Live cells & Phenotypic Screening, Causal pathways in cells and drug development

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Jan 22, 2026

Outline

1. From target engagement to effect
2. Cell as system biology
3. Phenotypic screening
4. Target deconvolution
5. Appreciating artefacts
6. More complex multimode approaches
7. What separates the drug from the poison?

Classic functional screening tools

PRESTO-Tango as an open-source resource for interrogation of the druggable human GPCRome

nature structural & molecular biology

Wesley K Kroeze[1,2,5], Maria F Sassano[1,2,5], Xi-Ping Huang[1,2,5], Katherine Lansu[1], John D McCorvy[1], Patrick M Giguère[1], Noah Sciaky[1] & Bryan L Roth[1–4]

G protein–coupled receptors (GPCRs) are essential mediators of cellular signaling and are important targets of drug action. Of the approximately 350 nonolfactory human GPCRs, more than 100 are still considered to be 'orphans' because their endogenous ligands remain unknown. Here, we describe a unique open-source resource that allows interrogation of the druggable human GPCRome via a G protein–independent β-arrestin–recruitment assay. We validate this unique platform at more than 120 nonorphan human GPCR targets, demonstrate its utility for discovering new ligands for orphan human GPCRs and describe a method (parallel receptorome expression and screening via transcriptional output, with transcriptional activation following arrestin translocation (PRESTO-Tango)) for the simultaneous and parallel interrogation of the entire human nonolfactory GPCRome.

VOLUME 22 NUMBER 5 MAY 2015 NATURE STRUCTURAL & MOLECULAR BIOLOGY

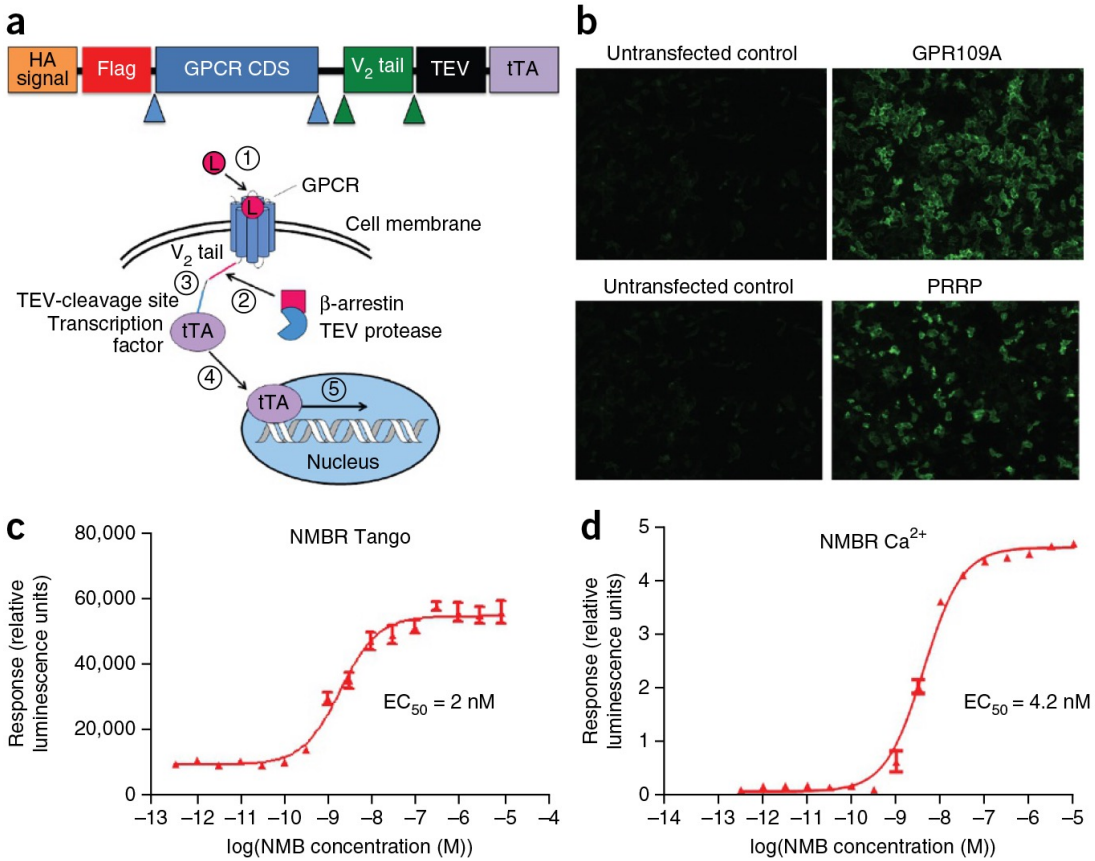
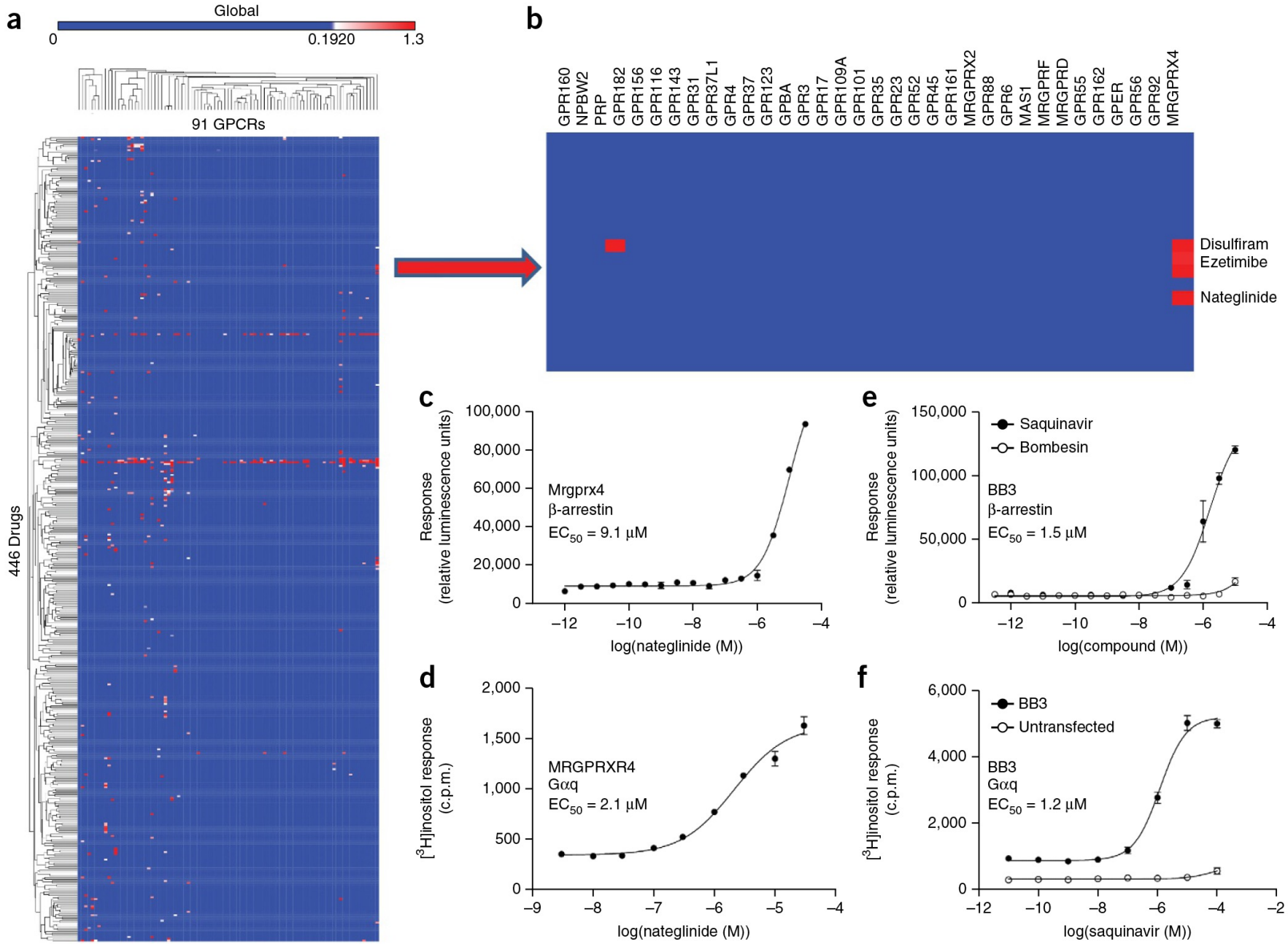
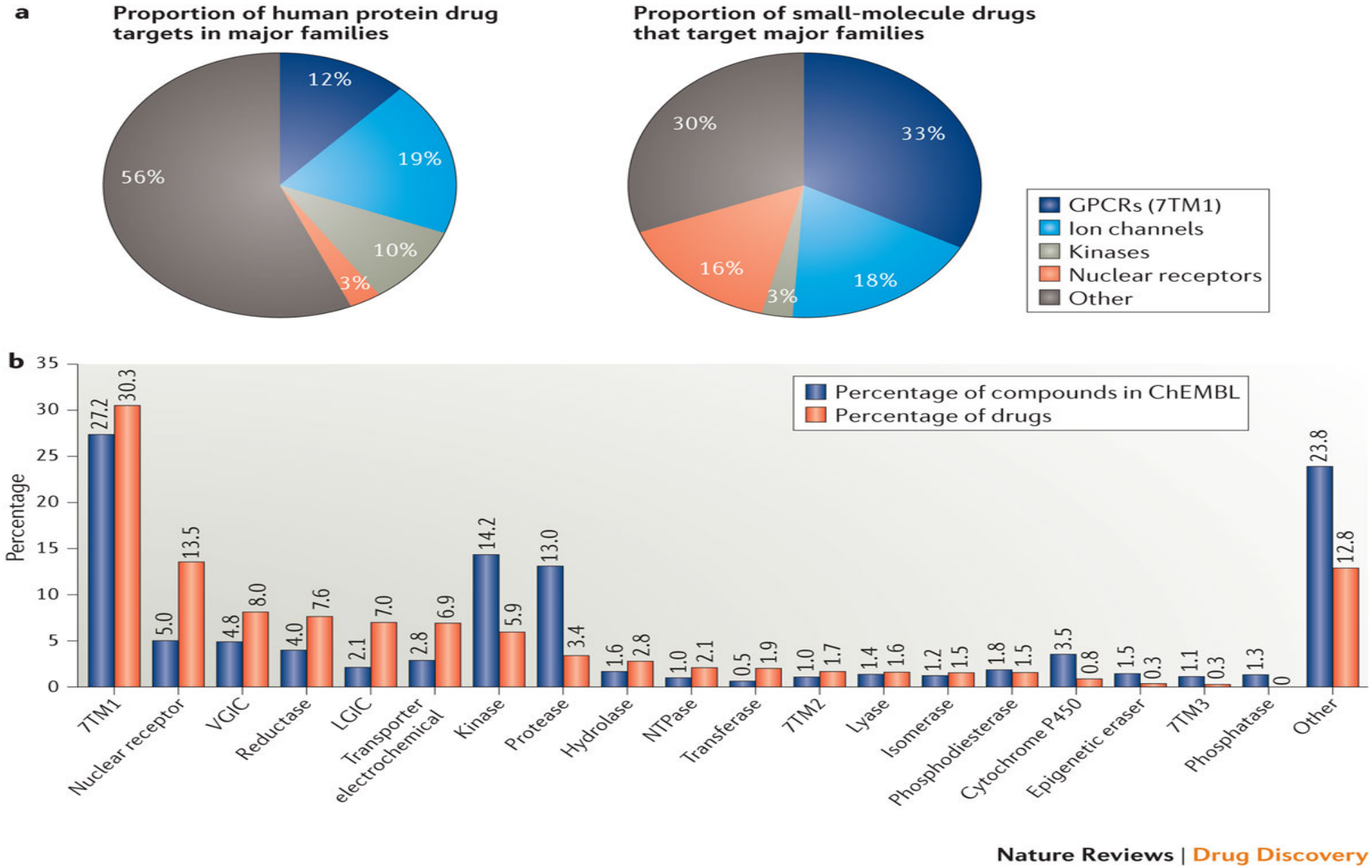


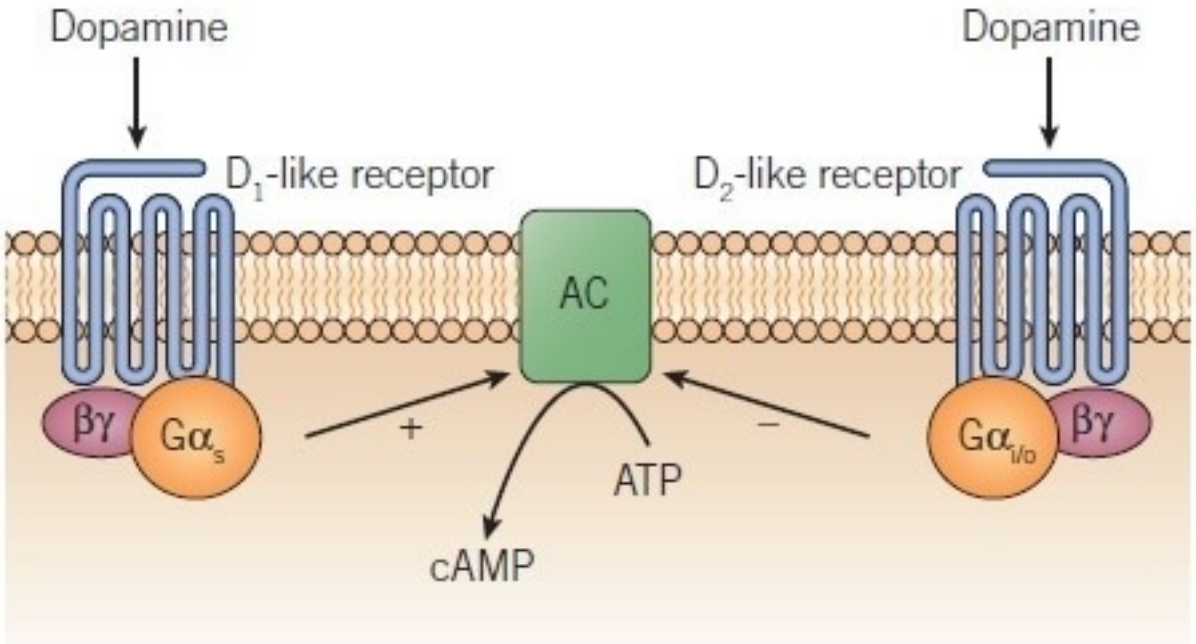
Figure 1 Design, principle and validation of selected Tango assays. (**a**) Top, modular design of Tango constructs. HA, hemagglutinin. Blue arrowheads, Cla I sites; green arrowheads, Age I sites. Bottom, general scheme for the β-arrestin (Tango) recruitment assay. Upon activation of the GPCR by an agonist (L) (1), β-arrestin is recruited to the C terminus of the receptor (2). This is followed by cleavage of the GPCR fusion protein at the TEV protease–cleavage site (3). Cleavage results in the release of the tTA transcription factor (4), which, after transport to the nucleus, activates transcription of the luciferase reporter gene (5). (**b**) Surface expression of two selected Tango constructs, as shown by immunofluorescence with an anti-Flag antibody. (**c**,**d**) Concentration-response curves of a prototypical nonorphan GPCR, the neuromedin B receptor (NMBR) stimulated by neuromedin B (NMB) in the Tango assay (**c**) and in a calcium-release assay (**d**). EC_{50}, half-maximal effective concentration. Data are shown as mean ± s.e.m. of typical experiments done in quadruplicate. Curves were fitted with GraphPad Prism 5.0.


a
Global
0
0.1920
1.3
91 GPCRs
446 Drugs
b
GPR160
NPBW2
PRP
GPR182
GPR156
GPR116
GPR143
GPR31
GPR37L1
GPR4
GPR37
GPR123
GPBA
GPR3
GPR17
GPR109A
GPR101
GPR35
GPR23
GPR52
GPR45
GPR161
MRGPRX2
GPR88
GPR6
MAS1
MRGPRF
MRGPRD
GPR55
GPR162
GPER
GPR56
GPR92
MRGPRX4
Disulfiram
Ezetimibe
Nateglinide
c
Response (relative luminescence units)
Mrgprx4
β-arrestin
EC50 = 9.1 μM
log(nateglinide (M))
d
[3H]inositol response (c.p.m.)
MRGPRXR4
Gαq
EC50 = 2.1 μM
log(nateglinide (M))
e
Response (relative luminescence units)
Saquinavir
Bombesin
BB3
β-arrestin
EC50 = 1.5 μM
log(compound (M))
f
[3H]inositol response (c.p.m.)
BB3
Untransfected
BB3
Gαq
EC50 = 1.2 μM
log(saquinavir (M))

