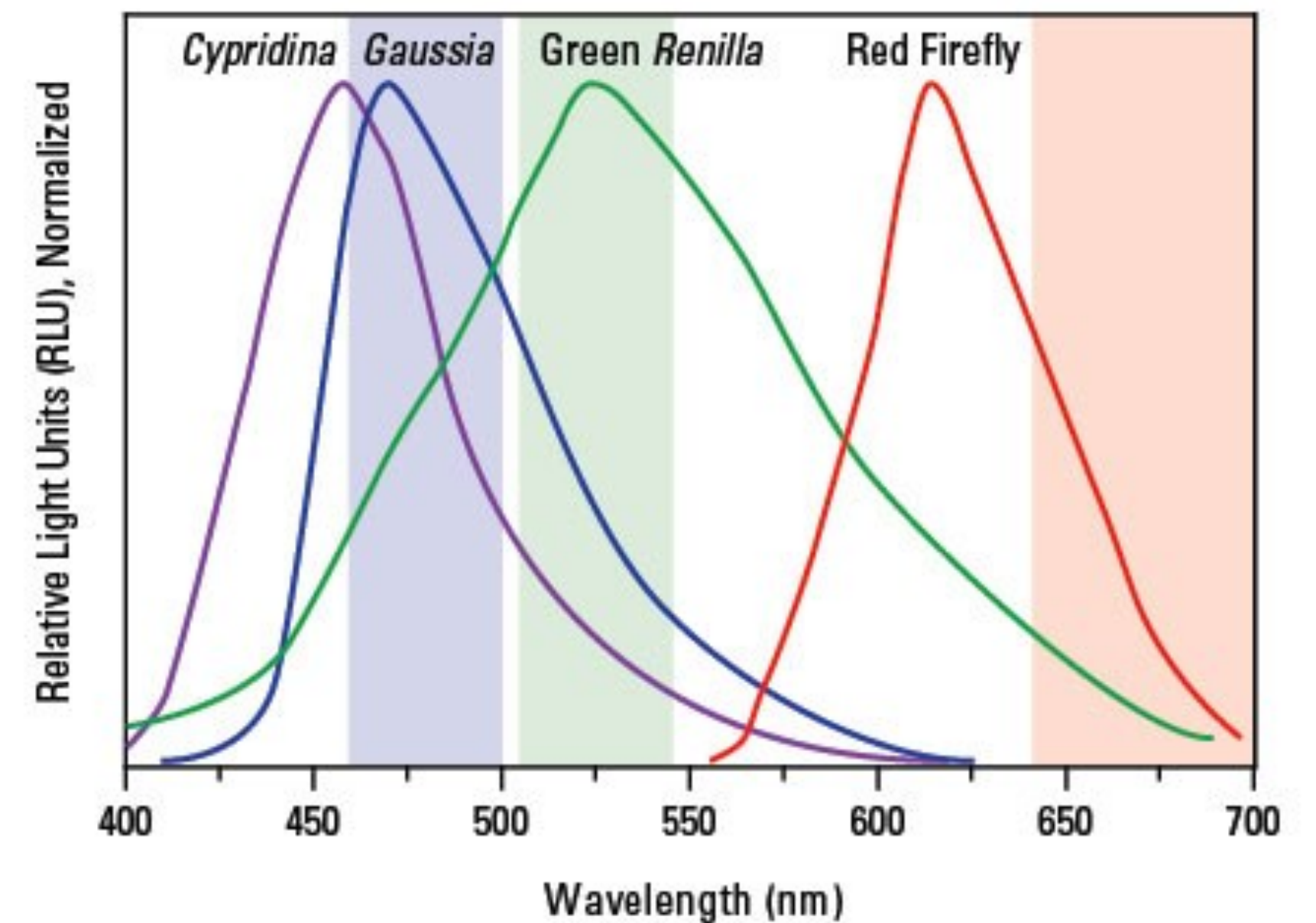
## Bioluminescence



**Sensitive method**

**Can be multiplexed with different luciferase enzymes**

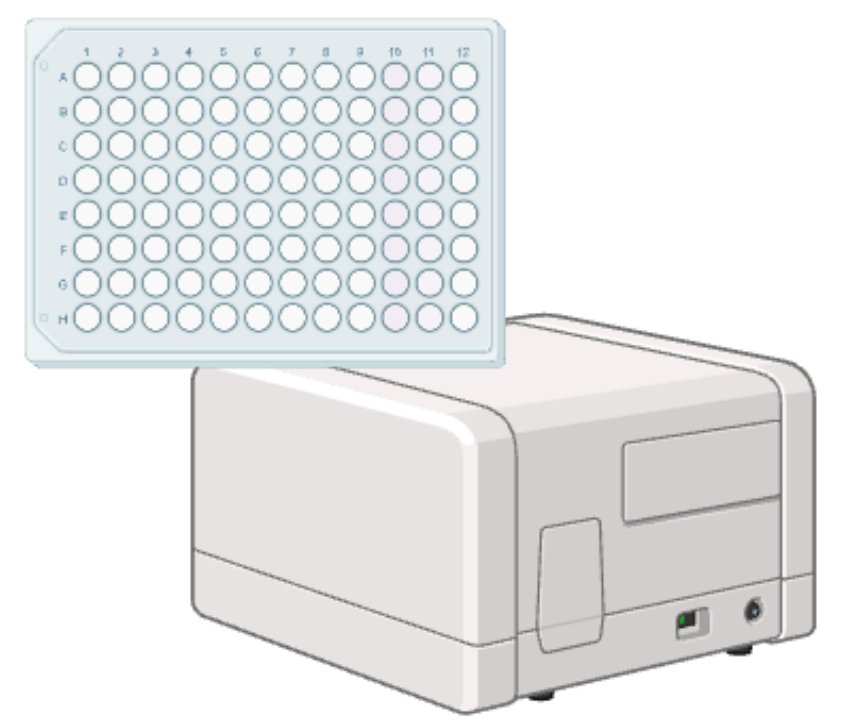
**Easy and quantitative readout**



# HTS in small molecule drug discovery

## Screening methods - cell based assays

### Cell viability assay



**ATP can be used as a direct measurement of cell viability using luciferase**

**Fast phenotypic screening**

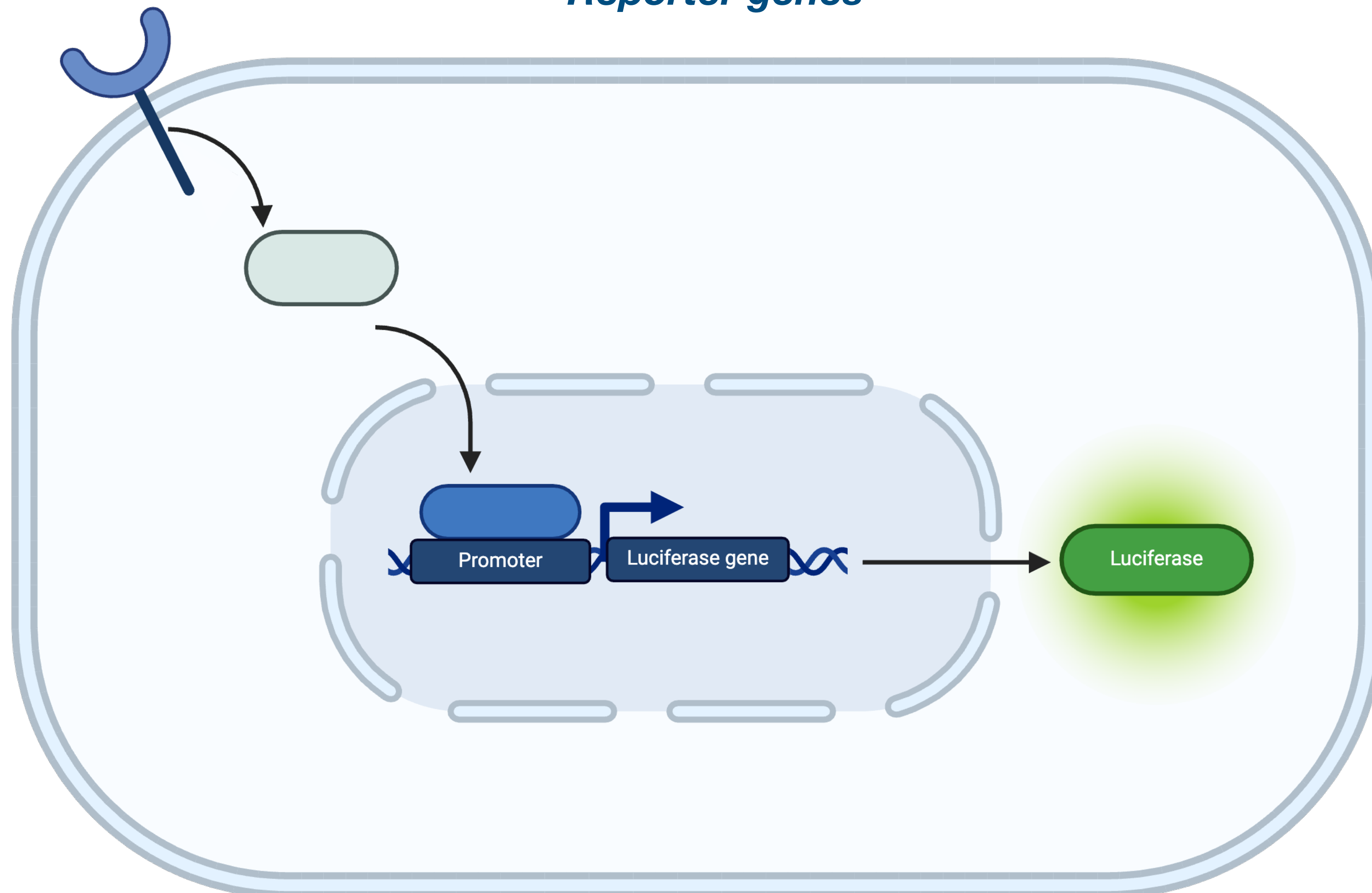
**Luciferases are also commonly used as reporters in cell**



# HTS in small molecule drug discovery

## Screening methods - cell based assays

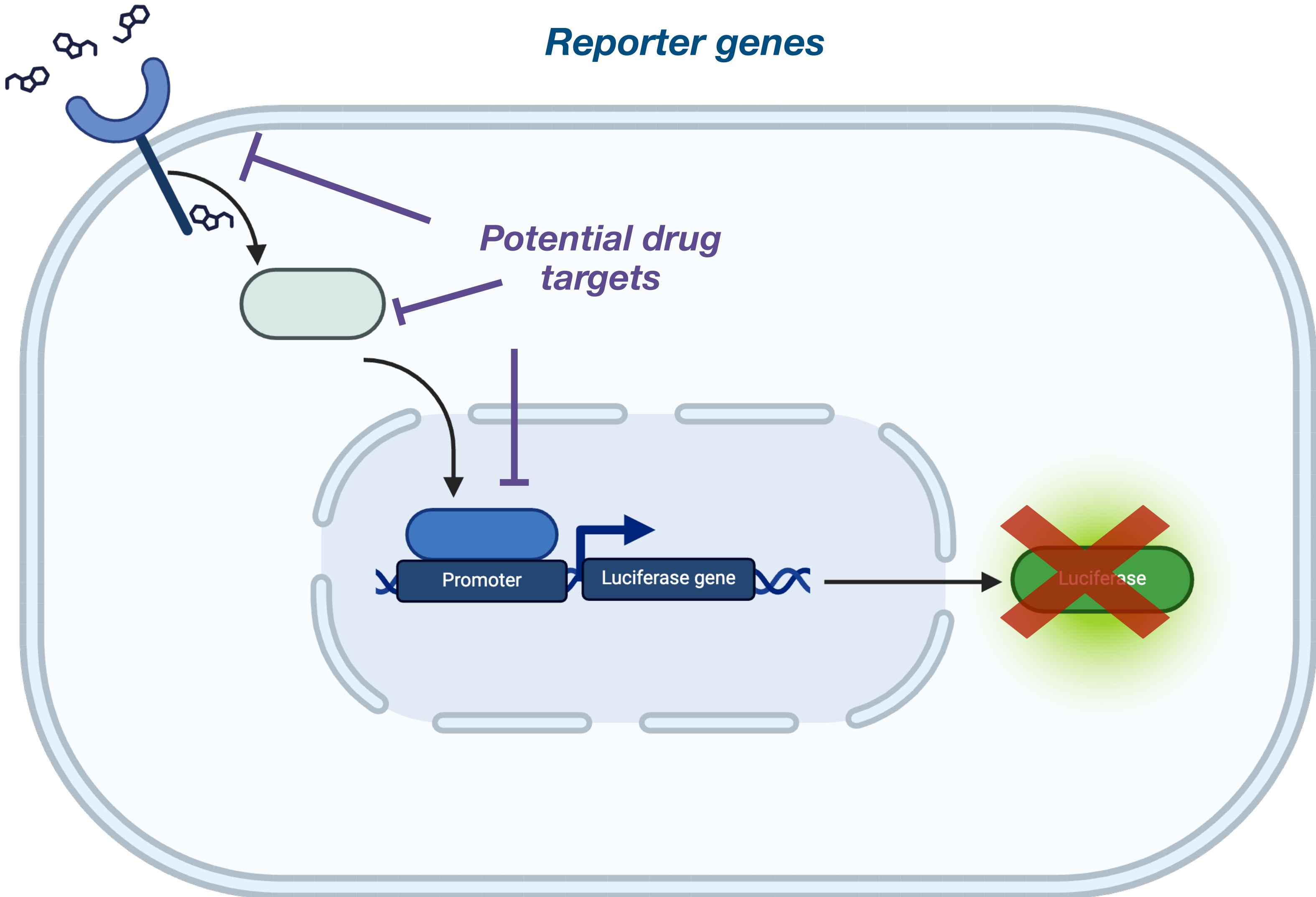
### Reporter genes



# HTS in small molecule drug discovery

Screening methods - cell based assays

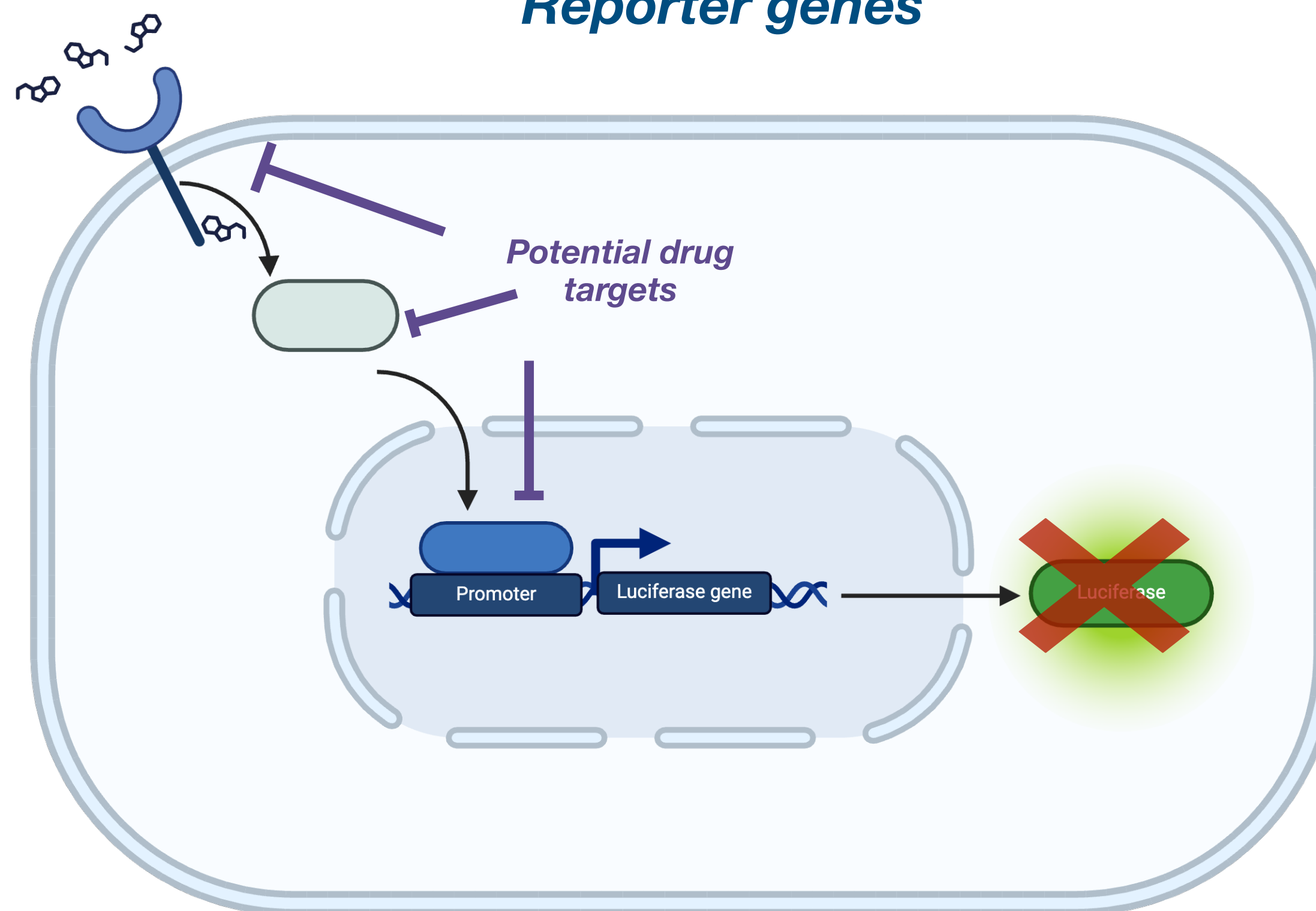
## Reporter genes



# HTS in small molecule drug discovery

Screening methods - cell based assays

## Reporter genes



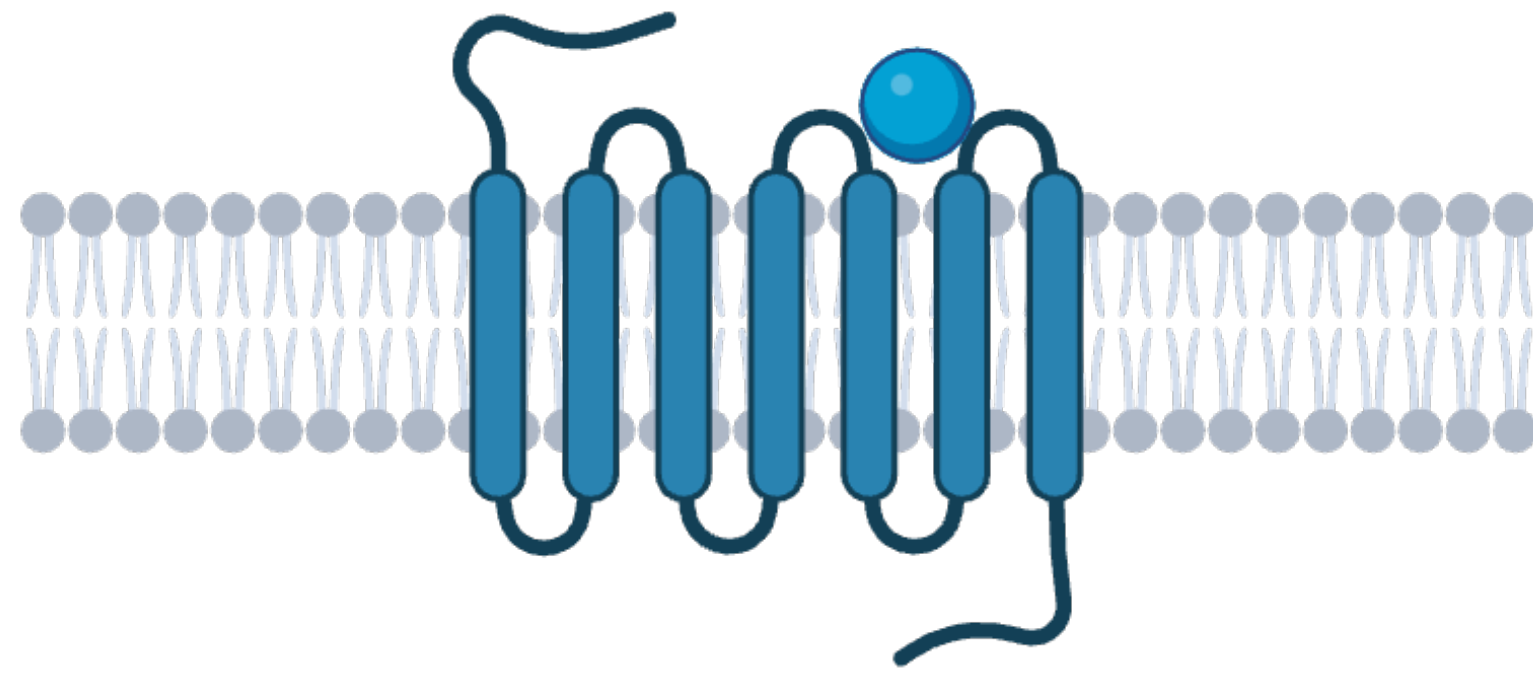
**Other common reporter genes are CAT (chloramphenicol acetyltransferase), GAL ( $\beta$ -galactosidase), LAC ( $\beta$ -lactamase), and GFP**

**Reporter gene can be sensitive to indirect effects because of the long incubation time**

# HTS in small molecule drug discovery

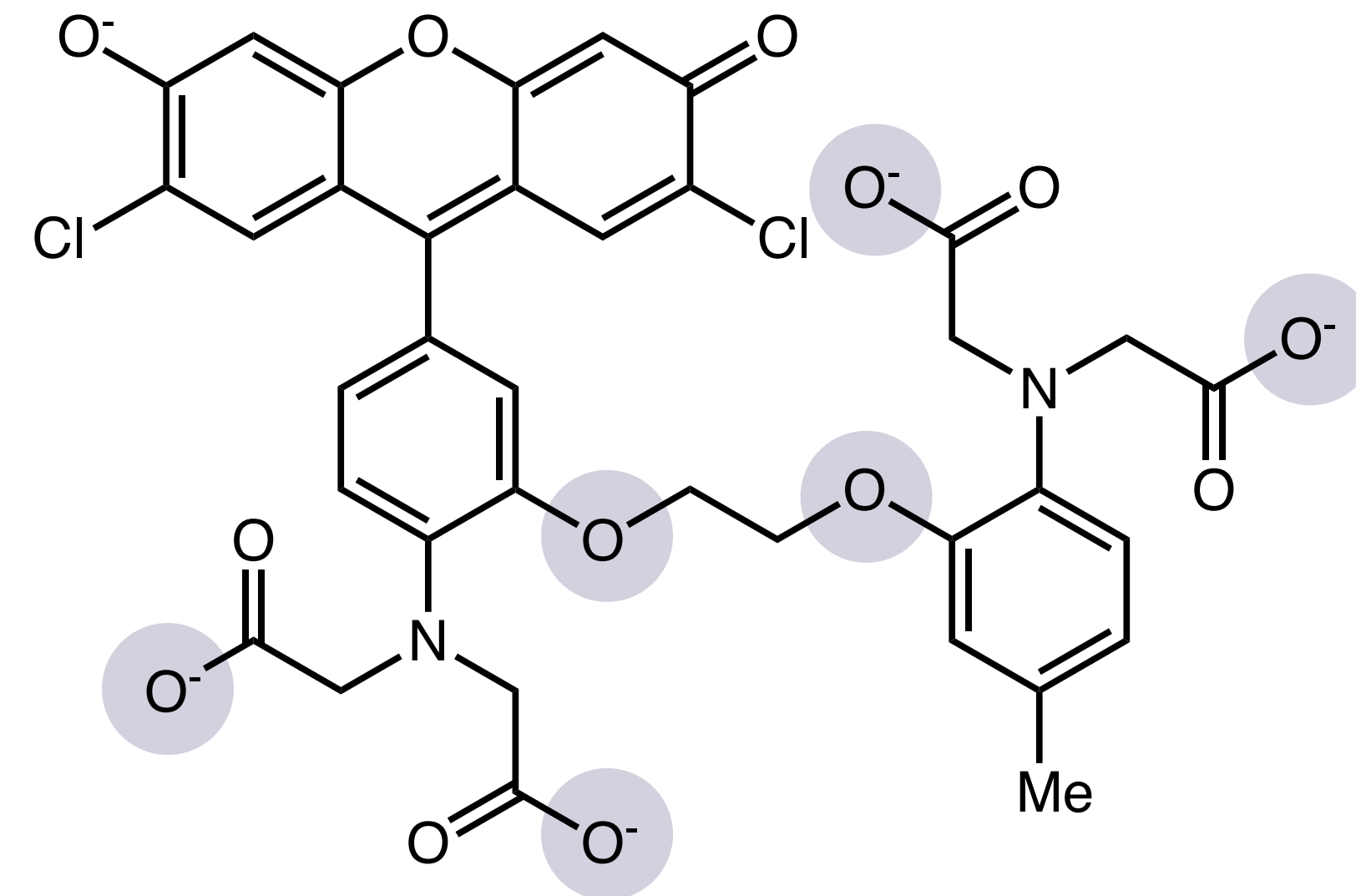
Screening methods - cell based assays

## Secondary messenger assays



Particularly useful for transporter proteins

Common for screening with GPCR



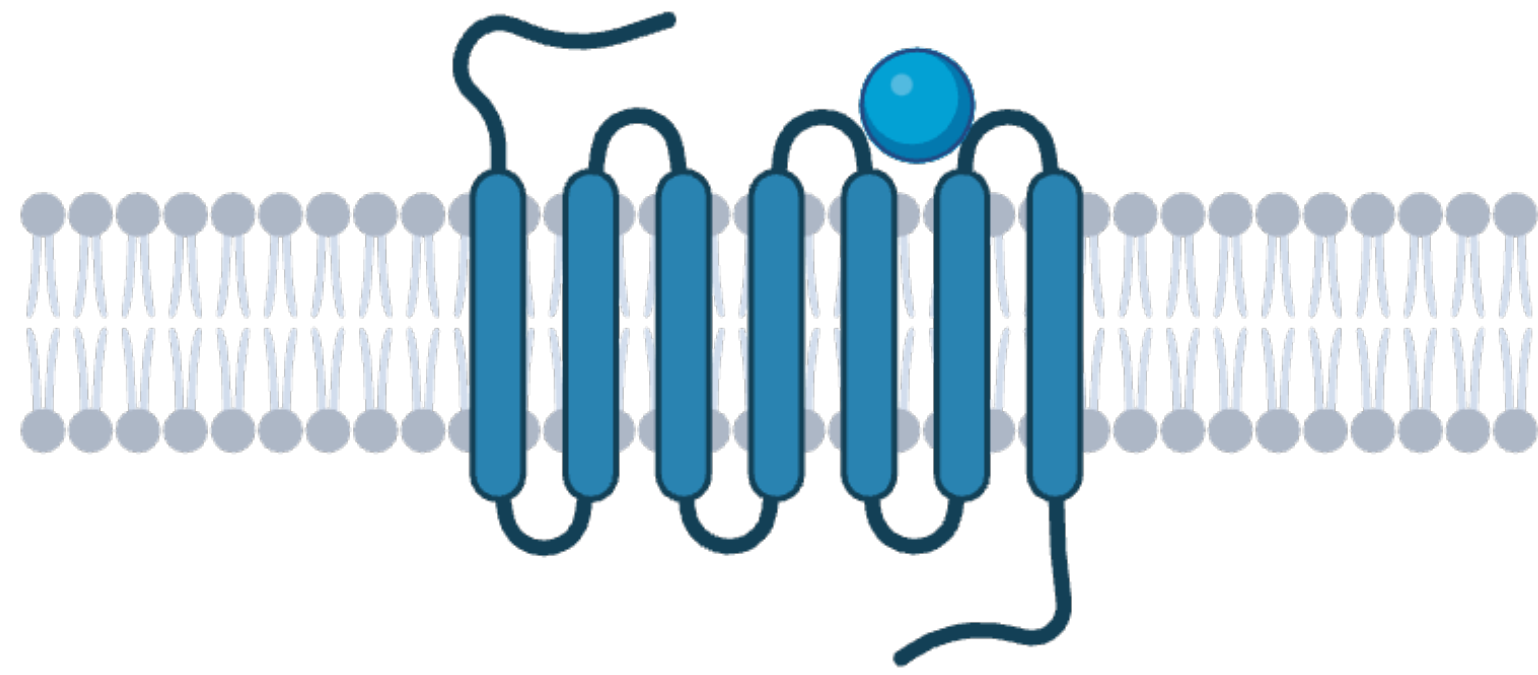
**Fluo-3**

526 nm emission after chelating with  $Ca^{2+}$

# HTS in small molecule drug discovery

Screening methods - cell based assays

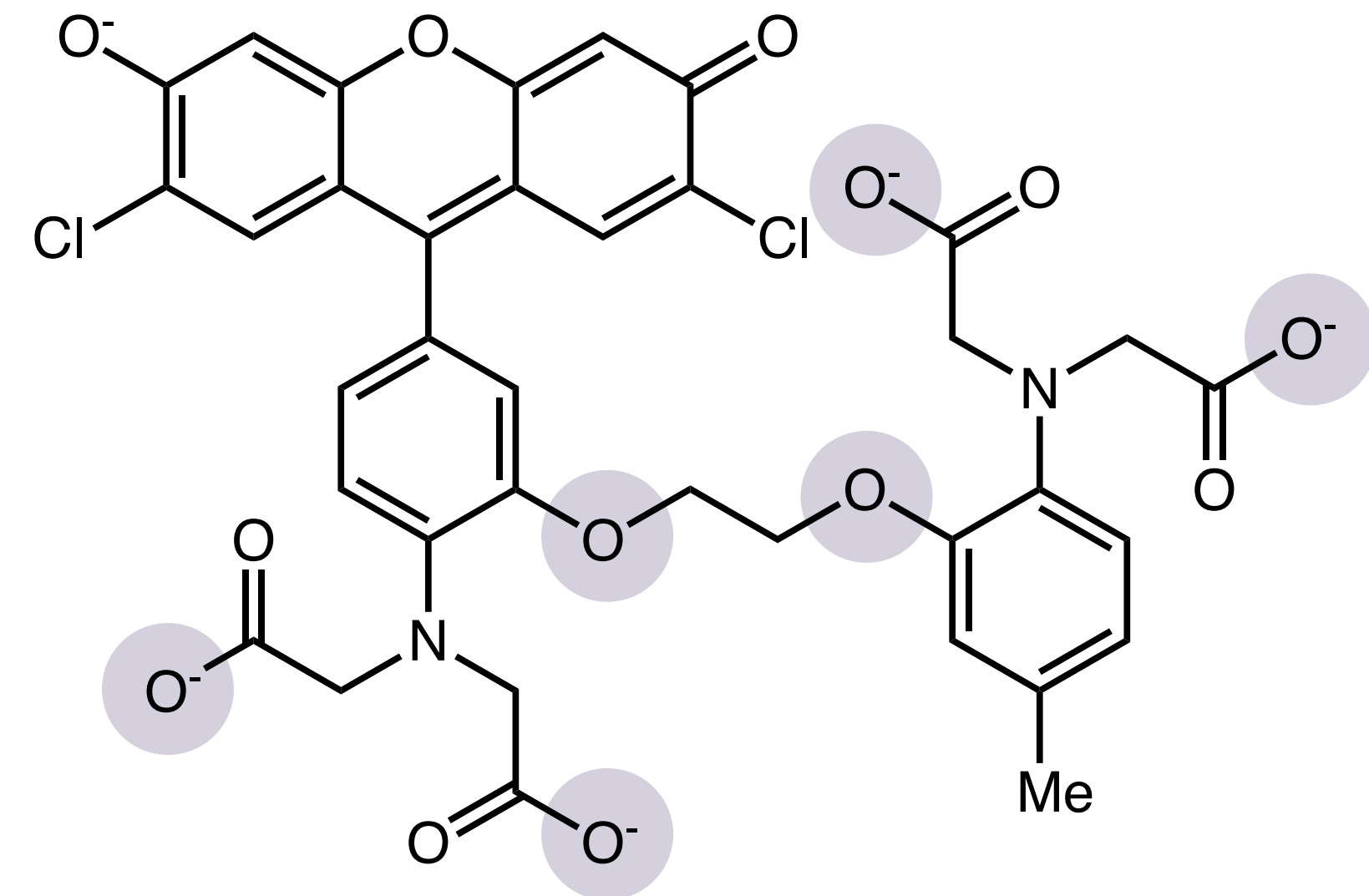
## Secondary messenger assays



Particularly useful for transporter proteins

Common for screening with GPCR

Fast and direct measure of  $Ca^{2+}$  level



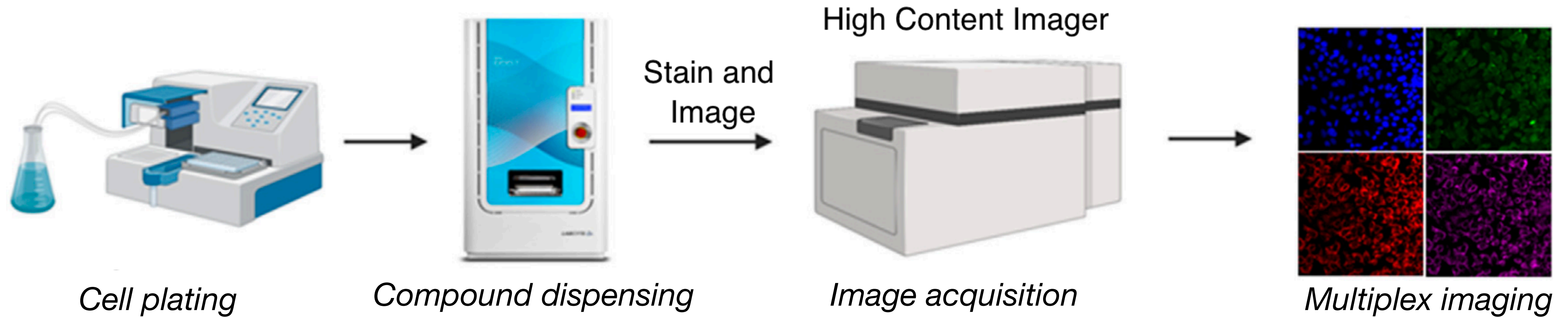
**Fluo-3**

526 nm emission after chelating with  $Ca^{2+}$

# HTS in small molecule drug discovery

Screening methods - cell based assays

## High content imaging



**Data processing**



From DNA binding dye:  
nuclear size, shape, intensity, texture

...

