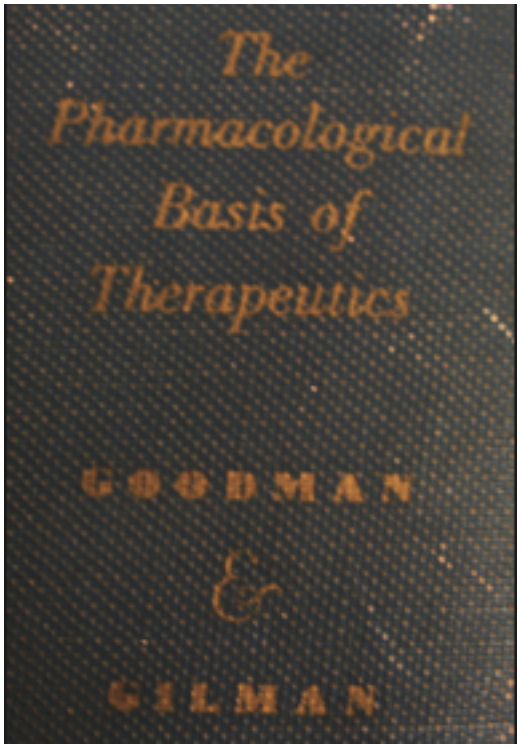
Lab Meeting 02/2/09

Compound Screening

Much of this comes from:

Landro et al.
HTS in the new millennium The role of pharmacology and flexibility.
Journal of Pharmacological and Toxicological Methods 44 (2000) 273 – 289

The classical approach for establishing new therapies is laid out in *The Pharmacological Basis of Therapeutics* written by Goodman and Gillman in 1941, and presently in its 11th edition. It is considered the bible of pharmacology. It describes validated intervention points for numerous disorders that can be tested and exploited for treating other conditions.



"A new text must be drafted to incorporate the target validation strategies of functional genomics into the drug discovery process. "

Among older techniques are those attempting to chemically engineer molecules that fit into a binding site that will prevent protein-protein interaction. These methods have been characterized with relatively poor success. The industry avoids this at least as a direct approach.

Classes of proteins that drugs target

45% cell membrane receptors, predominantly G protein coupled receptors

28% enzymes

11% hormones & growth factors

5% ion channels

2% nuclear receptors

2% DNAs

7% unknown

These represent about 500 targets but only a fraction have marketed therapies and of those, 83 of the top 100 marketed drugs target only 40 molecular interactions or pathways.

Landro et al., 2000

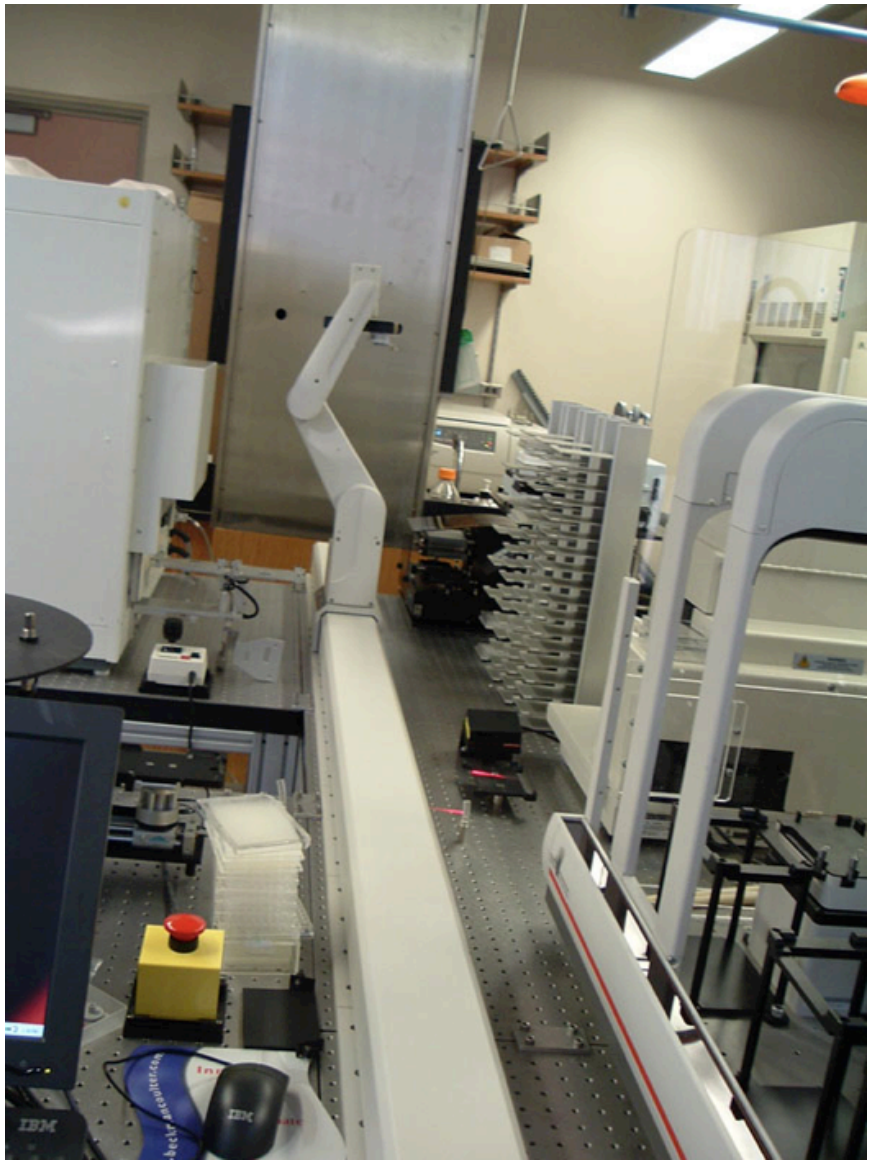
drug discovery via HTS

Identification of "lead chemical matter" is the first step in drug discovery.

"Lead chemical matter" refers to compounds of specific structures defining classes targeting your pathway; they or others in the class can be chemically modified to increase effectiveness (potency, solubility, permeability, delivery) and reduce toxicity.

Libraries today include many FDA approved drugs accelerating "bench to bedside". There are >1100 FDA approved drugs in the UCLA library.