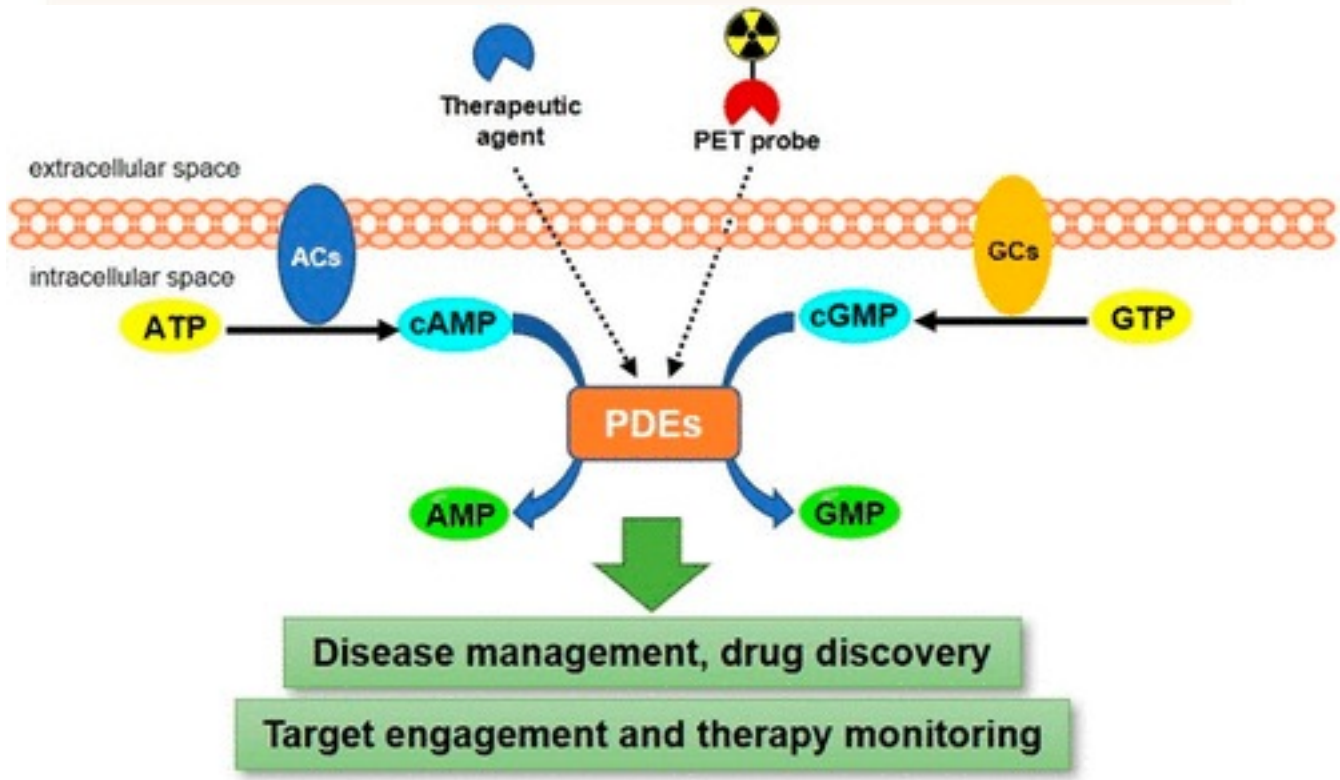


Santos et al., 2017

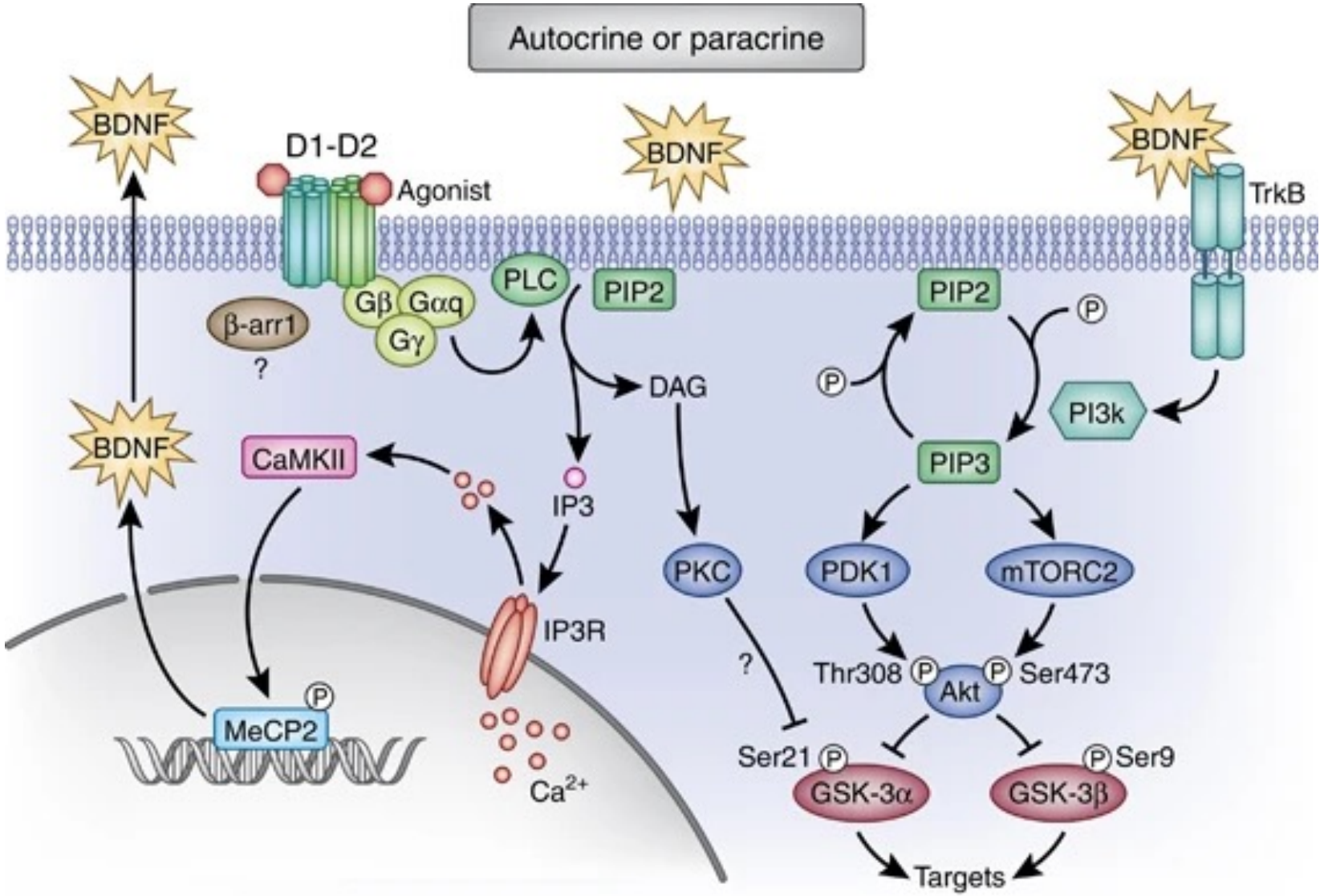
Receptors



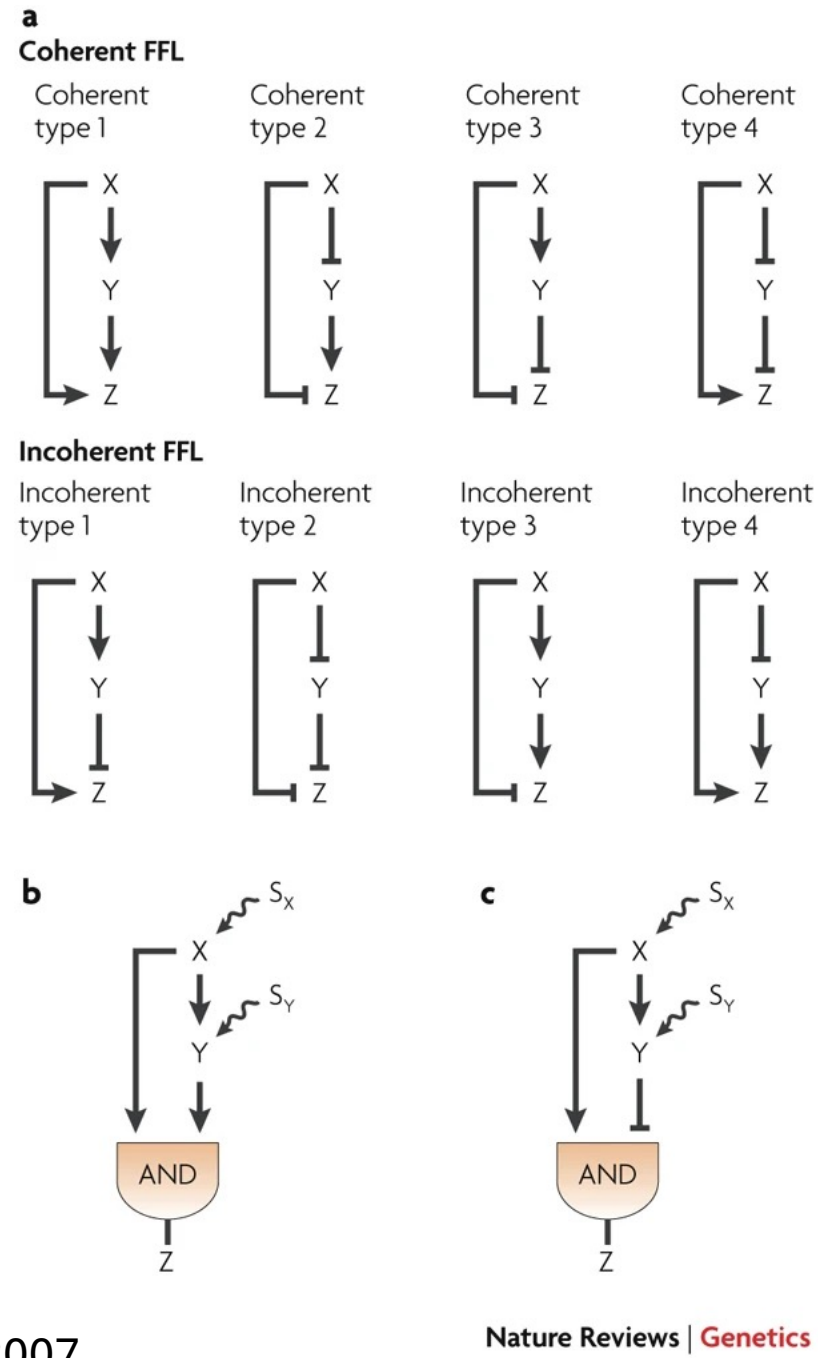
Enzymes

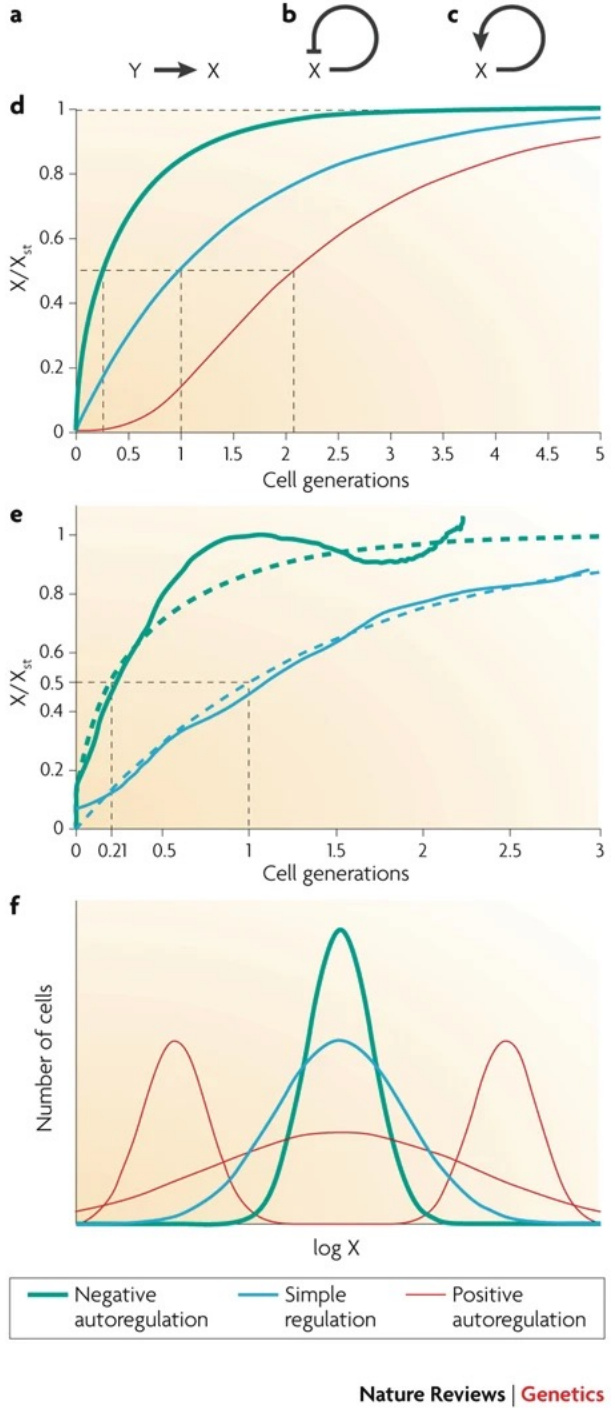


Cell signaling or Biology as cartoons



Perreault et al., 2013 NPP





Not obligated equal:
-Dynamic ON/OFF rate
-Kinetic
-Weight

Not limited to one intermediary
Not single input limited

Alon 2007

https://www.weizmann.ac.il/mcb/alon/download/network-motif-software

Cell signaling location

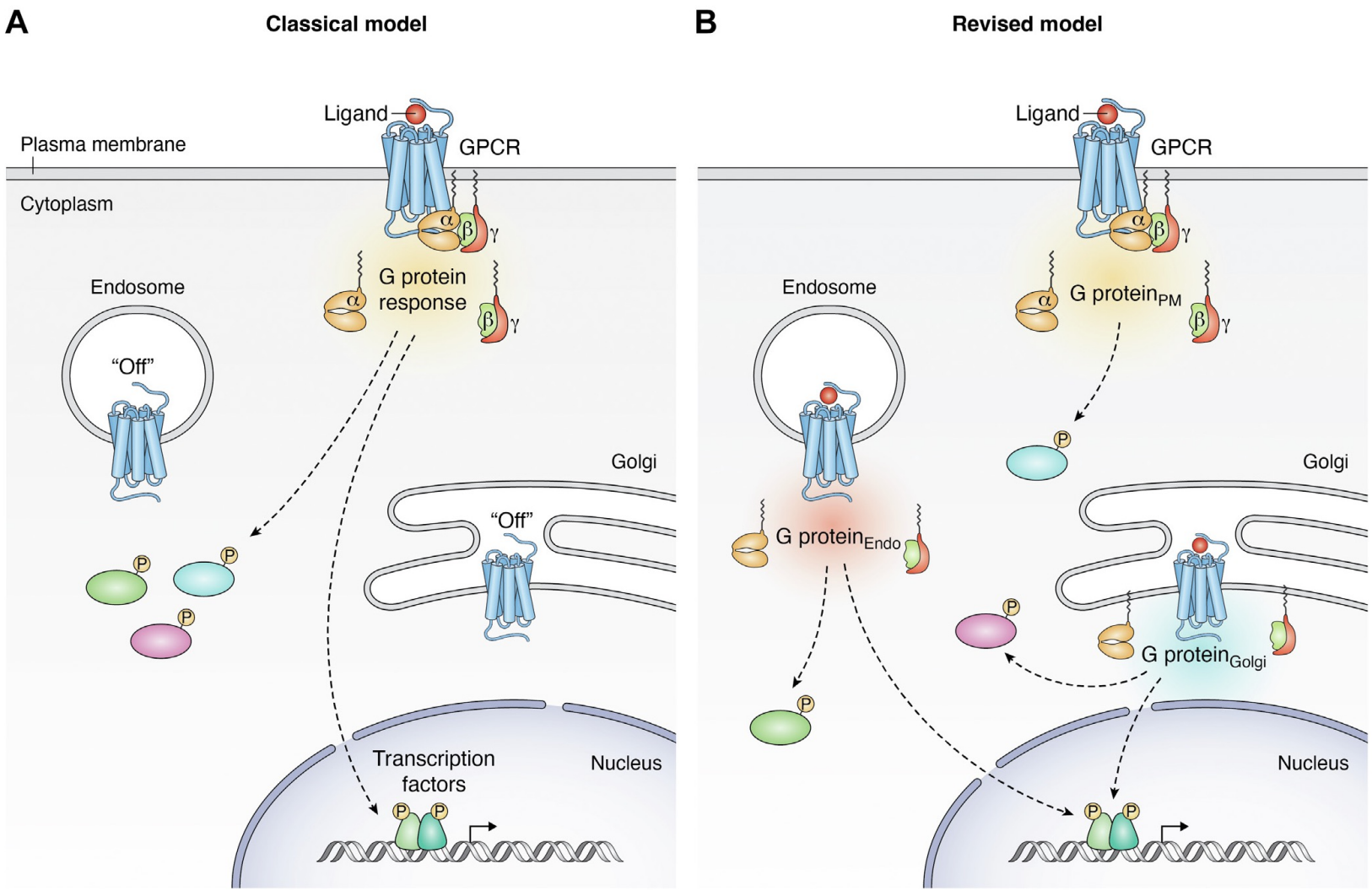


Figure 1. Spatial encoding of GPCR signaling. *A*, classical model: GPCRs initiate all G protein-dependent responses from the cell surface, and are turned "off" following internalization. *B*, revised model: Active GPCRs on intracellular membranes (endosomes, Golgi) stimulate a "second wave" of G protein-dependent signaling that gives rise to unique cellular responses, including protein phosphorylation and transcriptional reprogramming. endo, endosome; PM, plasma membrane; TF, transcription factor.