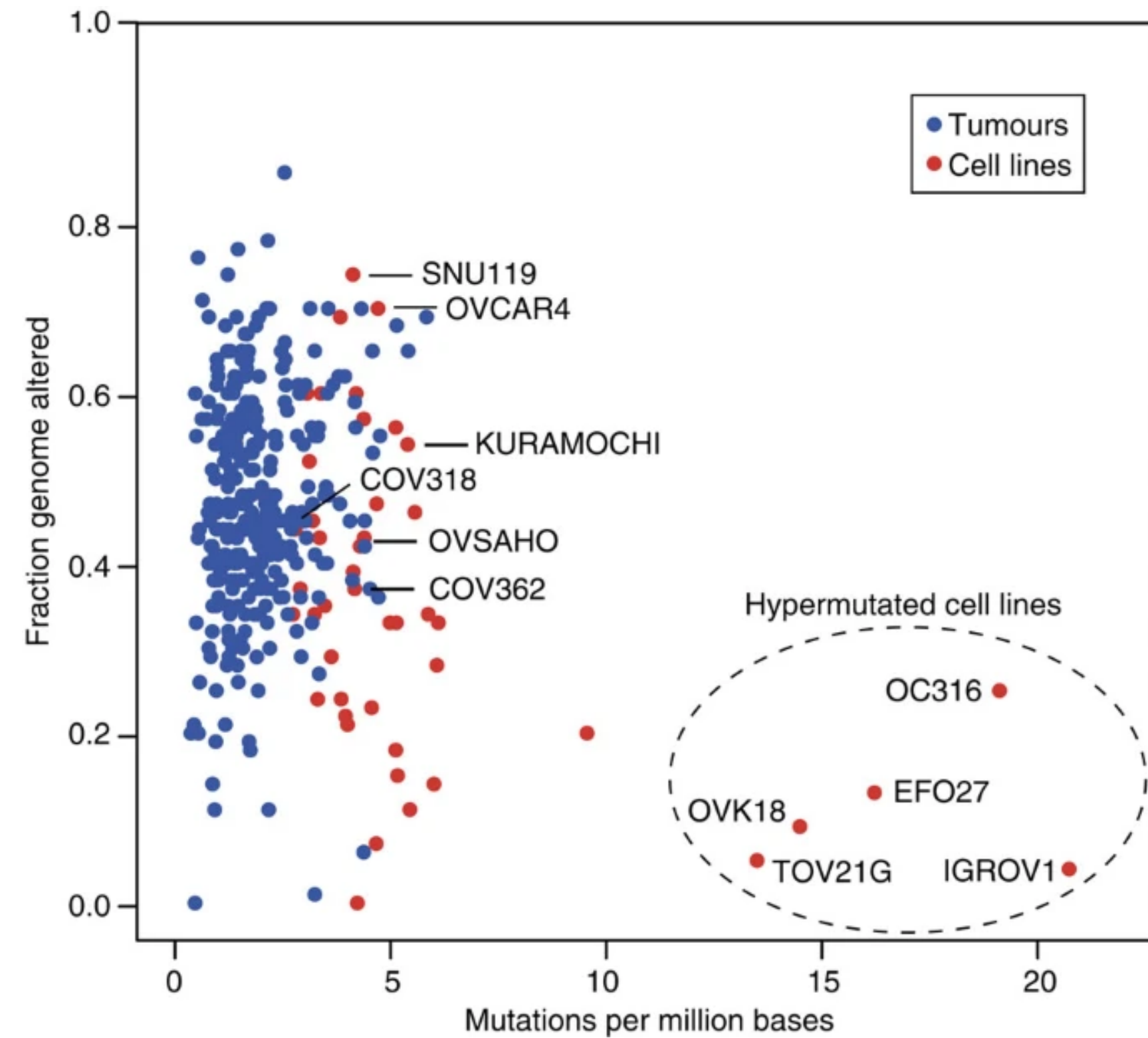
**Current trend towards screening with video sequences and imaging of whole organism**

# HTS in small molecule drug discovery

## Screening methods - cell based assays

### Choice of cell models

#### Mutations in HGSOV tumors and cell line models



**Commonly used cancer cell lines have more mutations than patient derived tumors**

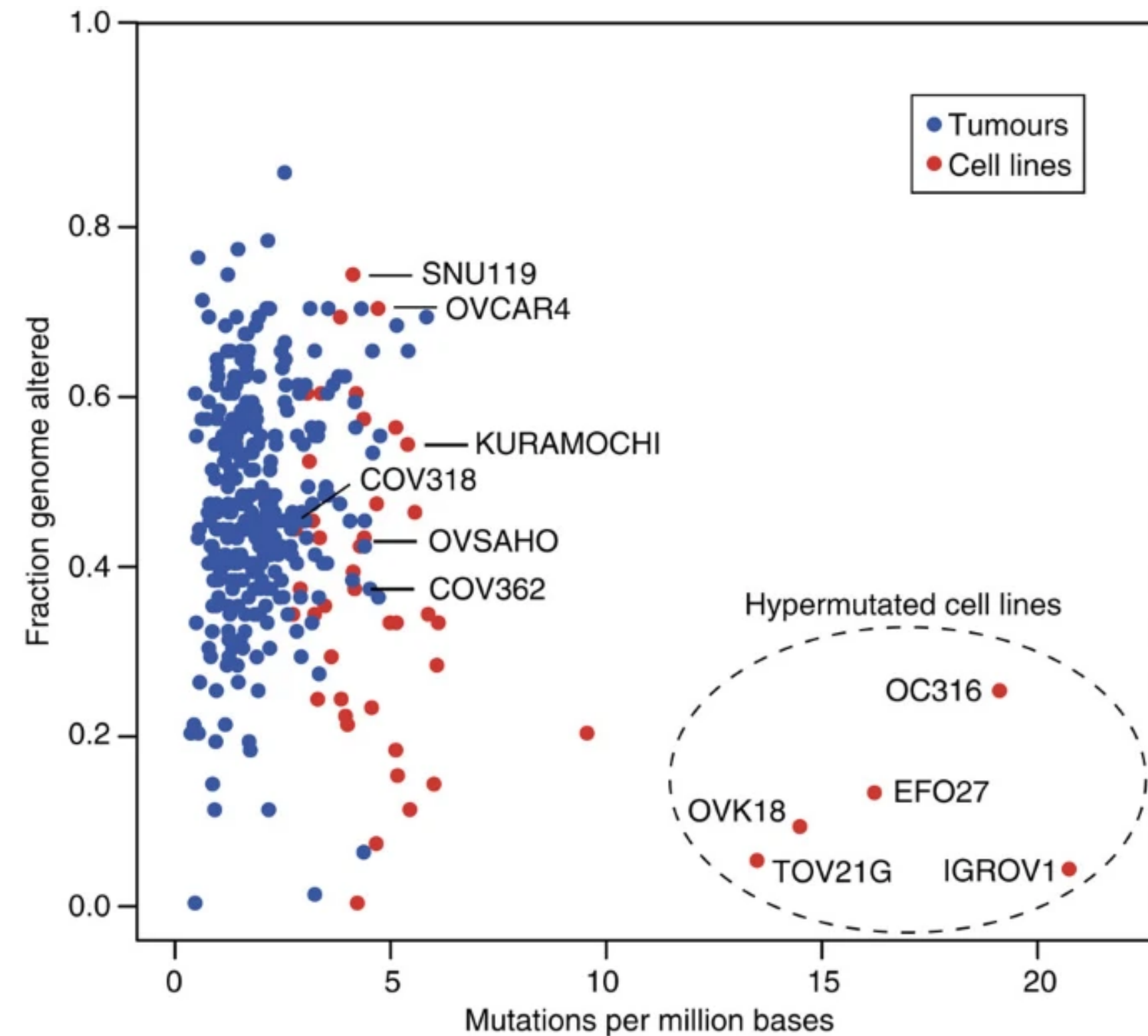
*HGSOV: High-grade serous ovarian cancer*

# HTS in small molecule drug discovery

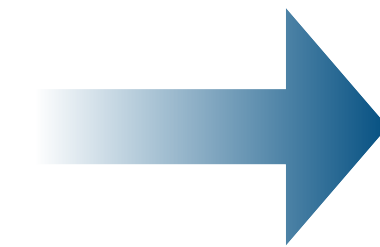
## Screening methods - cell based assays

### Choice of cell models

#### Mutations in HGSOV tumors and cell line models



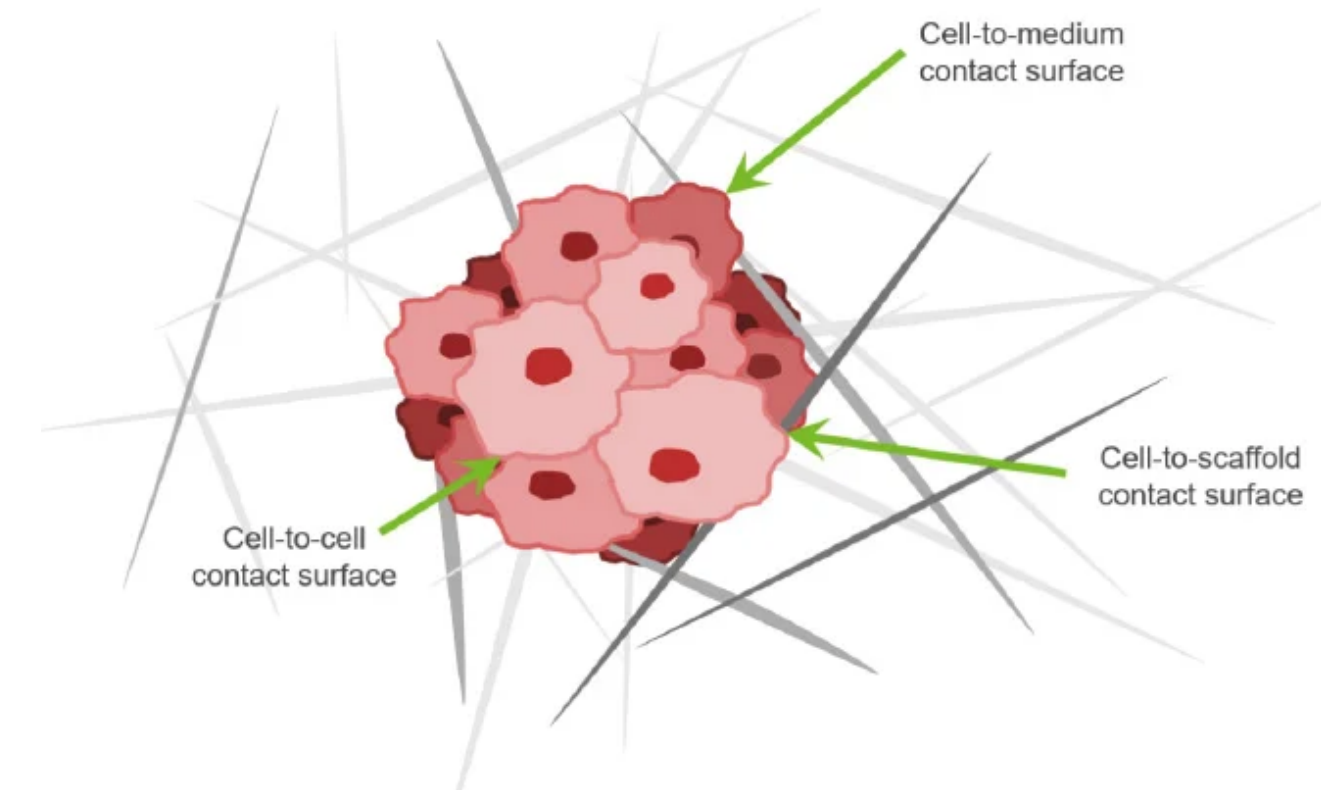
*HGSOV: High-grade serous ovarian cancer*



**Trend towards more disease-like models**

**Using primary/patient derived cells, 3D cell growth, and animal models**

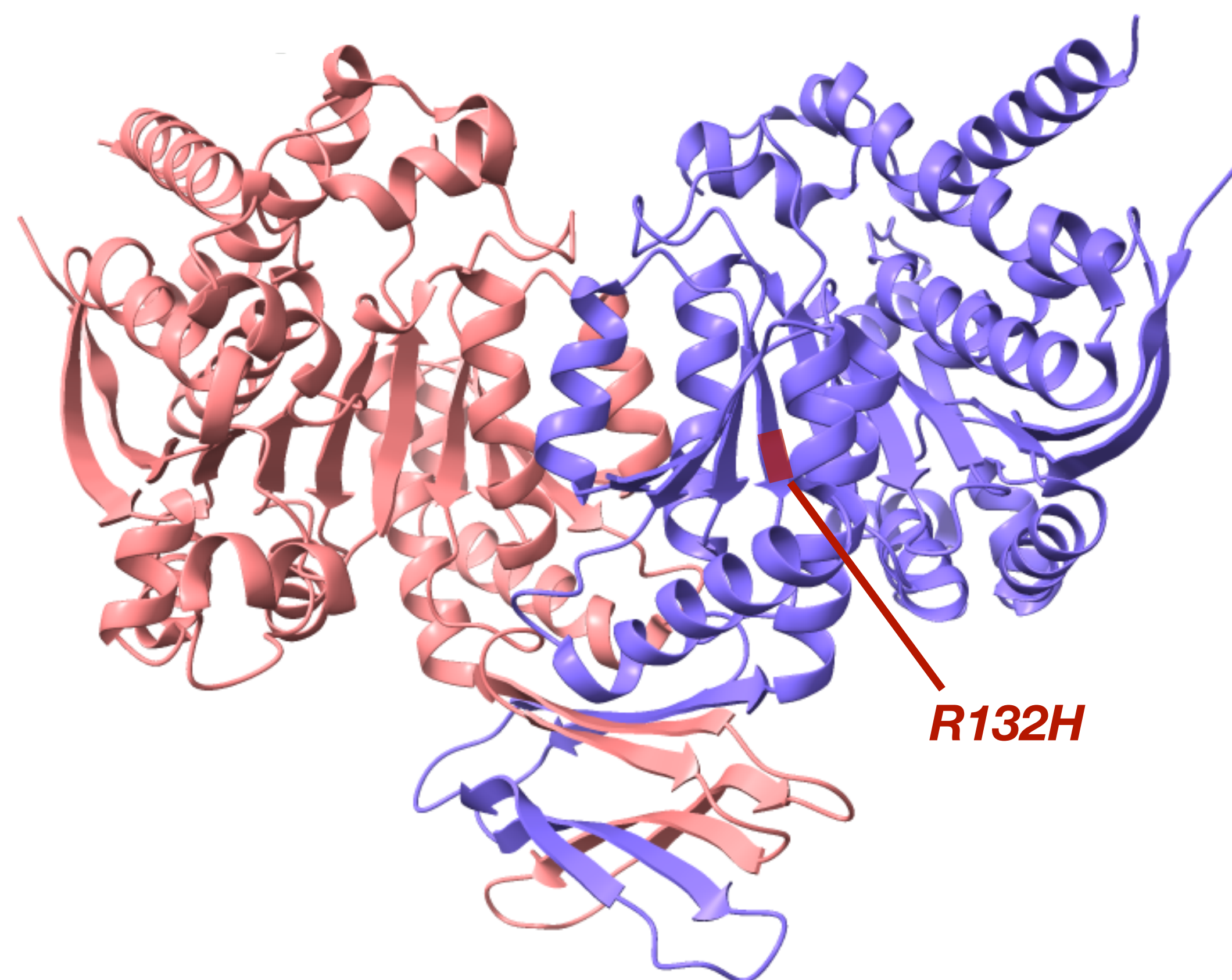
**Perfused cultures to study the long term effects**





# *HTS in small molecule drug discovery*

## *Case Study: mutant IDH inhibitors*



### ***IDH in cancer***

*An essential enzyme in the TCA cycle*

*IDH1 and IDH2 form **homodimers***

**Heterozygous point mutations** are prevalent in many cancers:

- 75% of brain cancers
- 20% acute myeloid leukemia
- also in colorectal, prostate cancer, etc.

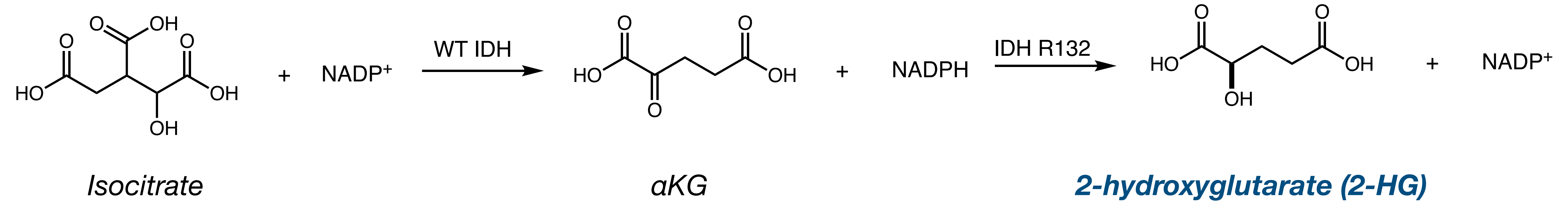
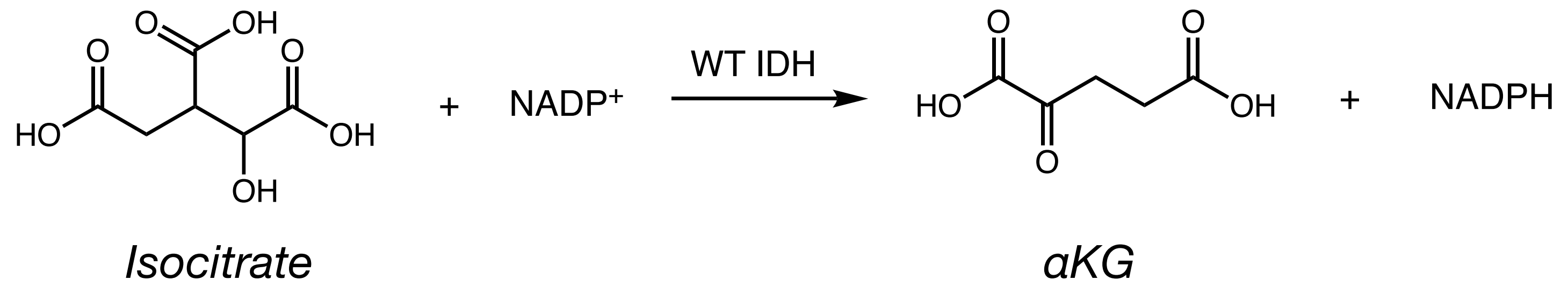
### ***What does the mutation do in cancer cells?***

Pirozzi, C. R., Yan, H. *Nat. Rev.* **2021**, 18

Coussens, N. P., Braisted, J. C., Peryea, T., et al. *Pharmacol Rev.* **2017**, 69

# HTS in small molecule drug discovery

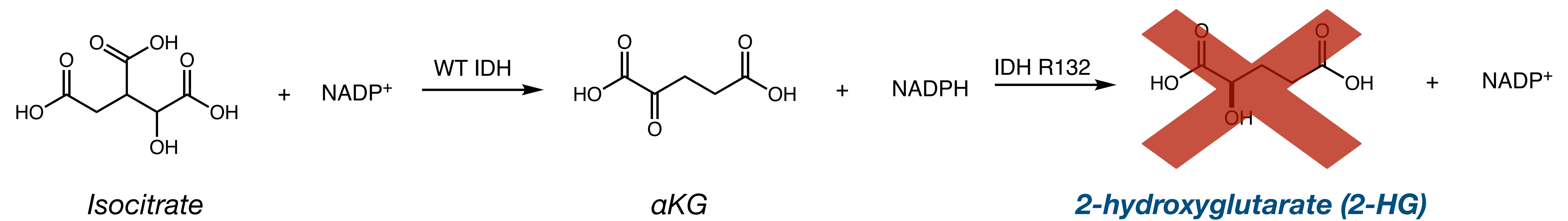
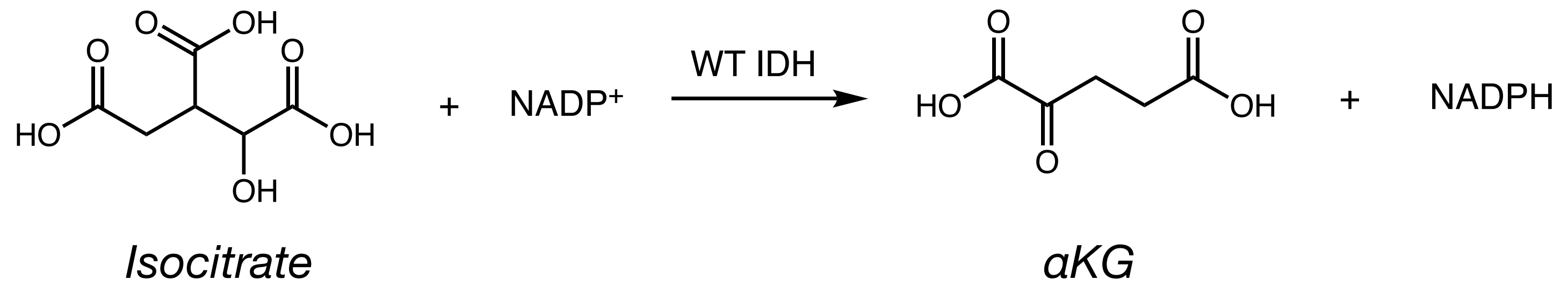
## Case Study: mutant IDH inhibitors



**2-HG has oncogenic roles and accumulates in IDH mutant cancer cells**

# HTS in small molecule drug discovery

## Case Study: mutant IDH inhibitors



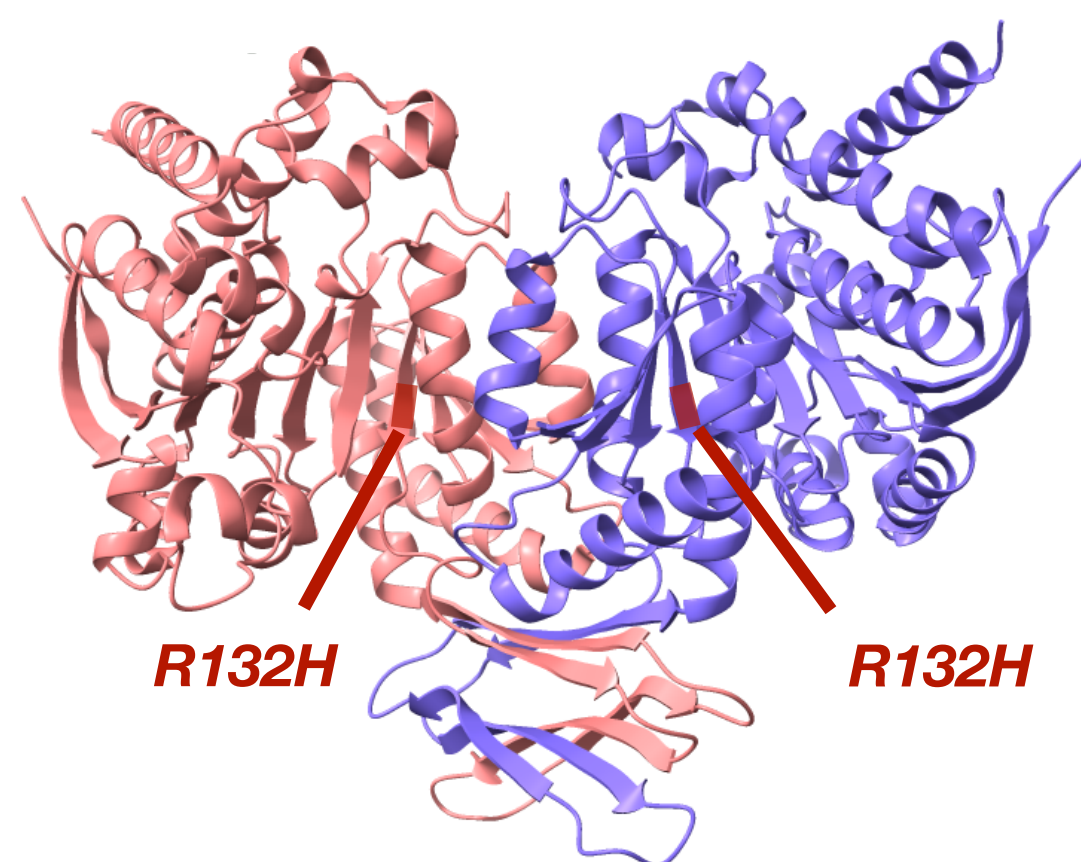
***Inhibiting mutant IDH would have few side effects and can potentially suppress tumor***

# HTS in small molecule drug discovery

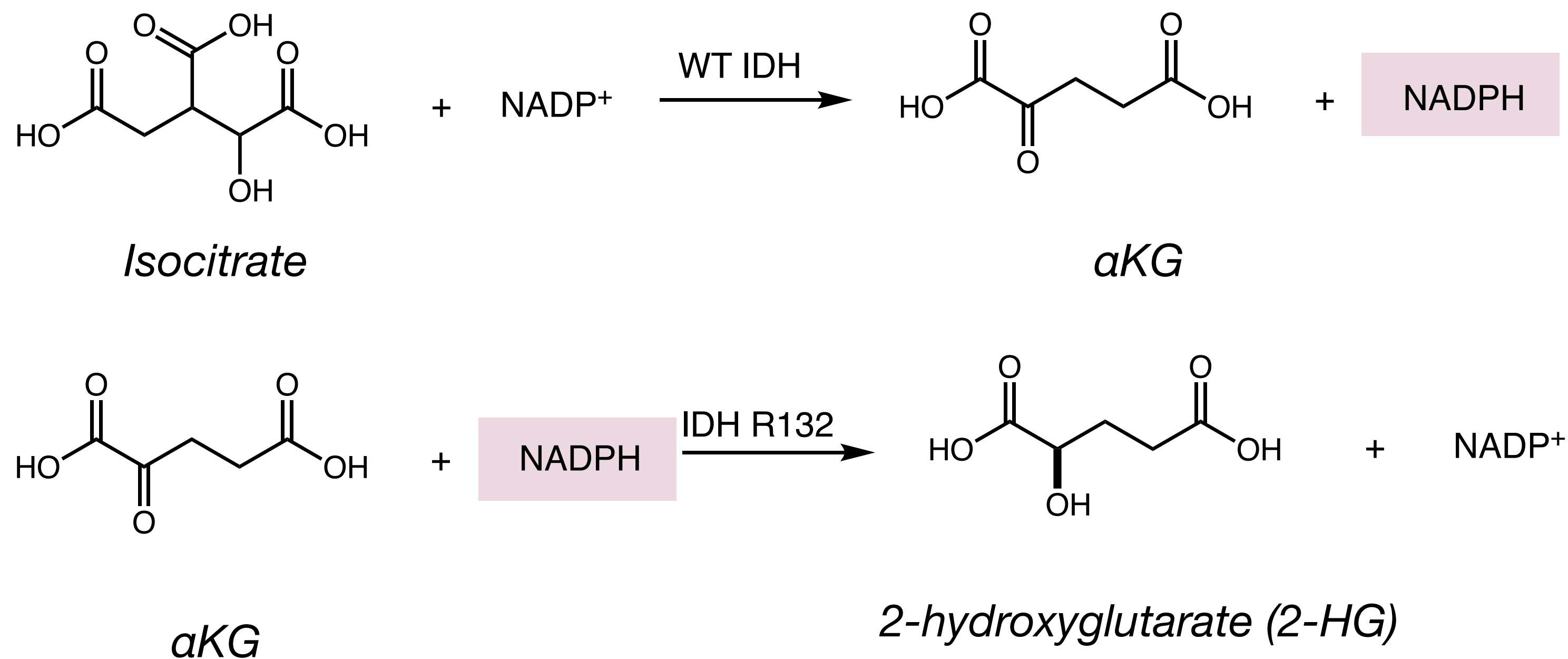
## Case Study: mutant IDH inhibitors - assay development