UCLA Molecular Screening Shared Resource (MSSR)



Well Formats (10,000 compounds):

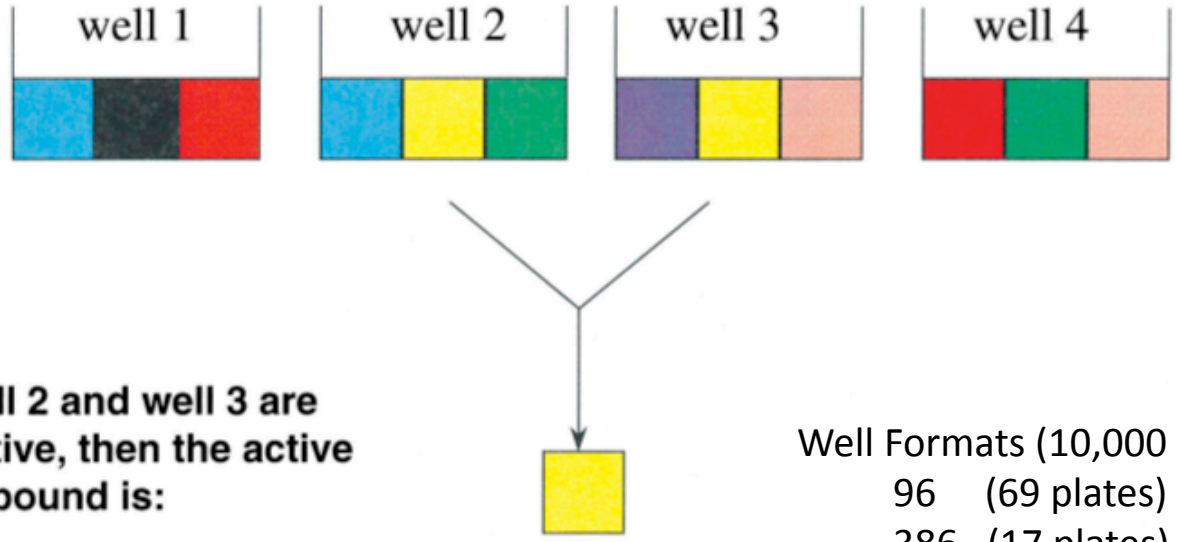
96 (104 plates)
386 (25 plates)
1536 (6.5 plates)

↓ Miniaturization

Six compounds compressed into four wells

How does compression work?

1) compounds appear twice with unique neighbors
2) any two positive wells contain only one compound in common
3) in this example, six compounds are compressed into four wells



if well 2 and well 3 are positive, then the active compound is:

Well Formats (10,000 compounds):

96 (69 plates)
386 (17 plates)
1536 (4.3 plates)

false signals

False Positives

Inactive compounds that falsely meet hit criterion and arise largely due to compression

- Biological Additivity

  Cumulative interaction of multiple weakly interacting compounds.

  Reduced by using compression with compounds of dissimilar chemical structure within a well.

- Bystander Effects

  When hit rates are high the number of inactive compounds appearing to meet hit criteria is inflated.

  Reduced by screening under conditions in which the assay hit rate is low.

False Negatives

Active compounds that meet hit criterion that go undetected

- Compression with two compounds of equal but opposite effects
- Compound not dissolved properly
- Compound used at a concentration outside of its effective range

False negatives are of greater concern than false positives because with negatives you have nothing to validate—you missed it entirely. But false negatives are less likely to occur.

Replicate analogues

Replicate analogues are replicates of compounds with similar structure

Spencer et al., 1998 showed that as the number of replicate analogues of a particular structural class increased, the probability of identifying that class as effective increased.

Remember that the goal of HTS is identifying lead compounds, so it isn't necessary to have all members of a class present.

Spencer et al. Biotechnology and Bioengineering 61, 61-67, 1998.

Lead compound summary

Golebiowskiy et al.,
Lead compounds discovered from libraries
Current Opinion in Chemical Biology 2003, 7:308–325

Reviewed > 100 HTS studies and presented a summary of all lead compounds.

Results

- 68 compounds
  - 26 antagonists
  - 36 inhibitors
  - 5 agonists
  - 1 inhibitor of protein binding
- 49 targets
- 43 sources
  - 36 pharmaceuticals
  - 7 universities

Detection technologies

Non-radiometric readouts

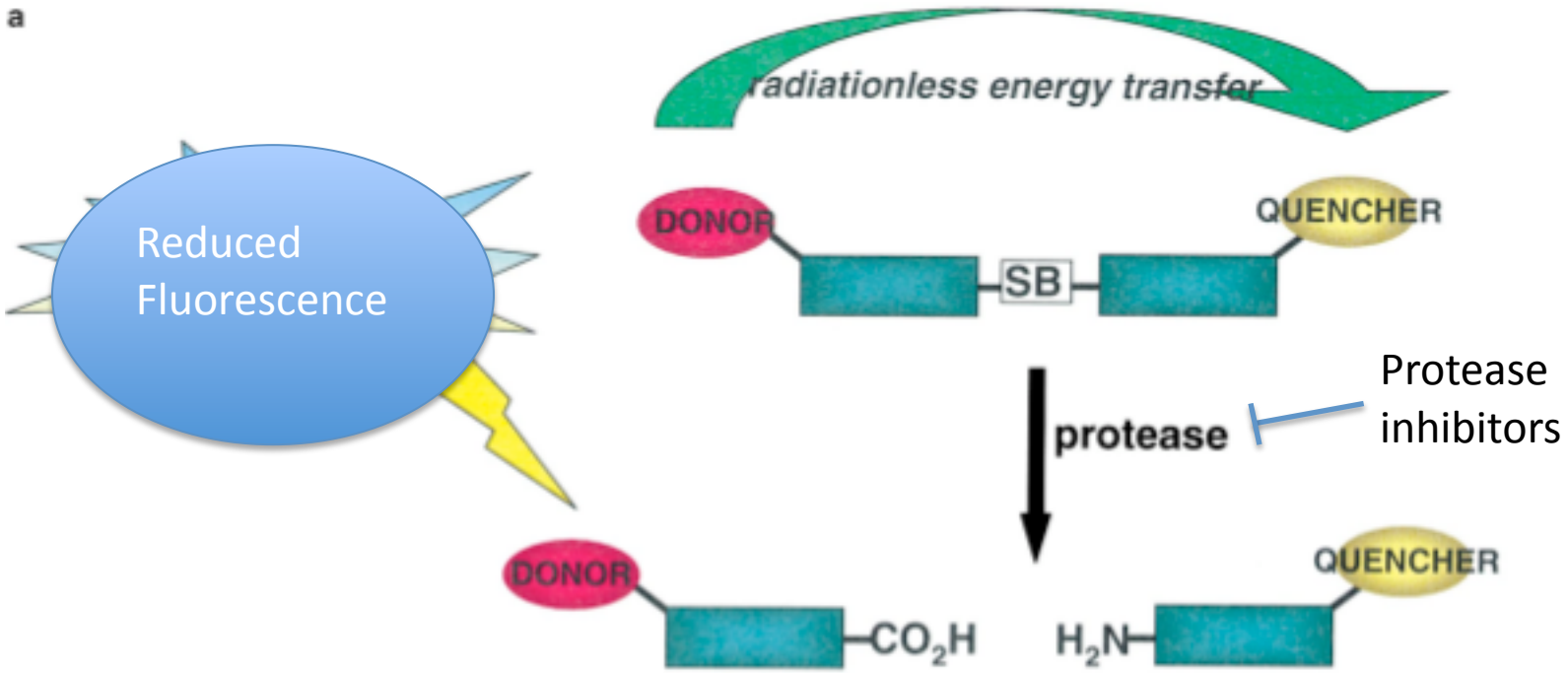
Absorbance
Colorimetric
Fluorescence
Luminescence

Radiometric readouts

Filtration and scintillation proximity.