Klauer et al., 2024 JBC

Drug action location

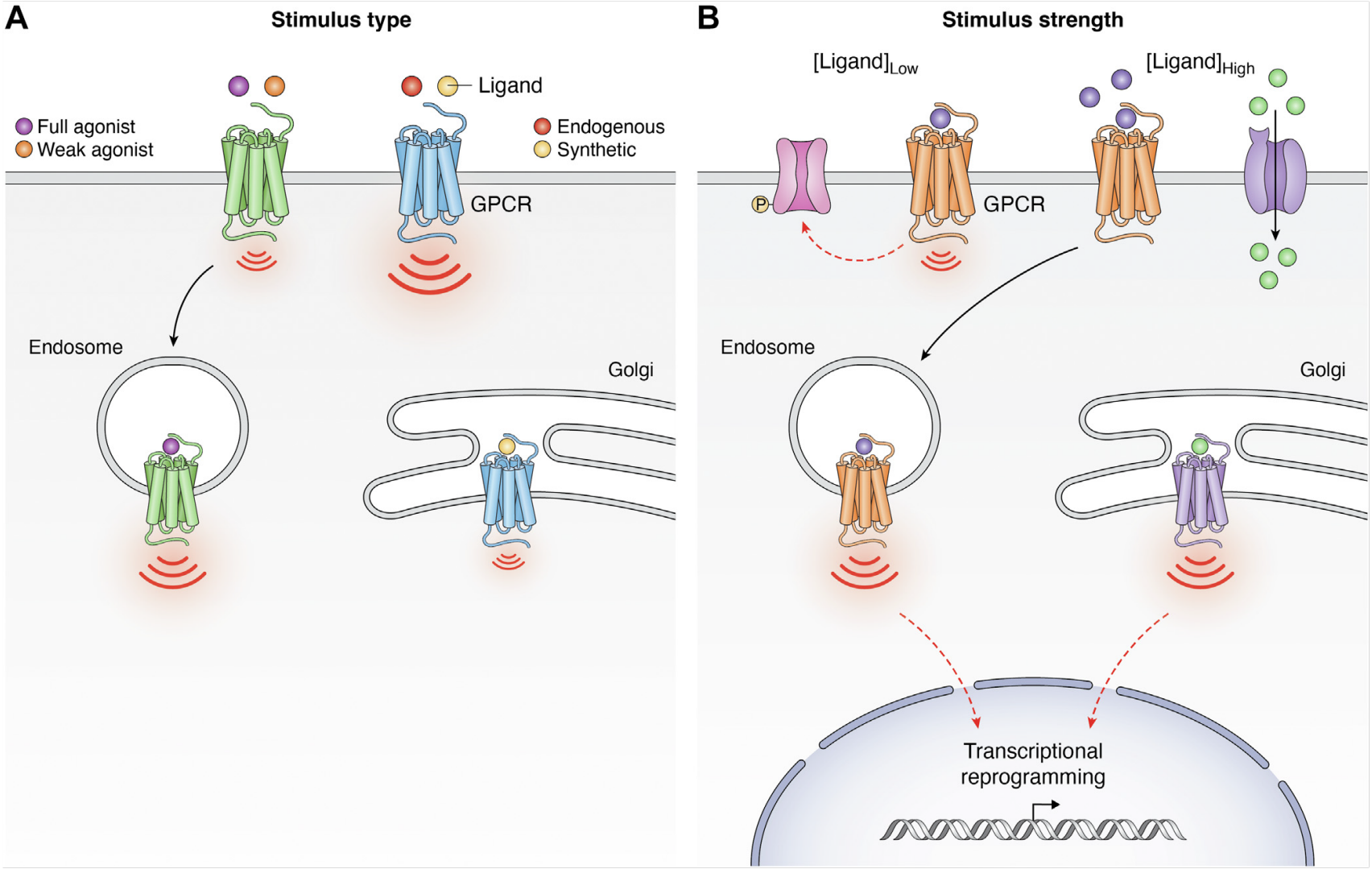
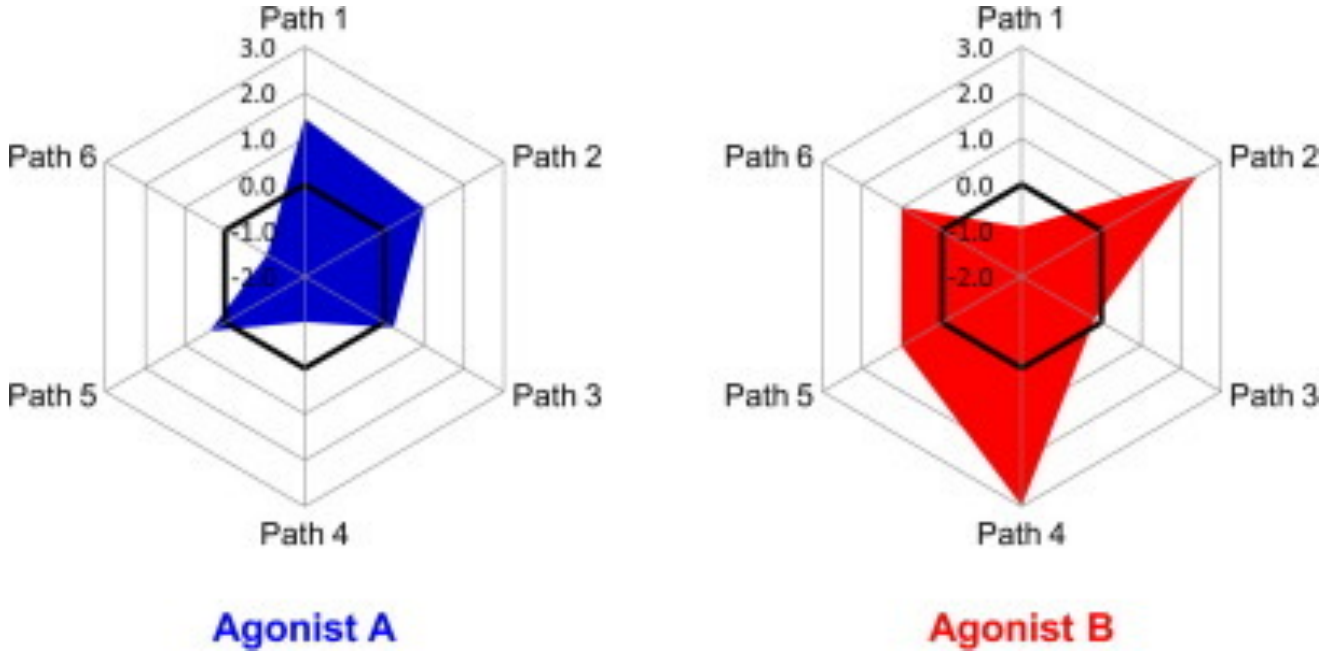
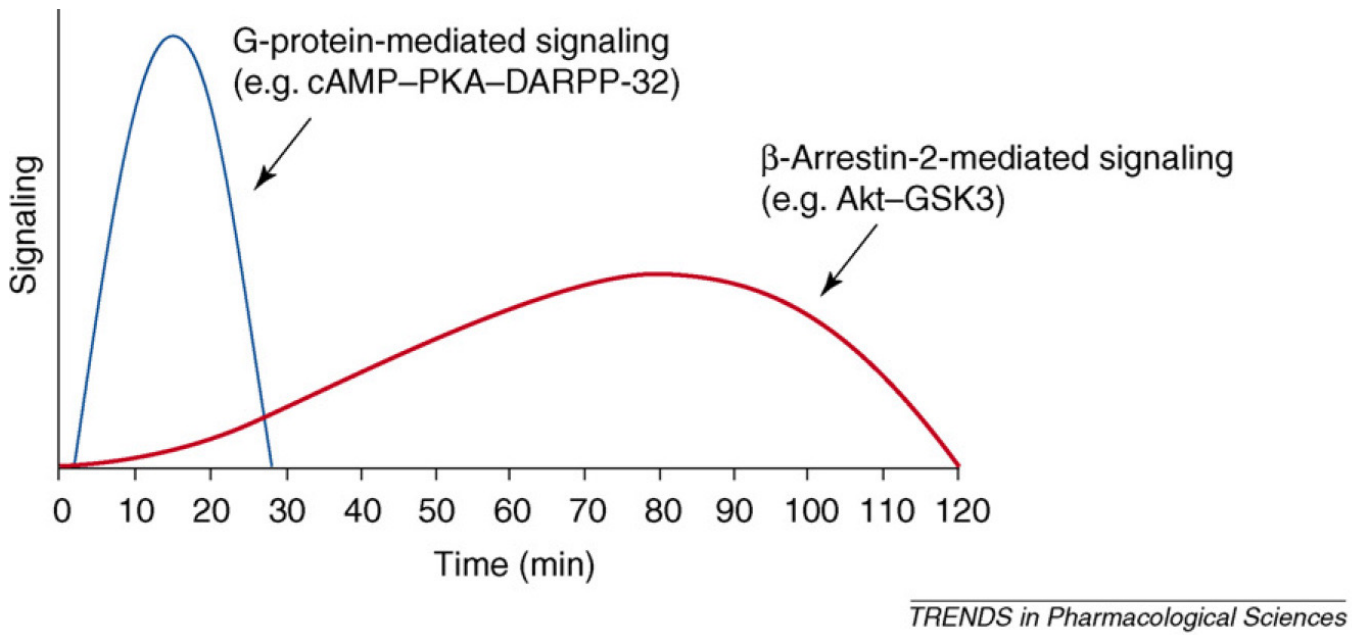


Figure 3. Speculative model for the role of intracellular GPCR signaling in ligand recognition. *A*, compartmentalized GPCR activation as a cellular mechanism to distinguish between endogenous agonists with distinct chemical properties or between endogenous and synthetic agonists. *B*, intracellular signaling as a cellular mechanism to interpret stimulus strength. Low dose of stimulation would activate the plasma membrane-delimited fraction of receptors, leading to localized and transient responses (*e.g.*, ion channel phosphorylation). Only high concentrations of ligand would drive efficient endocytosis or transport into the cell *via* transporters, and consequently would trigger extensive cellular reprogramming.