### Recombinant mutant IDH1 homodimer



### Measuring NADPH level

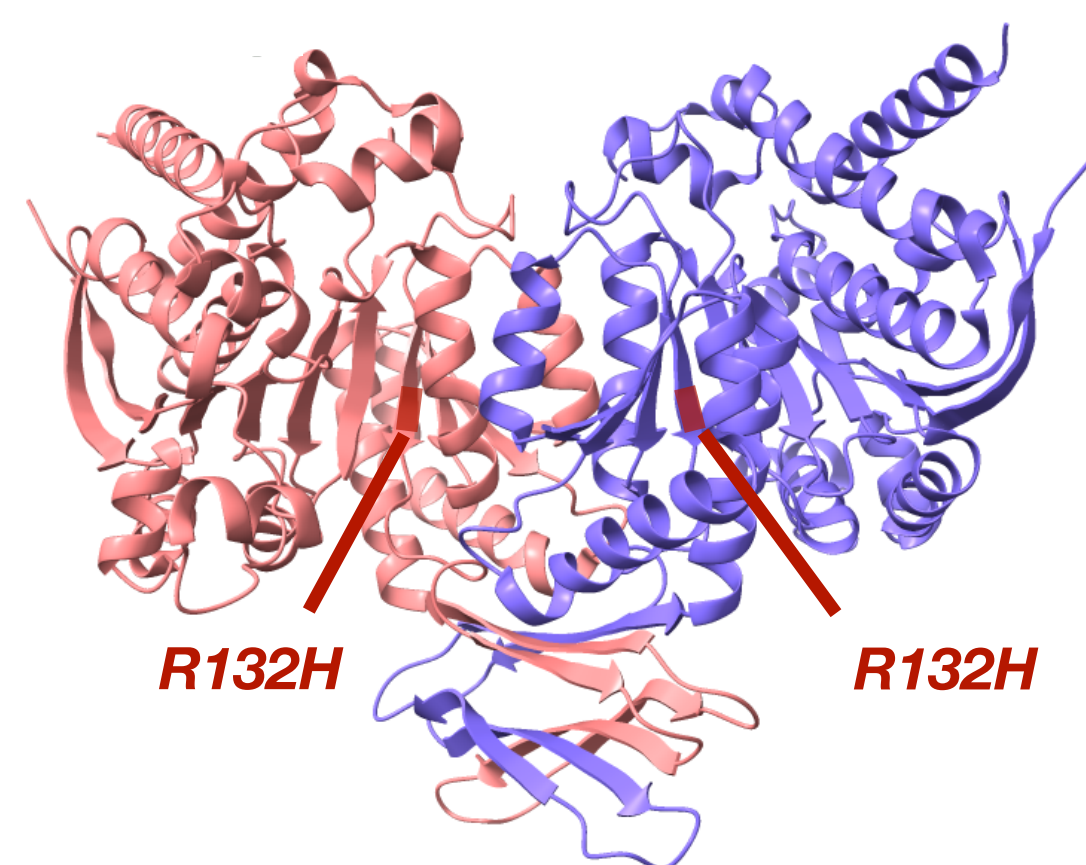


# HTS in small molecule drug discovery

## Case Study: mutant IDH inhibitors - assay development

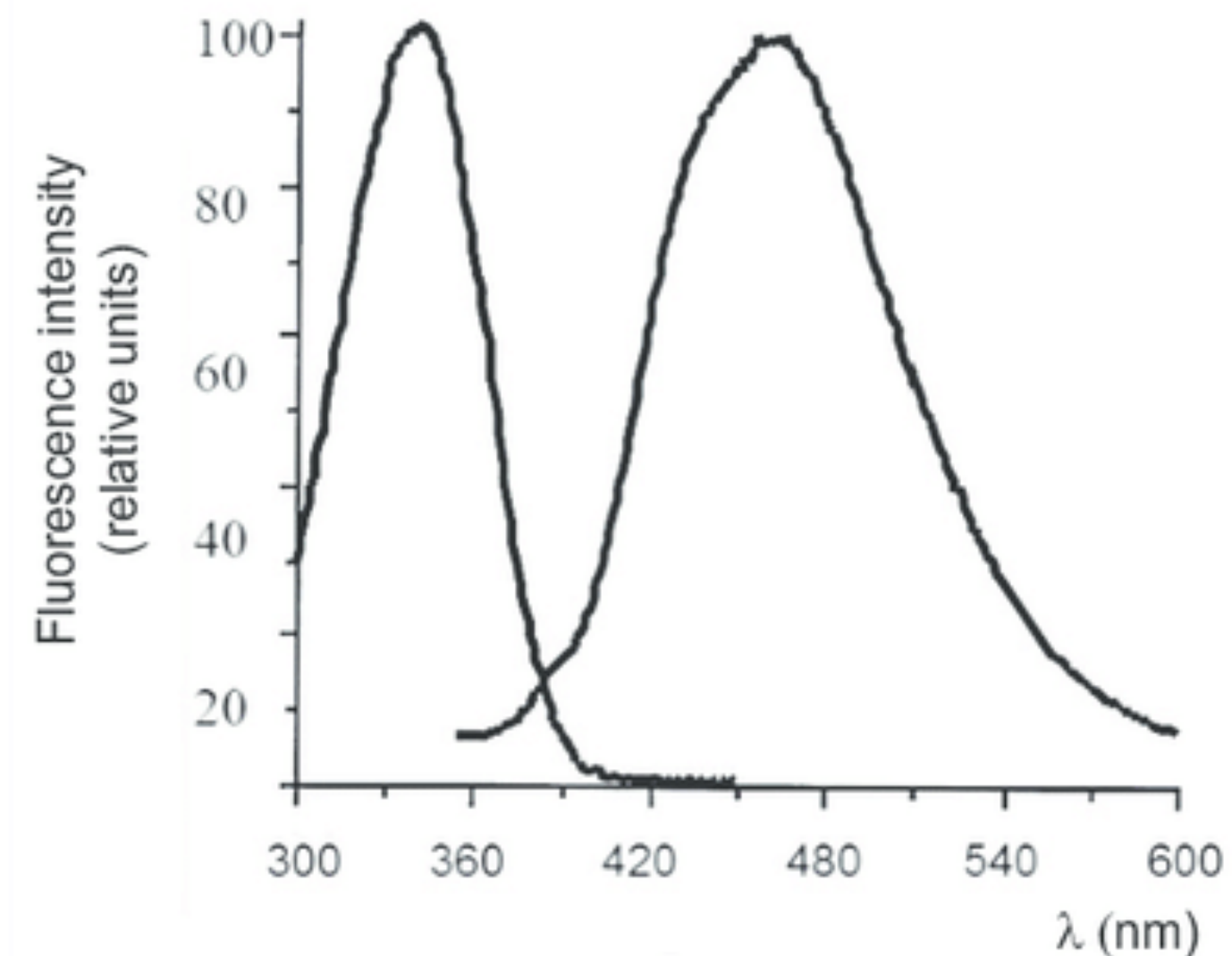


### Recombinant mutant IDH1 homodimer



### Measuring NADPH level

#### NADPH absorbance and emission



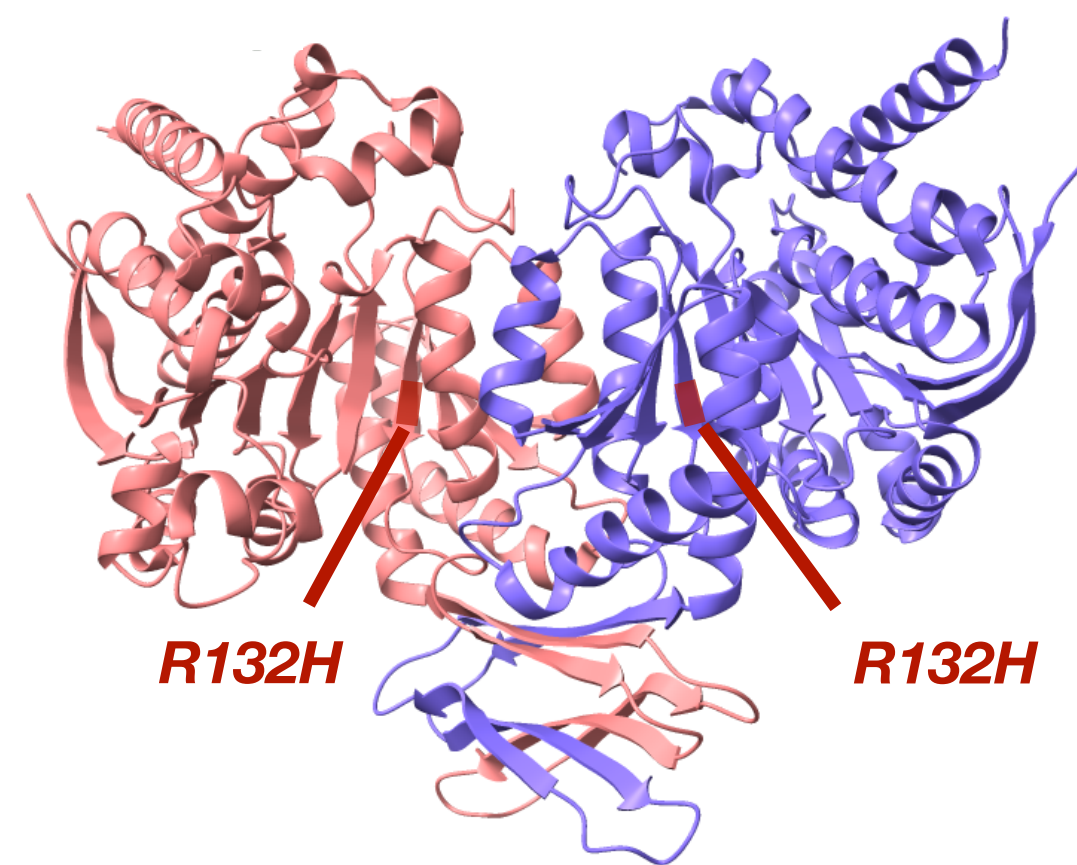
**Problem: compound interference**

# HTS in small molecule drug discovery

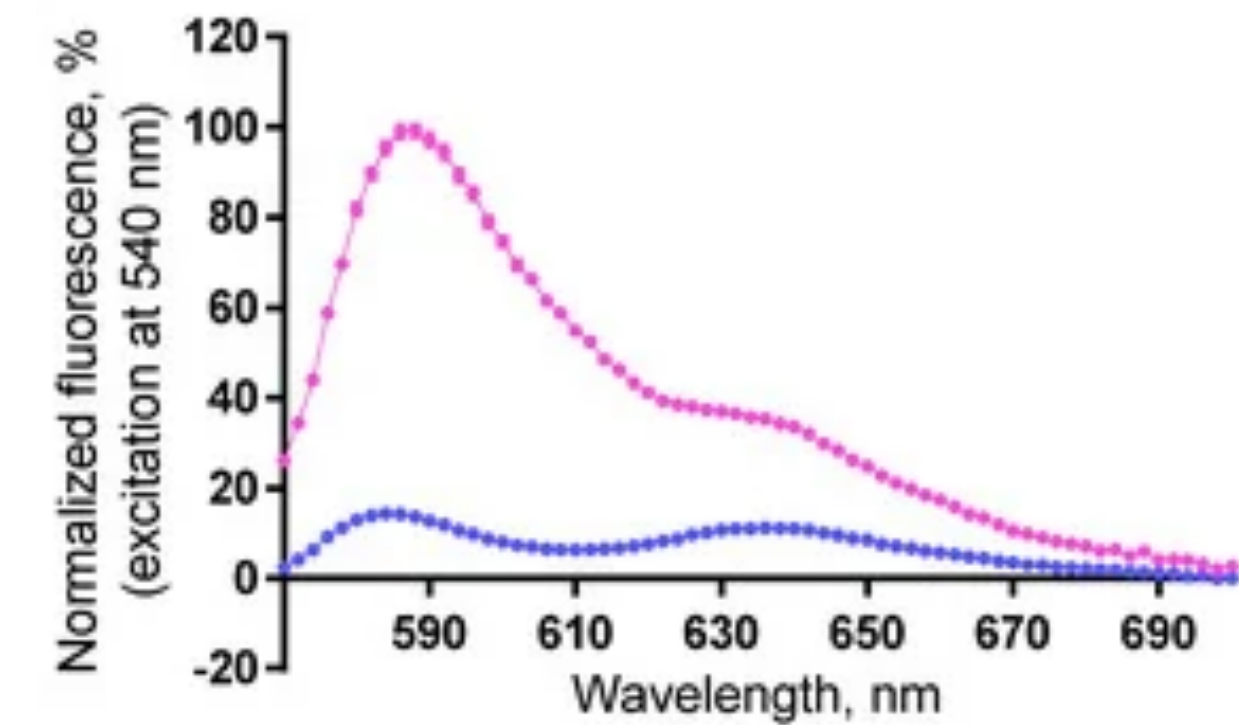
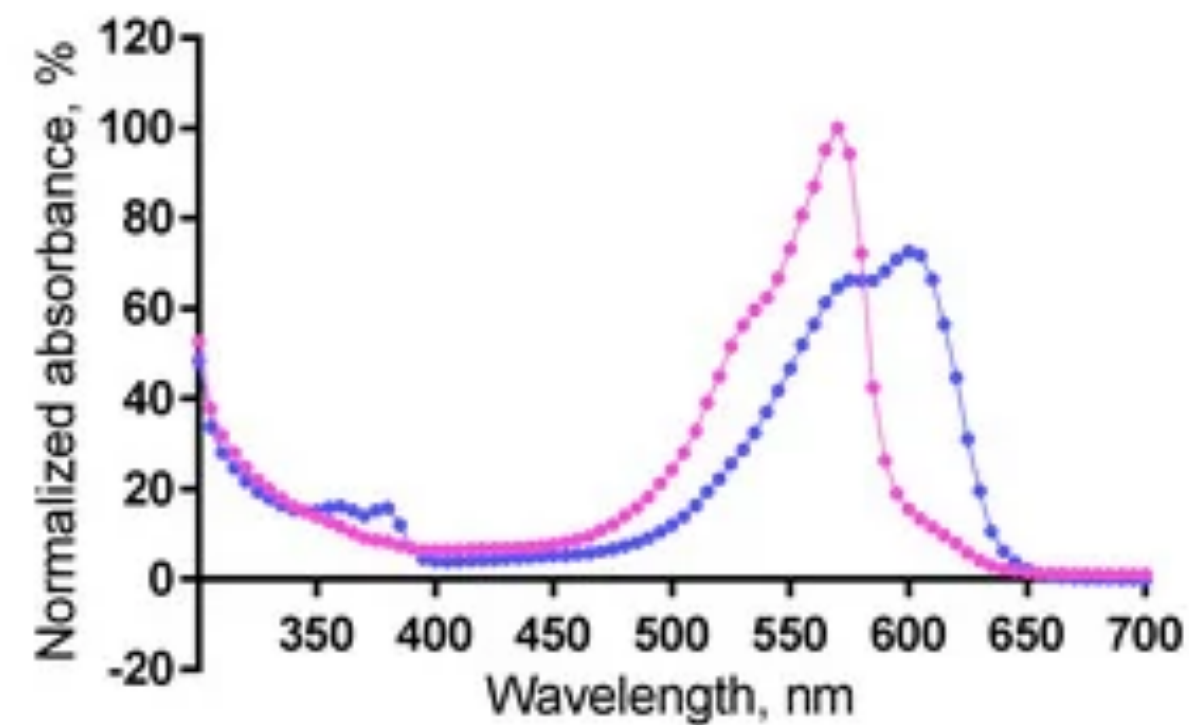
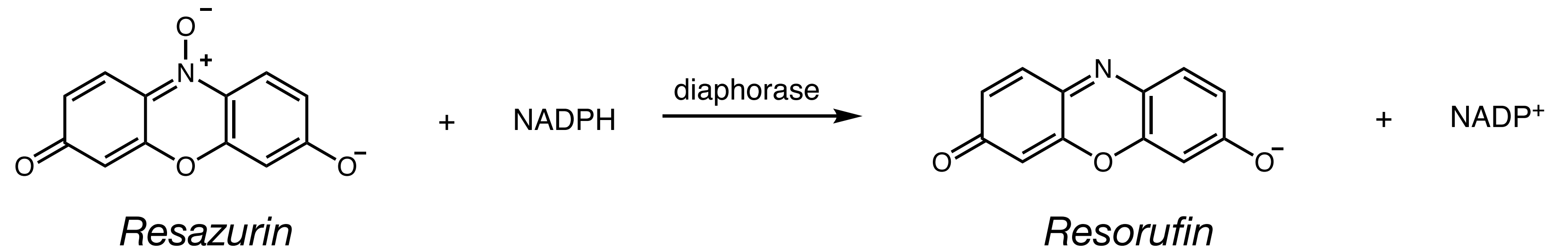
Case Study: mutant IDH inhibitors - assay development



## Recombinant mutant IDH1 homodimer



## Coupling dehydrogenases with diaphorase



— Resazurin — Resorufin

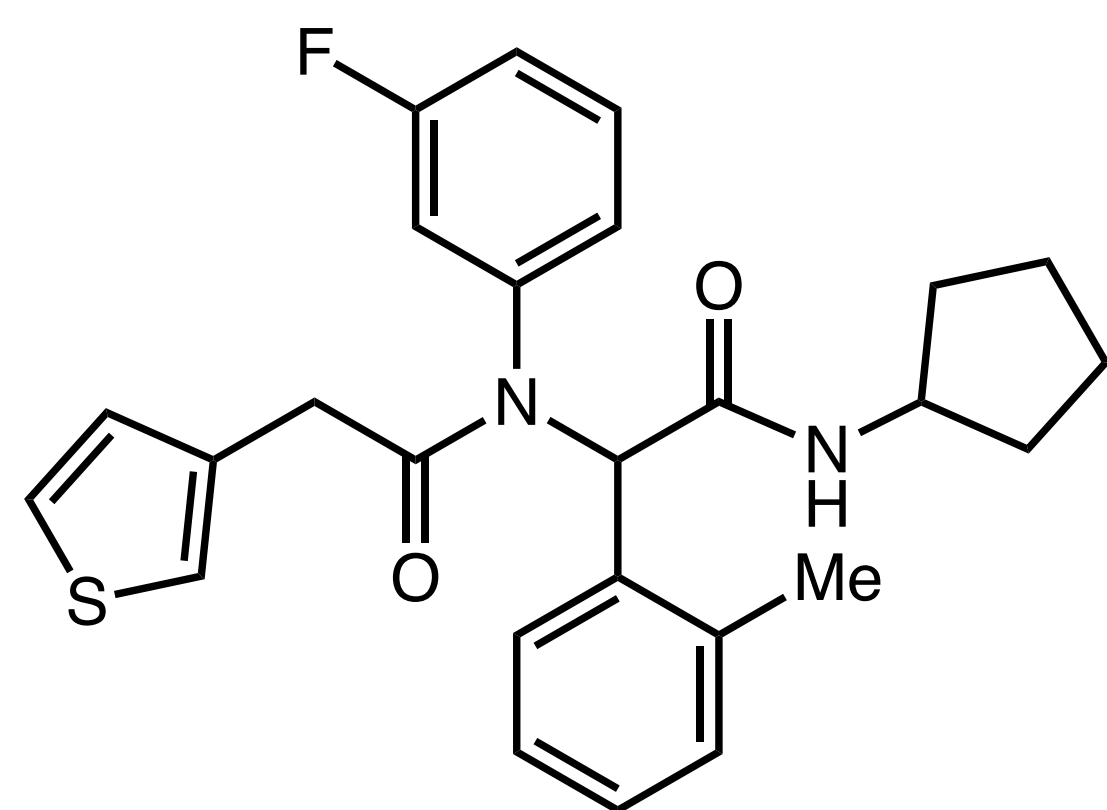
## Redshifting the fluorephore reporter

Popovici-Muller, J., Saunders, J. O., Salituro, F. G., et al. *ACS Med. Chem. Lett.* **2012**, 3

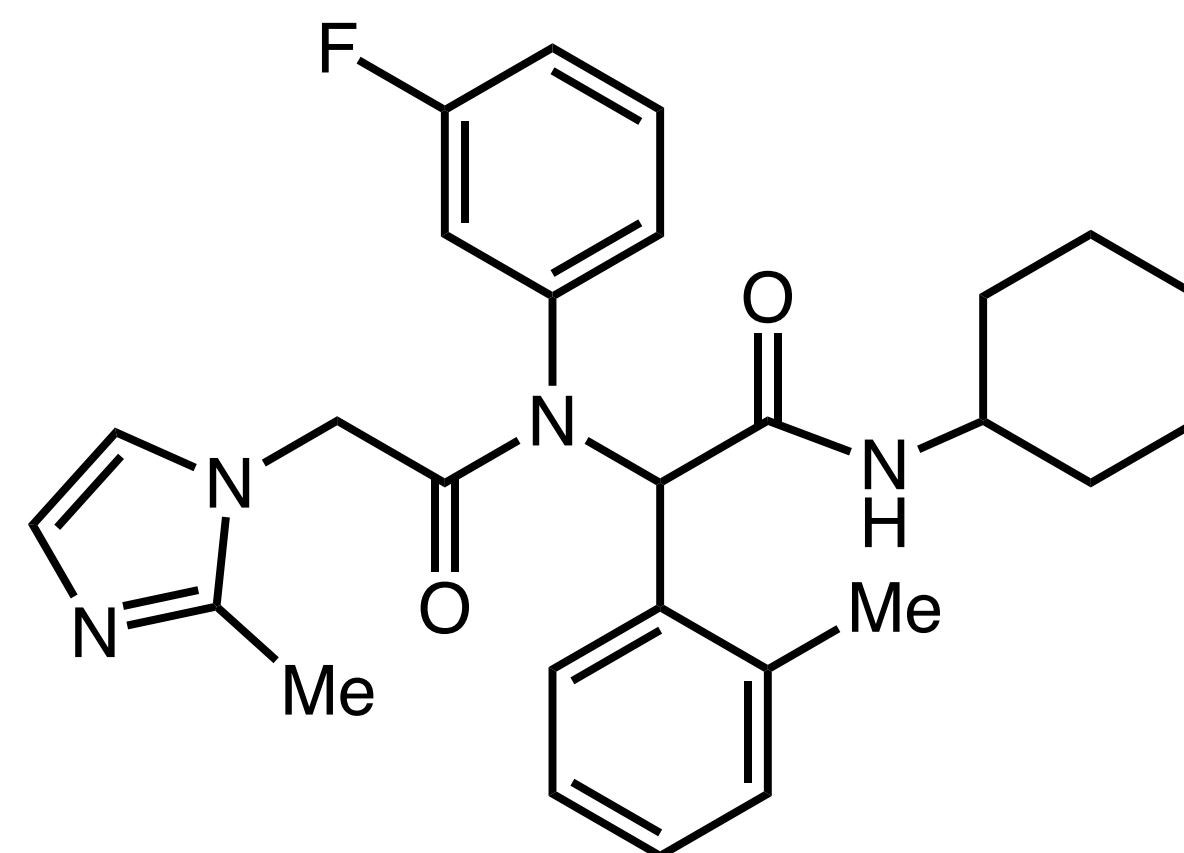
Davis, M., Shen, M., Simeonov, A., et al. *ADST.* **2016**, 14(3)

# HTS in small molecule drug discovery

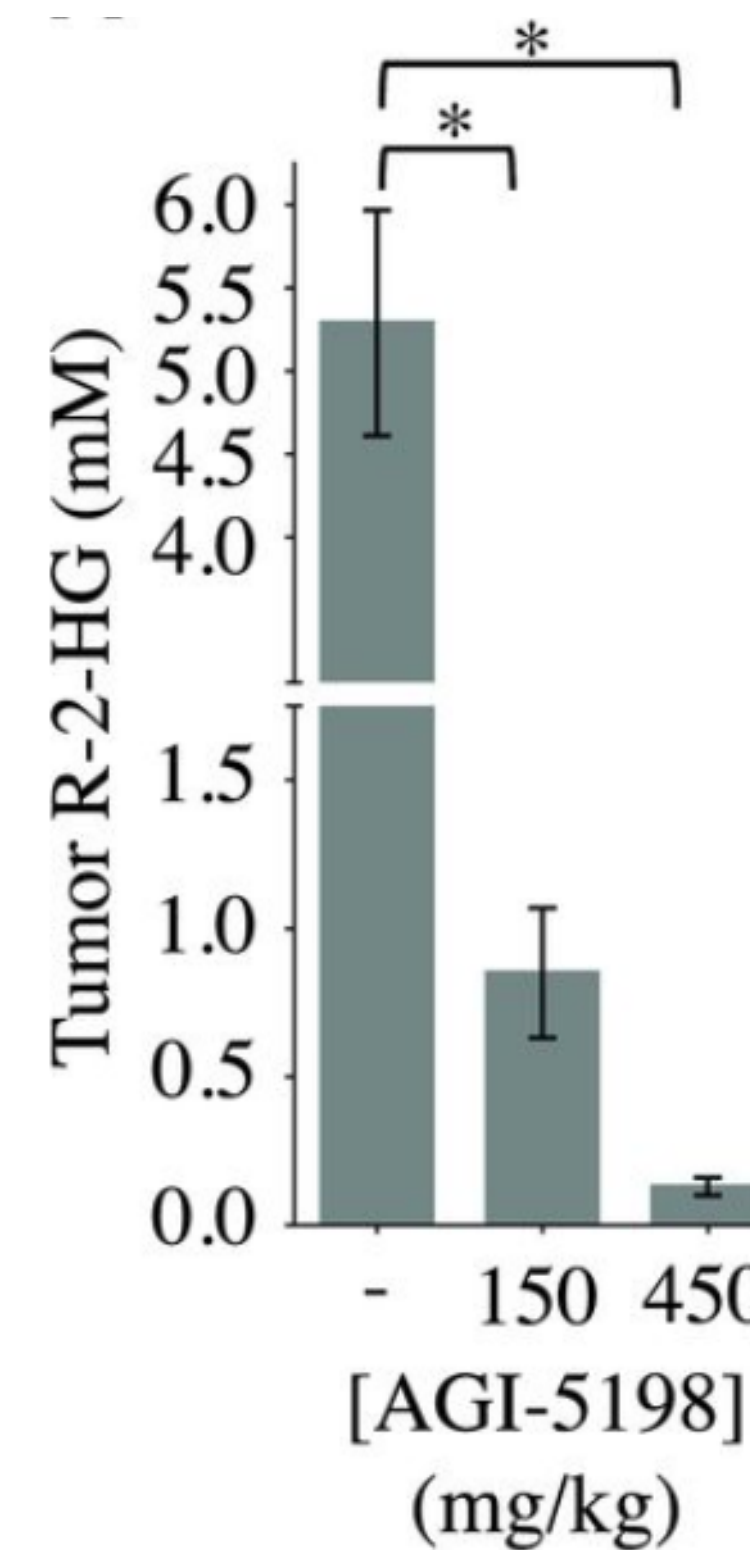
## Case Study: mutant IDH inhibitors - assay development



**HTS hit**



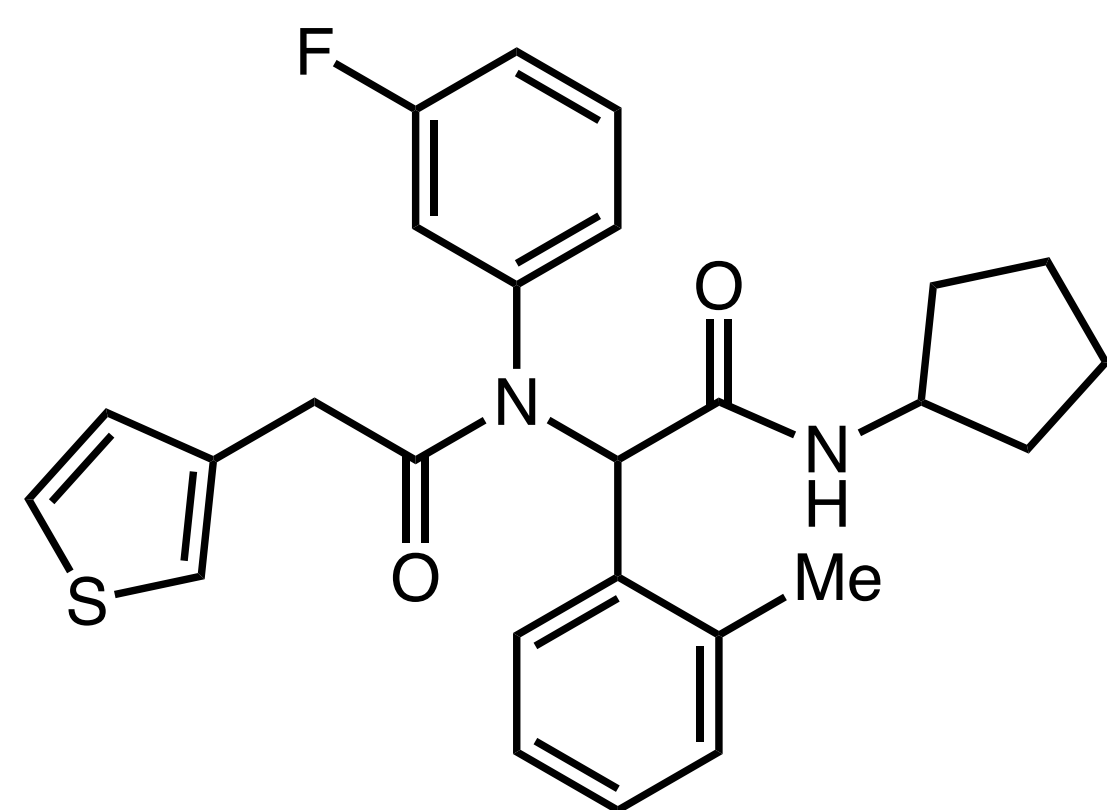
**AG-5198**



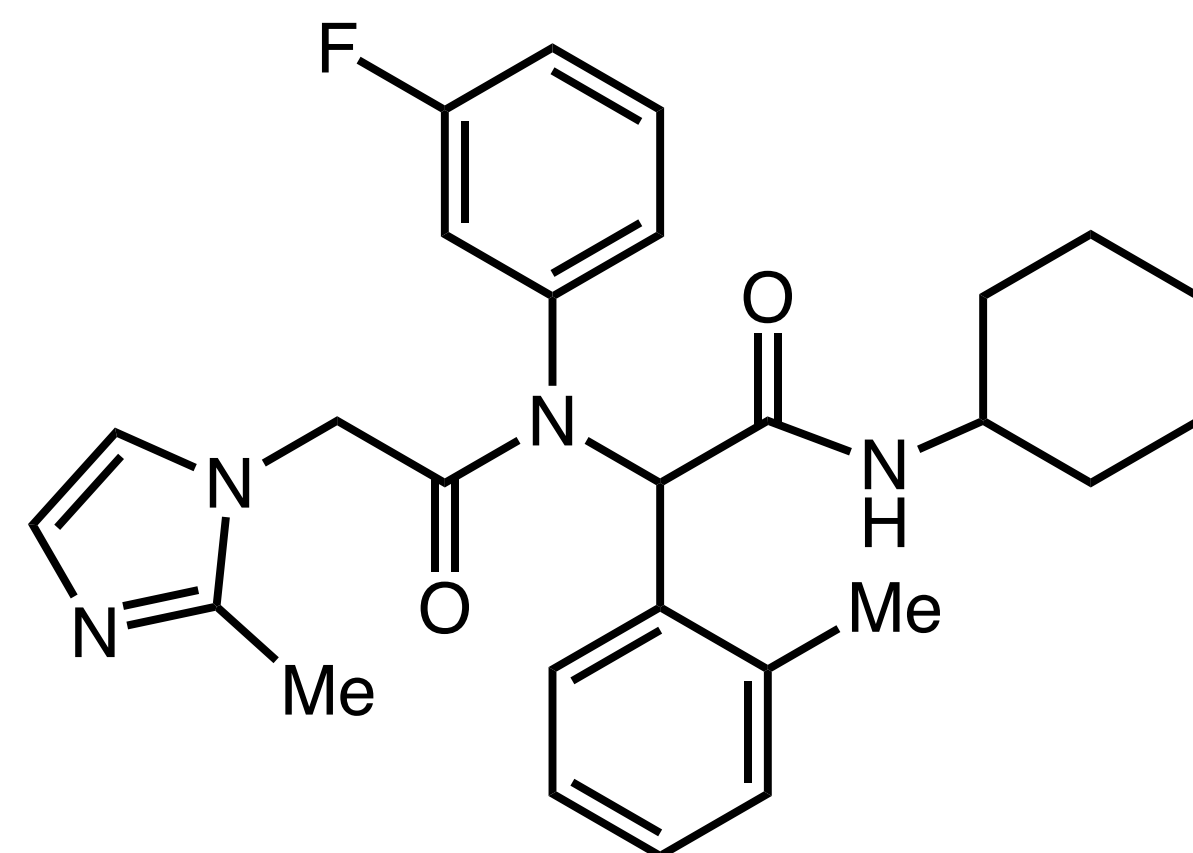
**But AG-5198 shows poor metabolic stability  
(data not disclosed)**