Non-radiometric readouts

FRET

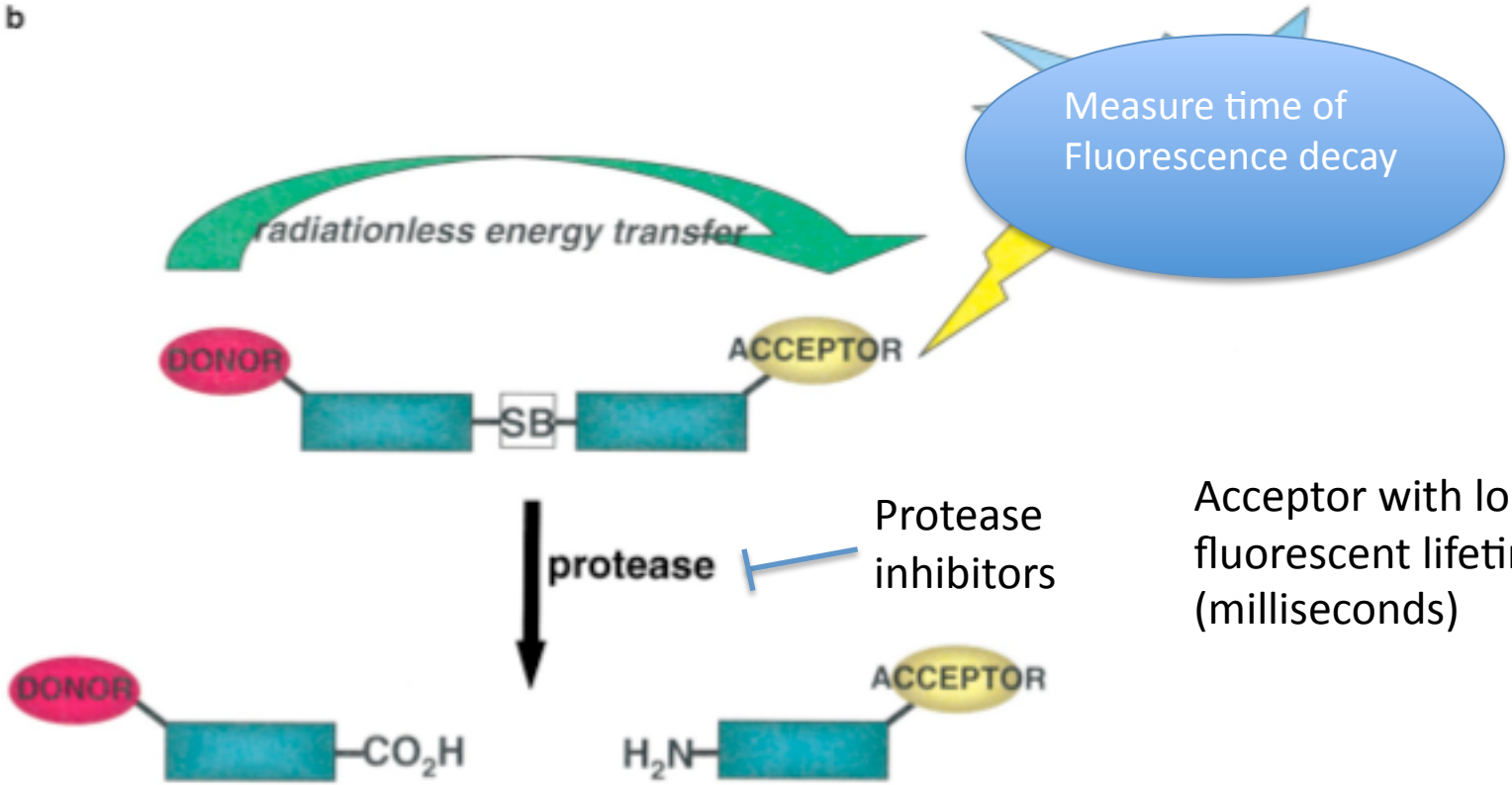


Separation by 8 residues optimal
SB=scissile bond

Disadvantages:

must know the protease and its cleavage site
absorption by library compounds reducing FRET signal