Pathway preference

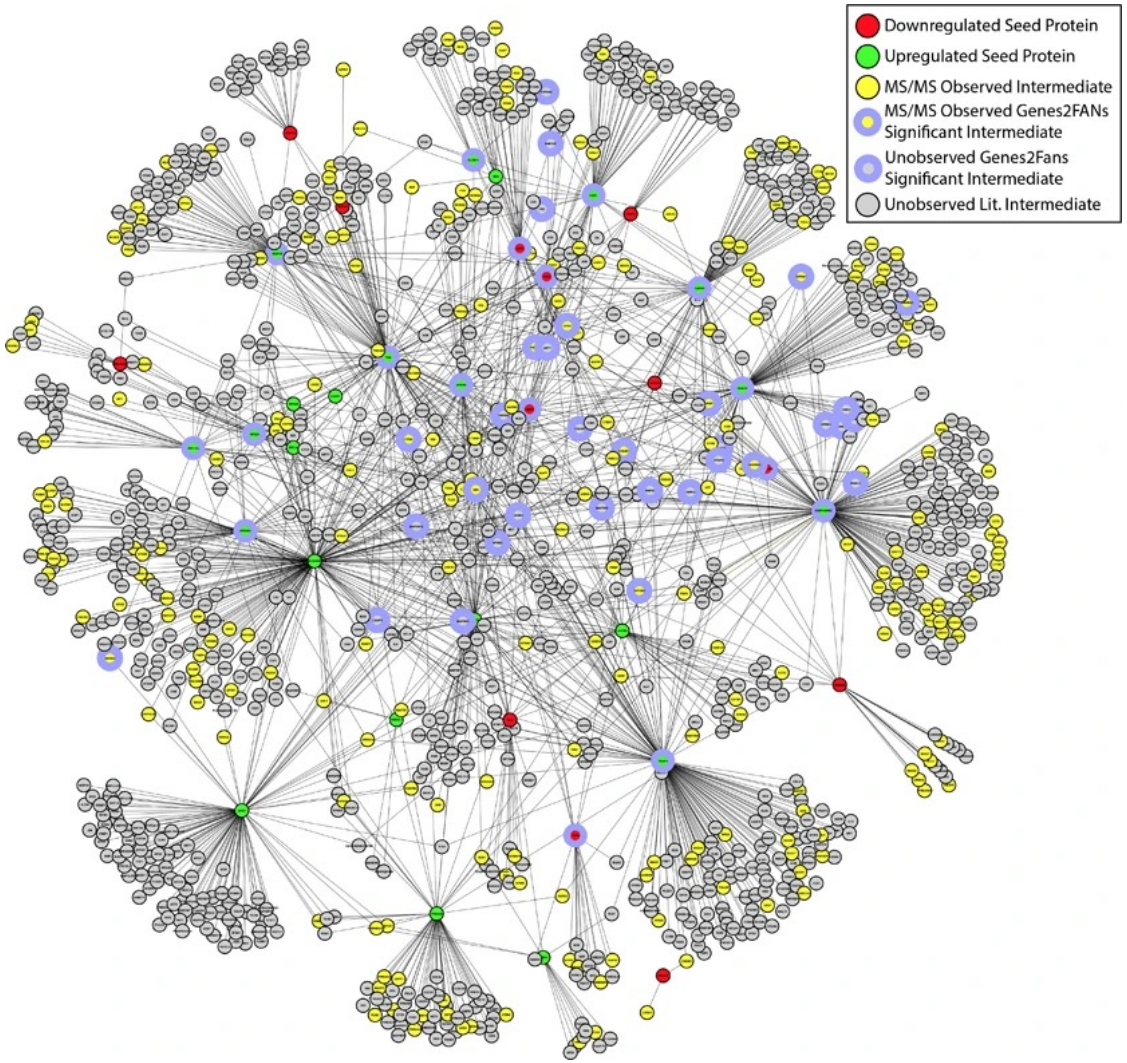


Differential pathway dynamic



TRENDS in Pharmacological Sciences

Cell biology as an interaction computational network



Deterministic but

1- complicated
2-stochatic chaotic aspects

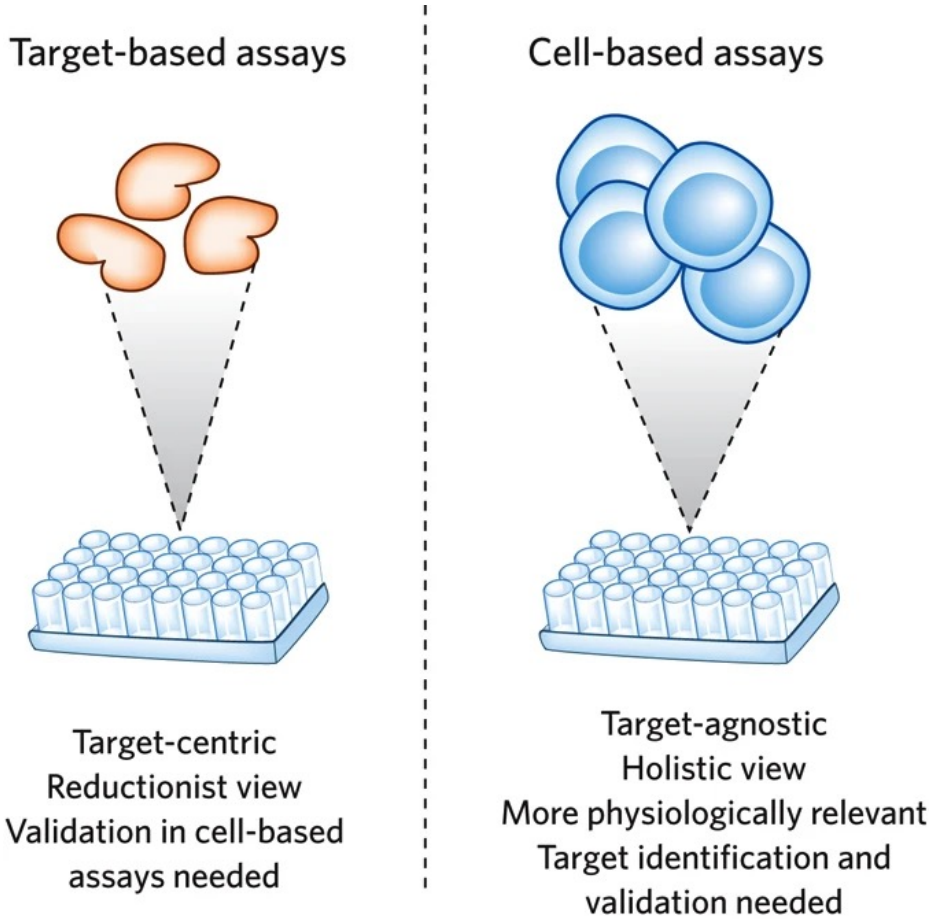
Tool limitations

1- We cannot measure all at once, all the time, everywhere.
2- Experimental system disruption (not quantum physic level but important)

Input vs output correlation may be more feasible than integrated deterministic predictions

Stockton and Devi, Neuropsychopharmacology

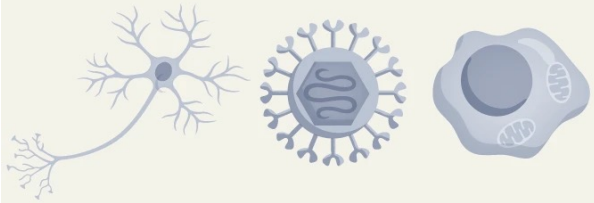
Target-based screening vs cell-based assays



Ursu et al., 2017

Range of phenotypic “drug discovery”

Cellular models



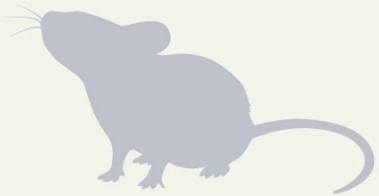
Ivacaftor for cystic fibrosis (oral)
- Potentiator of CFTR-mediated Cl^- transport
- Discovered using a gain-of-function assay in NIH/3T3 cells expressing ΔF508-CFTR mutant

Trametinib for metastatic melanoma (oral)
- Inhibitor of MEK1/MEK2 protein kinases
- Discovered as an inducer of CDK inhibitor protein $p15^{INK4b}$ in several cancer cell lines

Daclatasvir for hepatitis C virus infection (oral)
- Inhibitor of the HCV protein NS5A
- Discovered in a cell-based HCV replicon phenotypic screening assay

Risdiplam for spinal muscular atrophy (oral)
- Restores missing exon 7 in SMN2 mRNA
- Identified in a phenotypic screen evaluating survival in rat primary motor neurons

Animal models



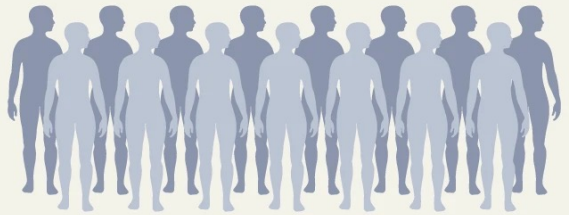
Lacosamide for partial-onset seizures (oral, intravenous)
- Enhancer of Na^+ channel slow inactivation
- Discovered through an in vivo screening campaign in rodent epilepsy models

Memantine for Alzheimer disease (oral)
- Antagonist of NMDA (main MoA) and $5\text{-}HT_3$ receptors; agonist of dopamine D_2 receptors
- Discovered in an in vivo rat model assessing dopaminergic activities

Minoxidil for hypertension (oral)
- Kir6.2/SUR2 K^+ channel activator
- Identified due to its hypotensive effect in an in vivo dog model evaluating anti-ulcer activity

Ezetimibe for high cholesterol levels (oral)
- Inhibitor of NPC1L1 sterol transporter
- Discovered in an in vivo hamster model of cholesterol absorption

Patients



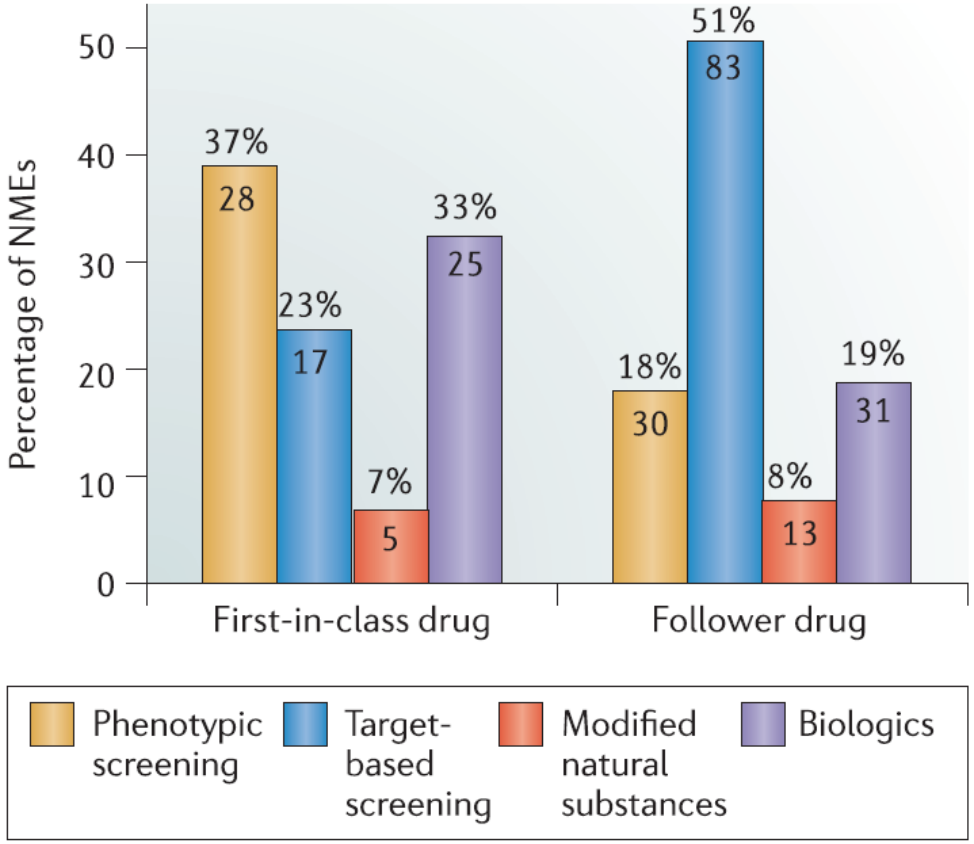
Amantadine for dyskinesia (oral)
- Potential targets include AADC, GDNF, PDE1, sigma 1 and $\alpha_4\beta_2$ nicotinic receptors
- Initially developed for influenza; treatment of a patient with Parkinson disease, who showed improved symptoms, led to its development for dyskinesia

Sildenafil for erectile dysfunction and pulmonary arterial hypertension (oral)
- PDE5 inhibitor initially developed for angina
- Observation of penile erections as a side effect led to its development for erectile dysfunction

Minoxidil for androgenic alopecia (topical)
- Topical form of minoxidil repurposed for alopecia after observing hypertrichosis as a side effect in patients treated with the oral form of the drug

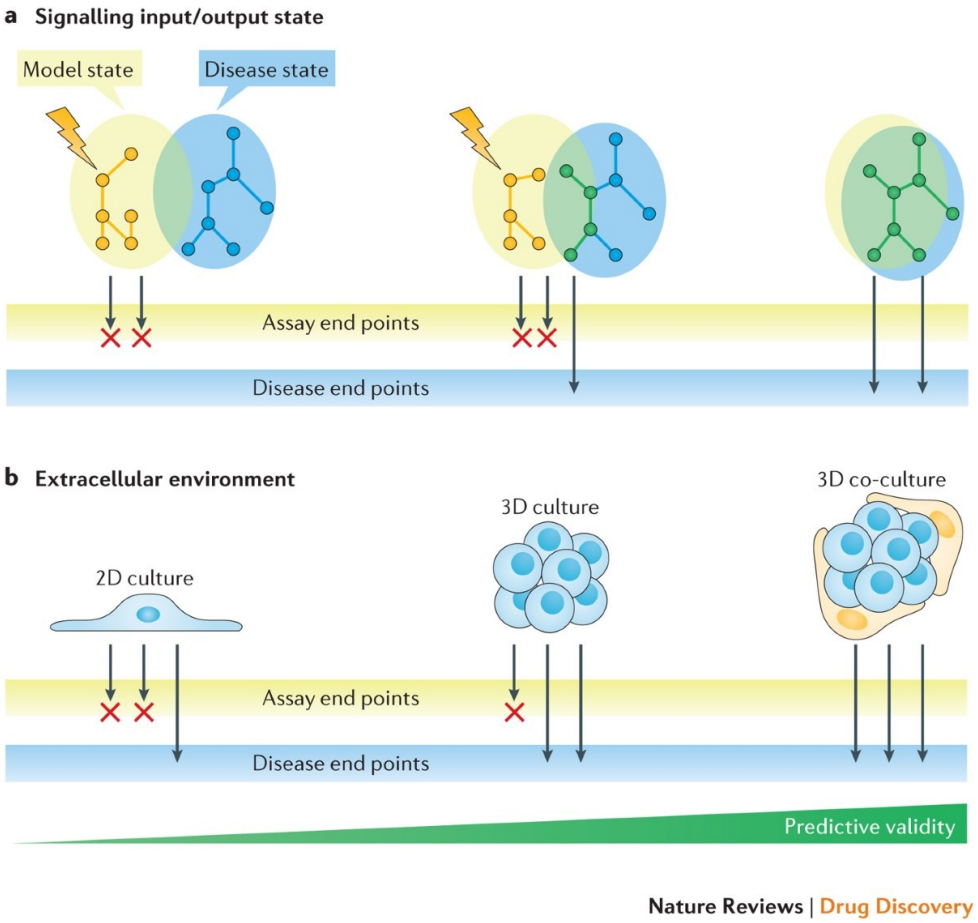
Vincent et al Nature Reviews Drug Discovery 2022

Proportion of clinical drugs discovered by phenotypic screening (1999 – 2008)