# HTS in small molecule drug discovery

## Case Study: mutant IDH inhibitors

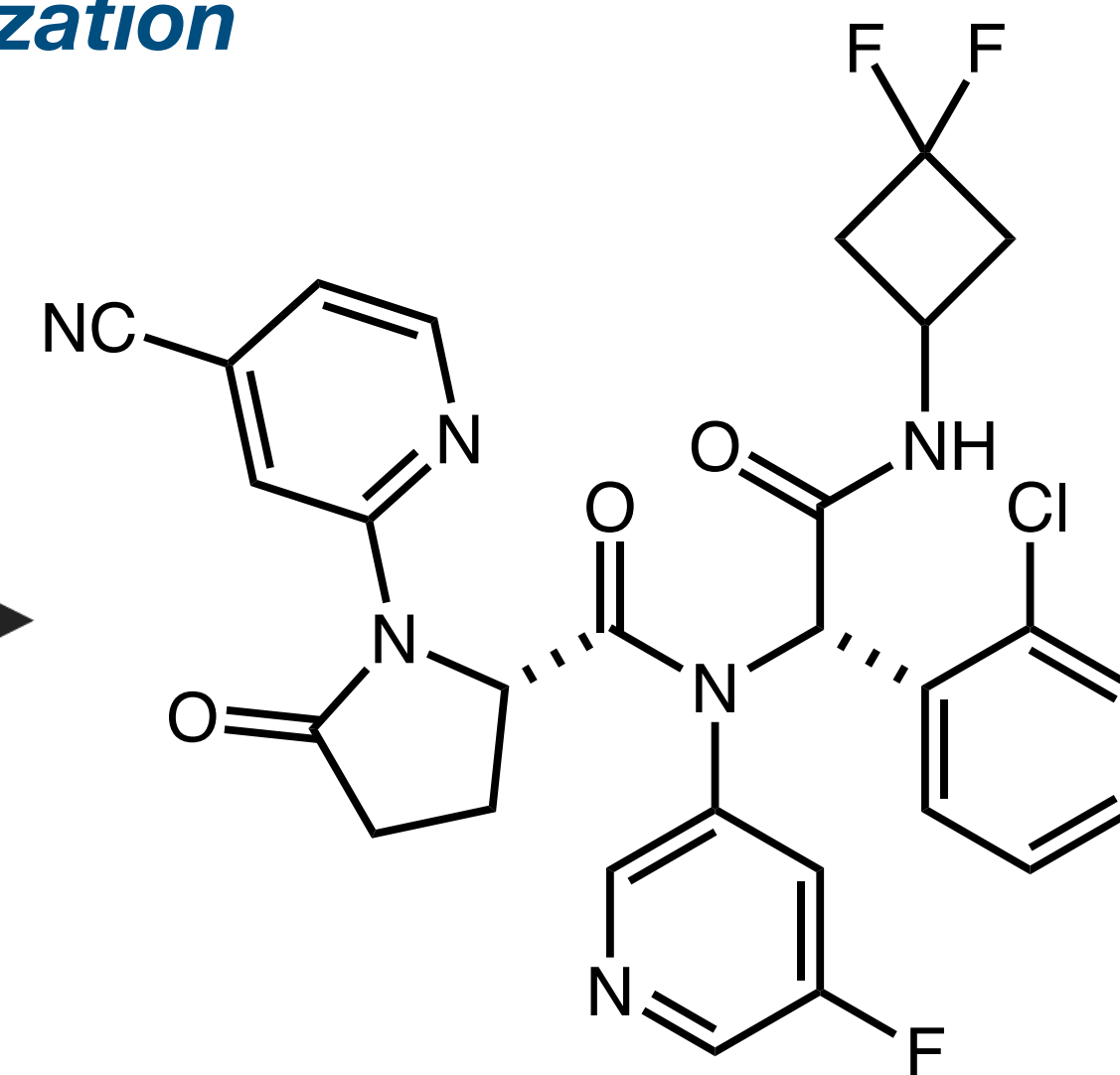


**HTS hit**



**AG-5198**

### Lead optimization



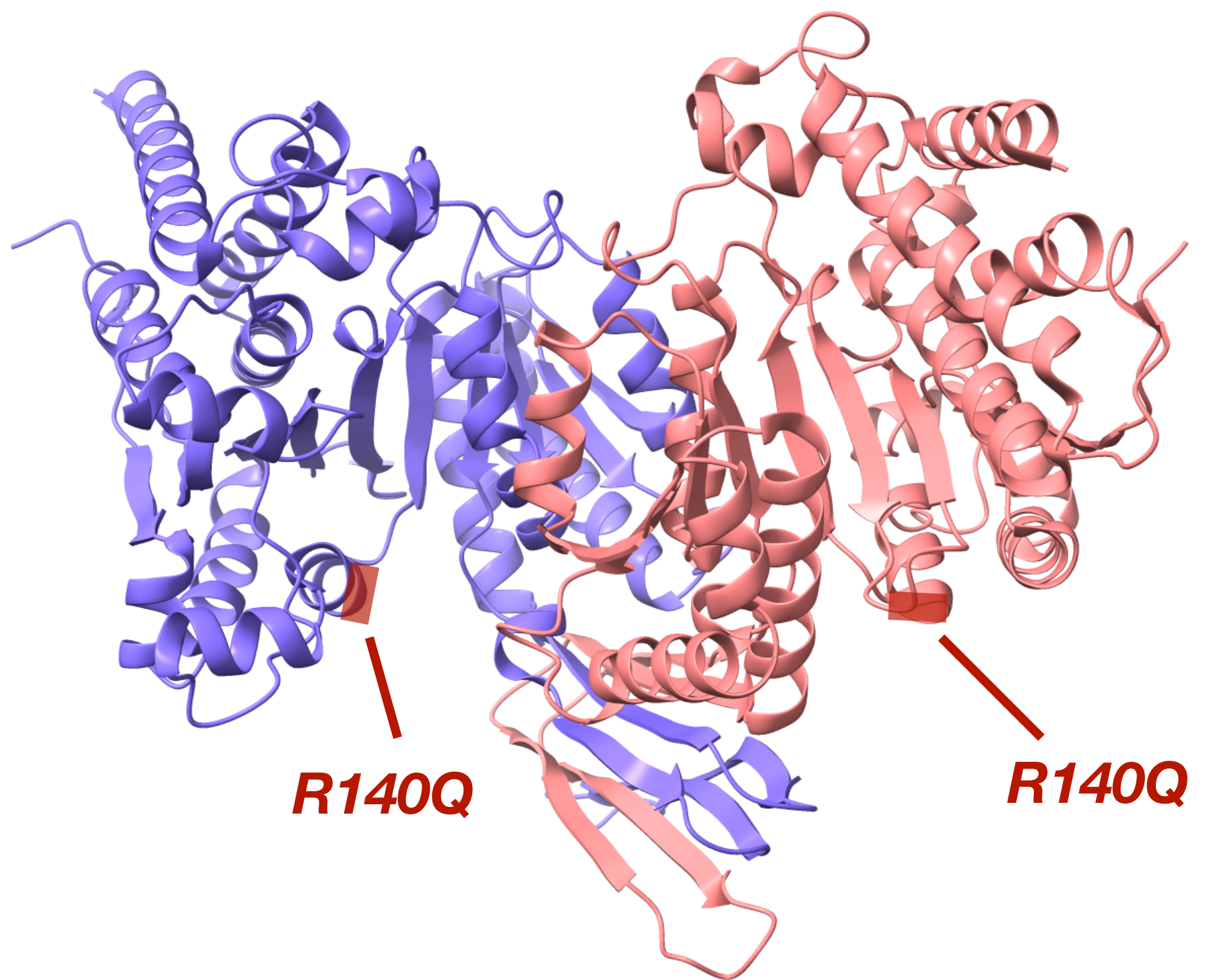
**AG-120**  
**Ivosidenib**



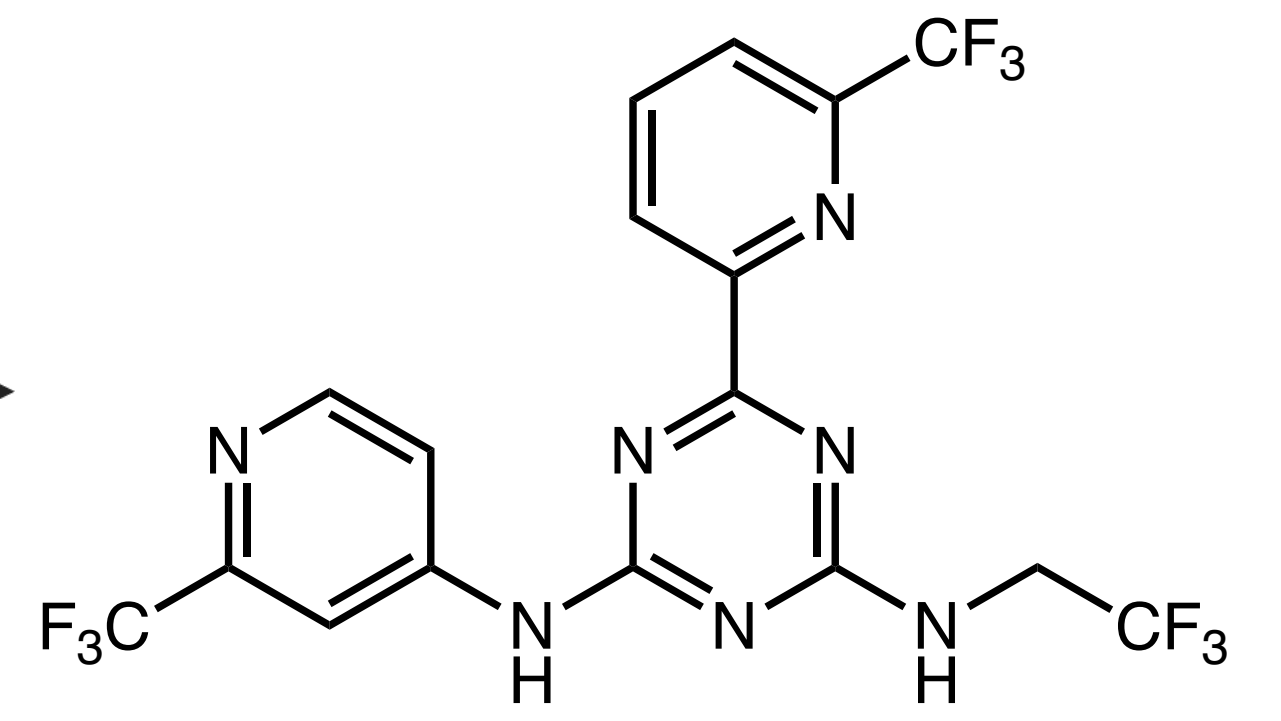
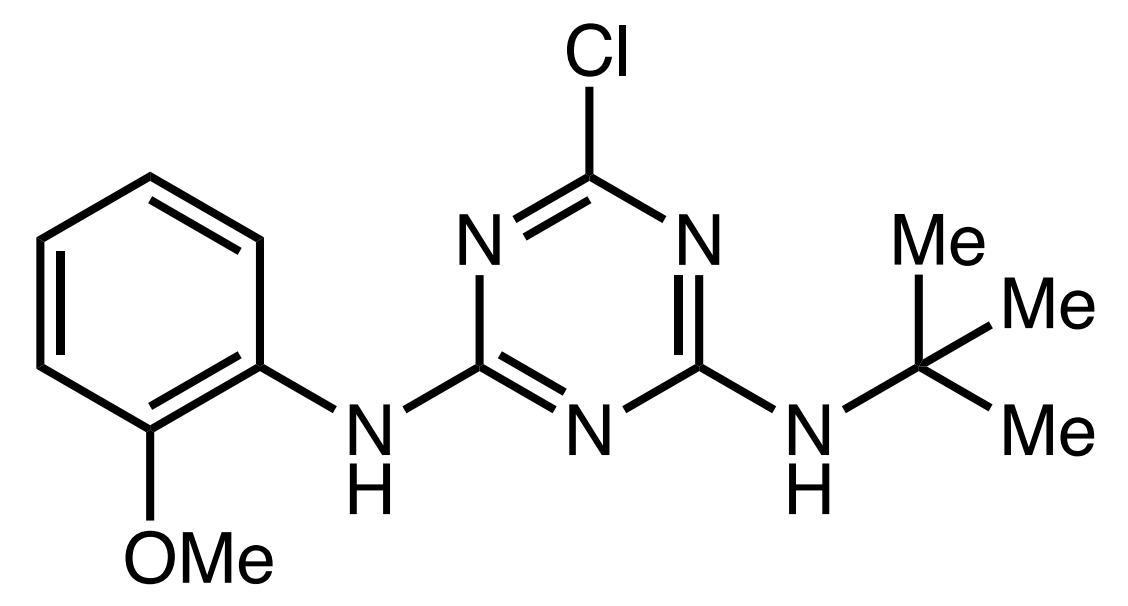
# HTS in small molecule drug discovery

## Case Study: mutant IDH inhibitors

**Mutant IDH2 homodimer**



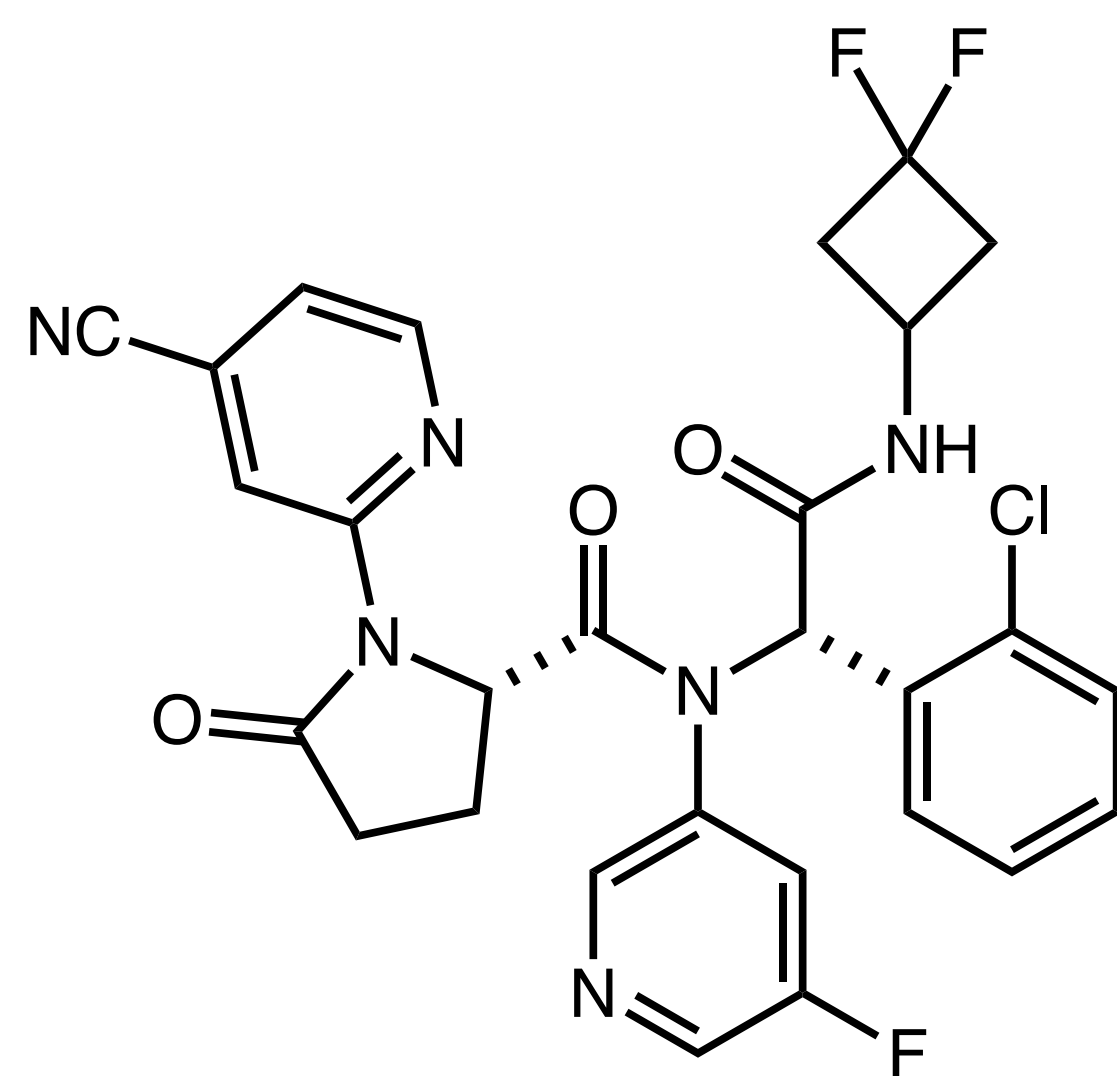
**IDH2 Inhibitor**



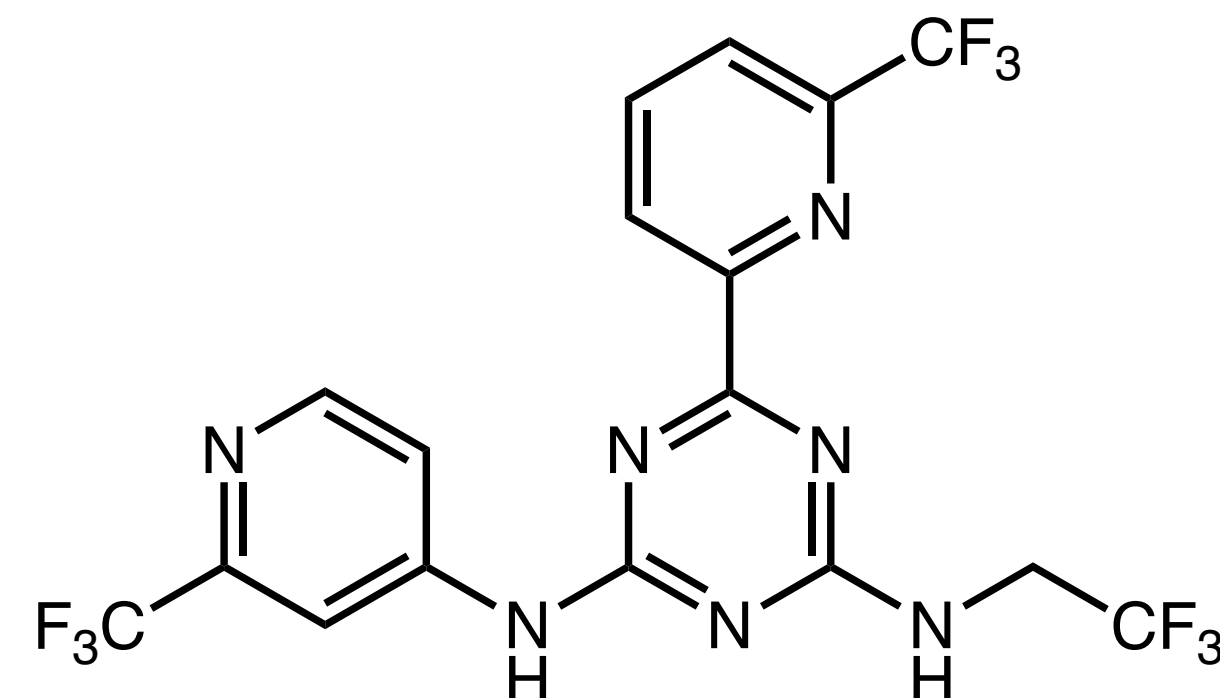
IDH2: catalyzes the same reaction as IDH1 but located in mitochondria

# HTS in small molecule drug discovery

## Case Study: mutant IDH inhibitors



**AG-120**  
**Ivosidenib**  
Mutant IDH1 inhibitor



**AG-221**  
**Enasidenib**  
Mutant IDH2 inhibitor

### Problems with existing inhibitors

Only specific to either IDH1 or IDH2

Isoform selective inhibitors may lead to acquired resistance

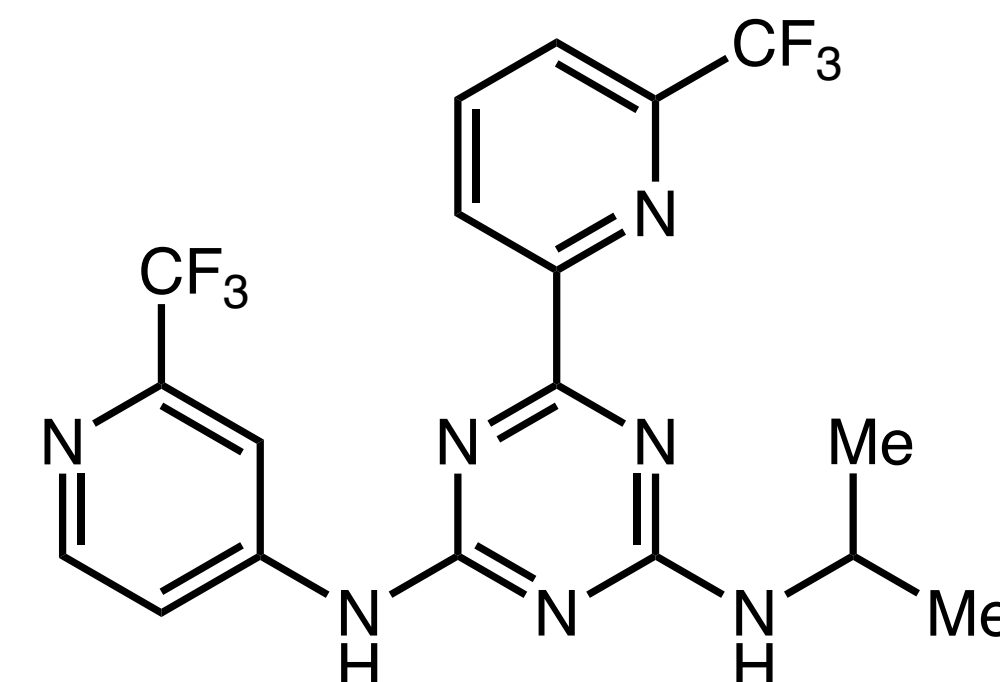
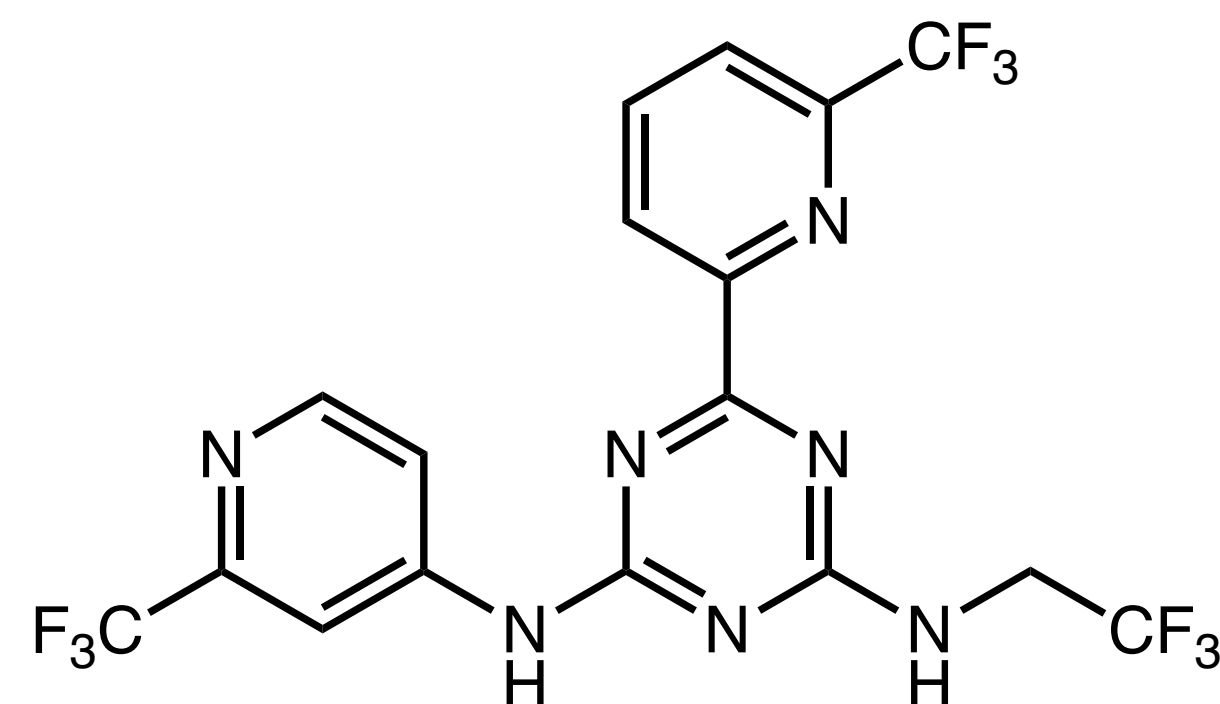
Both approved for acute myeloid leukemia (AML)

Low brain drug exposure limits potential efficacy for glioma

**Are there dual IDH inhibitors that also penetrate the brain?**

# HTS in small molecule drug discovery

## Case Study: mutant IDH inhibitors



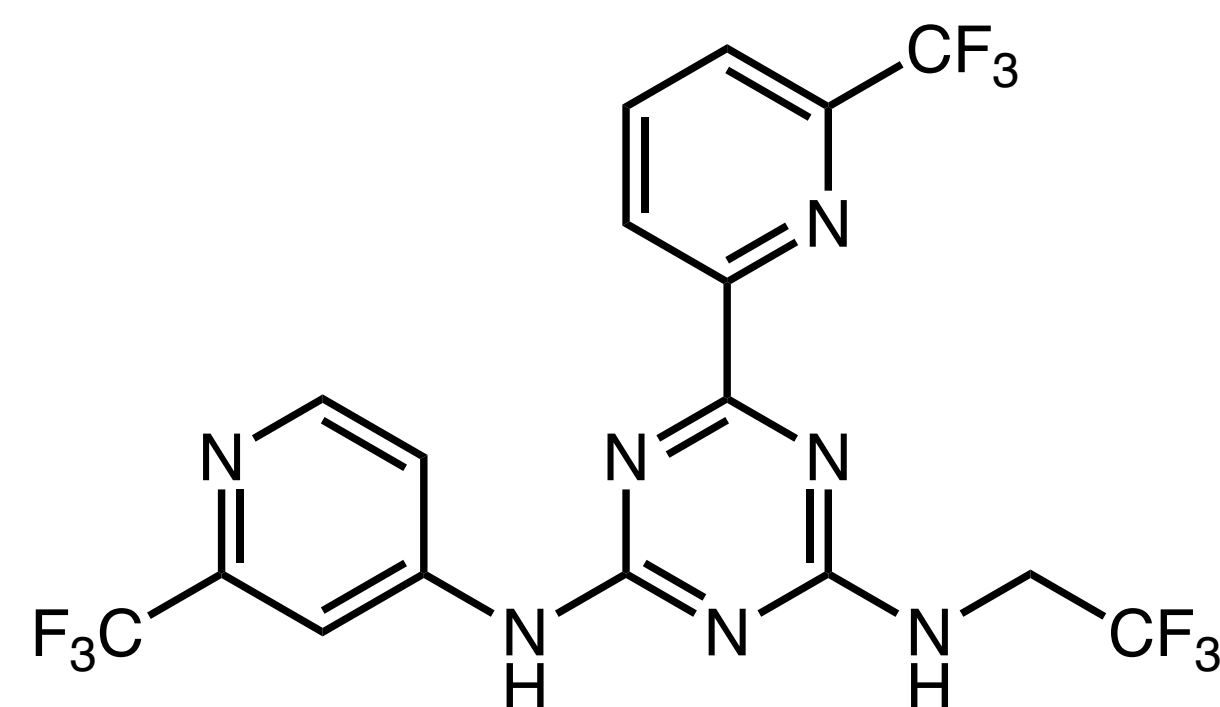
*AG-221 Enasidenib*

*AGI-12026*

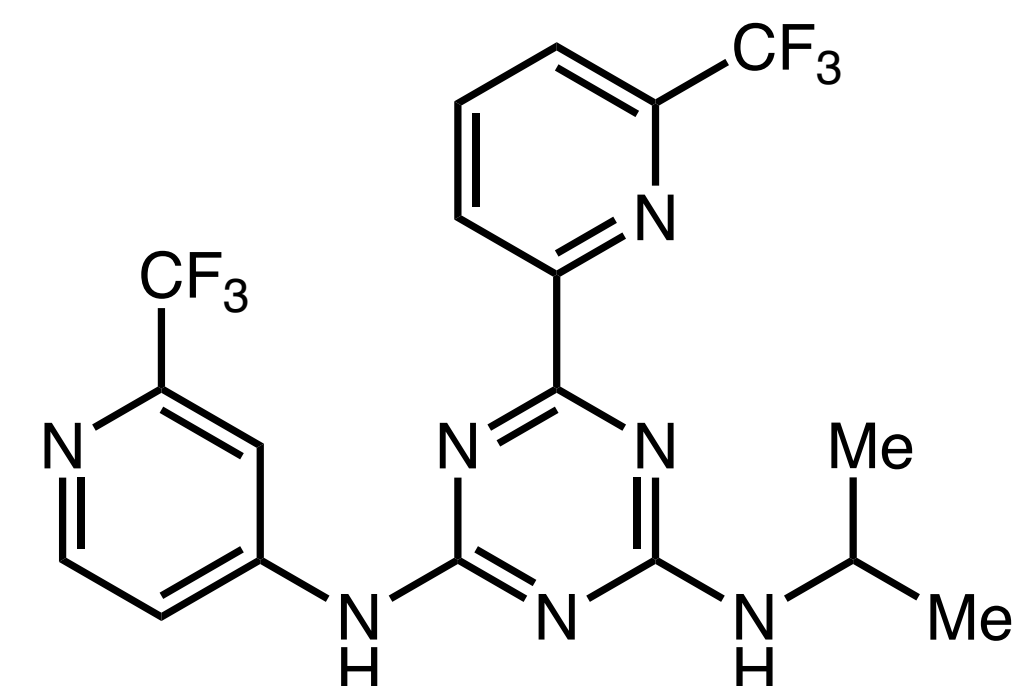
<i>IDH1-WT/IDH-R132H IC<sub>50</sub></i>	<i>0.677 μM</i>	<i>0.020 μM</i>
<i>IDH2-R140Q IC<sub>50</sub></i>	<i>0.009 μM</i>	<i>0.078 μM</i>
<i>Mean mouse brain-to-plasma ratio</i>	<i>0.14</i>	<i>2.5</i>

# HTS in small molecule drug discovery

## Case Study: mutant IDH inhibitors



AG-221 Enasidenib



AGI-12026

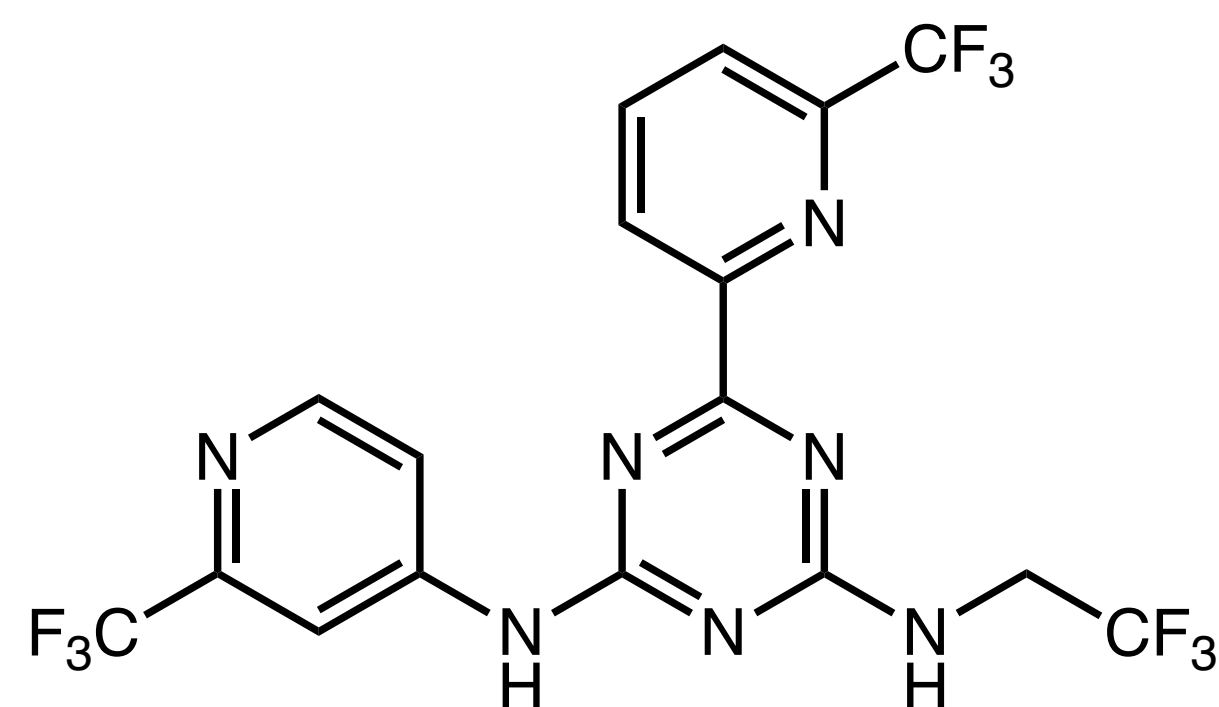
	AG-221 Enasidenib	AGI-12026
IDH1-WT/IDH-R132H IC <sub>50</sub>	0.677 μM	0.020 μM
IDH2-R140Q IC <sub>50</sub>	0.009 μM	0.078 μM
Mean mouse brain-to-plasma ratio	0.14	<b>2.5</b>