Time-resolved FRET signals

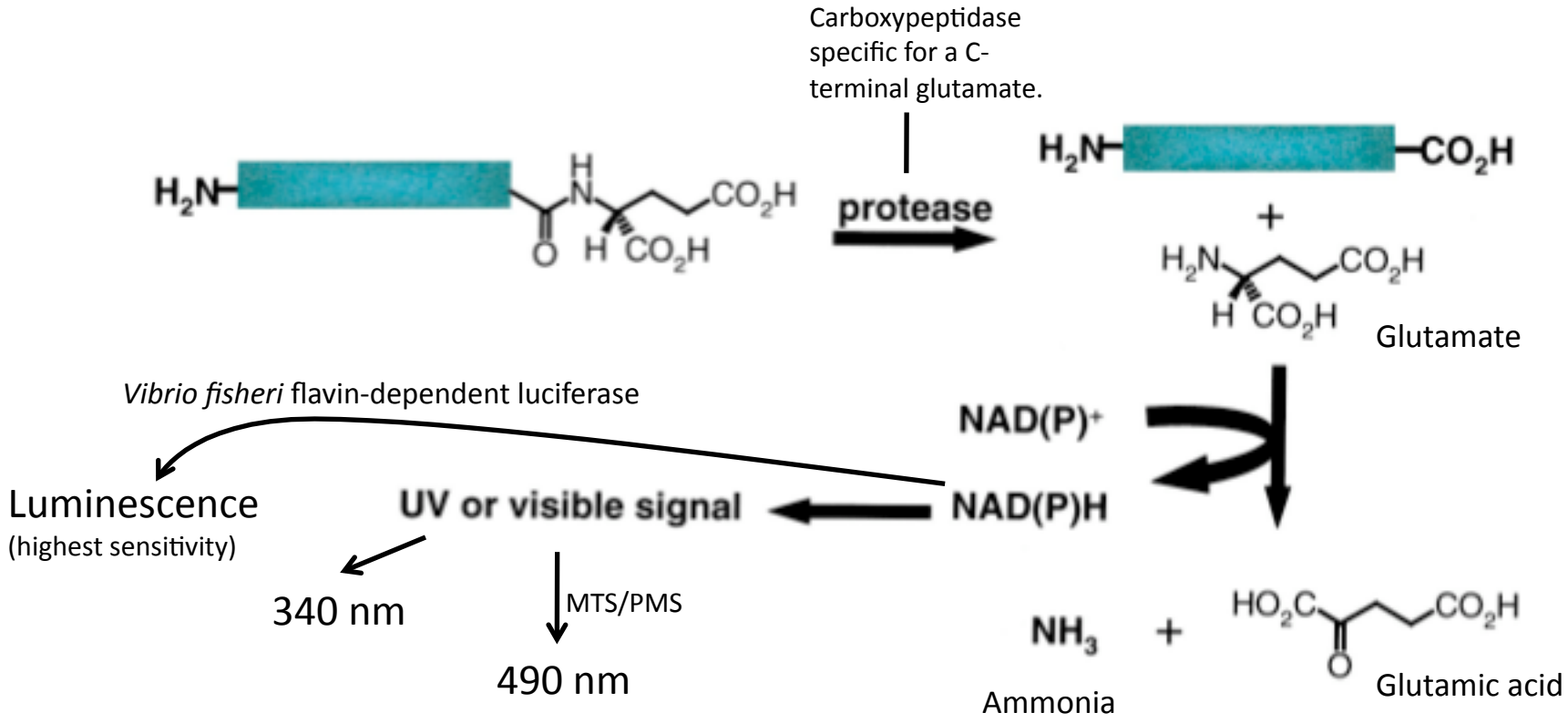


Acceptor with long fluorescent lifetime (milliseconds)

Advantage compared to donor quencher FRET:

Comparison across the plate of wells with similar times of decay leads to enhanced signal/noise ratios

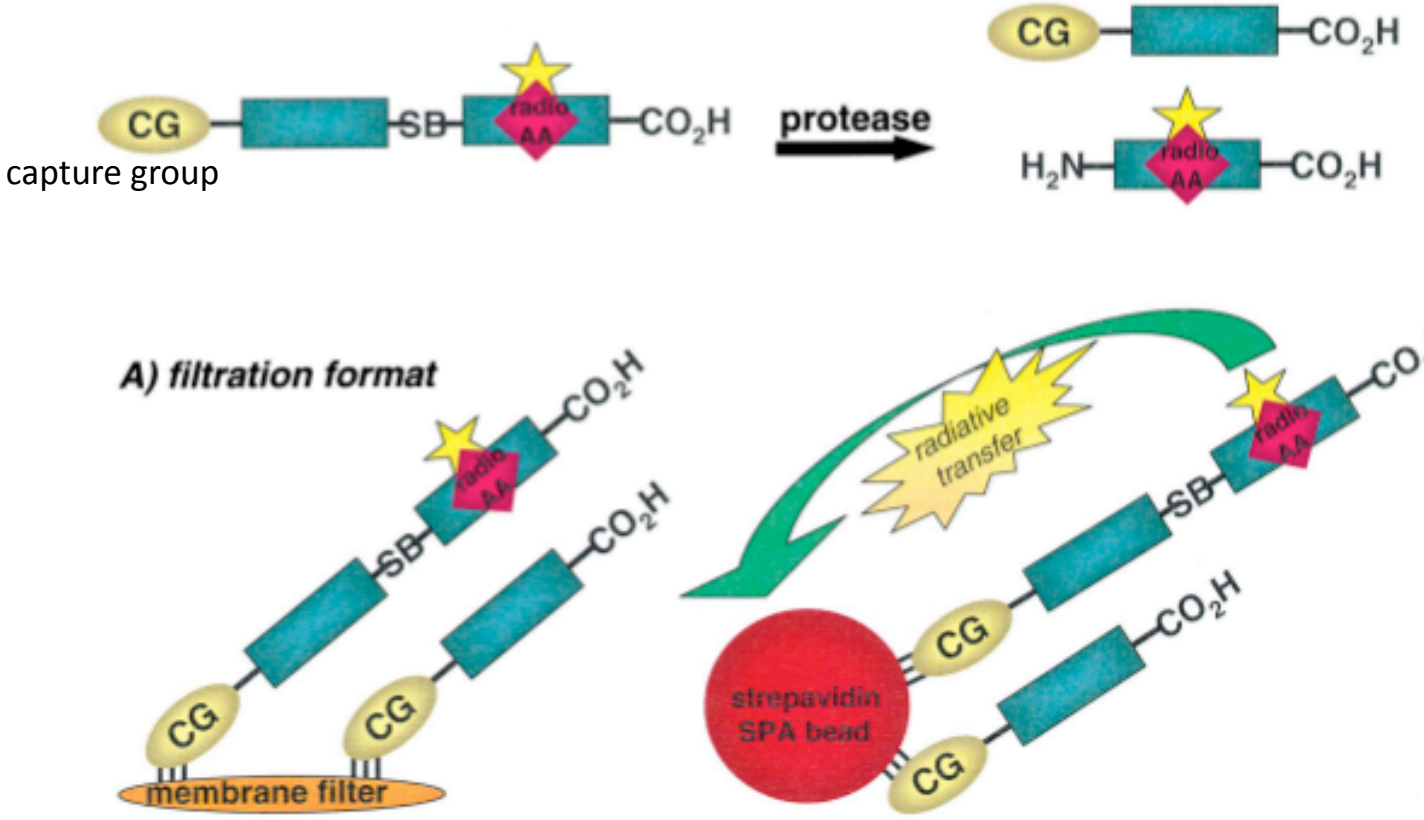
Enzyme Coupling



Assumption: irreversible 1st order reaction

Radiometric readouts

Filtration and SPA radiometric assays



Advantages:

- Easier assay development than FRET
- Can be a cell-free assay

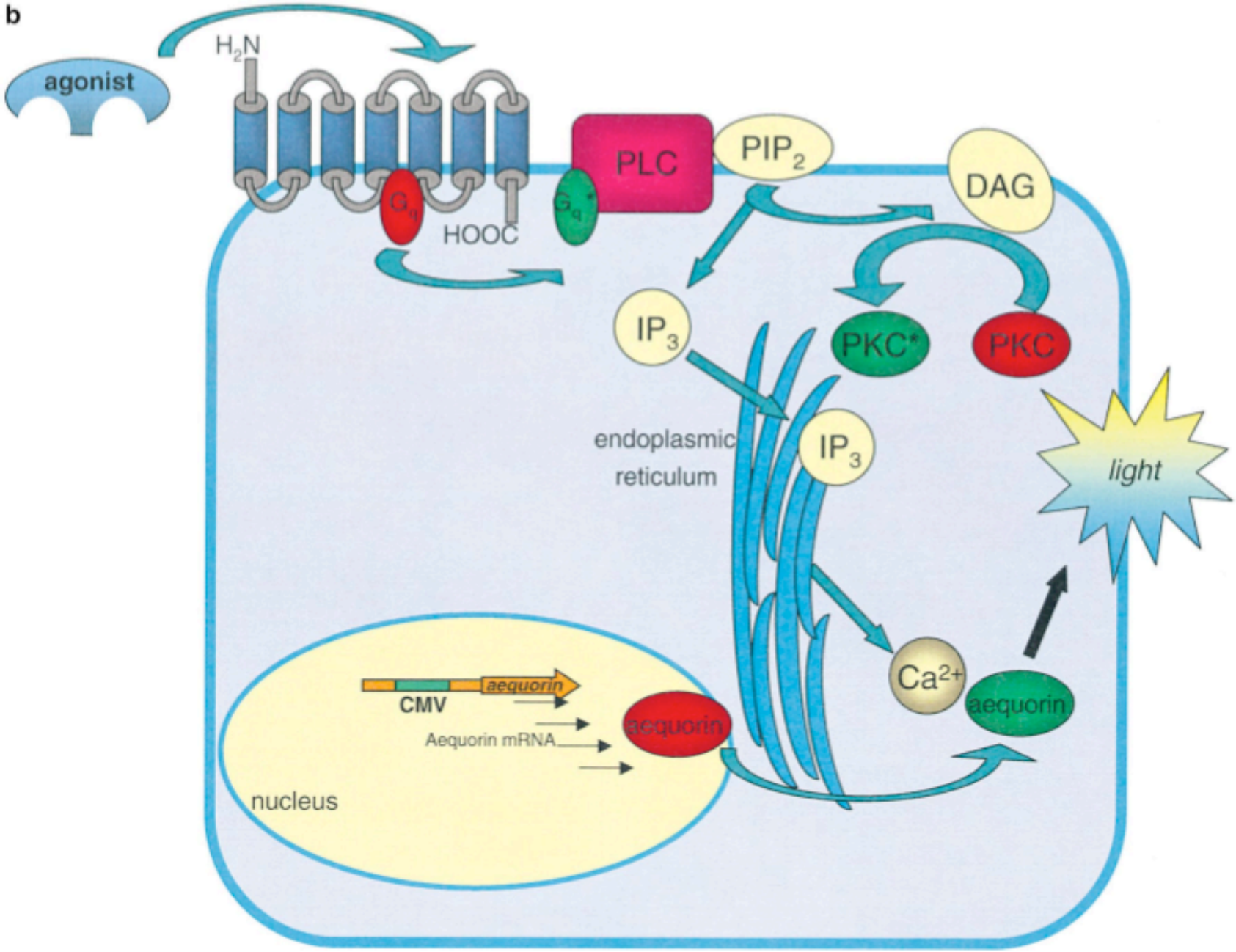
Disadvantages:

- Radioactive

Scintillation proximity assays (SPA)
Improved with "flash-plate" technology.

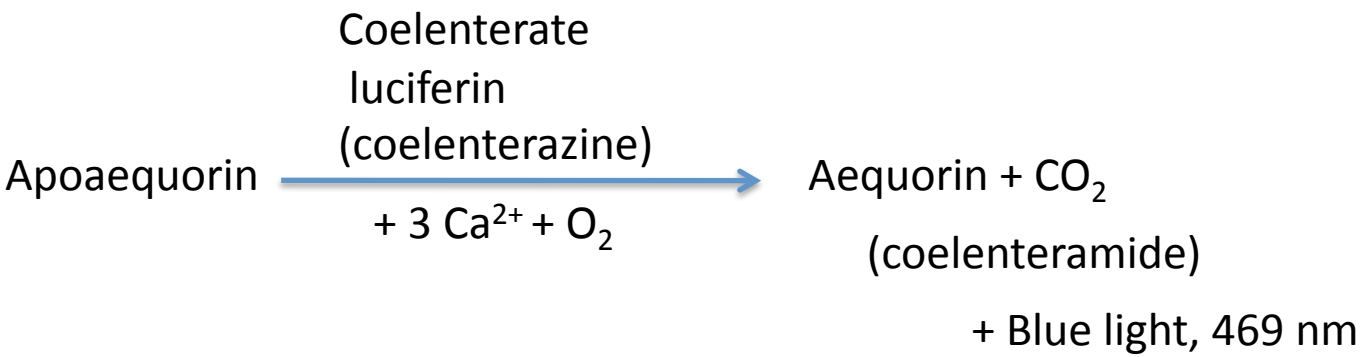
Specific assays: GPCR

Of all compounds with known targets, 45% are cell membrane receptors, predominantly G protein coupled receptors


b
agonist
H2N
HOOC
G1
G4*
PLC
PIP2
DAG
IP3
PKC*
PKC
endoplasmic
reticulum
IP3
light
Ca2+
aequorin
aequorin
CMV
Aequorin mRNA
nucleus

Aequorin as a Calcium Sensor

Apoaequorin is a 22 kDa jellyfish protein that catalyzes coelenterazine to coelenteramide in the presence of calicum and O_2 resulting in chemiluminescence.



The approximately third-power dependence of aequorin luminescence on Ca^{2+} concentration allows for a measurement of Ca2+ concentrations from 0.1 to >100 μM (Blinks, 1989).