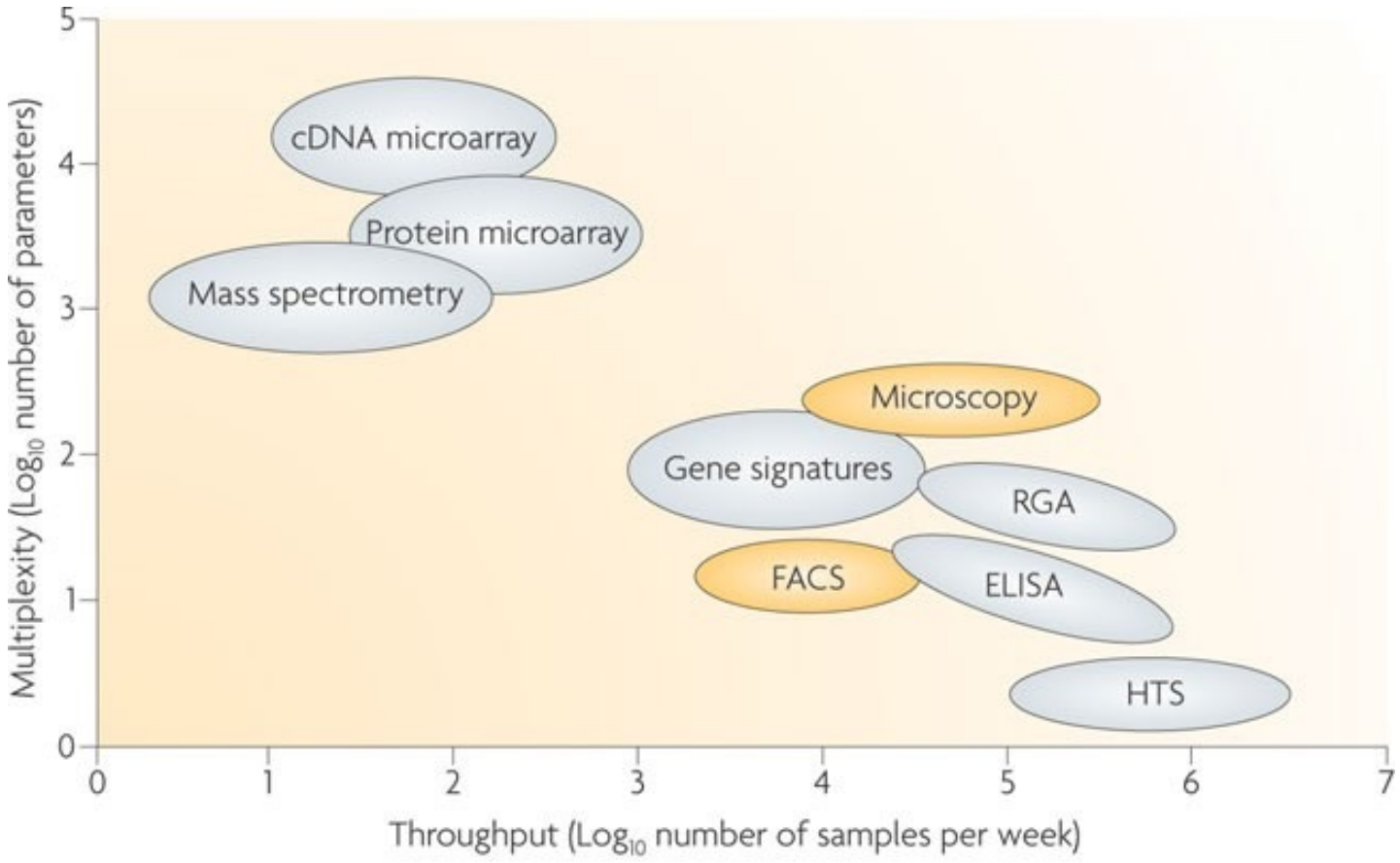
Swinney Clin Pharm Ther 2011

Challenges in choice of phenotypic assay: predictive validity



Moffat et al. Nat Rev Drug Discovery 2017

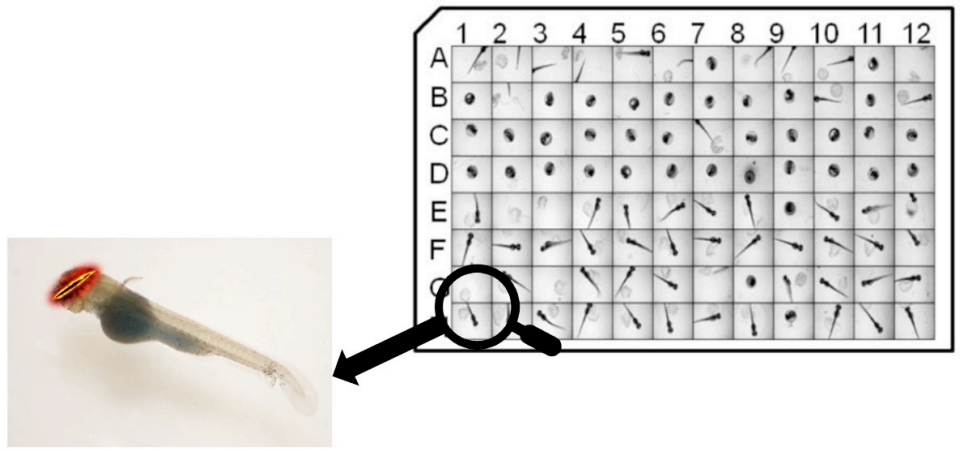
Choices of phenotypic assays



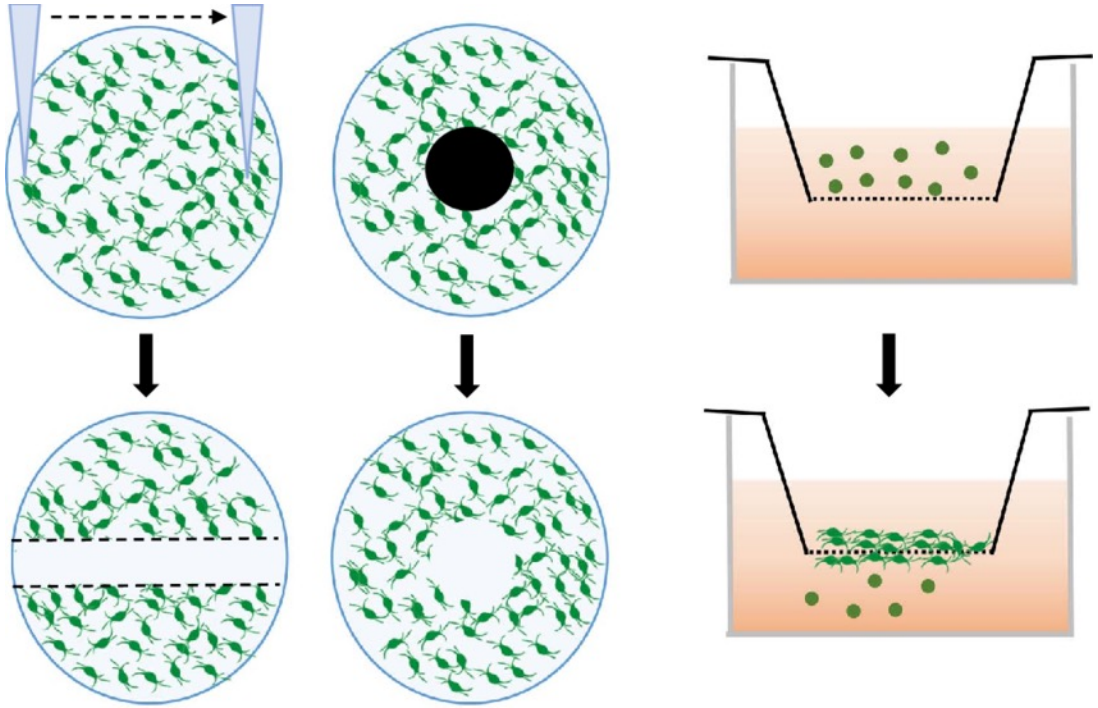
Nature Reviews | Drug Discovery

Feng et al., NRDD

Some phenotypic screening are specific to the goal of the drug screening campaign

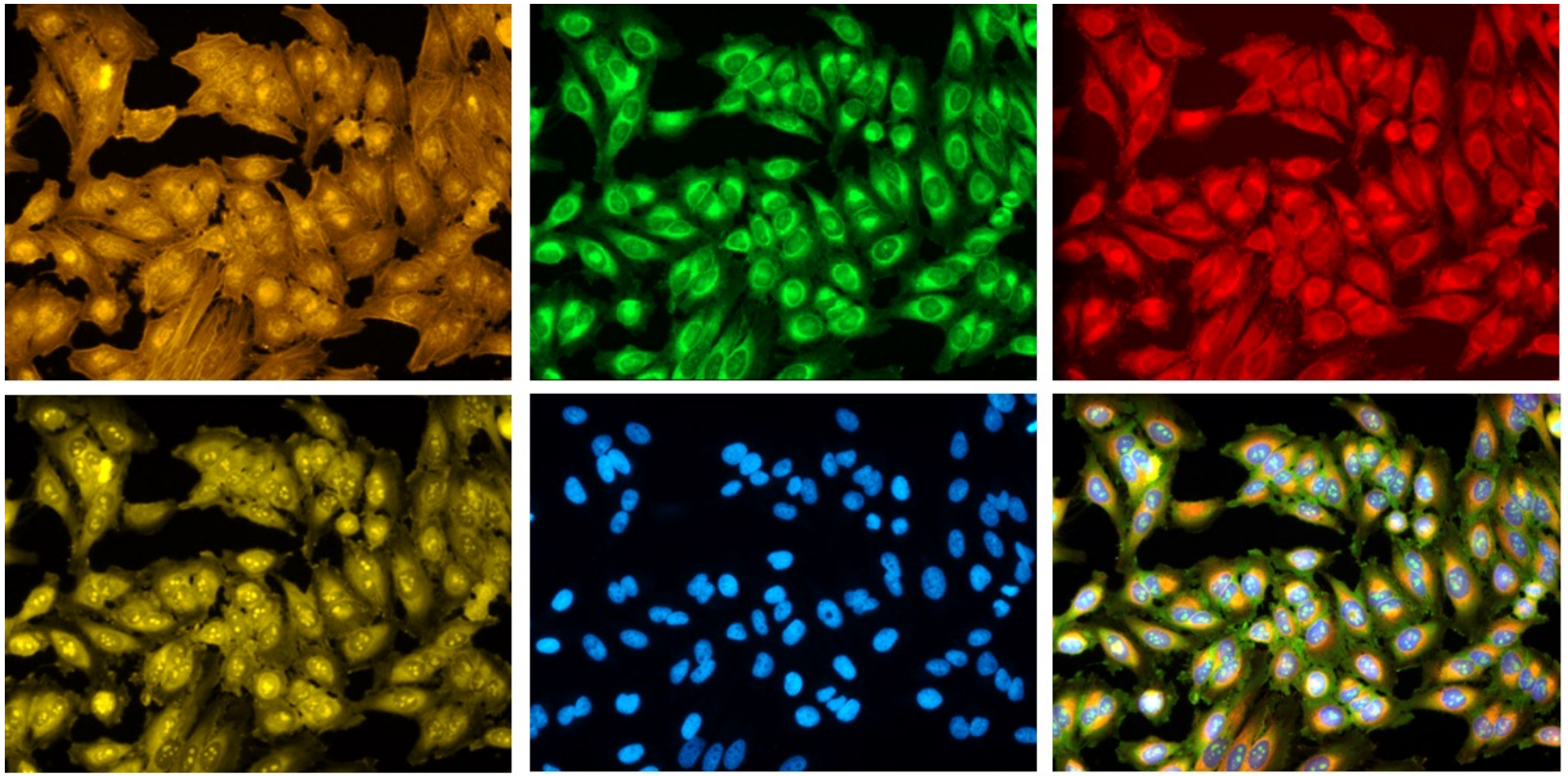


Effect of a drug on brain development, using zebrafish embryo model



Low-throughput wound healing assays

One type of screening: Cell painting microscopy: organelle/cell structure dyes



5 individual dyes that stain cell structures found in most cells

Eg: nuclei (blue)
Mitochondria (red)

Plus overlay (panel 6)

Images: Wikipedia

High Content Microscopy

Essays in Biochemistry (2019) EBC20180044
https://doi.org/10.1042/EBC20180044

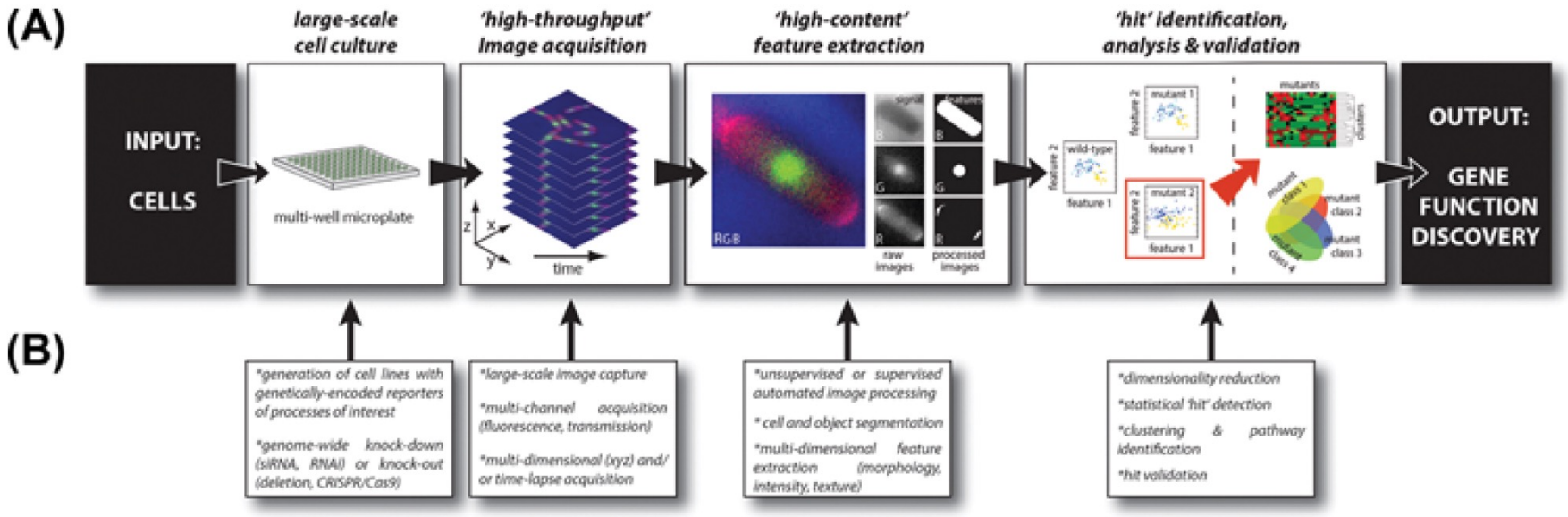


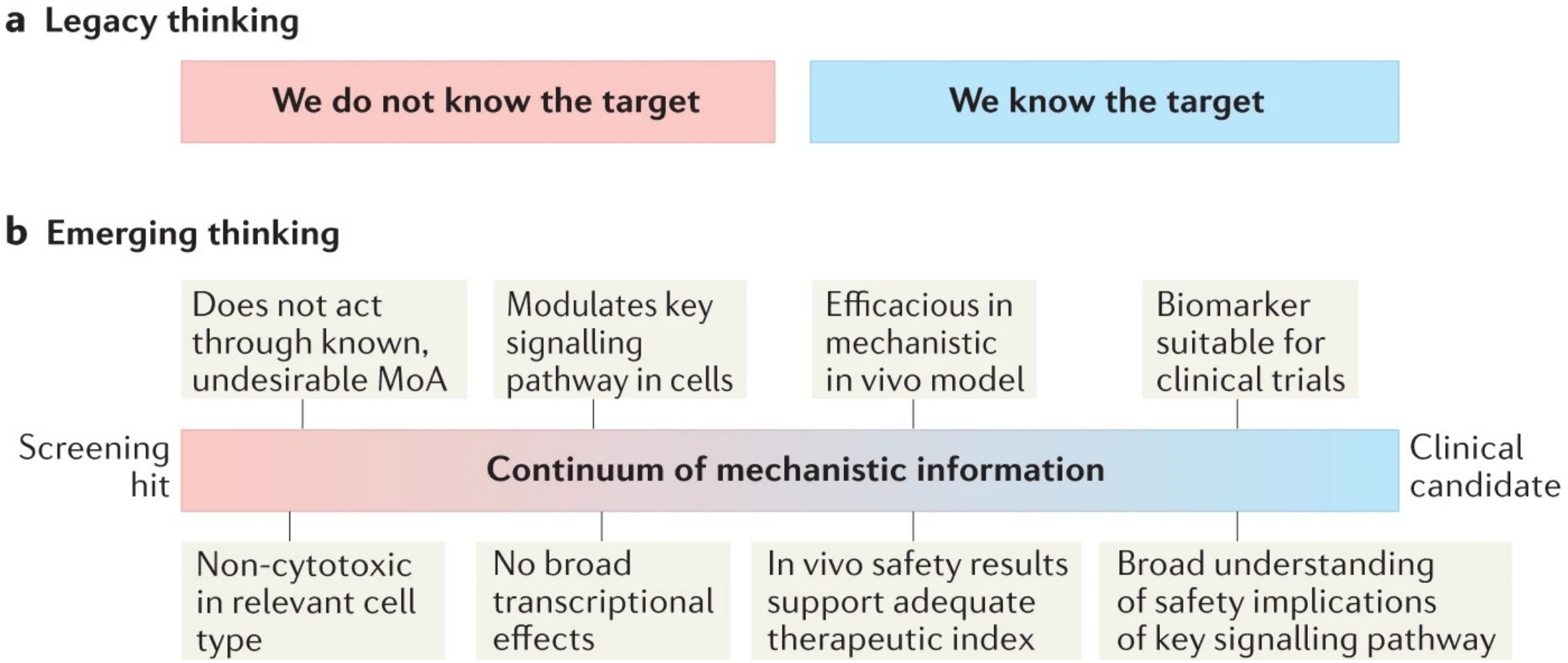
Figure 1. The principle of high-throughput/high-content microscopy screens to identify and study genes' functions at scale

(**A**) Typical high-throughput/high-content microscopy pipeline, where the inputs are cells and cell images, and the output is high-dimensional, single-cell feature data. (**B**) Implementation steps involved in the context of a single perturbation (KD/KO) genome-wide phenotypic screen, allowing to systematically discover gene functions at scale.

Target deconvolution

- FDA – MoA not required for drug approval, but “nice to have”
- With knowledge of the protein target we can:
  - Optimize selectivity
    - Improve binding affinity to our chosen target relative to other off-targets
  - Reduce toxicity
    - Avoid binding to proteins with known roles in toxicity

Improve our success for drug development by including mechanistic information (how does the drug “work”)



Moffat et al. Nat Rev Drug Discovery 2017

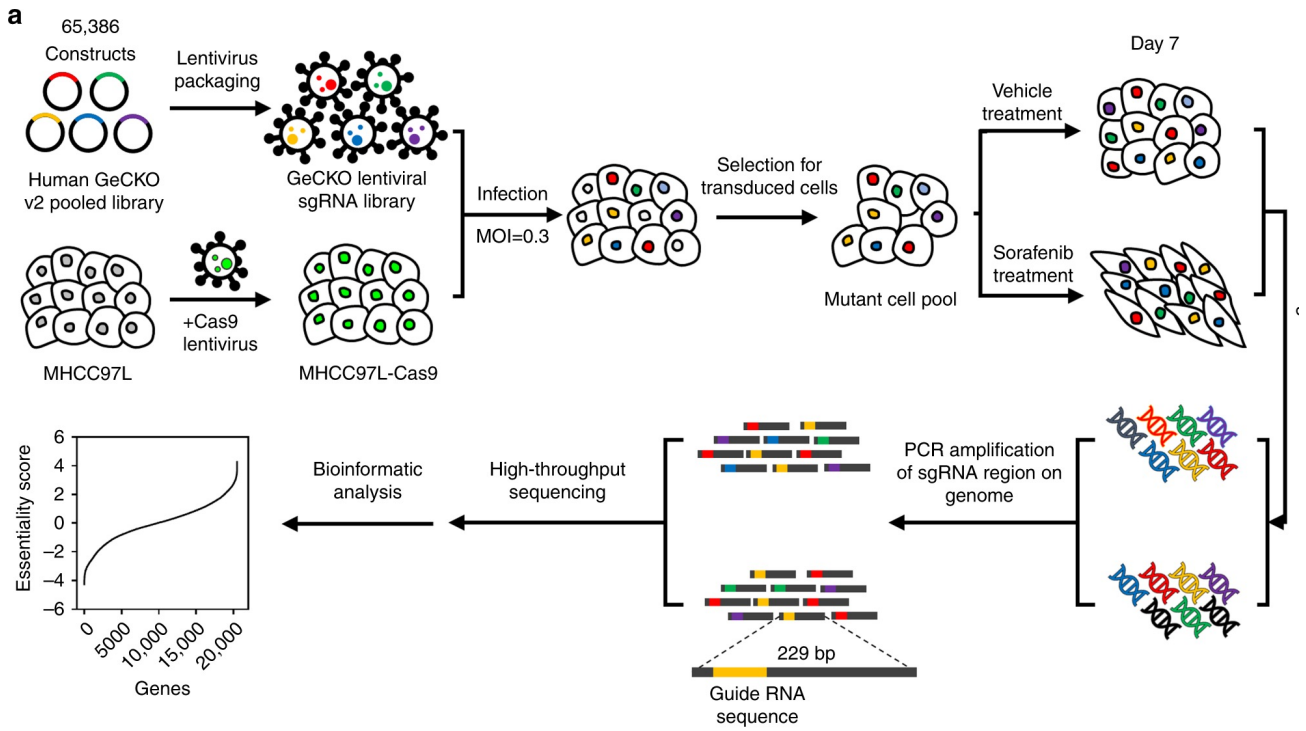
Genetic screens to uncover mechanistic information about pathways involved

- In the context of an intact cell, inactivate or activate a single gene Observe the phenotype that results from this intervention

- Can be conducted for all genes, one at a time, especially using inactivation.