Several triazine compounds showed some inhibition for both IDH1 and IDH2 and good brain-to-plasma ratio

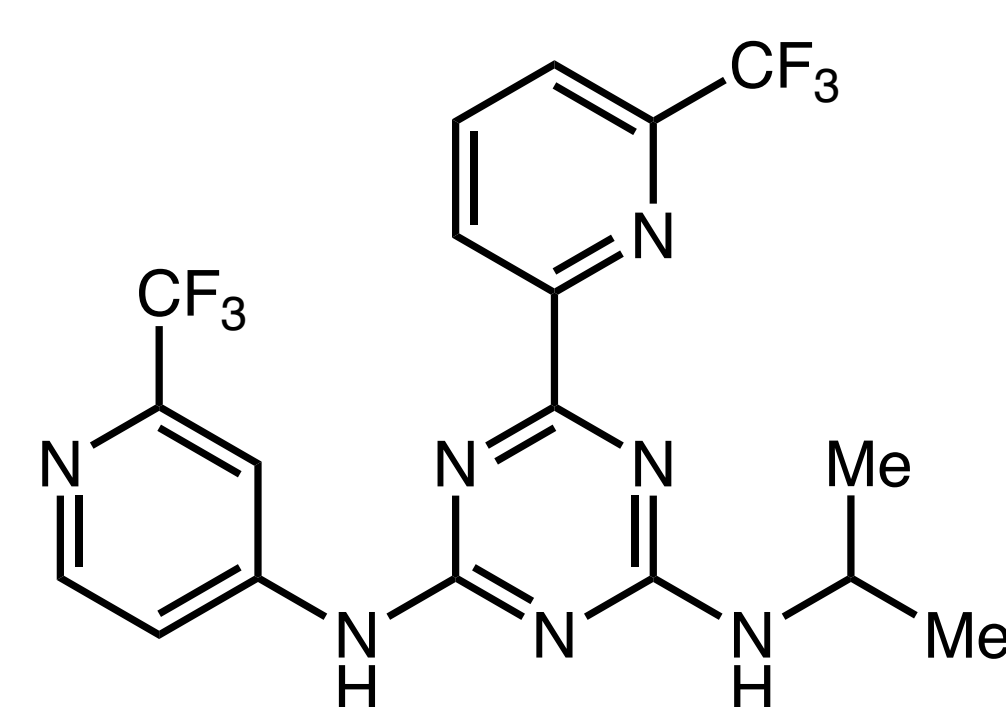
→ Screened through all triazine compounds

# HTS in small molecule drug discovery

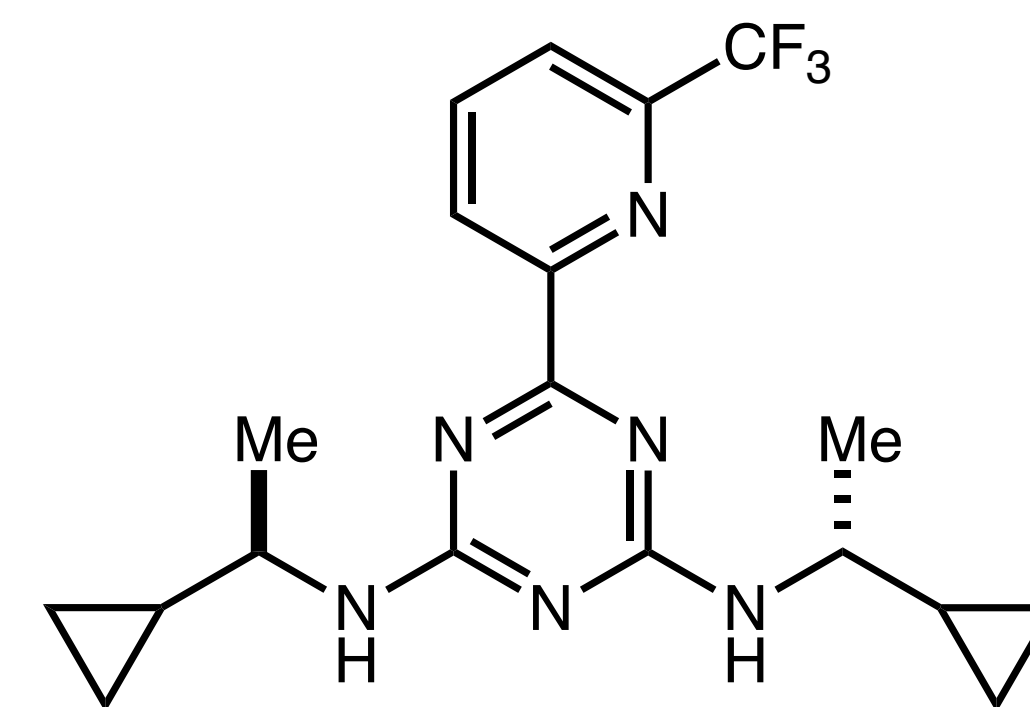
## Case Study: mutant IDH inhibitors



*AG-221 Enasidenib*



*AGI-12026*

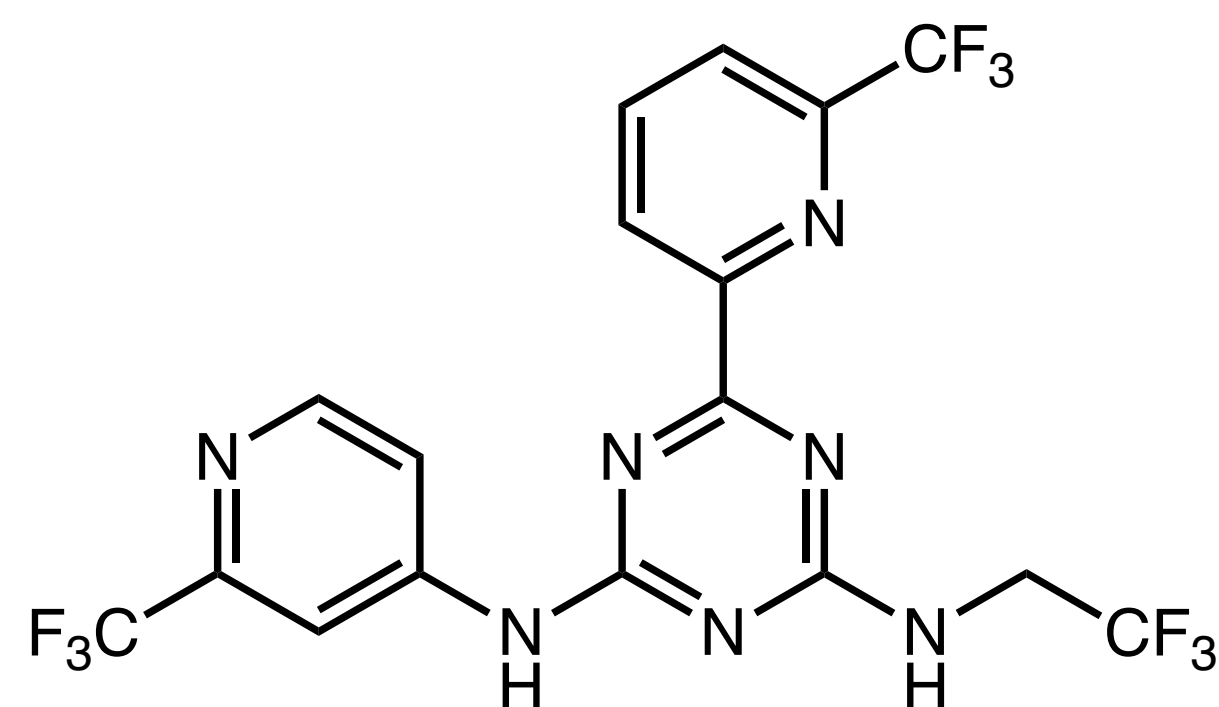


*HTS hit: AGI-15056*

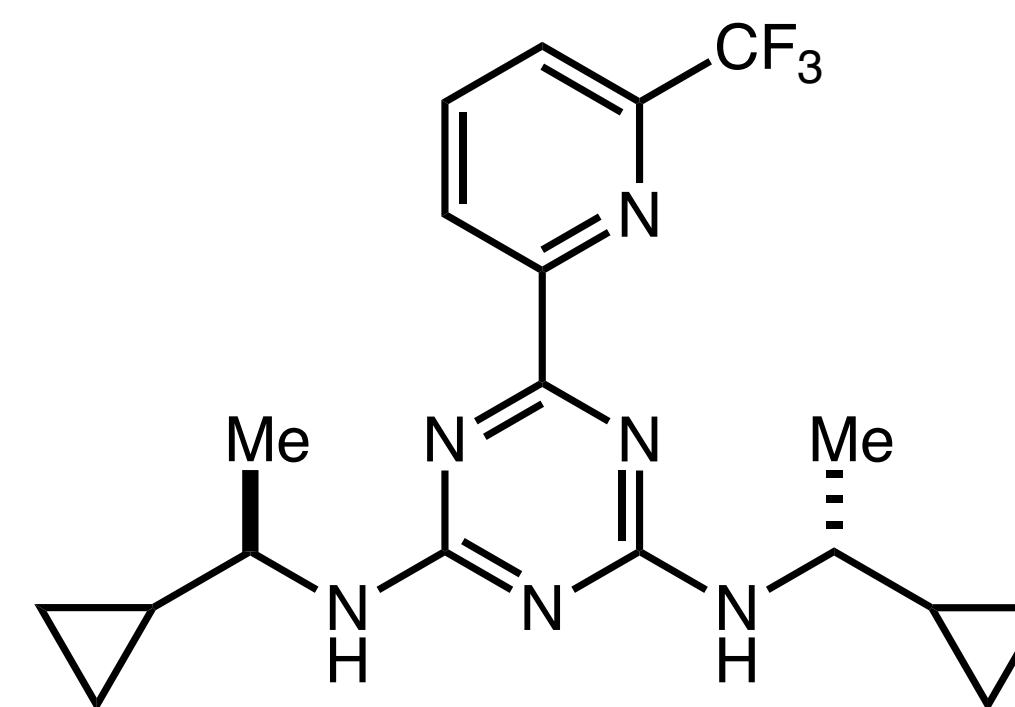
	<i>AG-221 Enasidenib</i>	<i>AGI-12026</i>	<i>HTS hit: AGI-15056</i>
<i>IDH1-WT/IDH-R132H IC<sub>50</sub></i>	<i>0.677 μM</i>	<i>0.020 μM</i>	<i>0.006 μM</i>
<i>IDH2-R140Q IC<sub>50</sub></i>	<i>0.009 μM</i>	<i>0.078 μM</i>	<i>0.048 μM</i>
<i>Mean mouse brain-to-plasma ratio</i>	<i>0.14</i>	<i>2.5</i>	<i>1.5</i>

# HTS in small molecule drug discovery

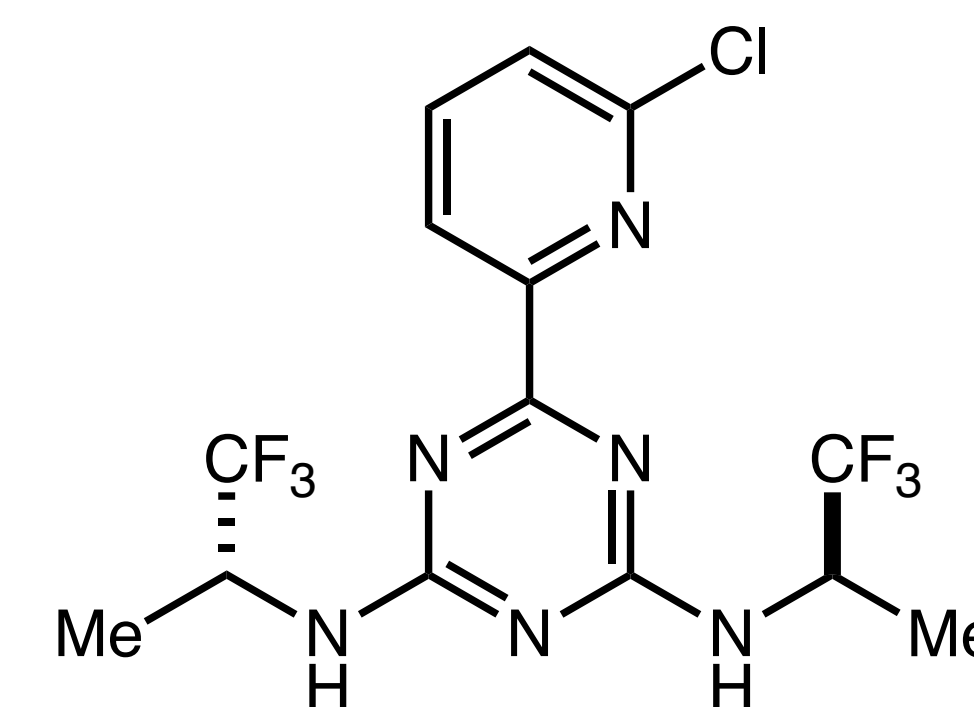
## Case Study: mutant IDH inhibitors



*AG-221 Enasidenib*



*HTS hit: AGI-15056*



**Lead optimization: AG-881**

	<i>AG-221 Enasidenib</i>	<i>HTS hit: AGI-15056</i>	<b>Lead optimization: AG-881</b>
<i>IDH1-WT/IDH-R132H IC<sub>50</sub></i>	<i>0.677 μM</i>	<i>0.006 μM</i>	<i>0.0006 μM</i>
<i>IDH2-R140Q IC<sub>50</sub></i>	<i>0.009 μM</i>	<i>0.048 μM</i>	<i>0.007 μM</i>
<i>Mean mouse brain-to-plasma ratio</i>	<i>0.14</i>	<i>1.5</i>	<i>1.33</i>

# HTS in small molecule drug discovery

## Case Study: mutant IDH inhibitors

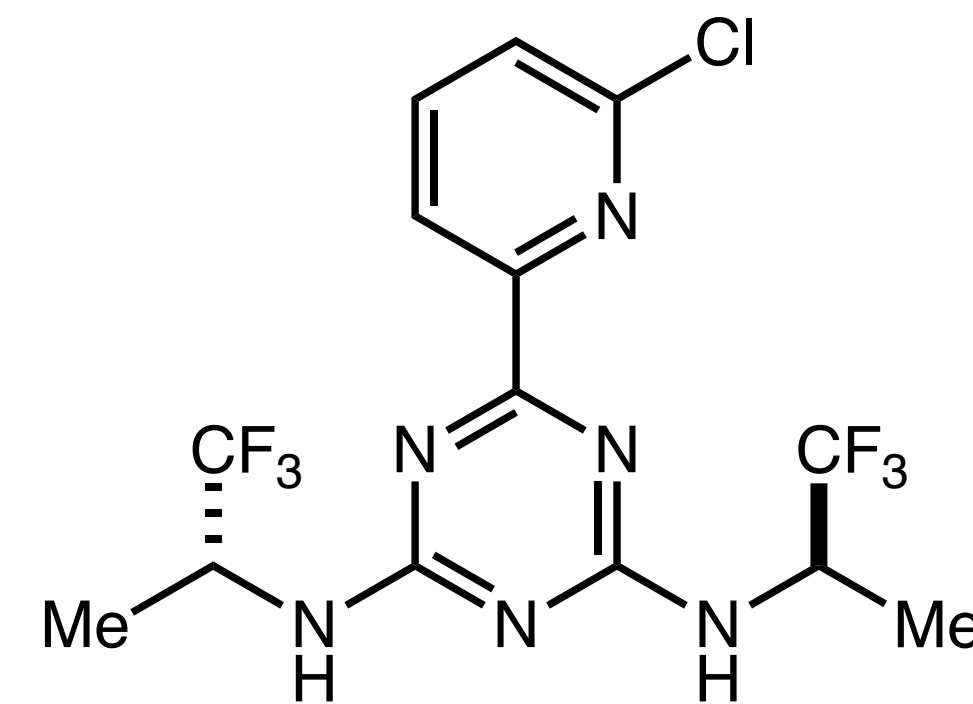
### FDA approves vorasidenib for Grade 2 astrocytoma or oligodendroglioma with a susceptible IDH1 or IDH2 mutation

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On August 6, 2024, the Food and Drug Administration approved vorasidenib (Vorango, Servier Pharmaceuticals LLC), an isocitrate dehydrogenase-1 (IDH1) and isocitrate dehydrogenase-2 (IDH2) inhibitor, for adult and pediatric patients 12 years and older with Grade 2 astrocytoma or oligodendroglioma with a susceptible IDH1 or IDH2 mutation, following surgery including biopsy, sub-total resection, or gross total resection.

Full prescribing information for Vorango will be posted on [Drugs@FDA](#).

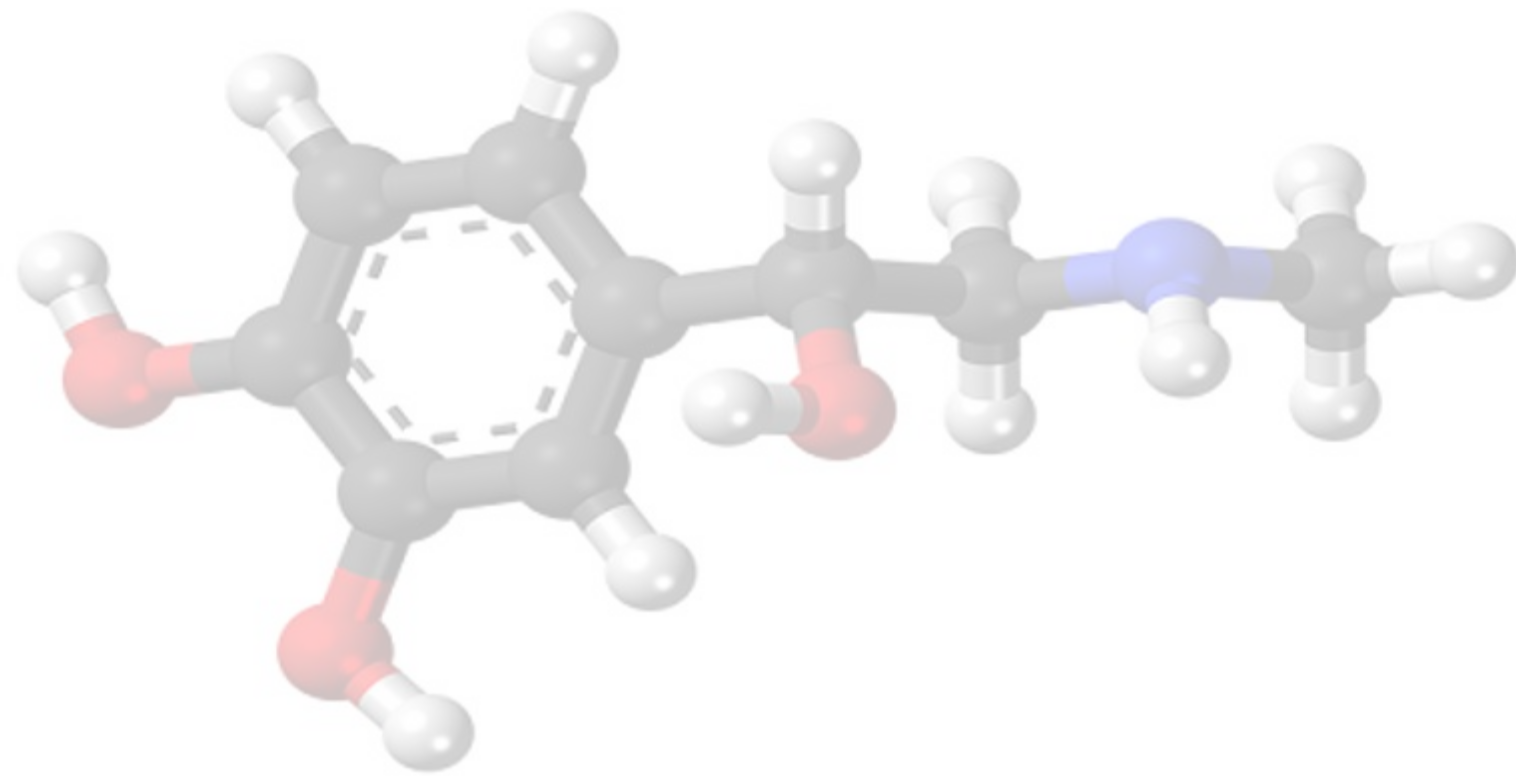
This is the first approval by the FDA of a systemic therapy for patients with Grade 2 astrocytoma or oligodendroglioma with a susceptible IDH1 or IDH2 mutation.