Plasmid available from Perkin Elmer

Aequorin luminescence is emitted as a flash

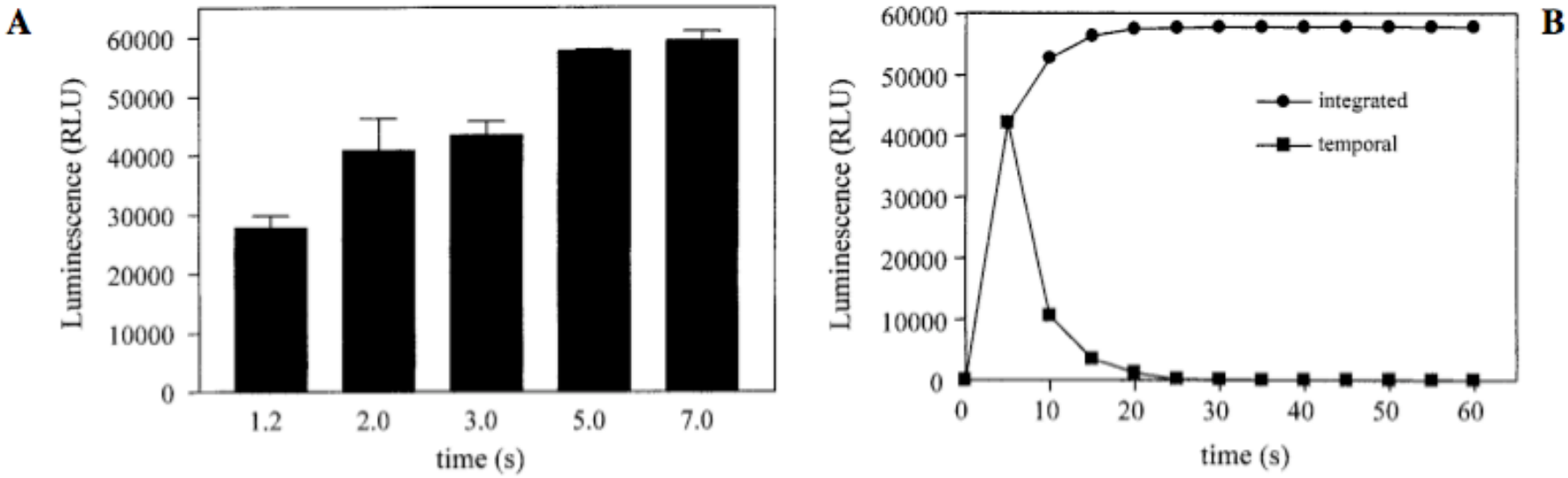
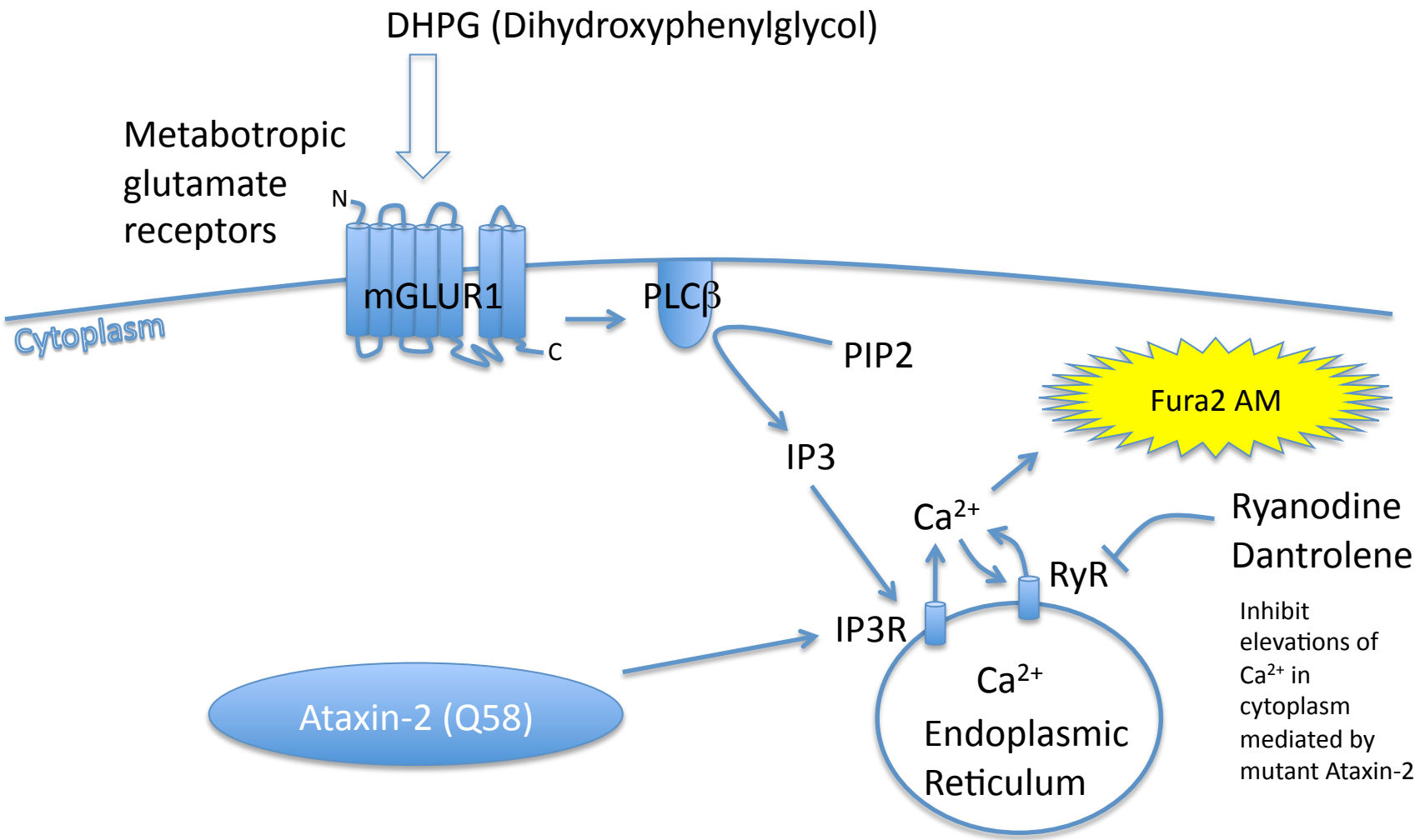
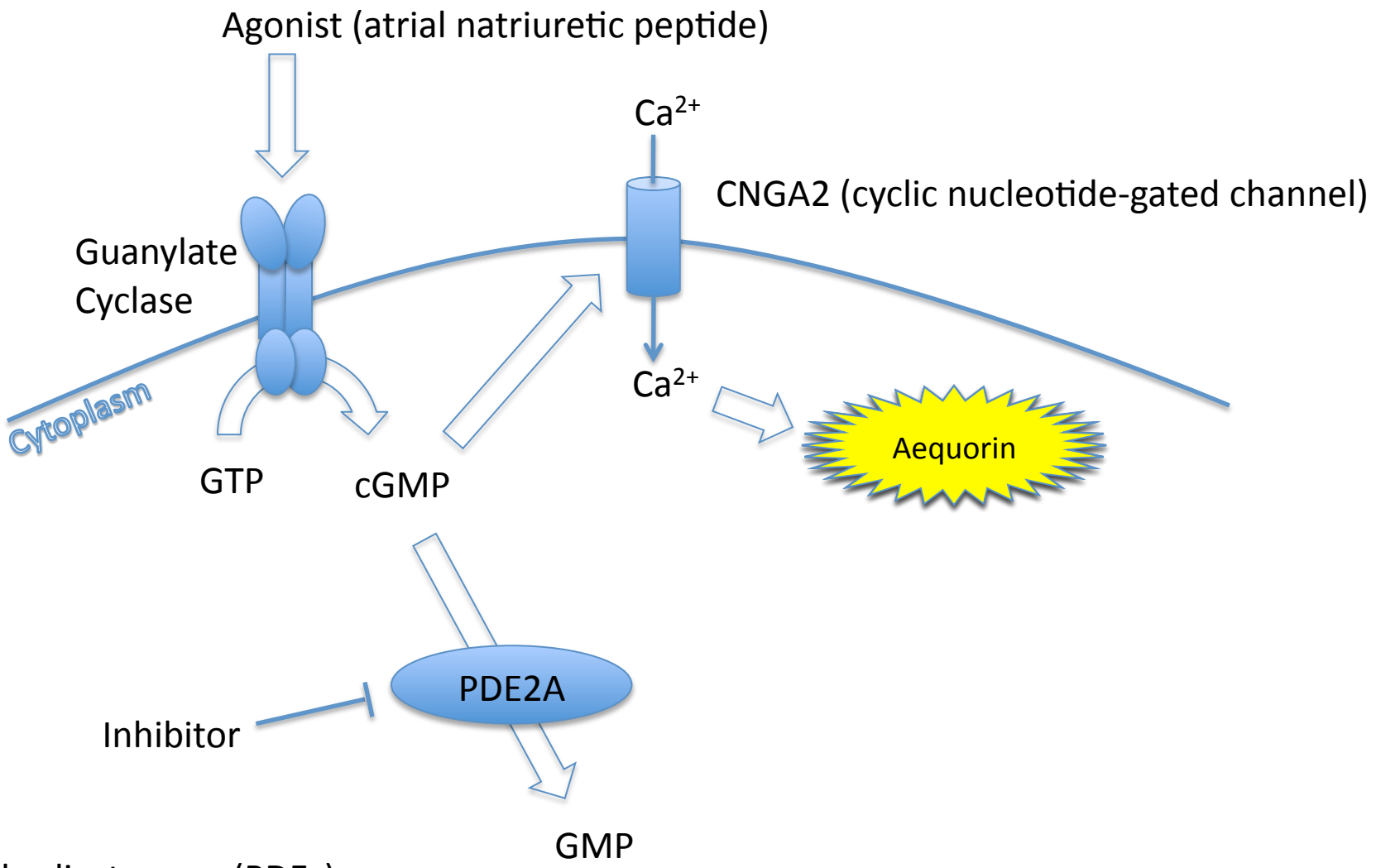