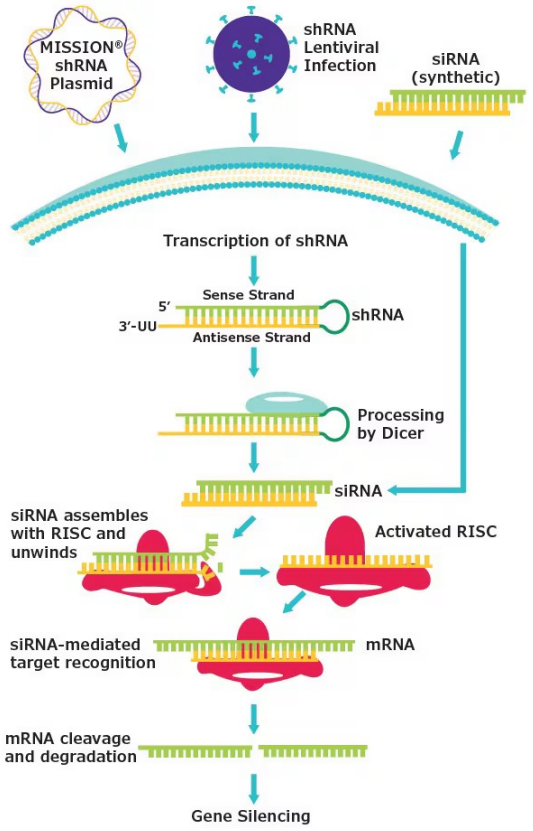
Interventions:
Genome-wide study of single-gene perturbations

- Create a cell population where at the start, each cell has a single gene inactivated. DNA tag on each cell.
- Allow these cells to compete over time → what is the resultant phenotype?
- Back-correlate the phenotypic changes to the gene involved

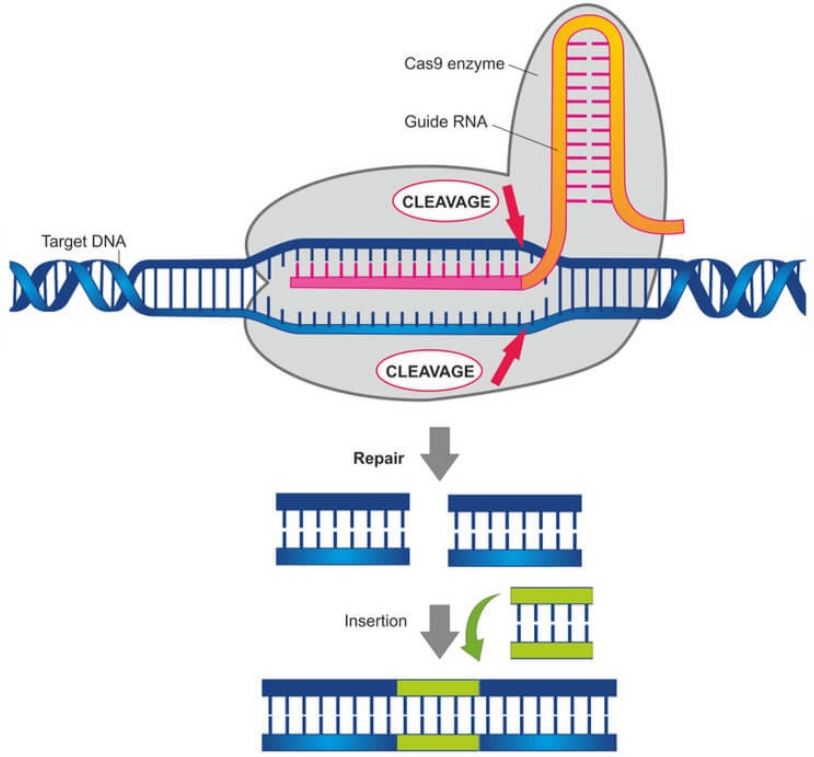


Disruptors of Gene expression

shRNA

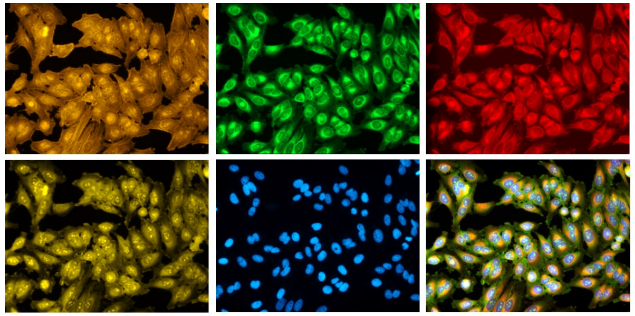


CRISPR

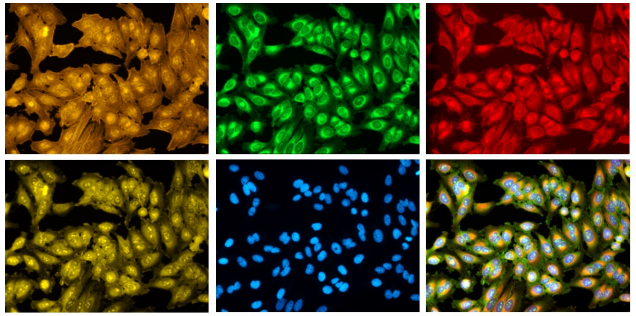


Combining phenotypic screening and genome-wide CRISPR screening

Perturb cells with small molecules



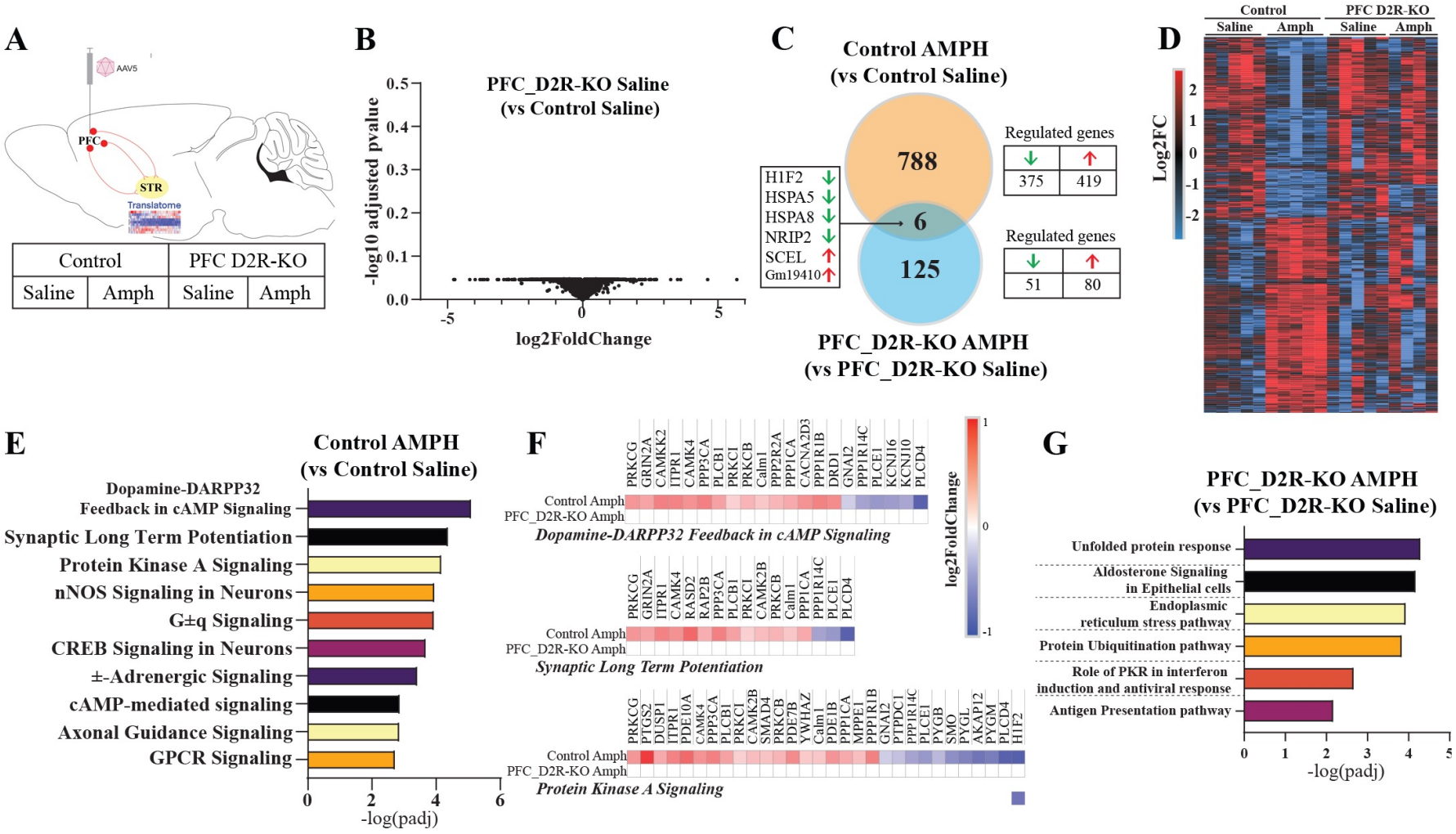
Perturb cells with gene editing



Compare embeddings to uncover similarities and attribute mechanisms to drugs

e.g. RxRx3, in colab #2 DR woith small molecule and CRISPR Screen

CRISPR + Drug

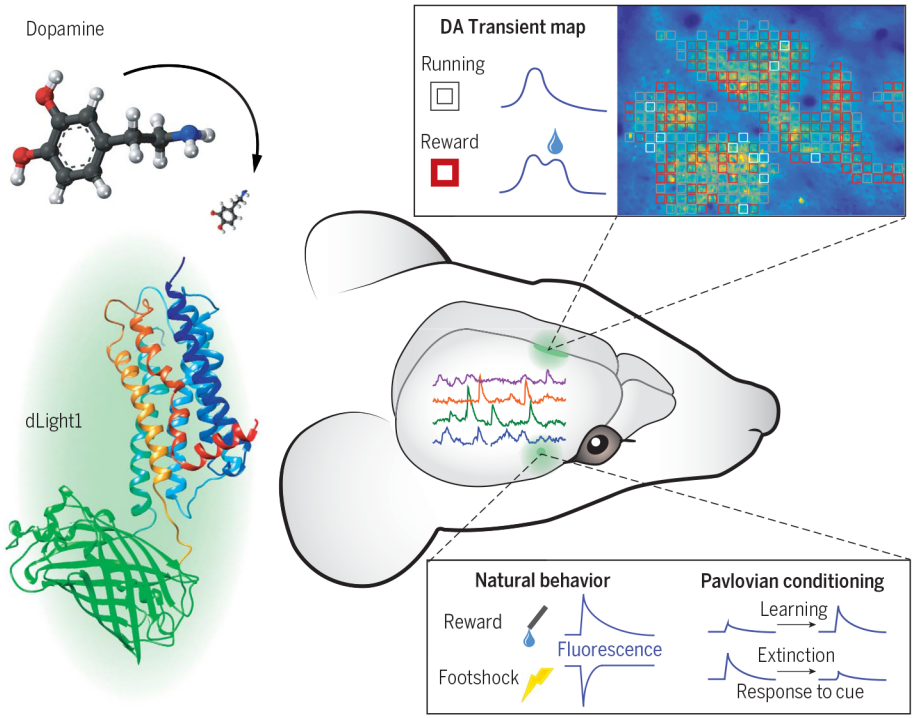


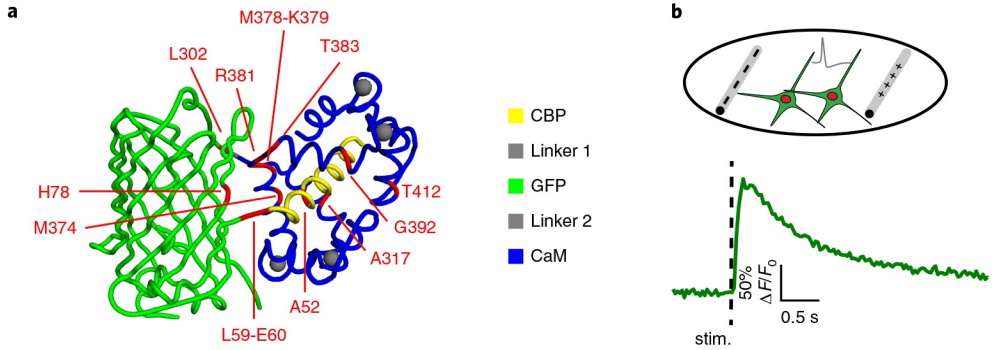
Quintana et al., unpublished

Monitoring specific cell responses in real time
Part 1 recombinant protein sensors

We can use light to detect dynamic variations of:

Calcium Levels
Specific receptor activation
Second messenger levels





Patriarchi et al., 2018 Science

Monitoring specific cell responses in real time
Part 2 Small aptamer sensors

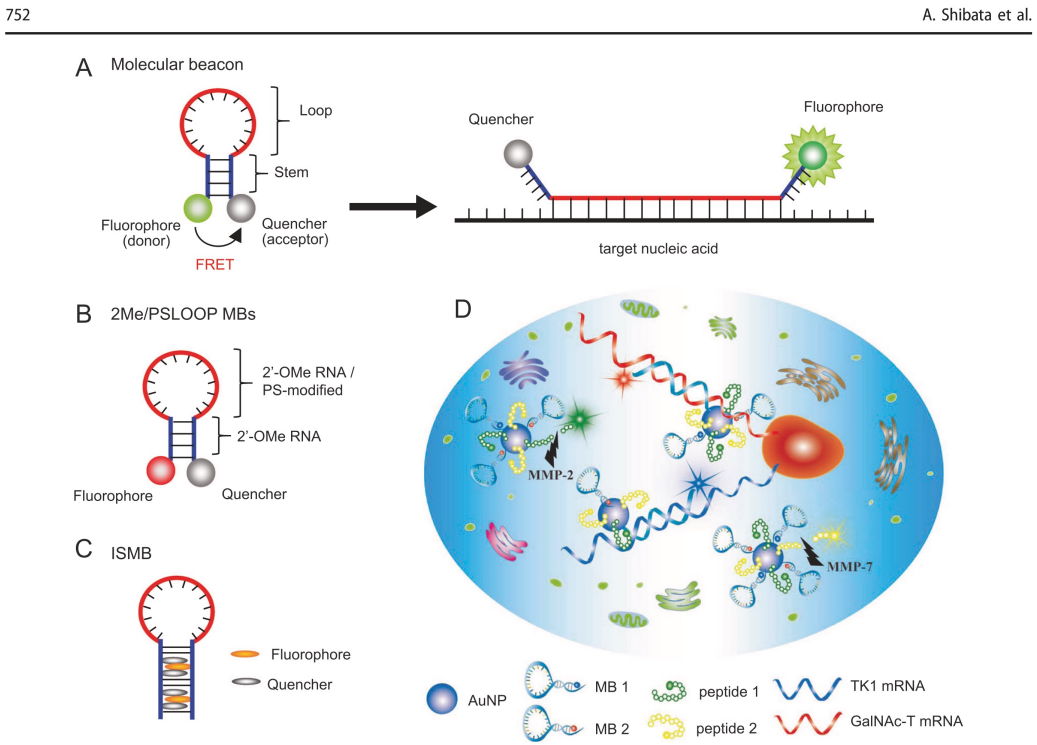


Fig. 1 **A** Structure and nucleic acid detection mechanism of MBs. Structures of (**B**) 2Me/PSLOOP MBs [20] and (**C**) ISMB [36]. **D** Illustration of the nanoprobe based on AuNPs for the detection and imaging of intracellular mRNAs and MMPs. The 5′-ends of MB1 and MB2 are labeled with Alexa Fluor 405 and Cy5, respectively. Peptide-1 is targeted to MMP-2, which is modified with FITC. Peptide-2 is targeted to MMP-2, which is modified with rhodamine B. When MB binds the target mRNA, the structure of MB is opened, and this "turns on" the fluorescence. In the presence of MMP, the peptide is specifically cleaved by MMP, which separates the fluorophore (FITC or rhodamine B) from AuNPs and generates a fluorescence signal. Reprinted with permission [37]. Copyright 2015 John Wiley and Sons