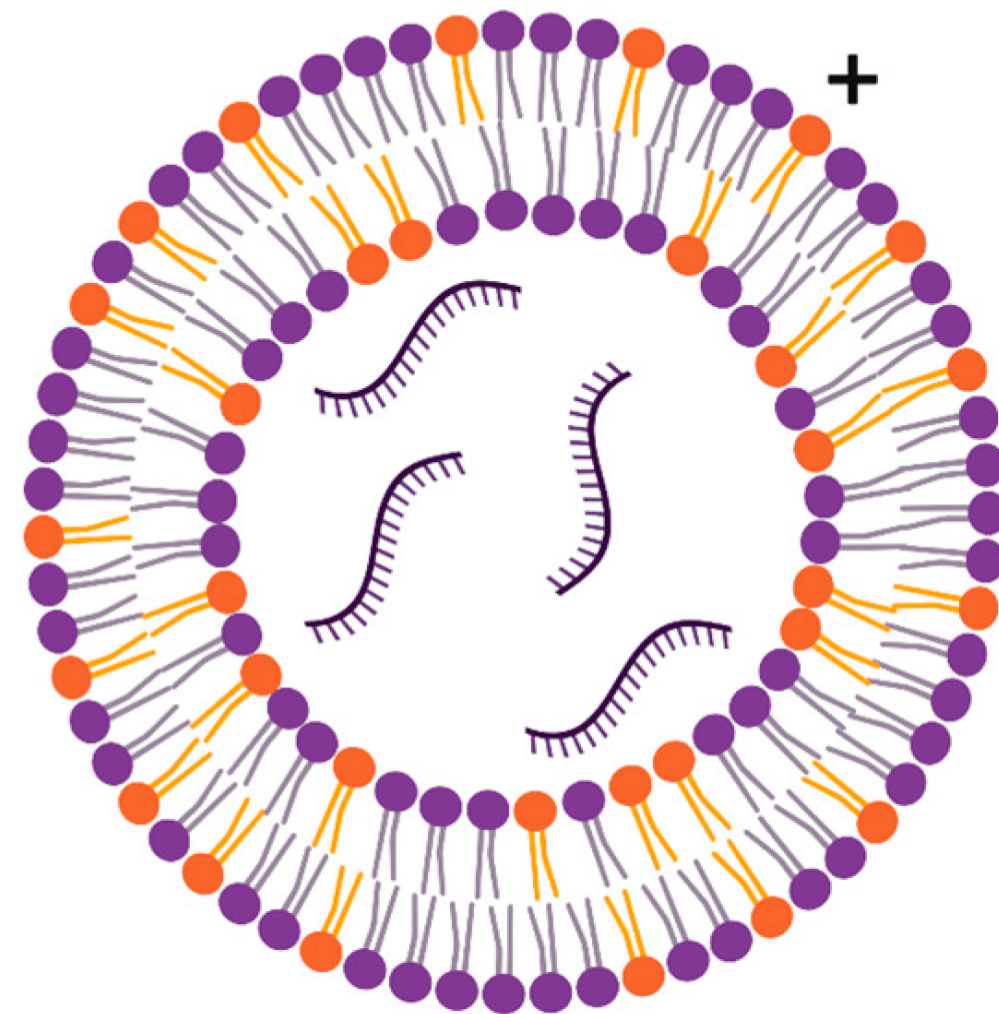
**AG-881**  
**Vorasidenib**

*Approved by the FDA on Aug 6, 2024  
For the treatment of low-grade glioma*

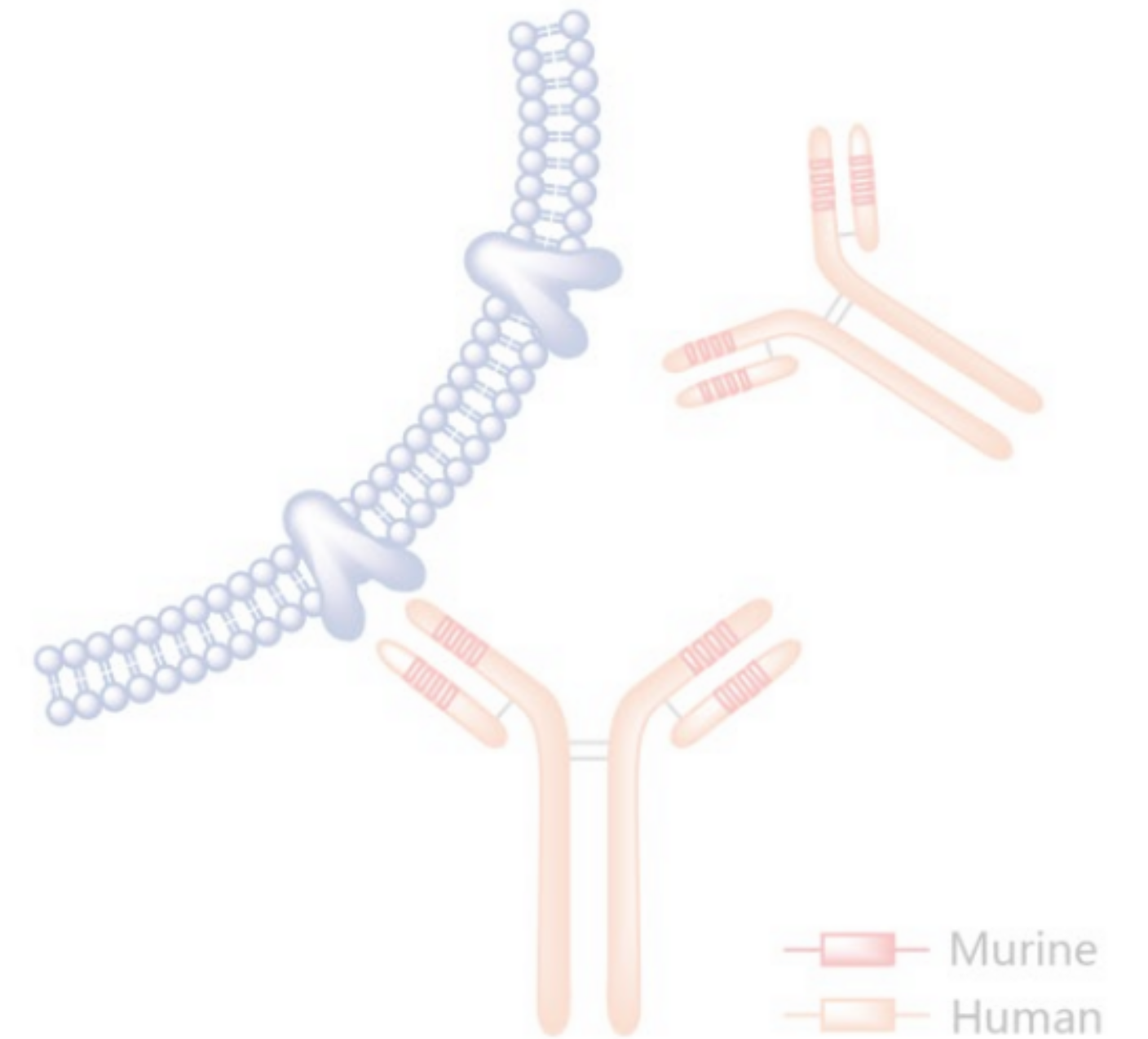
# *HTS strategies in drug discovery*



*Small molecule drugs*



*RNA based therapies*

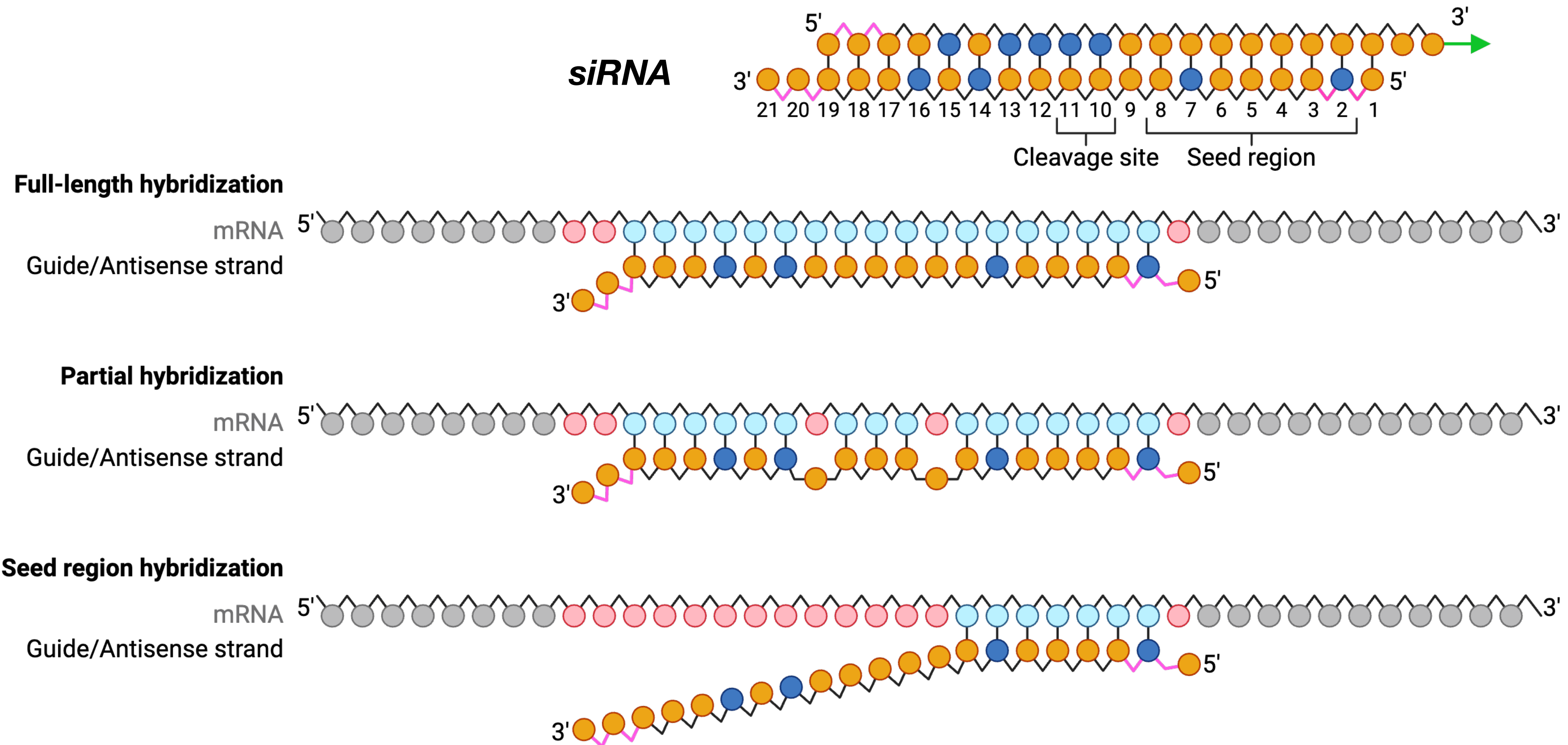


*Antibody based therapies*



# HTS in RNA based drug discovery

## siRNA sequence

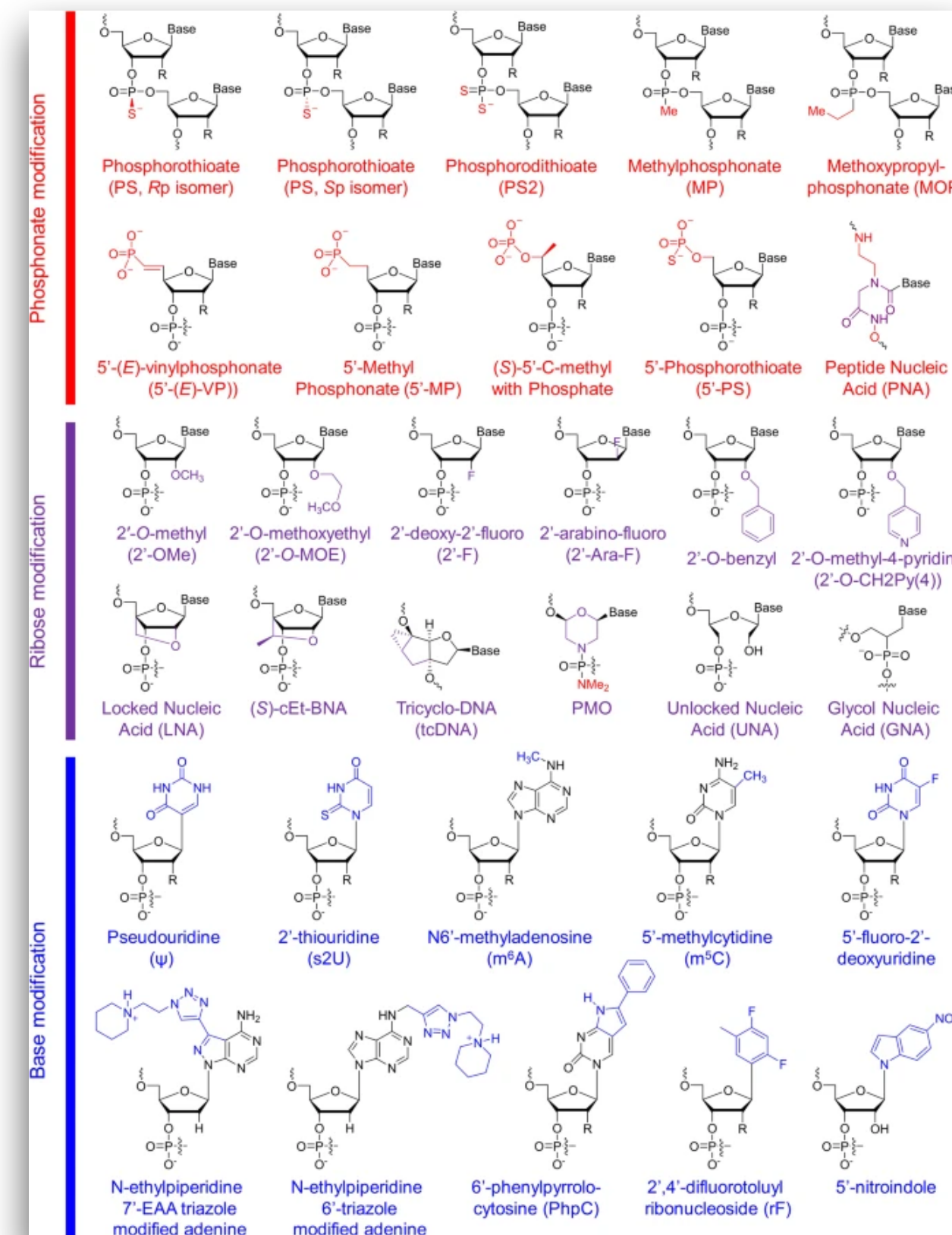
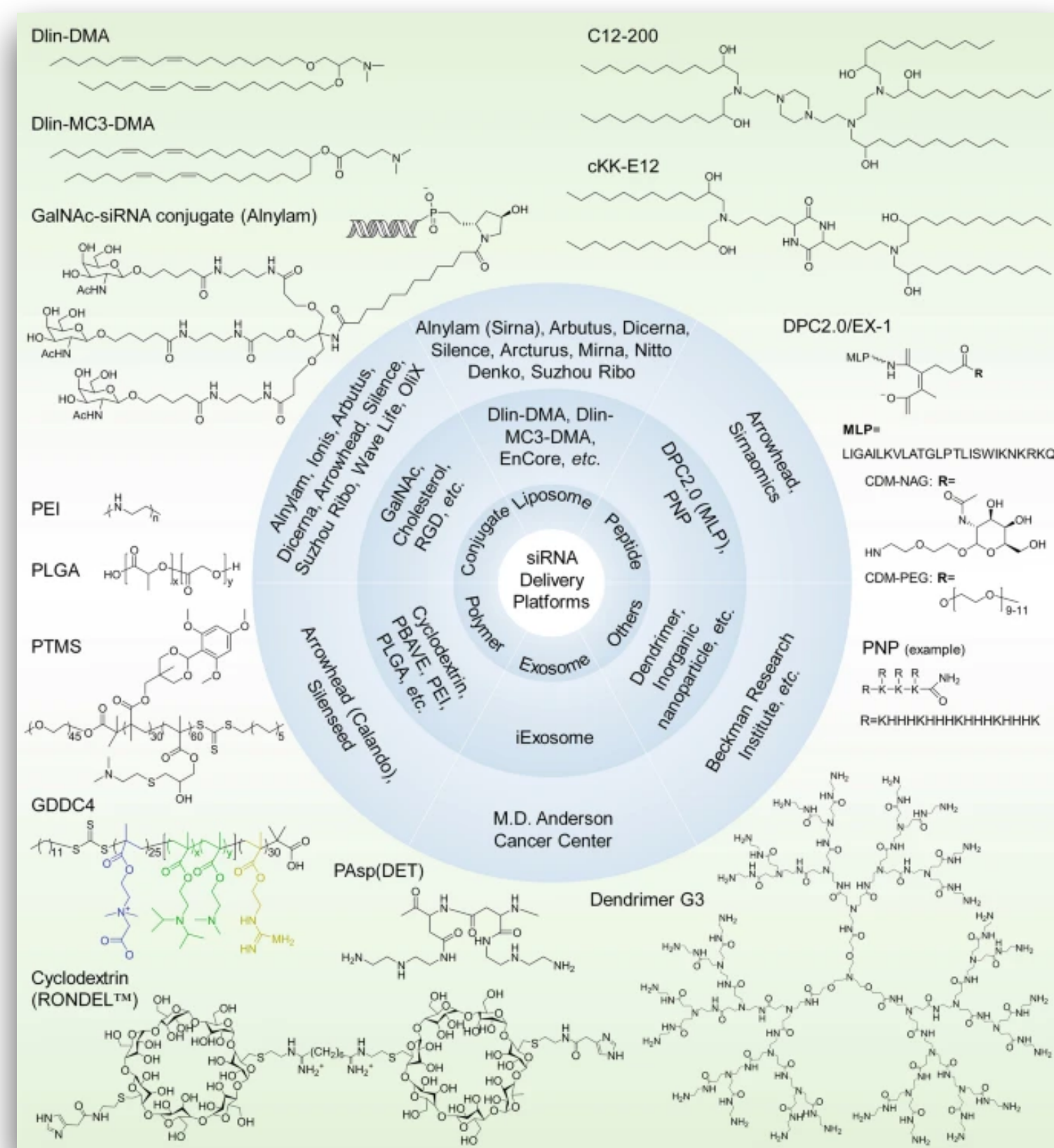


**Target specificity is determined largely by the sequence of the antisense strand**

**Computational algorithms are used to design siRNA**

# HTS in RNA based drug discovery

## Screening of siRNA drugs

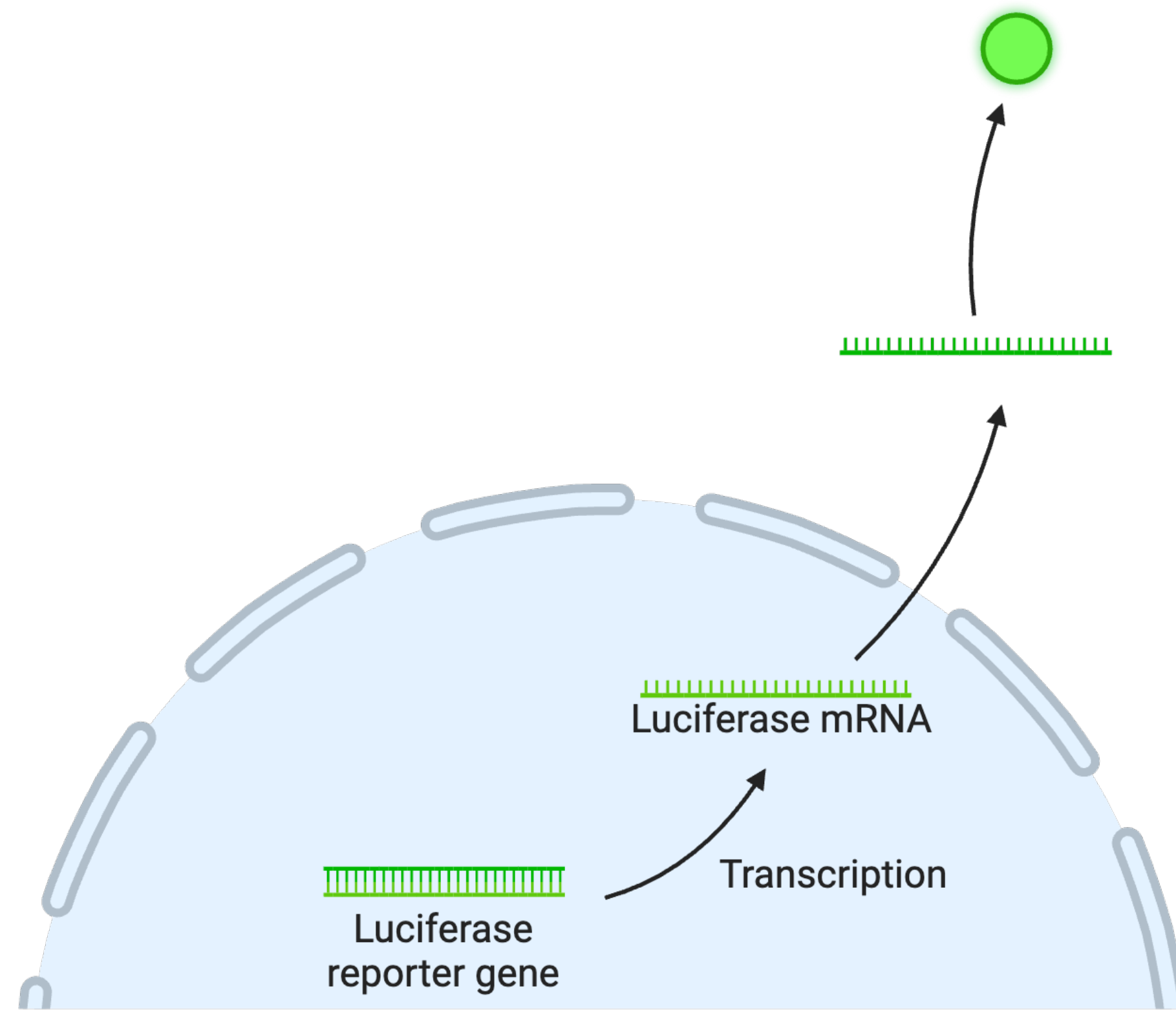


**Screenings are typically done for RNA delivery material and modifications to improve the stability and ADMET**

# *HTS in RNA based drug discovery*

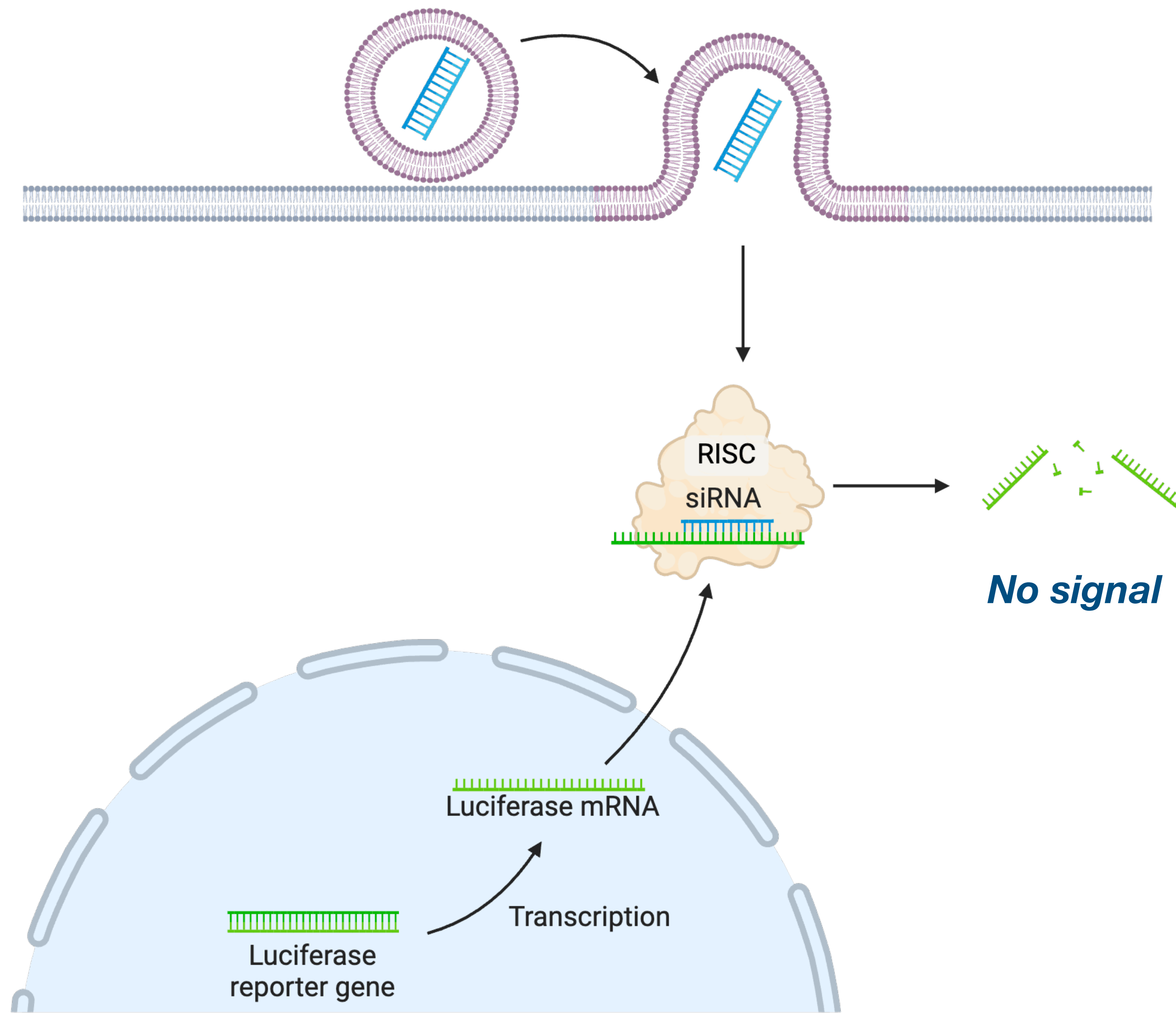
## *Screening of siRNA drugs*

***Reporter gene serves as a direct measurement of siRNA delivery and efficiency marker***



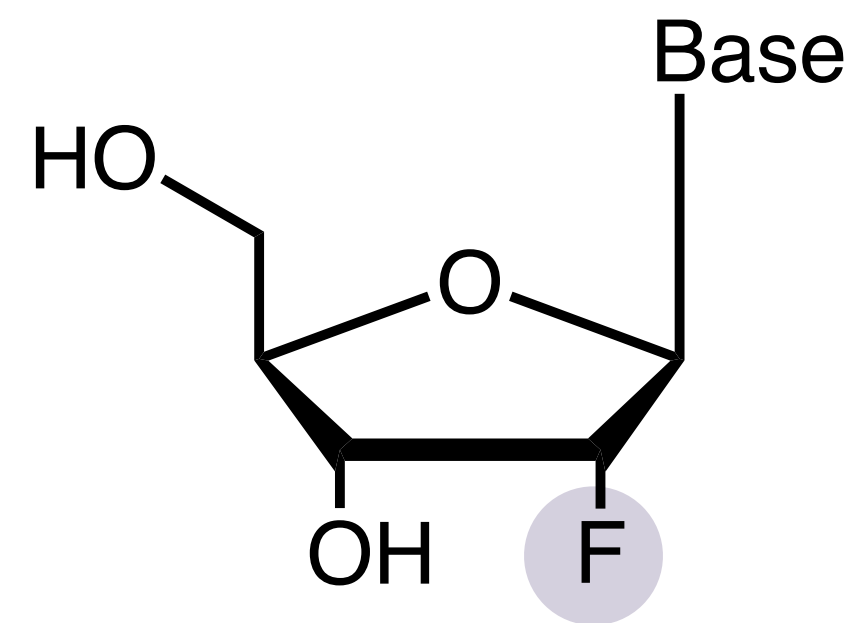
# HTS in RNA based drug discovery

## Screening of siRNA drugs



# HTS in RNA based drug discovery

Case study: iterative screening of RNA modifications

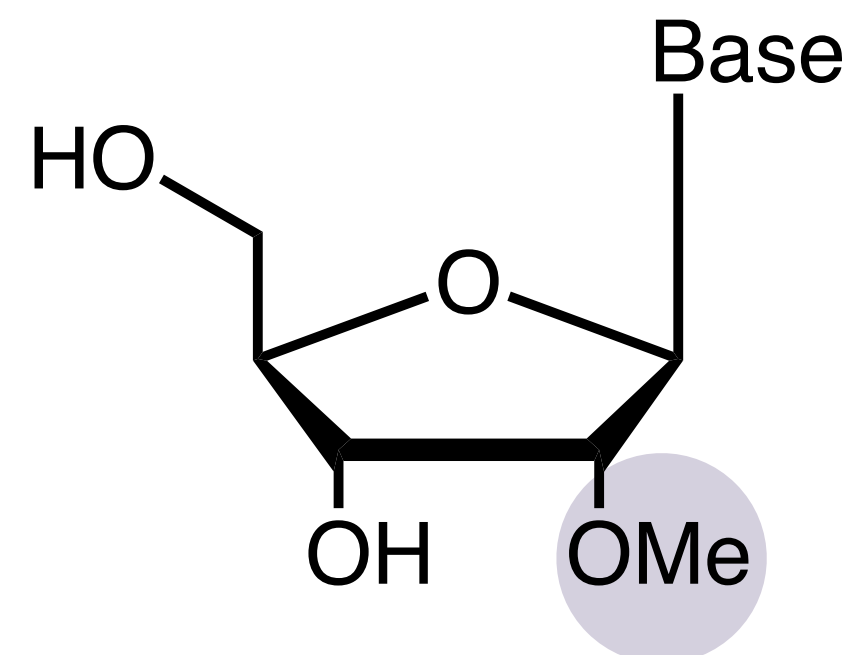


2'-F modified ribose

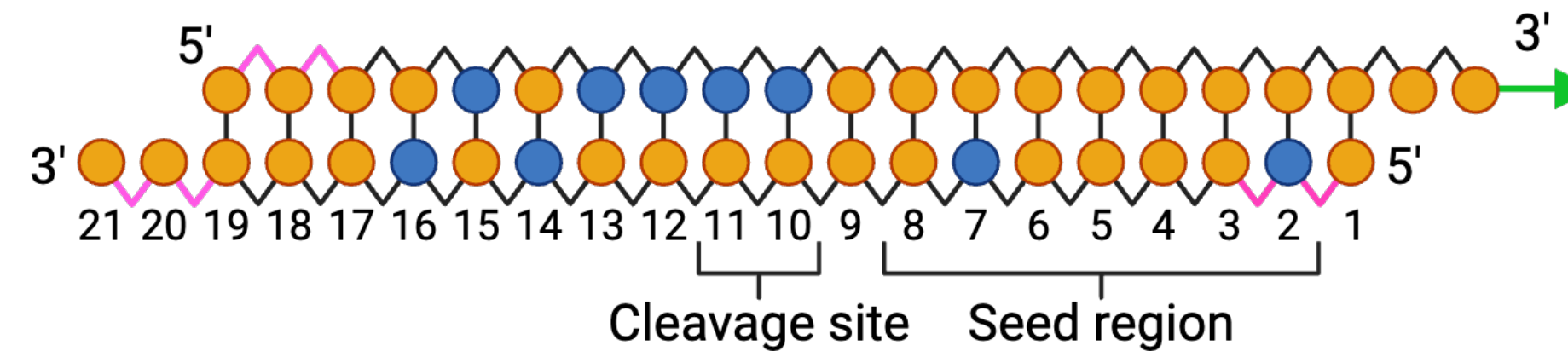
**Single-stranded 2'-F ribose can increase double-strand DNA breaks and impair cellular proliferation**



**Optimizing the number and placement of 2'-F and 2'-OMe**



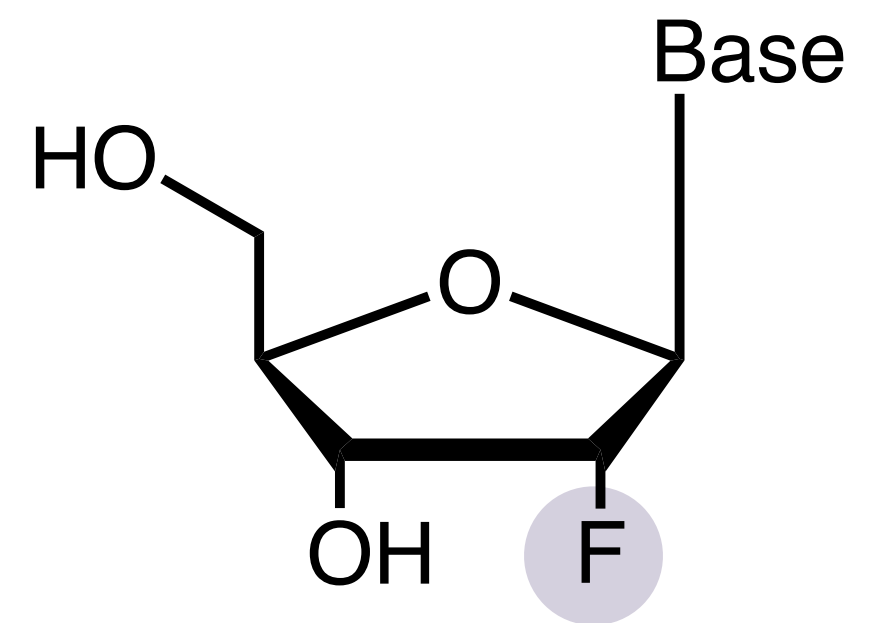
2'-OMe modified ribose



**Two modifications on a 21-mer and a 23-mer have  $2^{21}$  and  $2^{23}$  permutations**

# HTS in RNA based drug discovery

Case study: iterative screening of RNA modifications

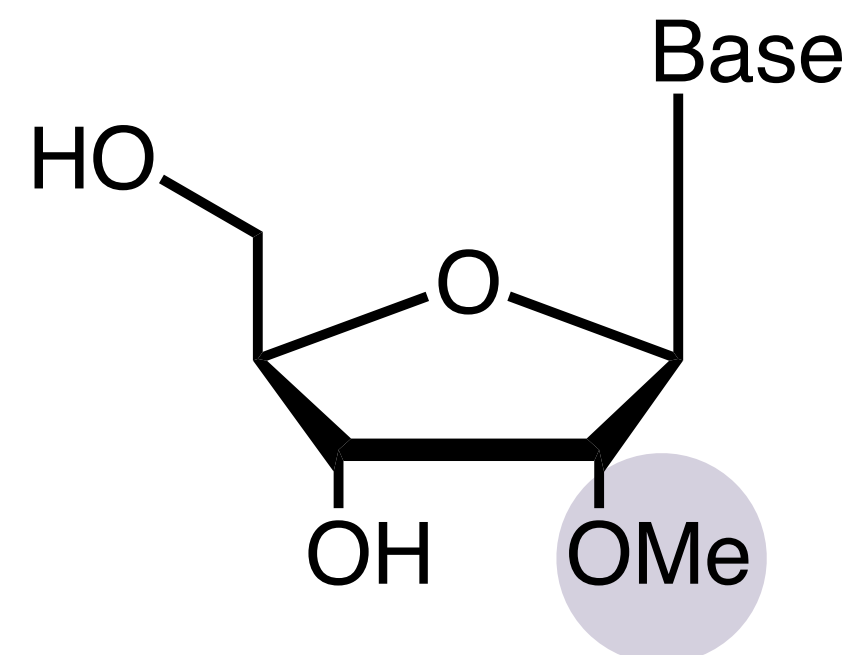


2'-F modified ribose

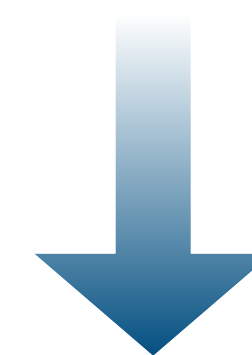
**Single-stranded 2'-F ribose can increase double-strand DNA breaks and impair cellular proliferation**