FIG. 3. Acquisition time for photon accumulation on luminescent camera sensor. (**A**) Optimal acquisition time setup. A total of 25,000 CHO cells expressing 5-HT_{2B} were injected on 5 μM 5-HT–containing plates and the luminescent signal (expressed in RLU) was monitored for 2 min with different sampling time (1.2–7 s). The total signal recorded during 2 min of measurement was plotted with its specific sampling time. Error bars indicate the values obtained for eight determinations. (**B**) A 5-s acquisition time was used to monitor the luminescent signal generated by the injection of CHO cells expressing Ox_2 receptor on 30 nM orexin B during 1 min. The real-time measurement by the FDSS6000 allows the monitoring of temporal kinetic changes (■) in intracellular calcium concentration and the subsequent determination and plotting of the integrated value of the signal (●).

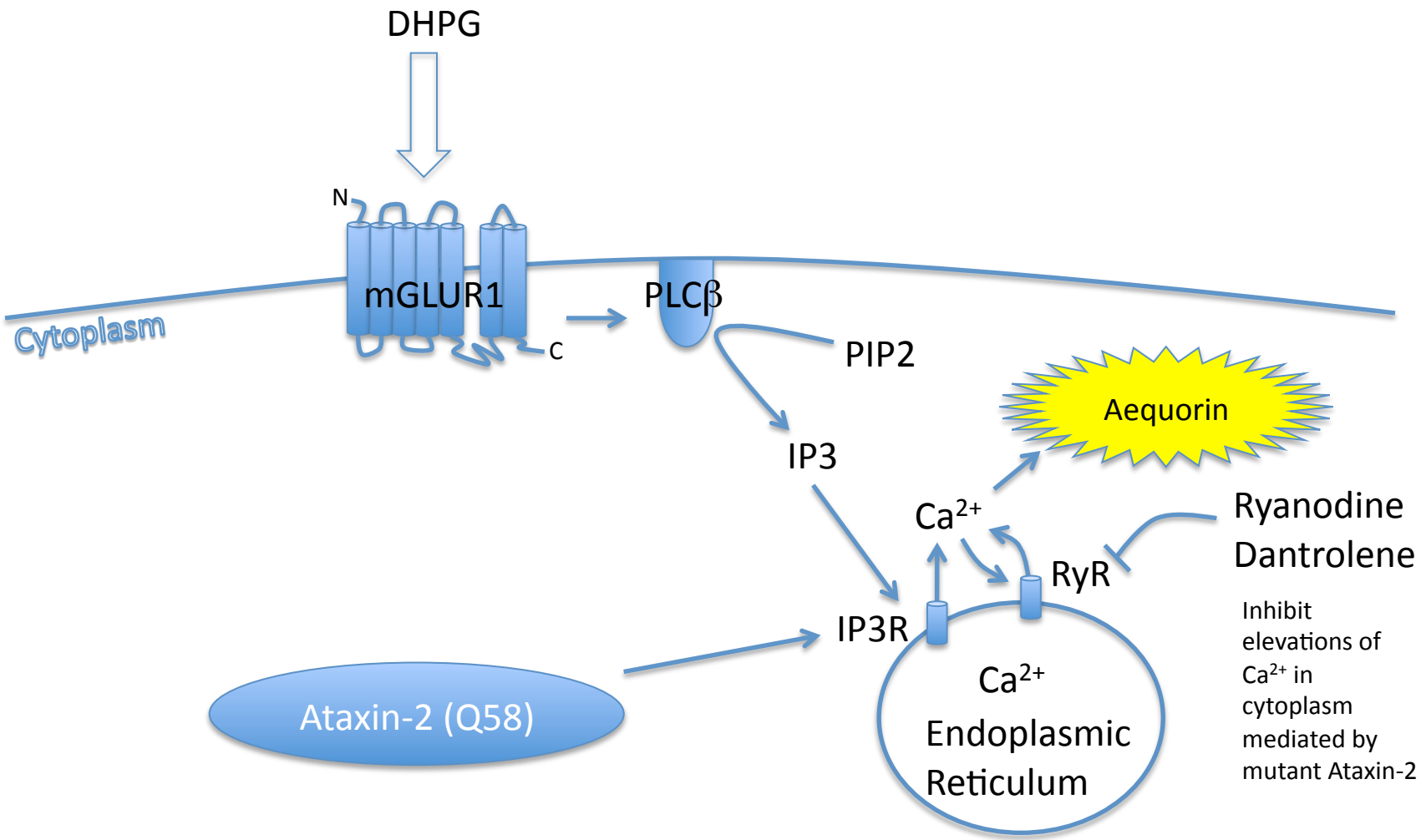
Le Poul et al., Adaptation of Aequorin Functional Assay to High Throughput Screening J Biomol Screen 7:57, 2002.