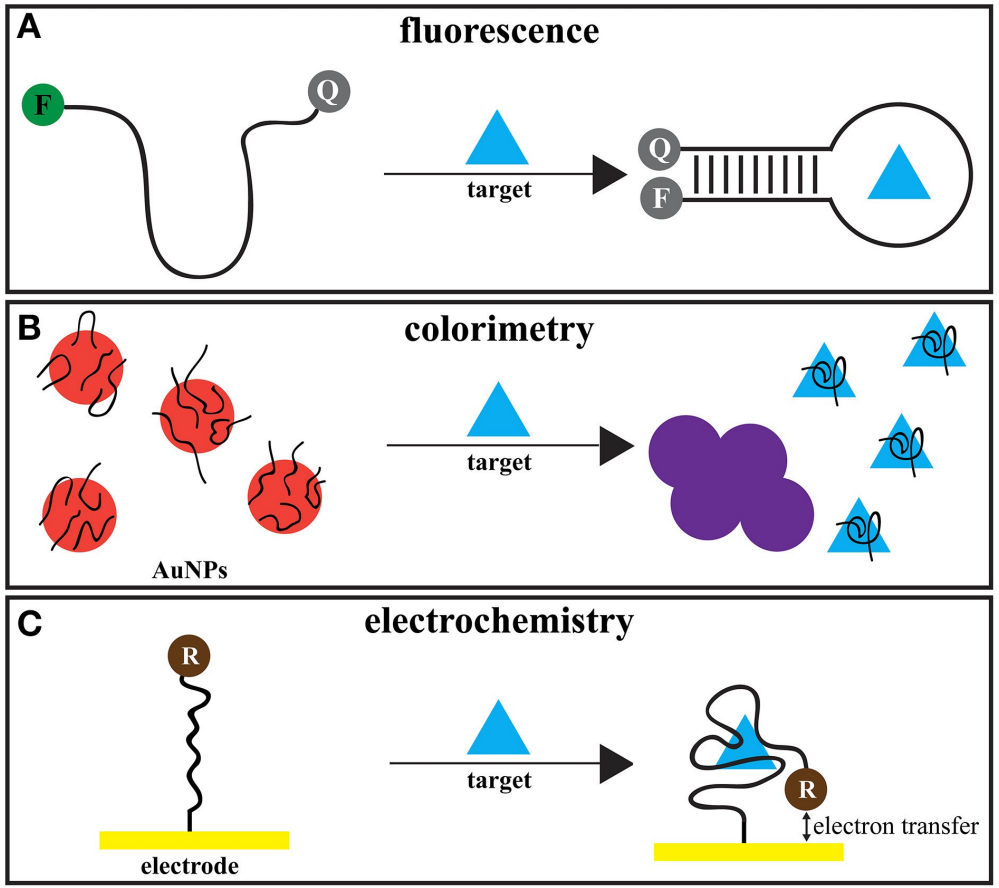
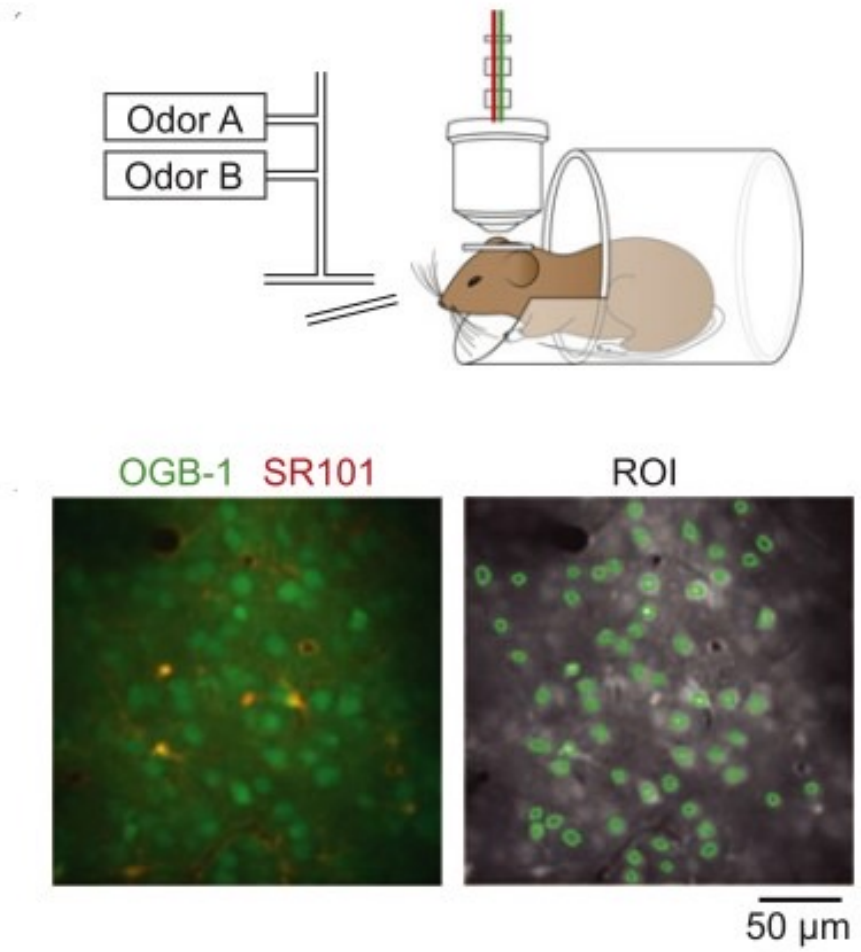
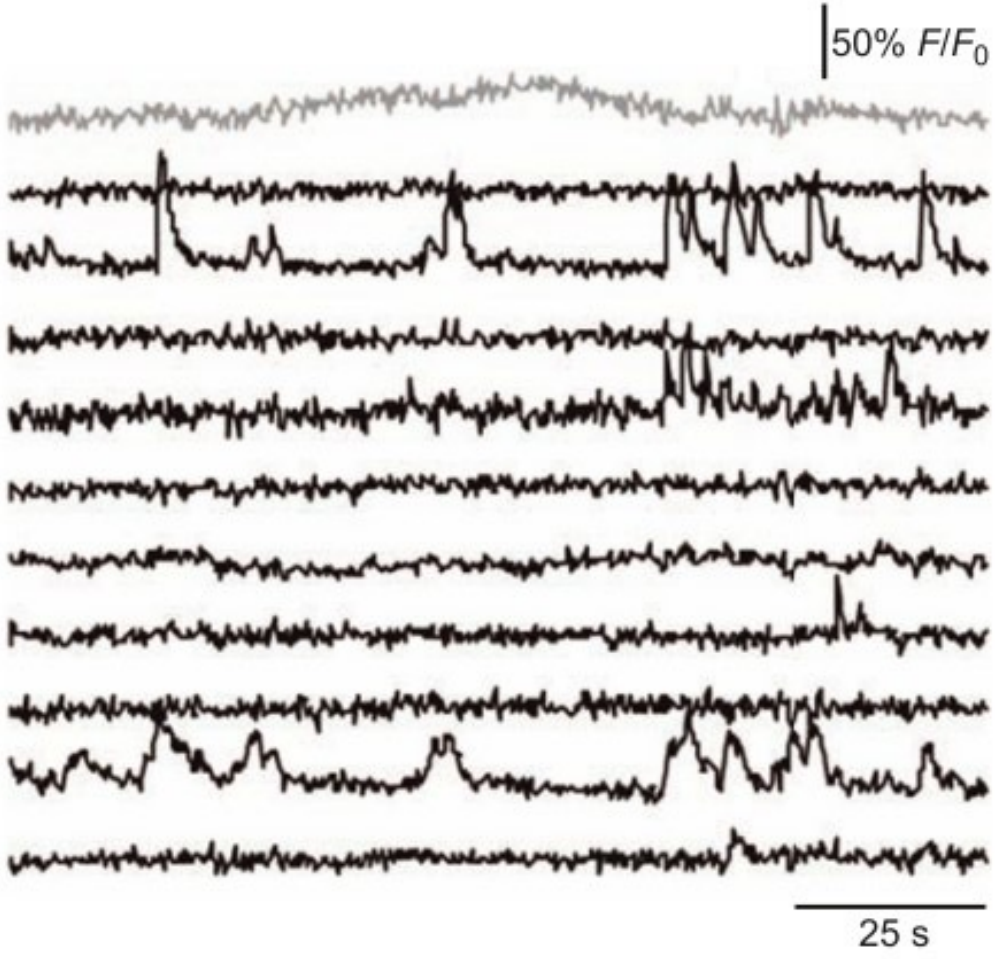


FIGURE1 |Smallmoleculeaptasensortypes. Depictedisanexample forasensorforthethreemostwidelyusedtypesforsmallmoleculetargets.F, fluorophore.Q,quencher.AuNPs,goldnanoparticles.R,redox probe. **(A)** The aptamerislabeledwithafluorophoreandanappropriatequencher.Upon bindingtothetarget,theconformationalchangeoftheaptamerbrings fluorophoreandquencherintoclosecontact,therebyquenchingthe fluorescence. **(B)** Theaptamerisunspecificallyabsorbedontothesurface ofAuNPsandthuspreventstheiraggregation.Uponbindingto thetarget, theAuNPsaggregate.Thisleadstoavisiblecolorchangefrom redtoblue. **(C)** Theaptamerisimmobilizedontoanelectrodeandlabeledwith aredox probe.Theconformationalchangeupontargetbindingbrings theprobeclose enoughtotheelectrodetoallowelectrontransferandthus, an electrochemicalreadout.

Monitoring specific cell responses in real time
Part 3: Can generate complex signals





Types of artefacts

Plate and sample

Uneven seeding / cell clumping.

Sample preparation artefacts (e.g. dust, lint, precipitates, bubbles, debris ....

Fixation/staining artifacts

Edge effects altered cell number or phenotype in outer wells.

Cellular stress from handling (e.g. delays in media changes, temperature shock).

Optical and imaging

Autofluorescence

Bleed-through / spectral cross-talk

Photobleaching and phototoxicity

Focus errors

Shading / vignetting and illumination non-uniformity

Camera noise and hot pixels

Compound related

Intrinsically fluorescent compounds

Quenchers or absorbers

Cytotoxic compounds

Aggregates/precipitates of compound.

Segmentation and analysis

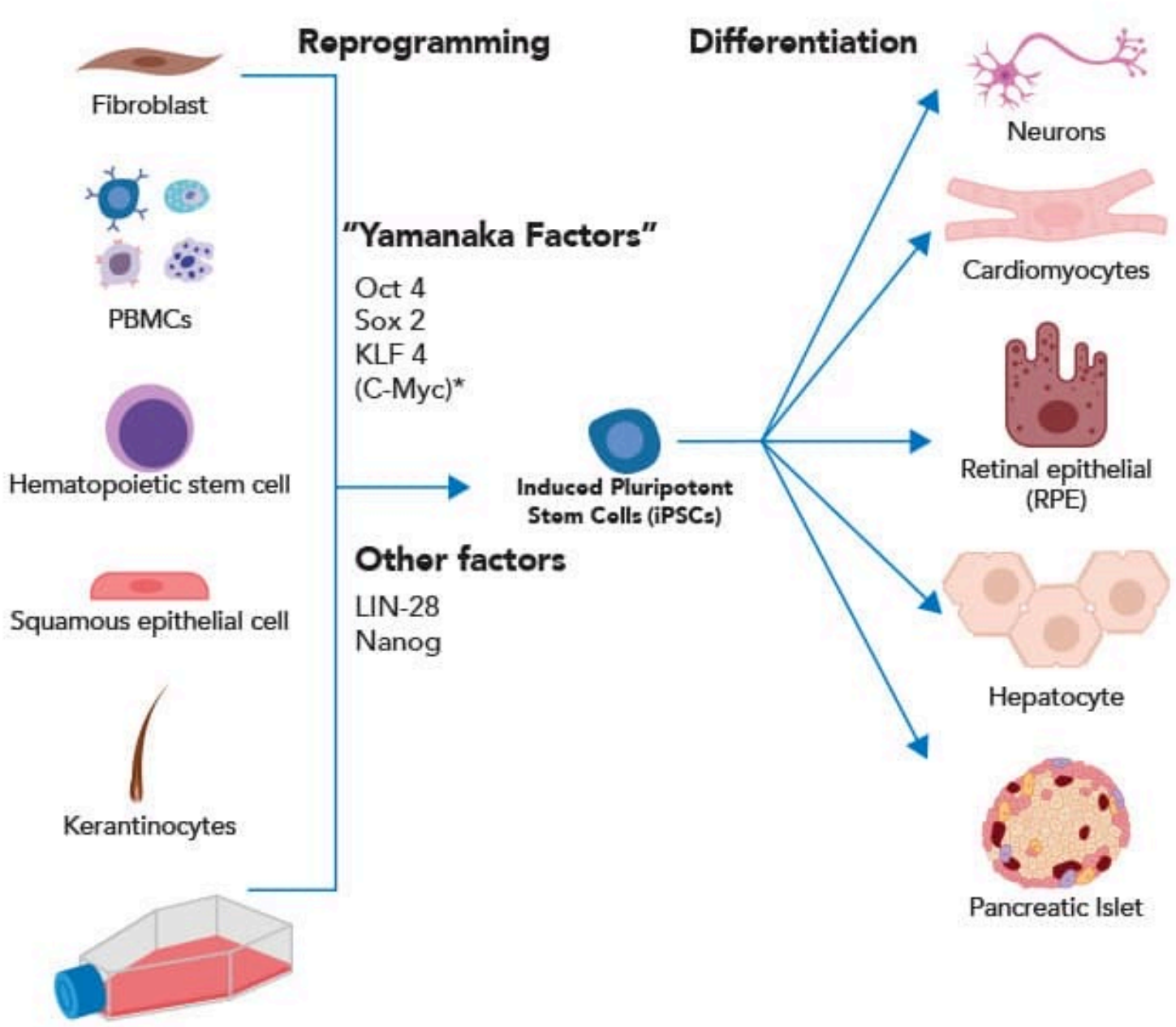
Over- or under-segmentation (e.g. merged nuclei in dense culture).

Halo and ring artifacts around nuclei or cells.

Plate/batch effects.

Outlier wells from dispensing errors.