**Optimizing the number and placement of 2'-F and 2'-OMe**



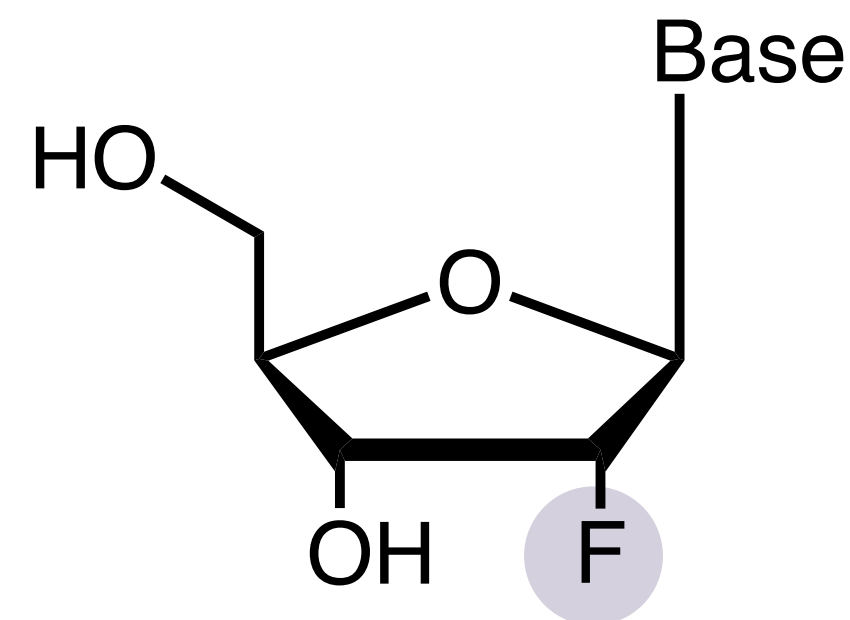
2'-OMe modified ribose



**Computational iterative screening**

# HTS in RNA based drug discovery

Case study: iterative screening of RNA modifications

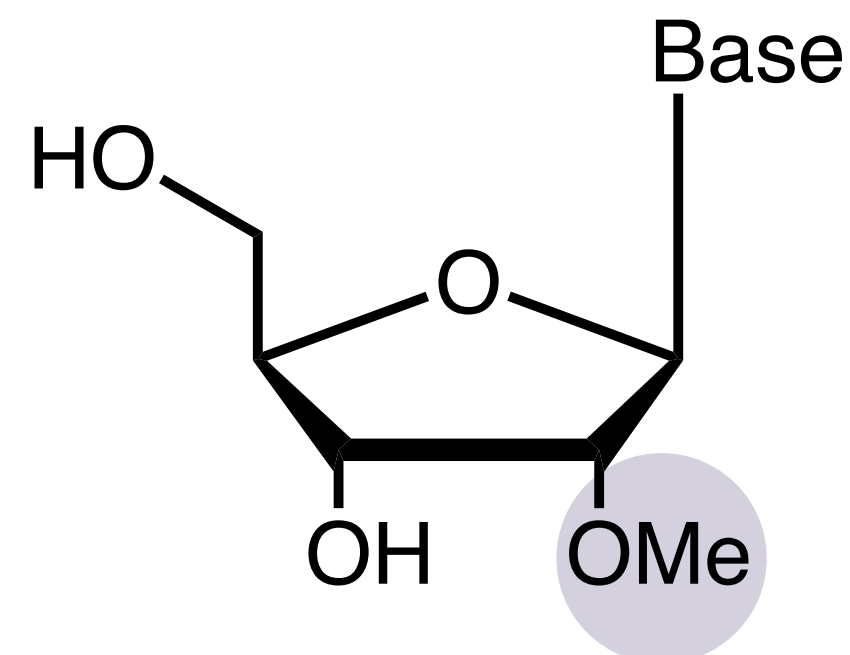


2'-F modified ribose

**Single-stranded 2'-F ribose can increase double-strand DNA breaks and impair cellular proliferation**



**Optimizing the number and placement of 2'-F and 2'-OMe**



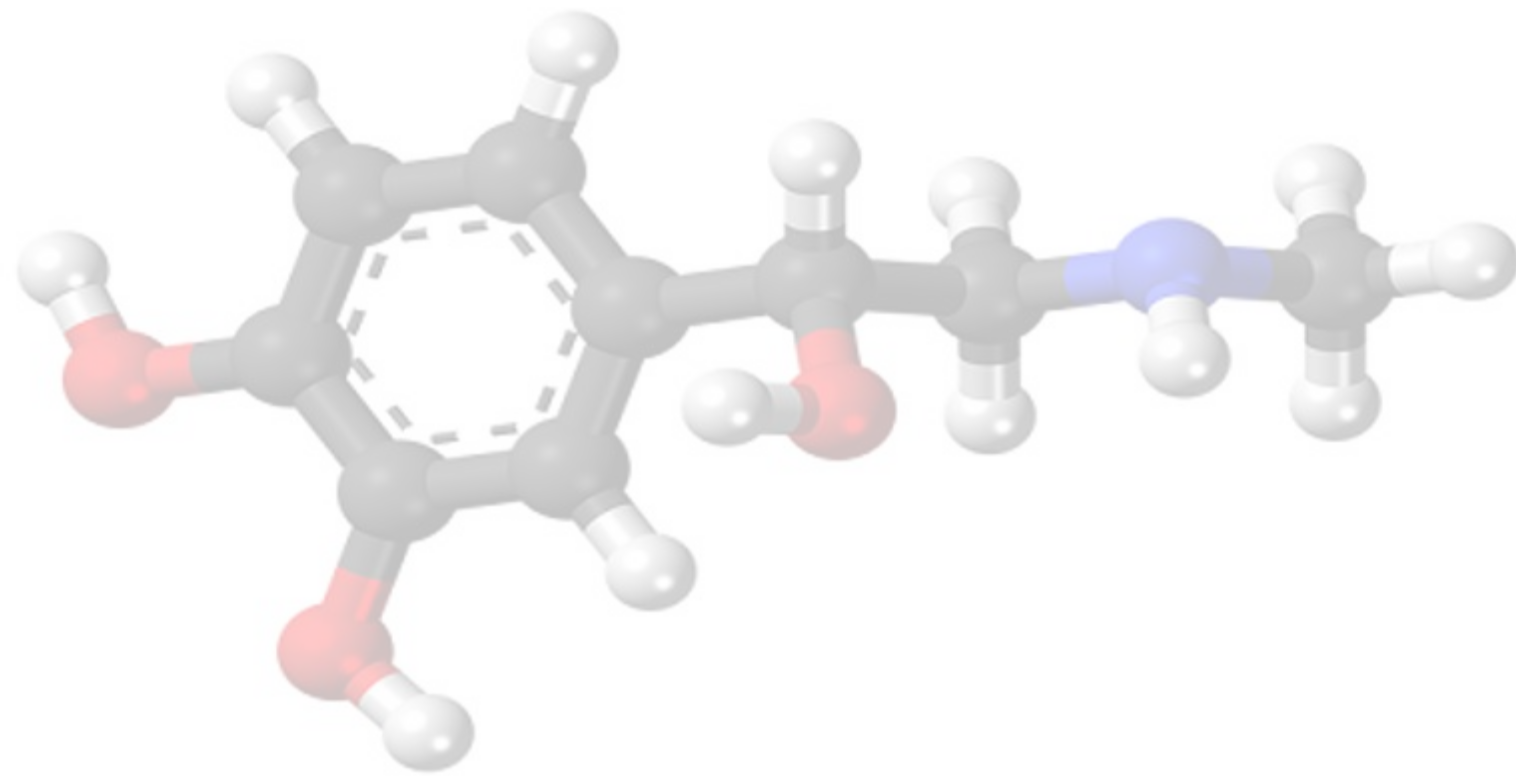
2'-OMe modified ribose



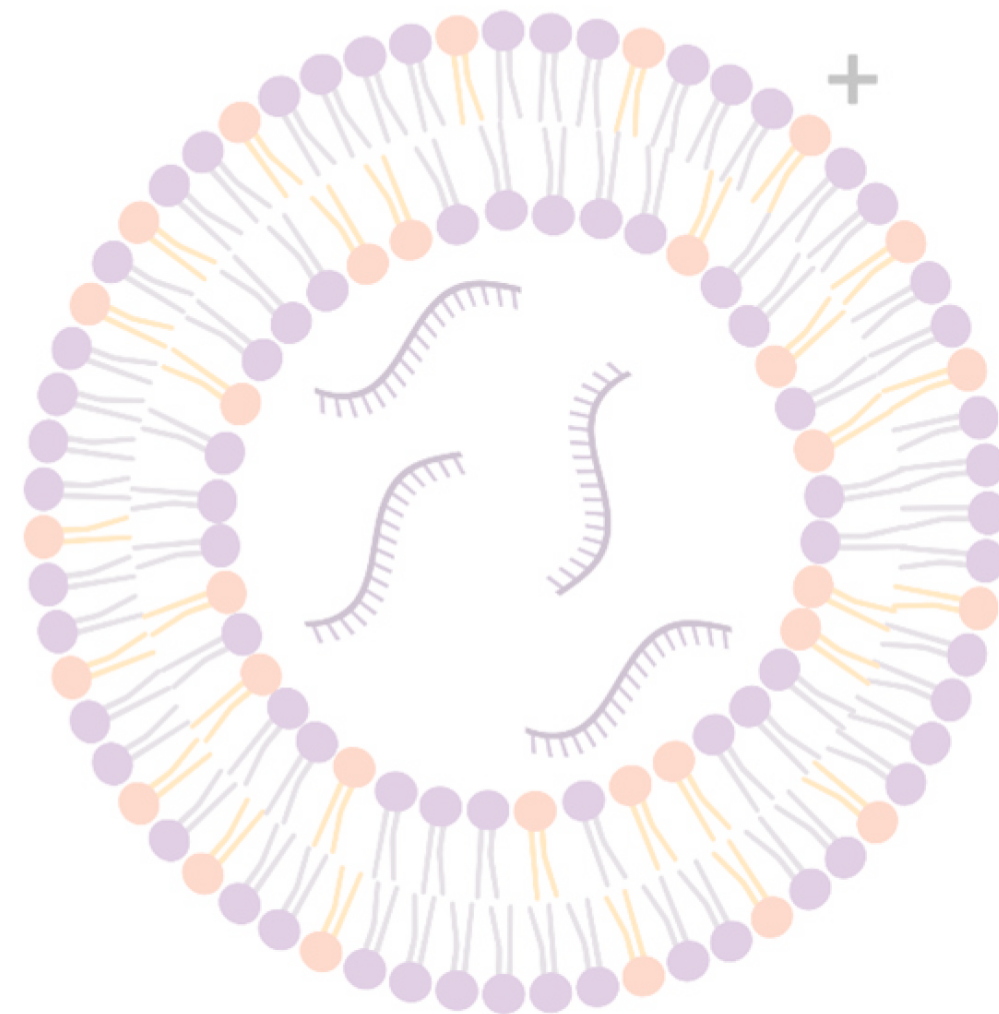
**Computational iterative screening**

**Iterative screening is also being used in small molecule drug discovery**

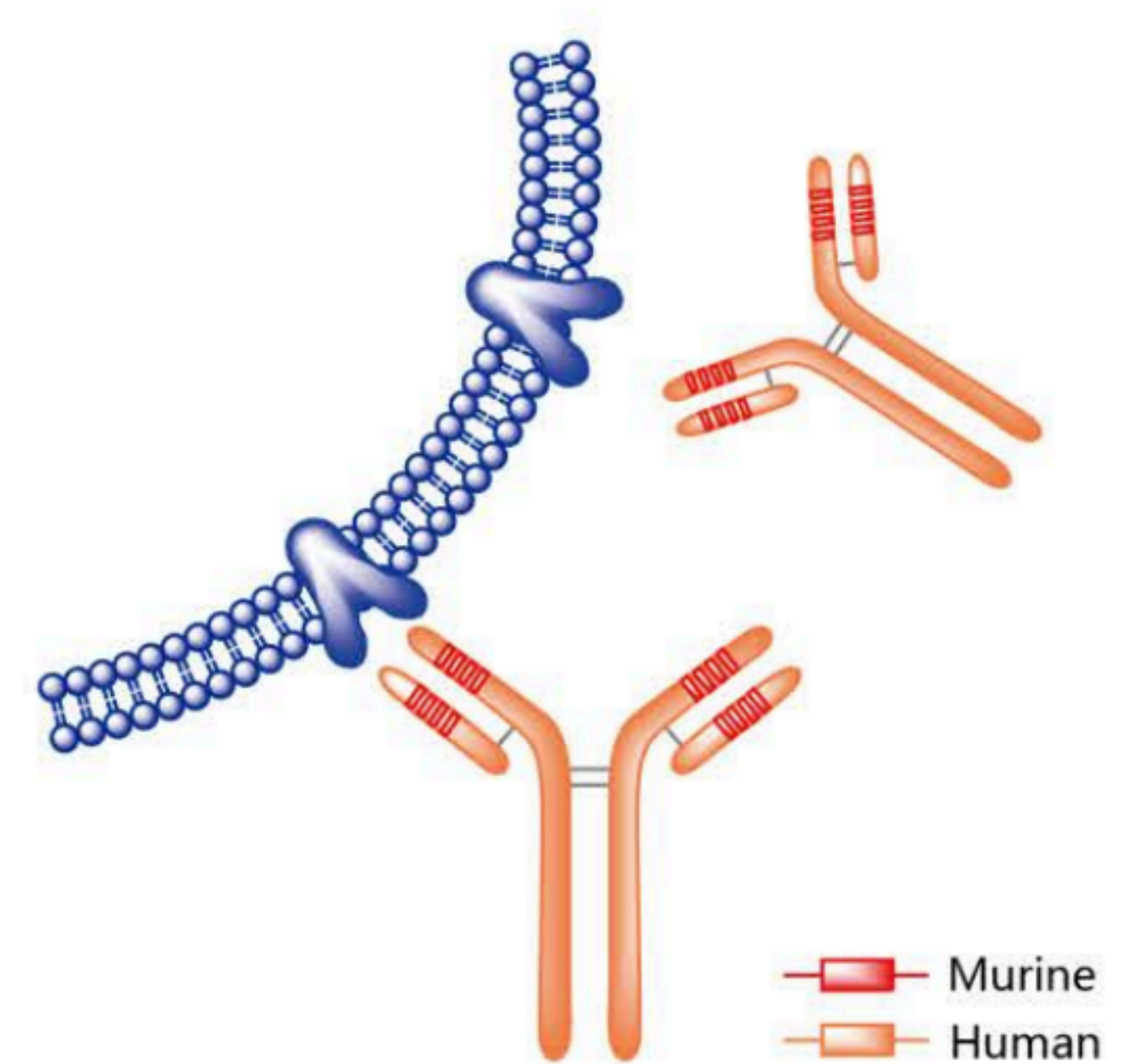
# *HTS strategies in drug discovery*



*Small molecule drugs*



*RNA based therapies*

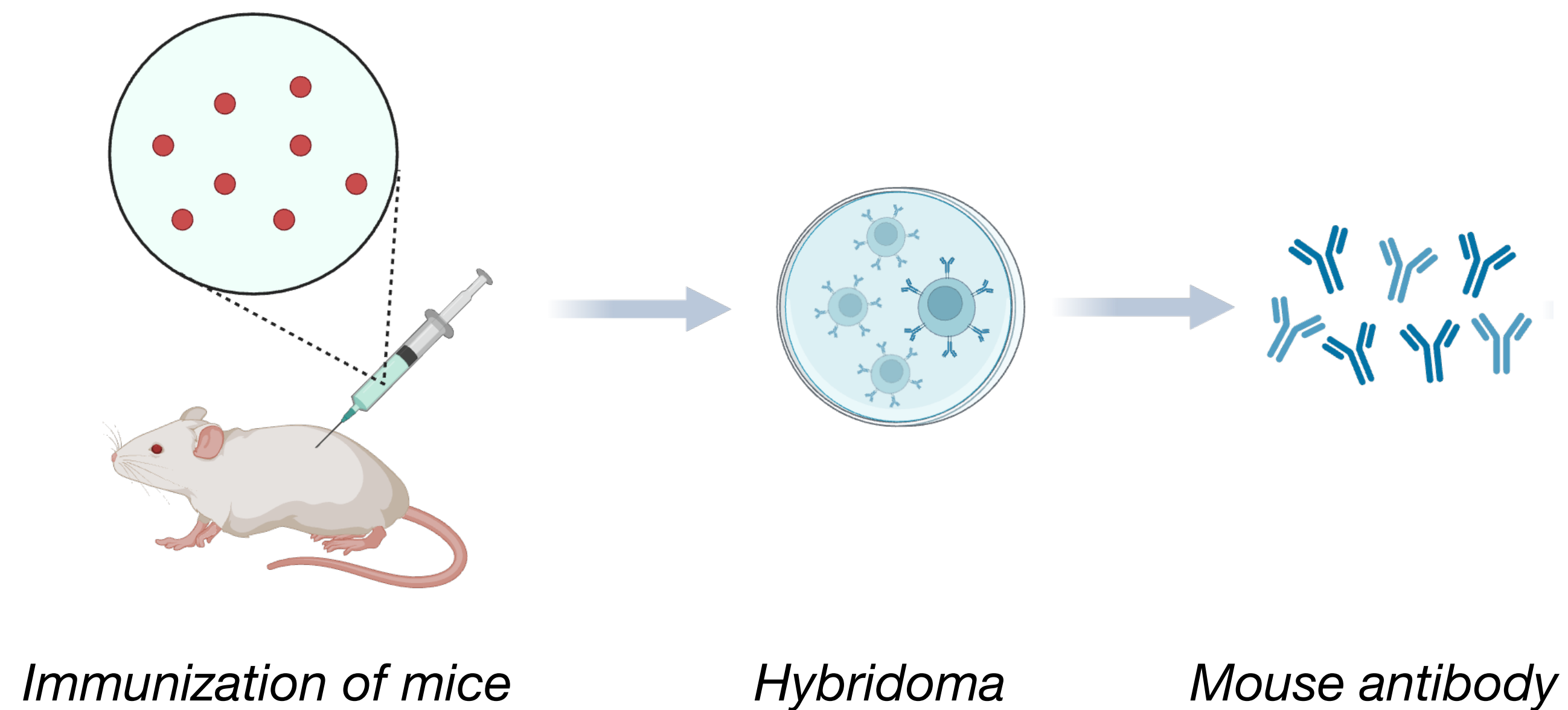


*Antibody based therapies*



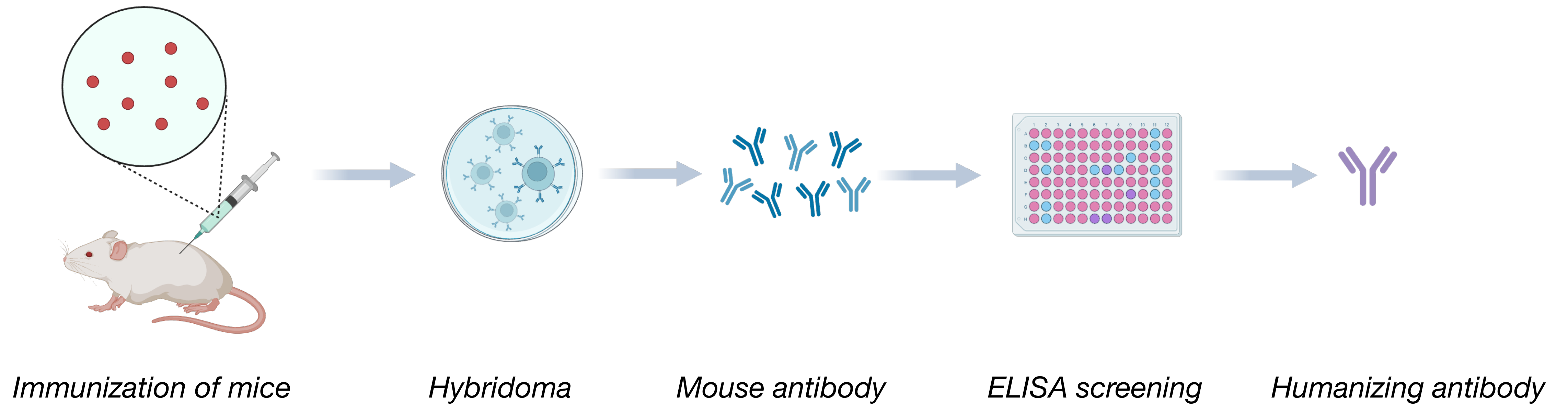
# *HTS in antibody based drug discovery*

## *Mouse hybridoma*



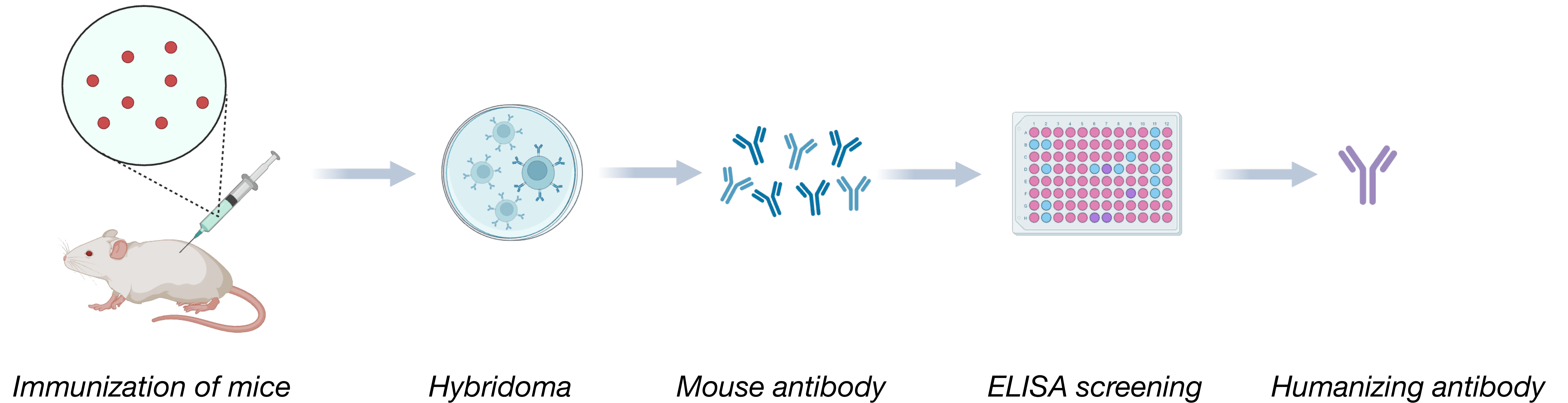
# *HTS in antibody based drug discovery*

## *Mouse hybridoma*



# *HTS in antibody based drug discovery*

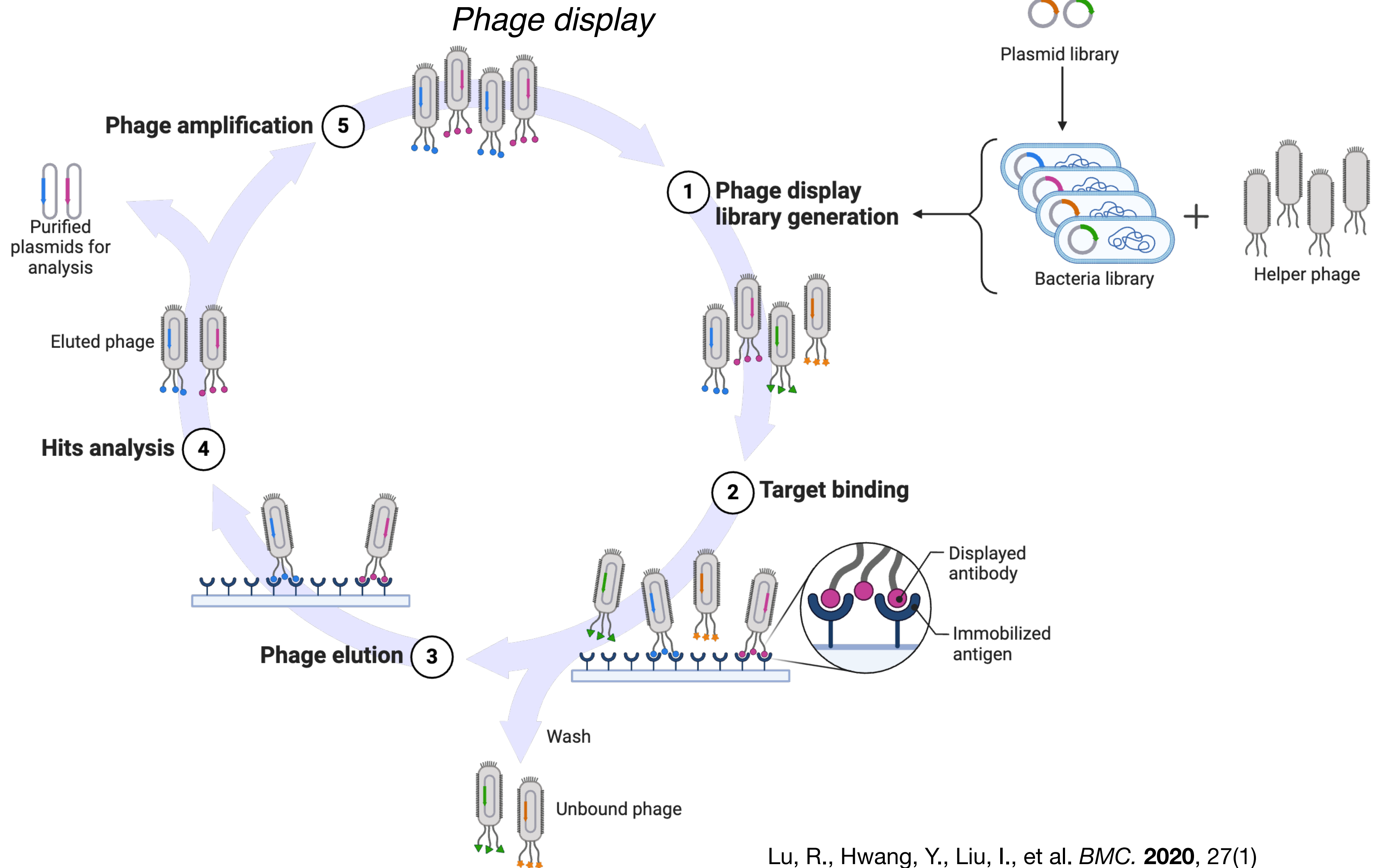
## *Mouse hybridoma*



***Very common method of producing antibody therapies among the approved drugs***

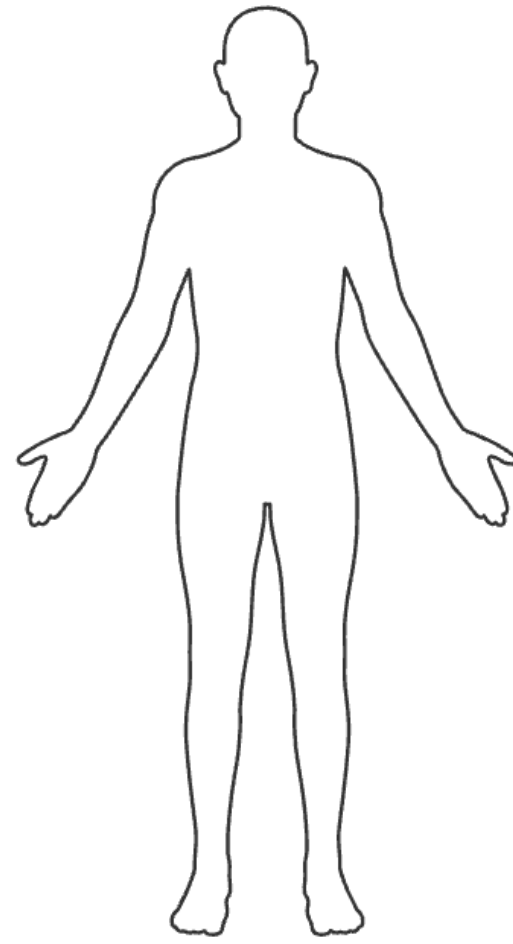
***Transgenic mouse have also been used to directly obtain human antibody from mouse***

# HTS in antibody based drug discovery



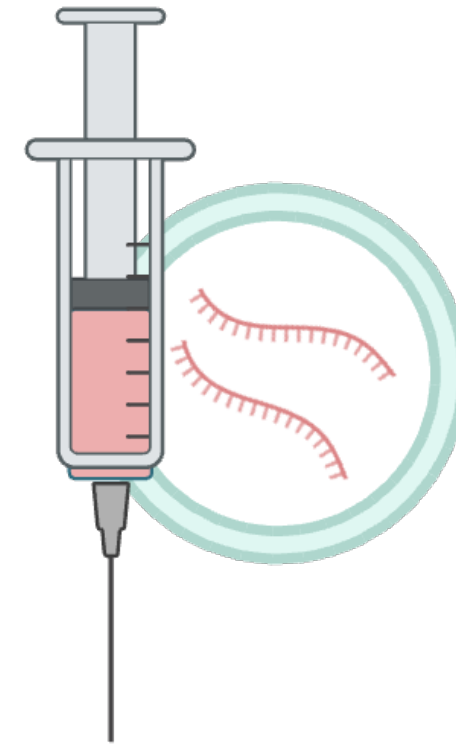
# *HTS in antibody based drug discovery*

## *Phage library*



### ***Naïve libraries***

*Non immunized donor*  
*Natural diversity library*



### ***Immune libraries***

*Immunized donor*  
*Biased towards a specific target*



### ***Synthetic/semisynthetic libraries***

*based on computational design*

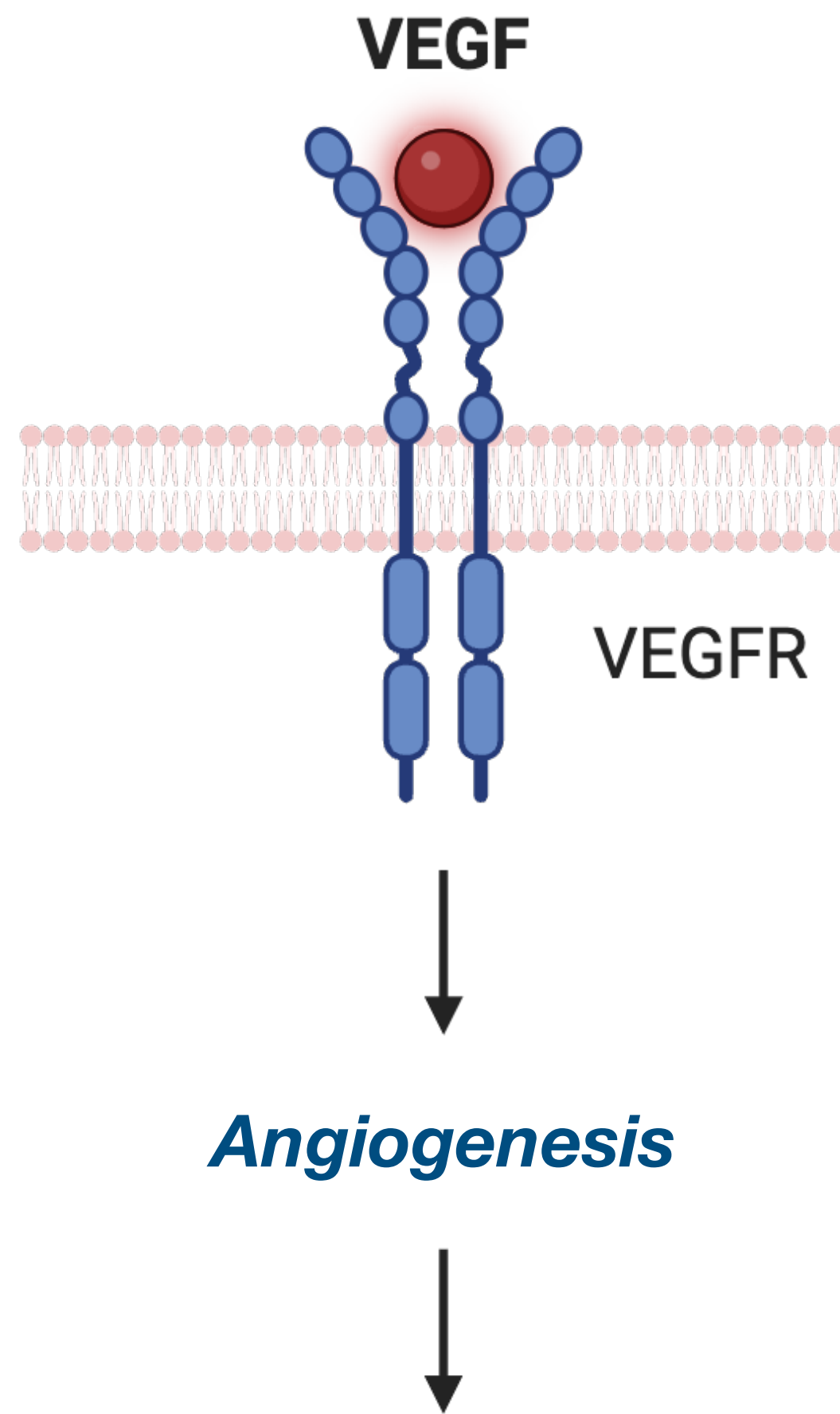
***Derived from human peripheral blood mononuclear cells (PBMCs)***