DHPG (Dihydroxyphenylglycol)
Metabotropic glutamate receptors
N
mGLUR1
C
Cytoplasm
PLCβ
PIP2
IP3
Fura2 AM
Ca2+
Ryanodine
Dantrolene
RyR
IP3R
Ataxin-2 (Q58)
Ca2+
Endoplasmic Reticulum
Inhibit elevations of Ca2+ in cytoplasm mediated by mutant Ataxin-2



Phosphodiesterases (PDEs) are relevant to calcium channel regulation and heart disease

Wunder et al., 2009


DHPG
N
mGLUR1
C
Cytoplasm
PLCβ
PIP2
IP3
Aequorin
Ca2+
Ryanodine
Dantrolene
RyR
IP3R
Ataxin-2 (Q58)
Ca2+
Endoplasmic
Reticulum
Inhibit elevations of Ca2+ in cytoplasm mediated by mutant Ataxin-2

Uses of an Atxn2(Q58) Aequorin cell line

- Validate Atxn2 function of compounds discovered via compound screening
- Evaluate effects of atxn2 binders (Fox1, IP3R) or modifiers (CNCA1A) on atxn2 function.
- Screening for new compounds altering atxn2 function
  - Validation screen would include a matched control cell line lacking exogenous Atxn2 so to eliminate compounds altering aequorin chemiluminescence independent of Atxn2

Why Aequorin is better than fluorescent dyes (Fura2 AM or Calcium Green 1)

- Apparently, aequorin can be a sensor for greater ranges of CA2+ concentrations than fluorescent dyes.
- Absorption by compounds can interfere with fluorescence of dyes.
- Chemiluminescence of Aequorin is orders of magnitude brighter than fluorescence dyes.
- Stable expression is preferred to adding a substrate, except you still have to add colenterazine.

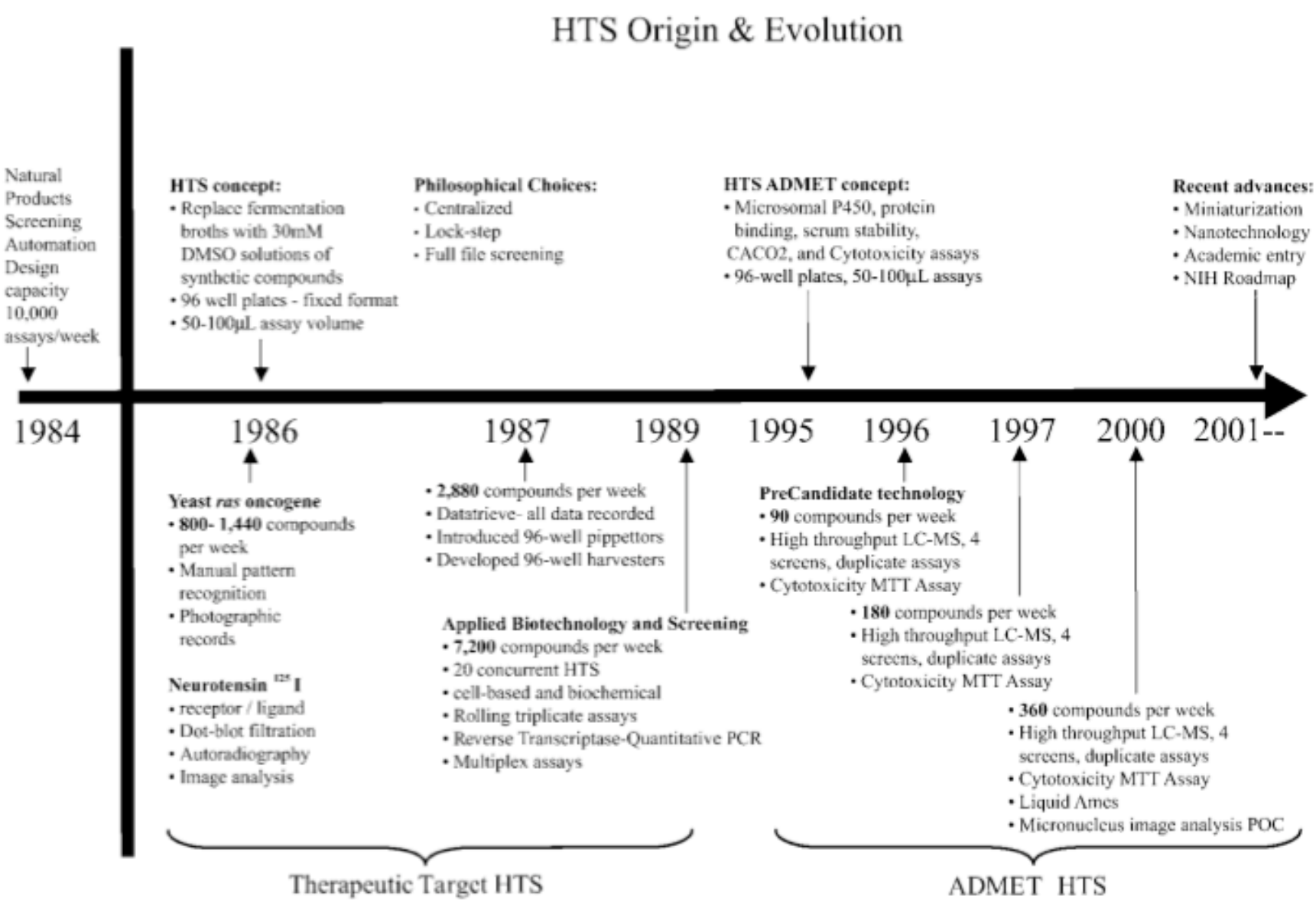


Figure 1 Chronological sequence of key decisions and developments pertaining to the origin and evolution of HTS.

Pereira & Williams. Origin and evolution of high throughput screening. British Journal of Pharmacology (2007) 152,

Virtual screening

Campbell McInnes
Virtual screening strategies in drug discovery
Current Opinion in Chemical Biology 2007, 11:494–502

Jalaie M, Shanmugasundaram V
Virtual screening: are we there yet?
Mini Rev Med Chem. 2006 Oct;6(10):1159-67. Review.

Screening for hypothetical molecules that are not in your possession but which can be obtained by purchase, or which do not exist but can be obtained by synthesis.

Based on computational prediction of binding affinity.