# HTS in antibody based drug discovery

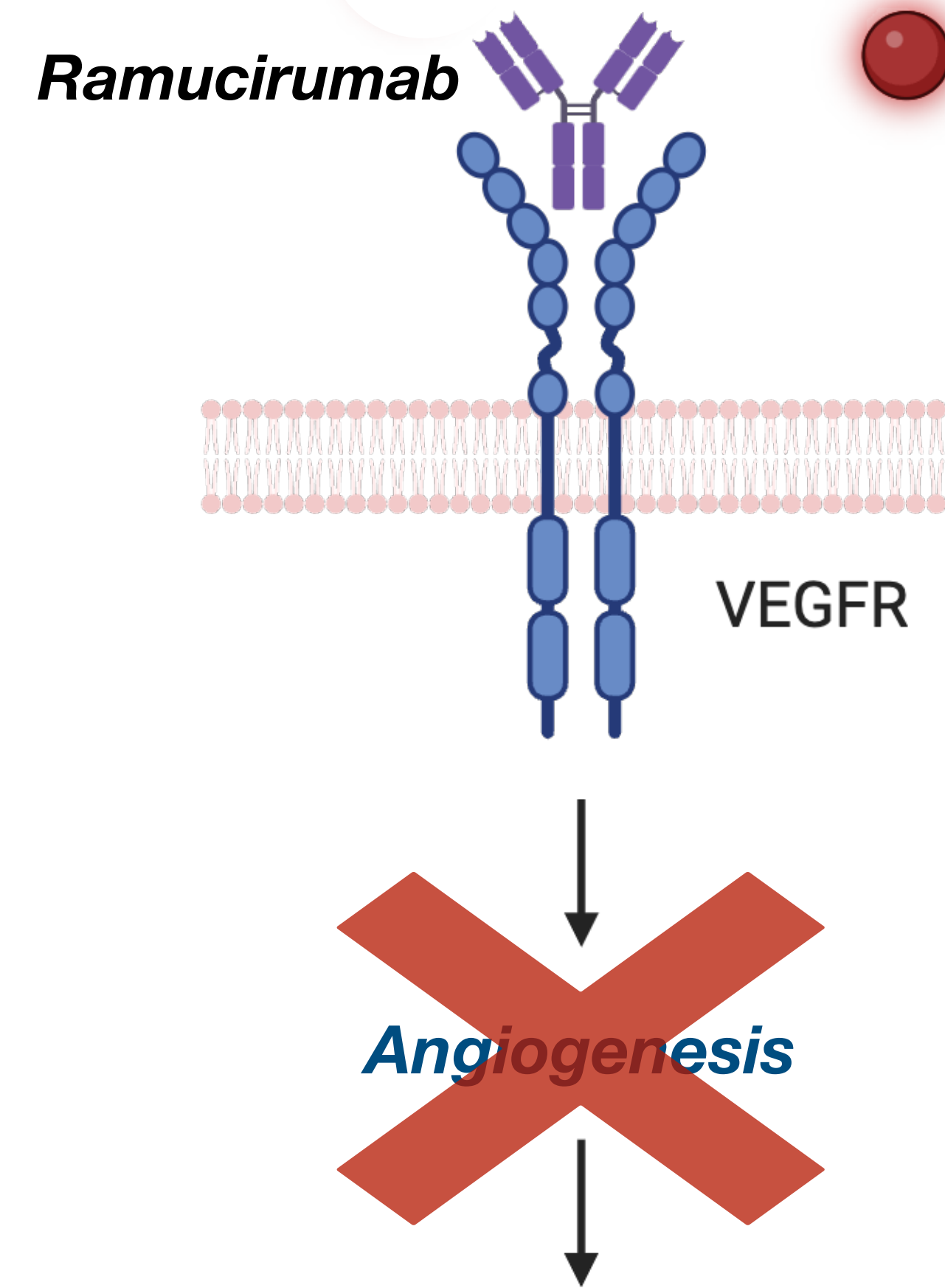
## Case study: Ramucirumab



**ImClone Systems**



**Tumor growth and metastasis**



**Tumor growth and metastasis**

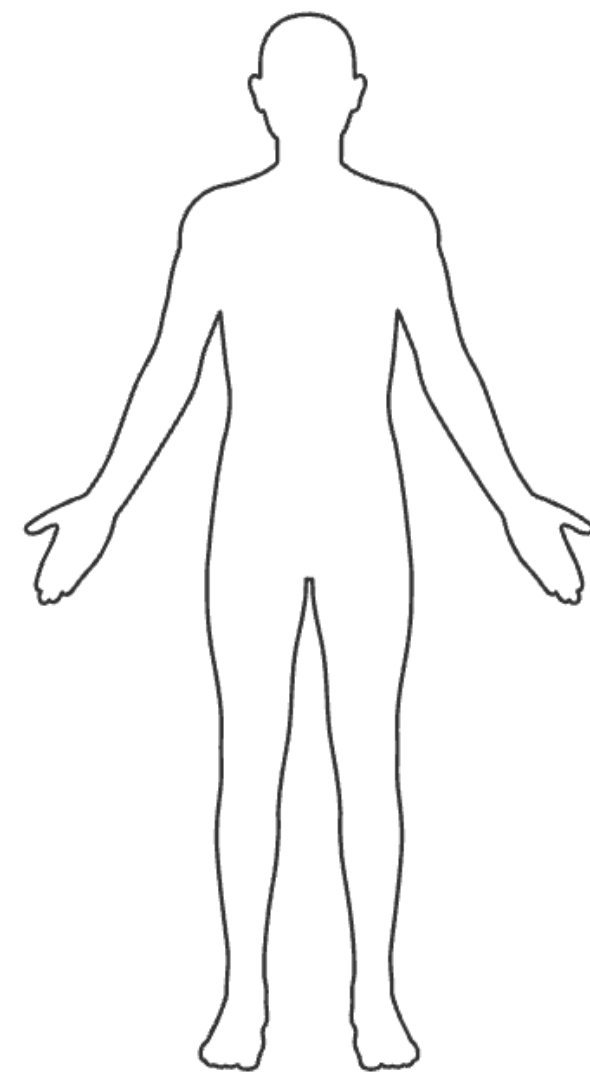
# HTS in antibody based drug discovery

Case study: Ramucirumab

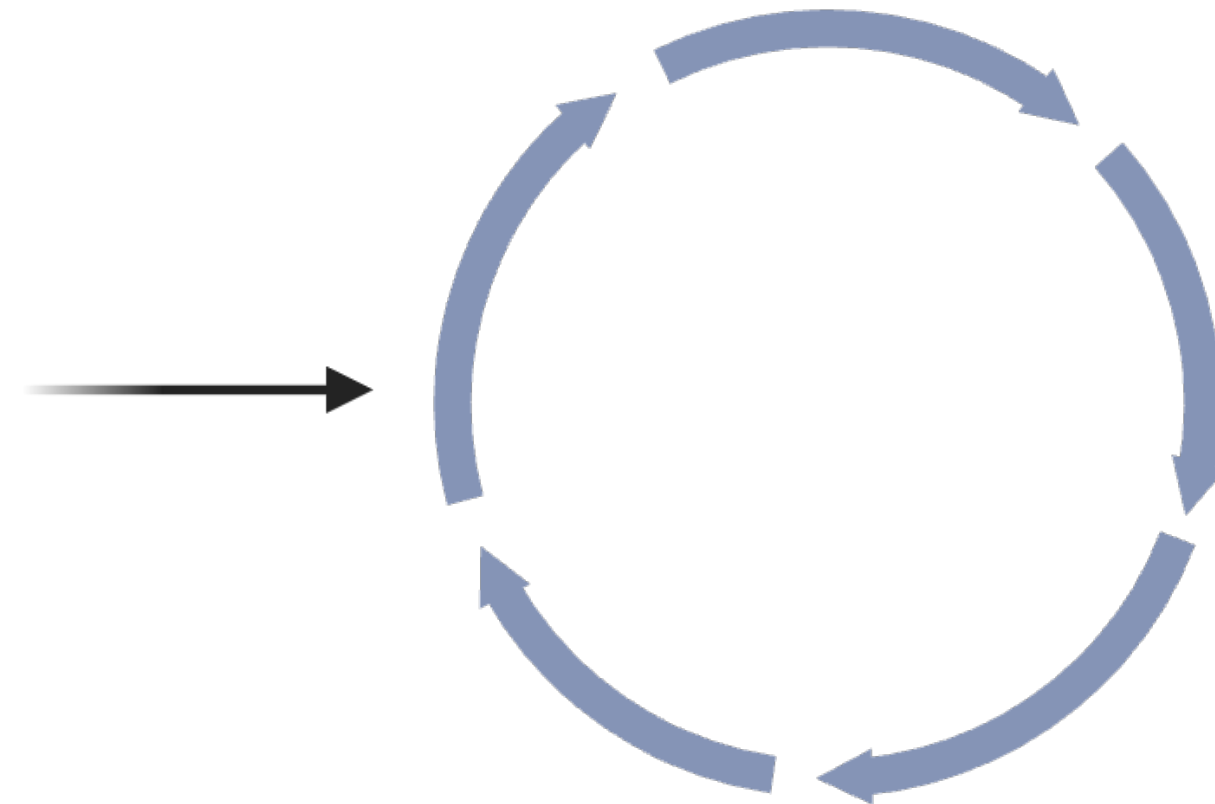


**ImClone Systems**

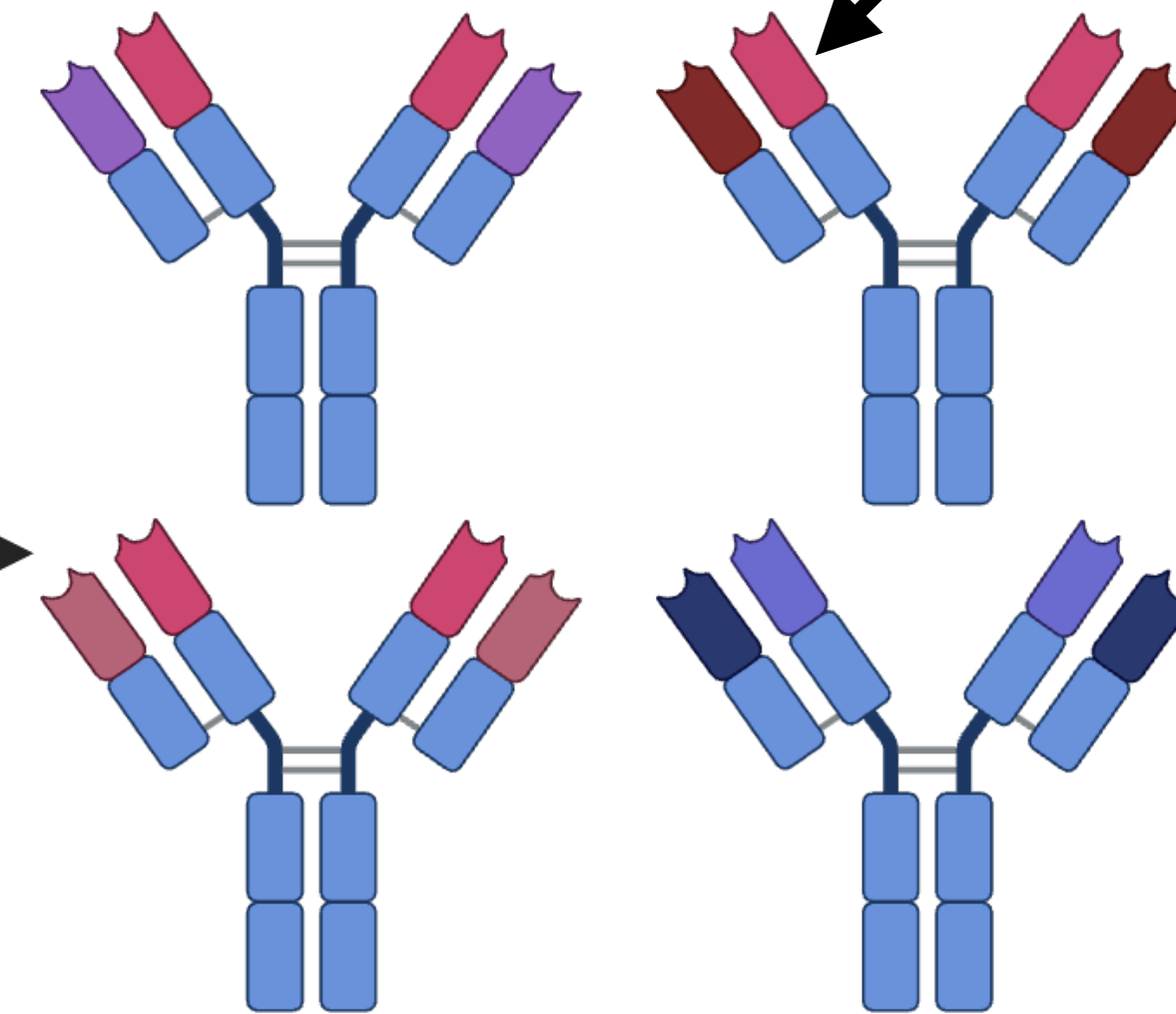
*3 of the hits have identical variable heavy (VH) chain*



*Naïve library with  $3.7 \times 10^{10}$  clones*



*3 rounds of screening*



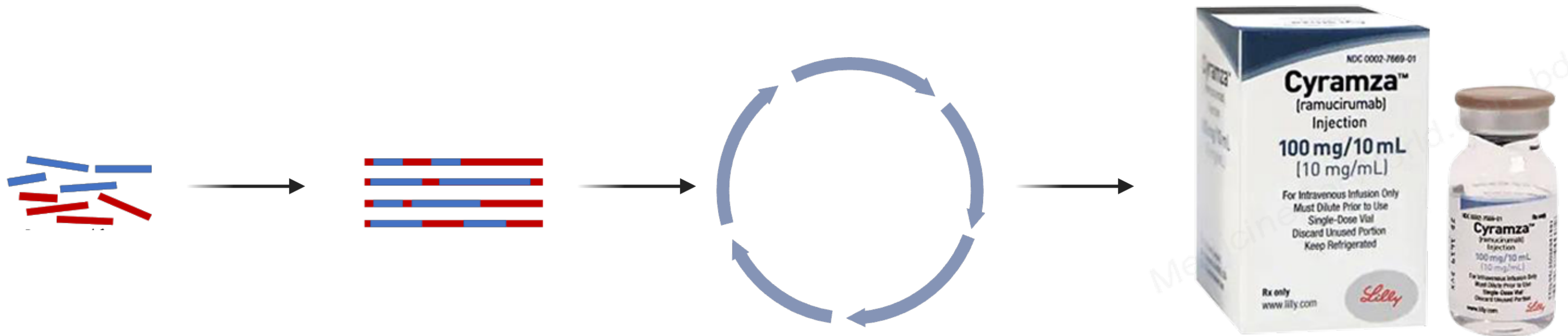
*Four hits with  $IC_{50}$  2-20 nM*

# HTS in antibody based drug discovery

## Case study: Ramucirumab



**ImClone Systems**



*Shuffled variable light (VL) chain while maintaining VH the same, generating  $1.5 \times 10^8$  clones*

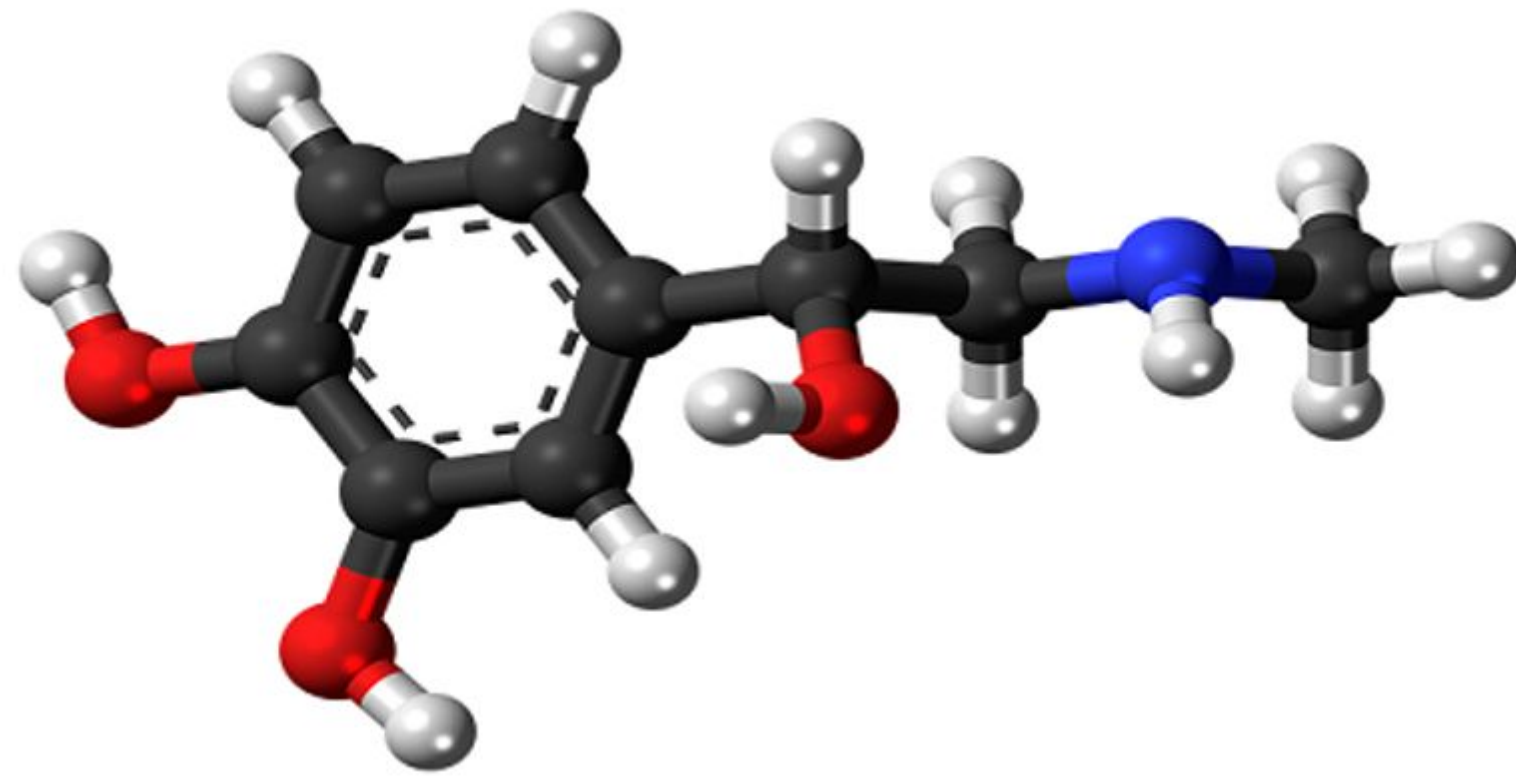
*4 rounds of selection*

*IMC-1121B  
Ramucirumab*

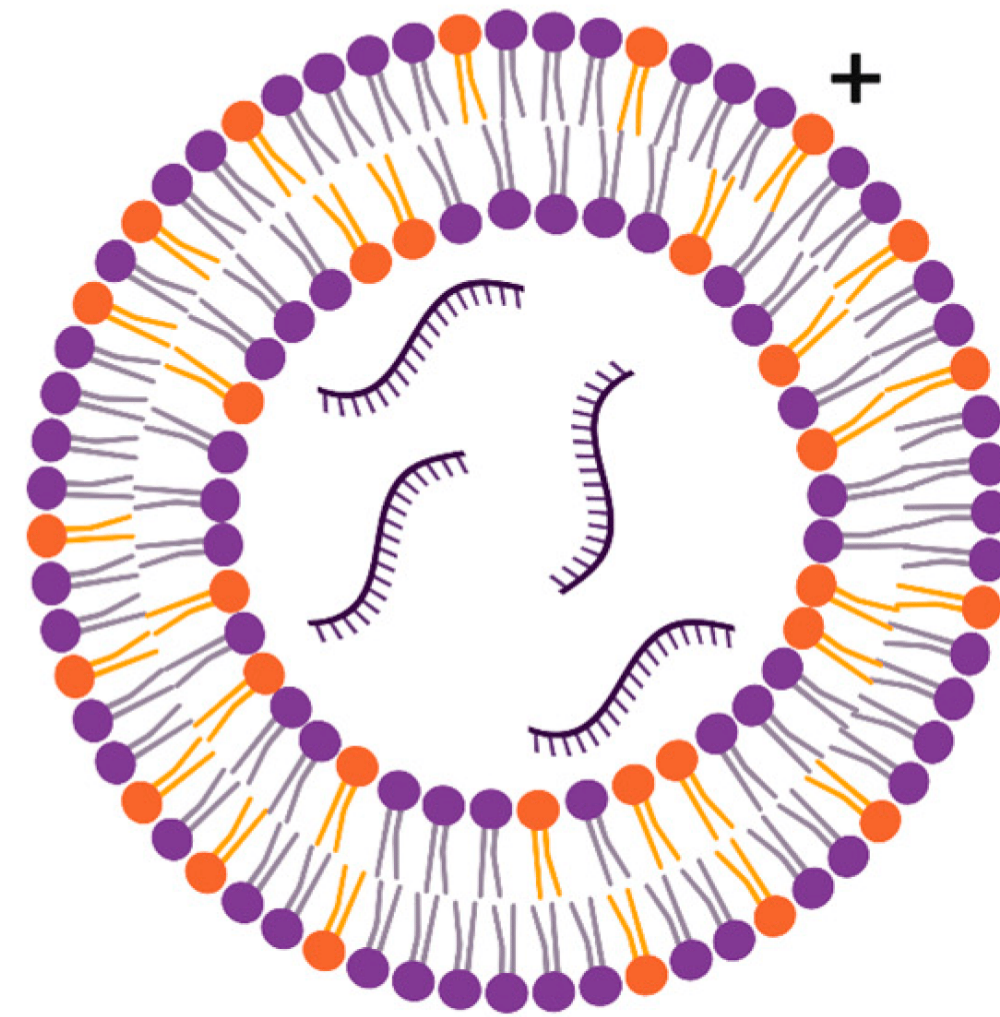
Lu, R., Hwang, Y., Liu, I., et al. *BMC*. **2020**, 27(1)  
Lu, D., Jimenez, X., Zhang, H., *Int. J. Cancer*,. **2001**, 97  
Lu, D., Shen, J., Vil, M. D., et al. *JBC*. **2003**, 278(44)



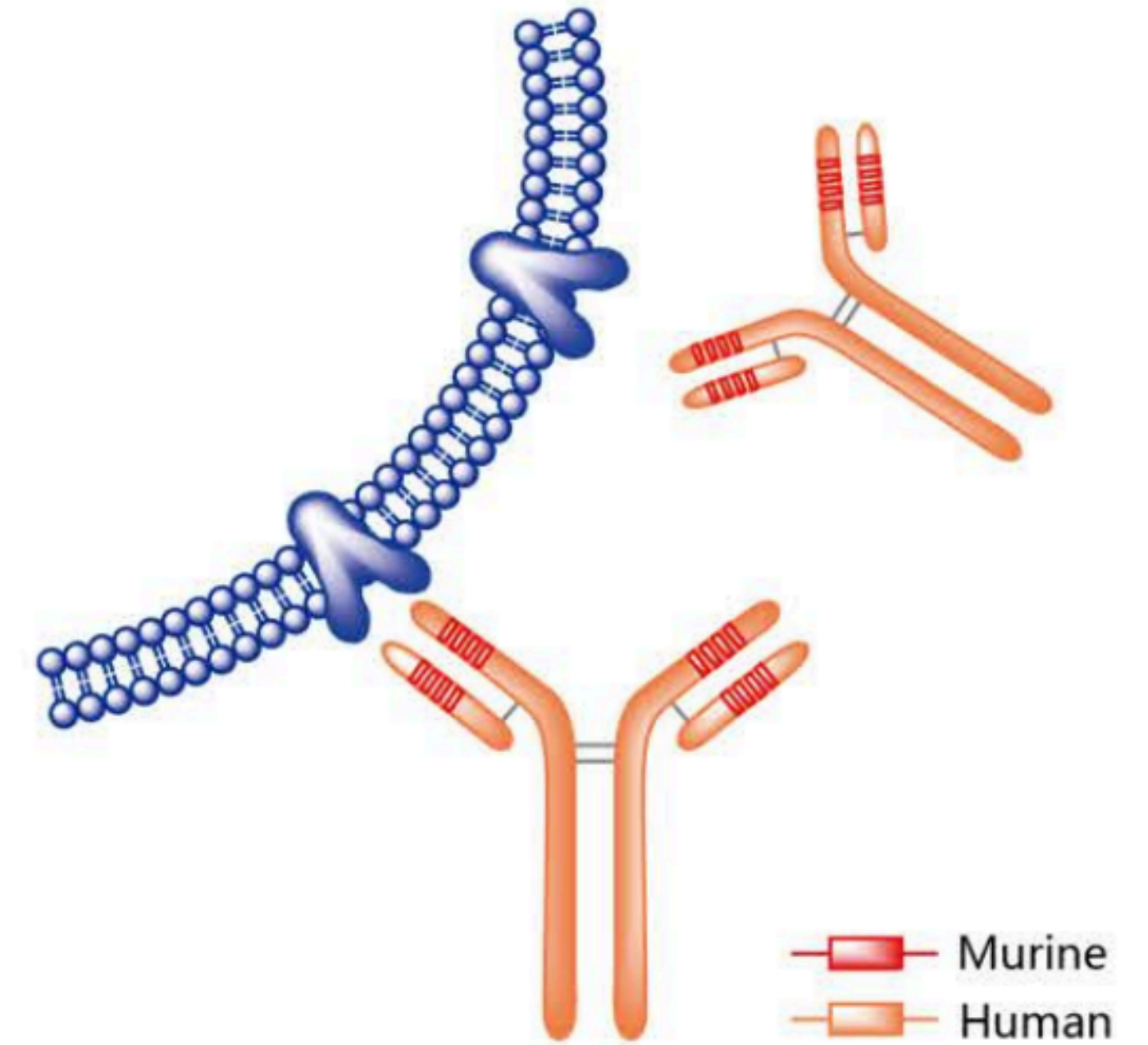
## *HTS strategies in drug discovery*



***Small molecule drugs***



***RNA based therapies***

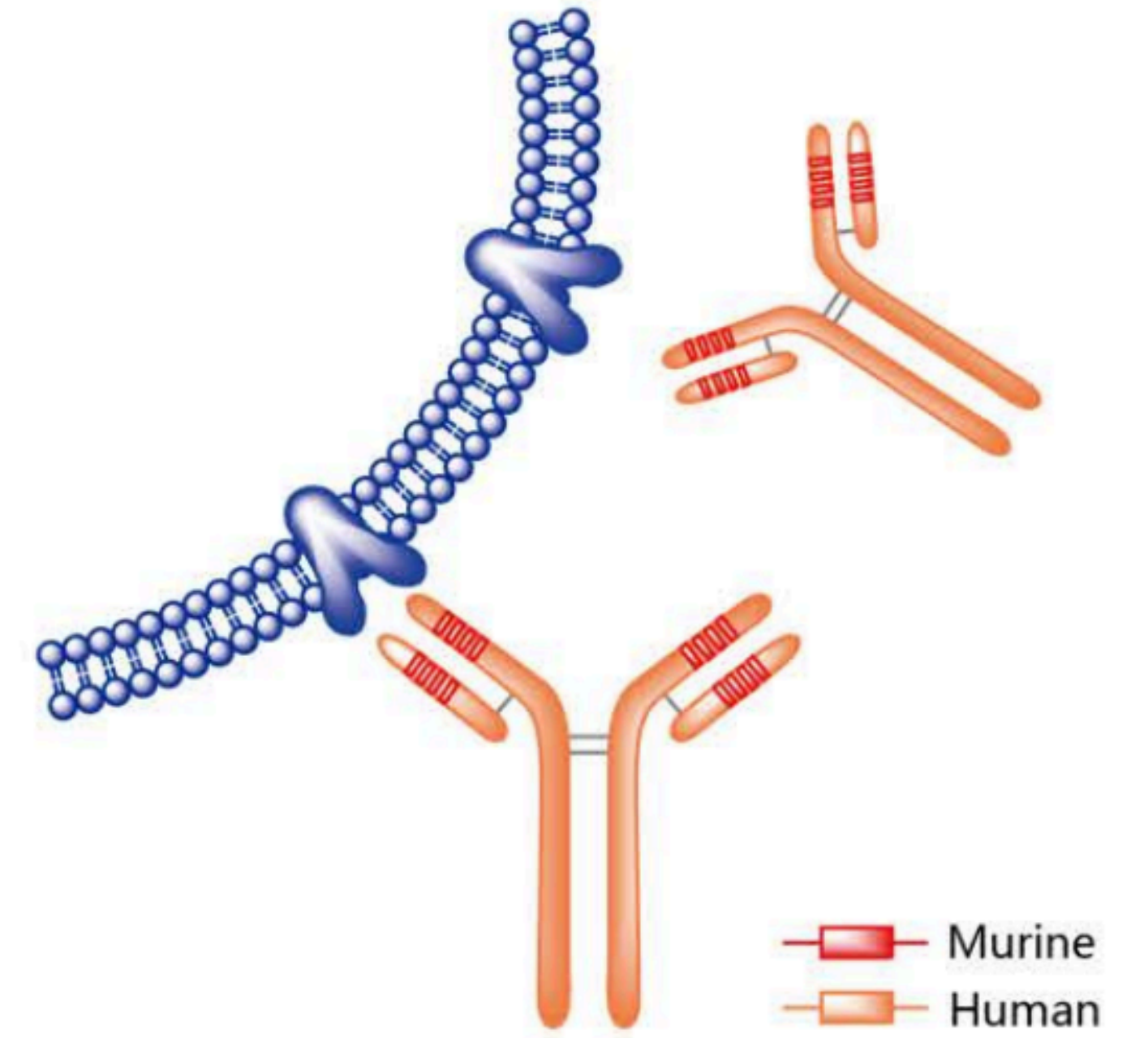
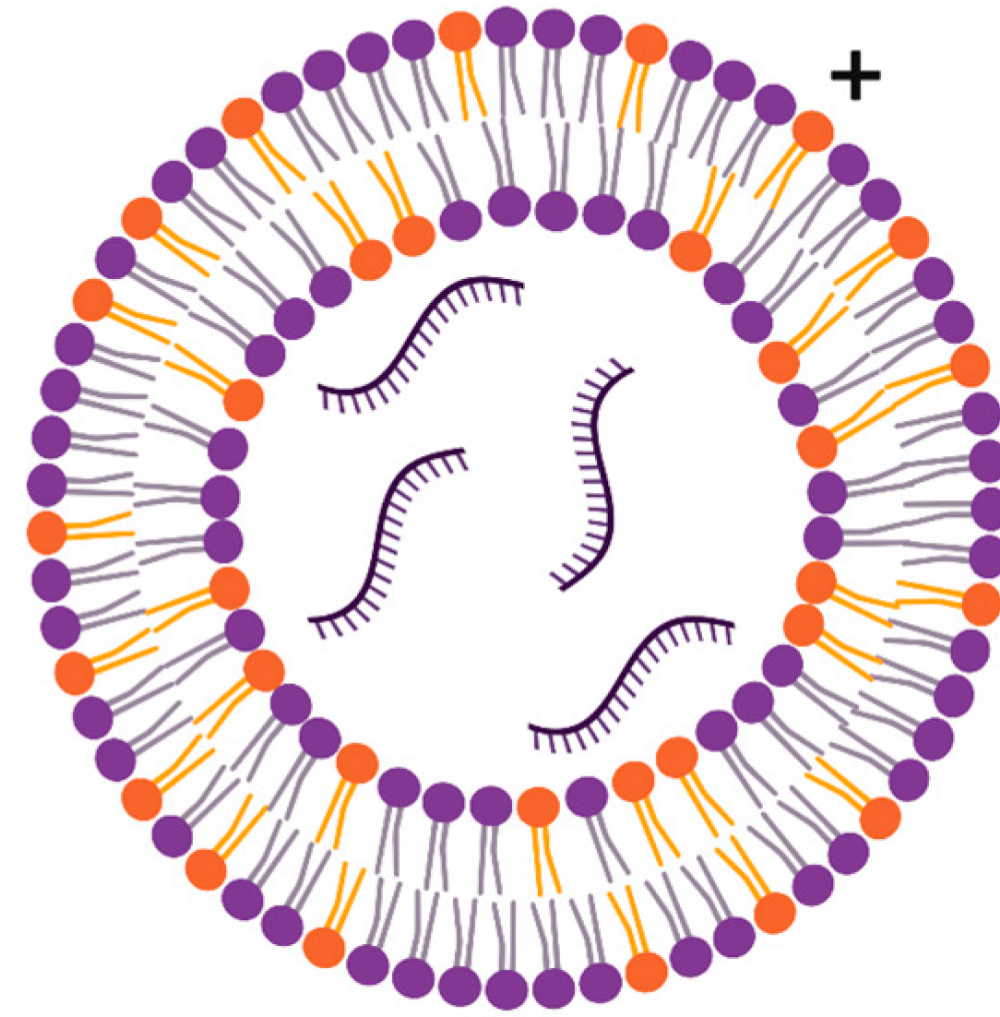
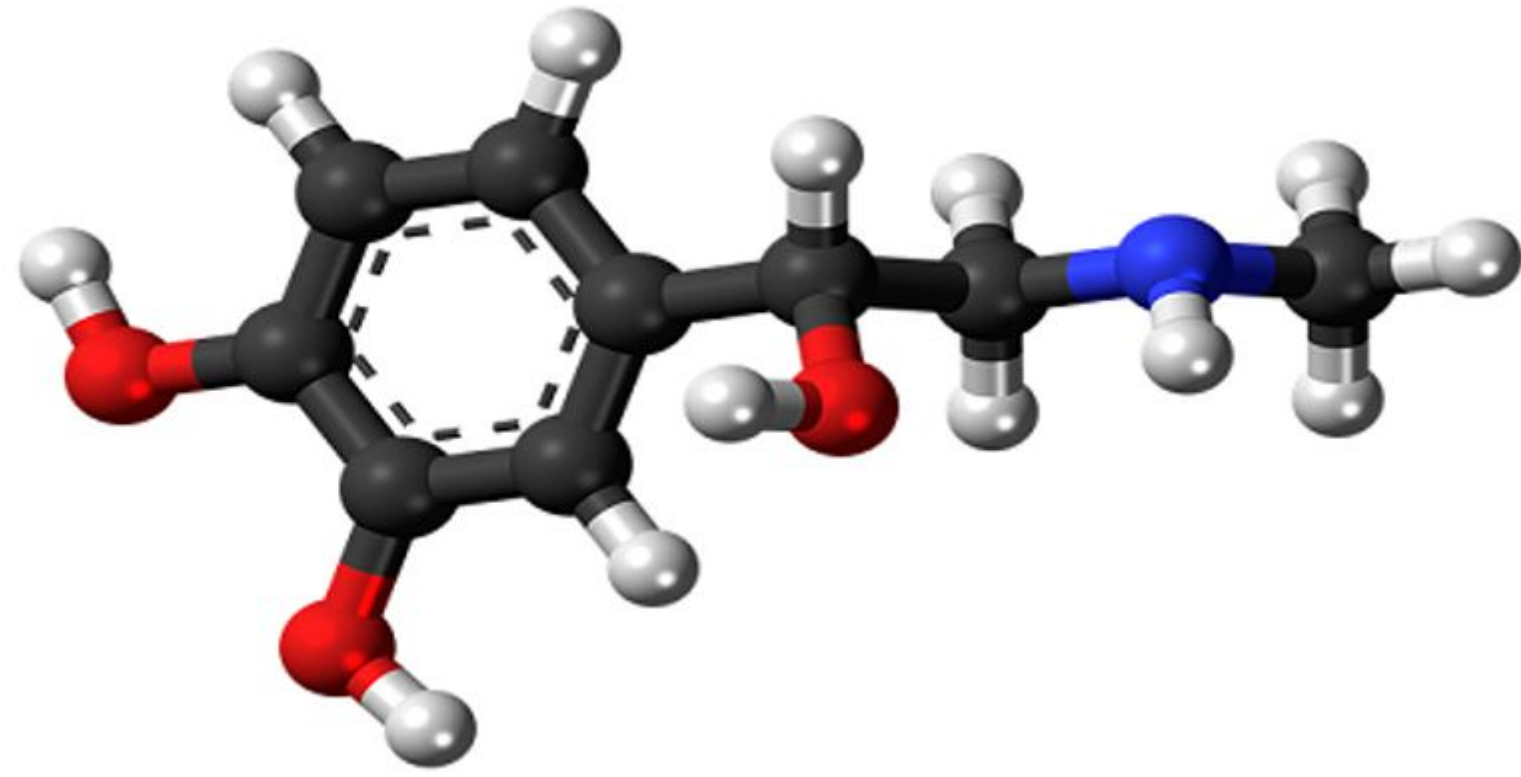


***Antibody based therapies***

***Screening library and screening methods are crucial for the success of HTS***

***Need to balance between using advanced technology and the cost of time and money that comes with it***

# *HTS strategies in drug discovery*



***Questions?***