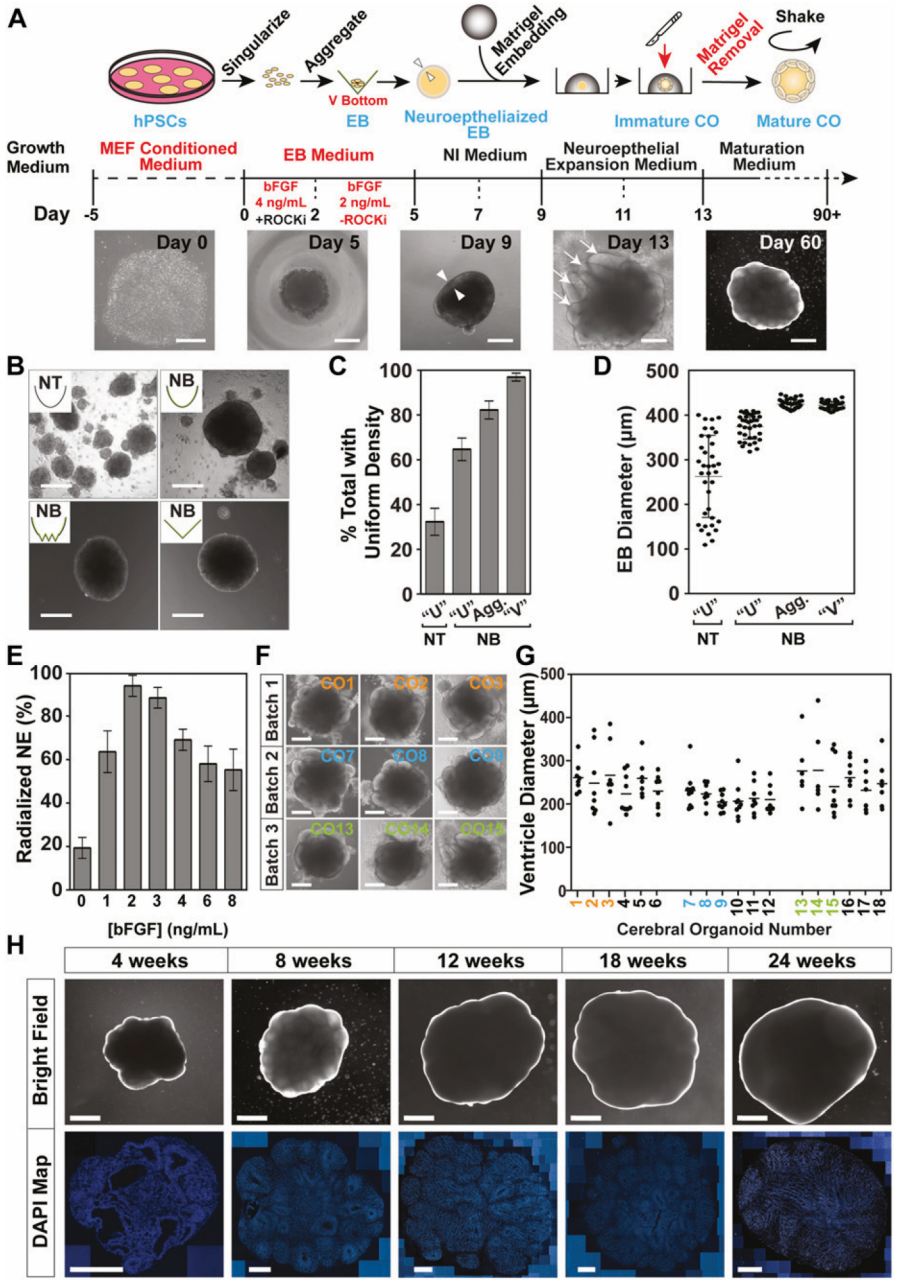
More complex in vitro cell systems



Replicates human genomic landscape

Expensive and time consuming

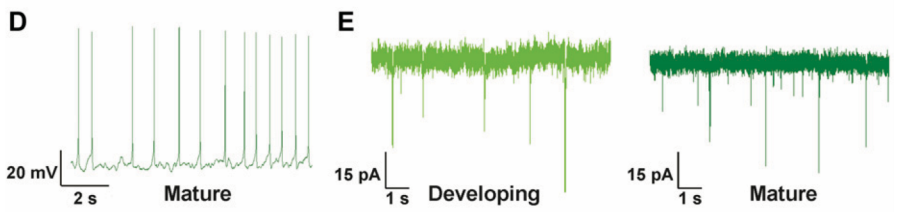
Novus Biological



Multicellular Organoids

Multi-cell type 3D systems

Even more time consuming
Developmental issues
Incomplete or inaccurate representation of real organs



Sivitilli et al., 2020

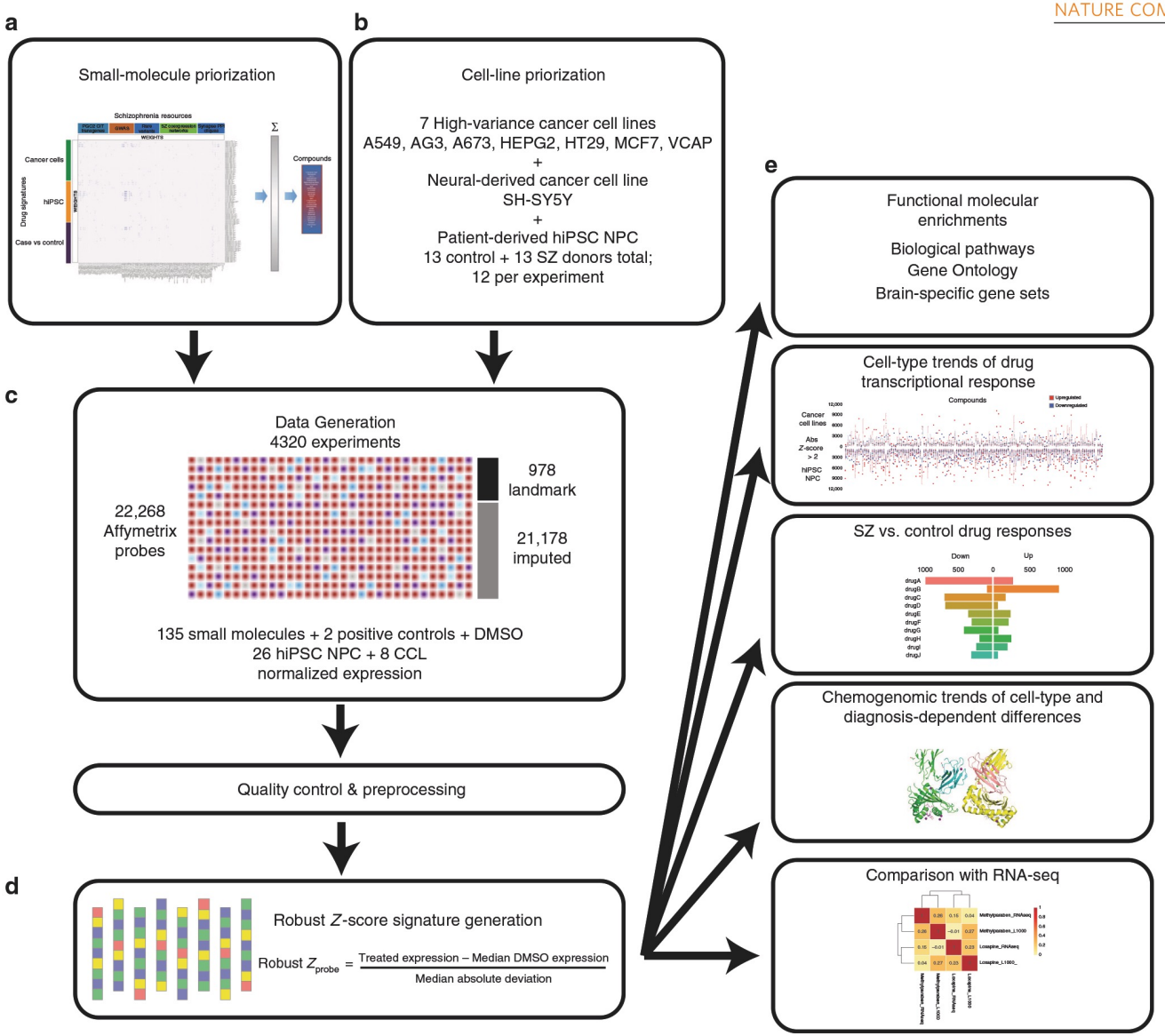
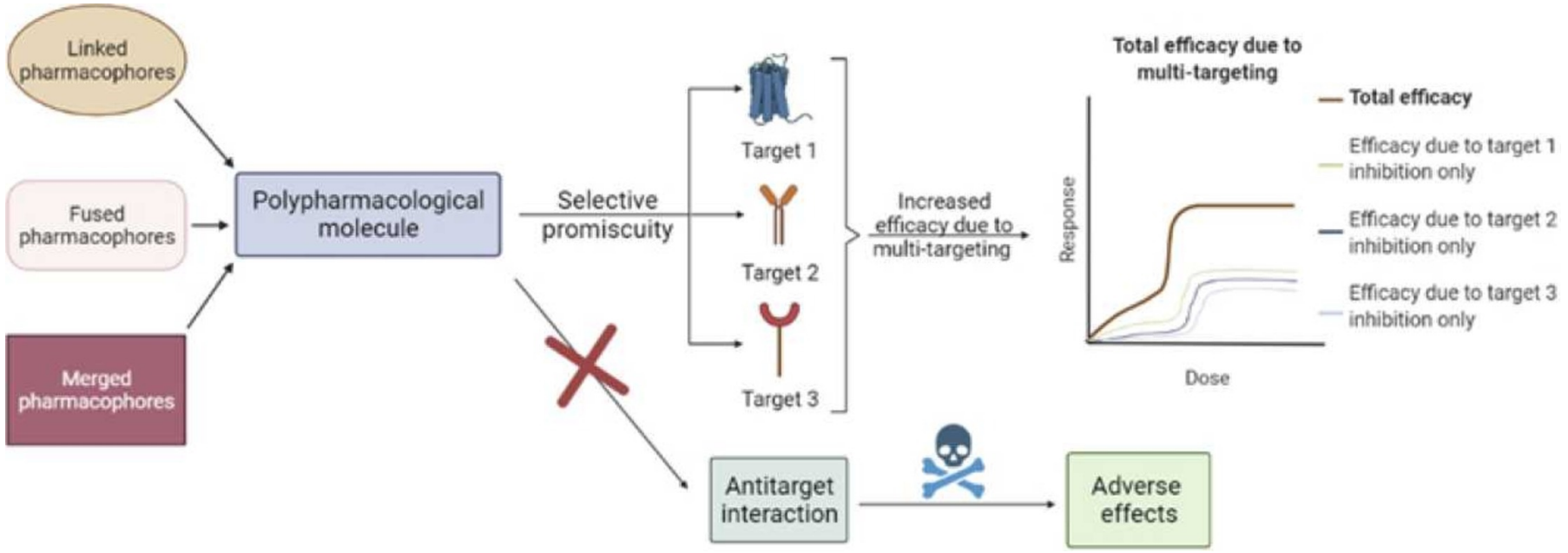


Fig. 1 Summary schematic of experimental and analytic pipeline. **a** One hundred and thirty-five drugs were prioritized for screening based on connectivity with diverse aspects of SZ-related biology. **b** Cells used for screening comprised seven CCLs (A549, AGS, A673, HEPG2, HT29, MCF7, and VCAP) that were prioritized using LINCs datasets, one additional neural CCL (SH-SY5Y) and hiPSC NPCs from 13 SZ and 13 control individuals (12 each per drug). **c** Data were generated using the L1000 platform to yield 6650 drug-perturbation transcriptomic profiles (135 drugs tested across 26 hiPSC NPCs and 8 CCLs). After data quality control, normalized expression was converted to **d** robust *Z*-scores based on comparison with isogenic DMSO-treated experiments, and used as inputs for **e** functional molecular enrichments, cell-type-specific (hiPSC NPCs and CCLs) trends, diagnosis-dependent (SZ and control) responses, and chemogenomics analyses. Global transcriptomic responses of two drugs were tested across hiPSC NPCs from three SZ and three control individuals by RNA-seq, as part of a validation of the L1000 results

What separates the drug from the poison?

- Major mechanisms of toxicity…
- Off-target
- On-target exacerbation
- Acute toxicity
- Chronic effect
- Dose

Drugs with multiple drug targets



Kabir and Muth, Pharmacol Res 2022

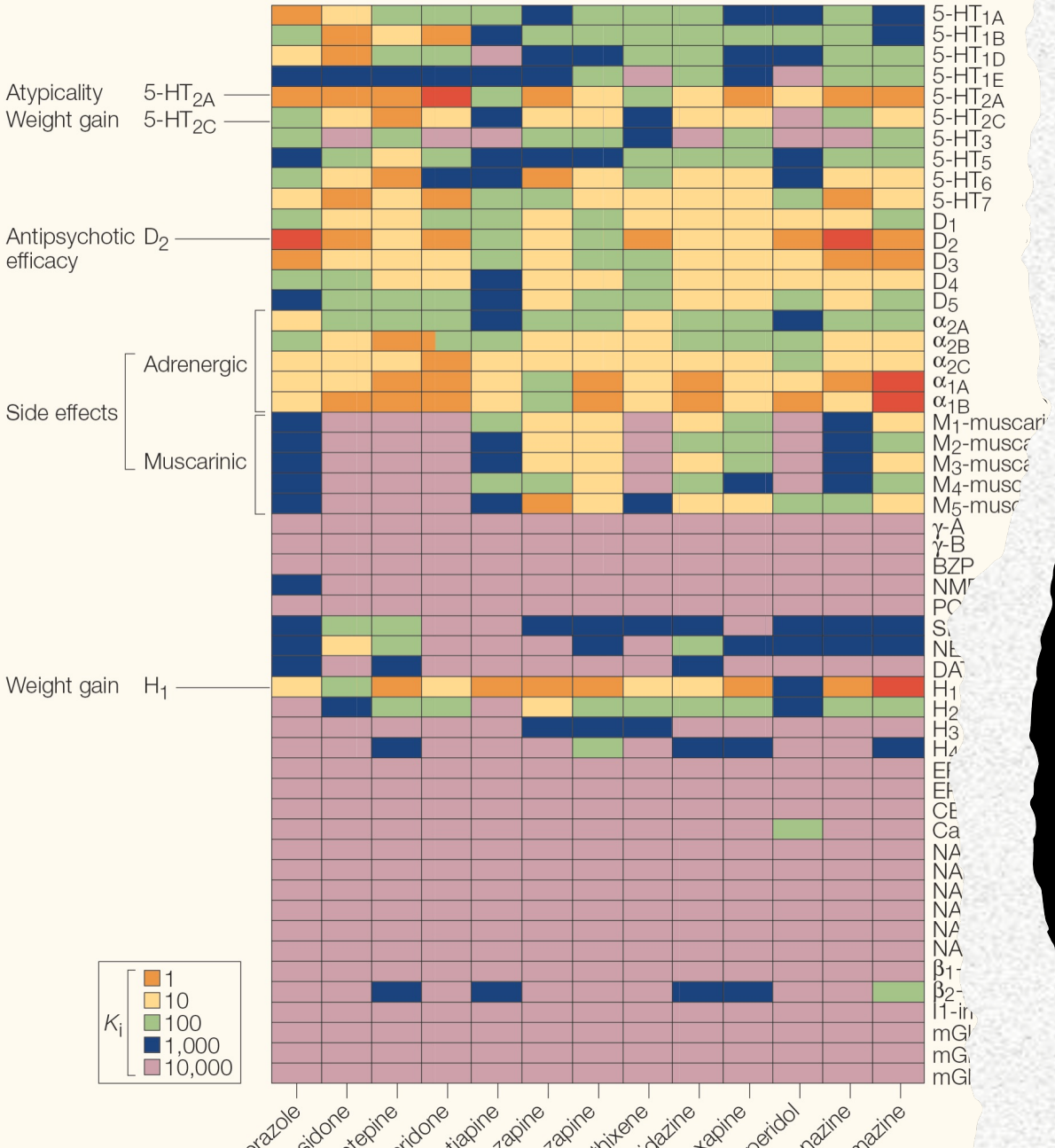
Antipsychotics as an example

Table 1 Receptor binding profiles and plasma half-life ($t_{1\backslash2}$) of antipsychotic drugs

Drug class	Second-generation antipsychotics								First-generation antipsychotics			
Receptor	**AMI**	**ARI**	**ASE**	**CLO**	**OLA**	**PALI**	**RIS**	**QUE**	**SER**	**ZIP**	**HAL**	**PER**
D_2	1.3[b]	0.66[a,b]	1.3[b]	210	20	2.8	3.77	770	2.7	2.6	2.6	1.4[b]
5-HT_{1A}	>10,000[c]	5.5[a,b]	2.5[b]	160	610	480	190	300	2,200	1.9[a,b]	1,800	421
5-HT_{2A}	2,000[c]	8.7[b]	0.06[b]	2.59	1.5	1.2	0.15	31	0.14	0.12	61	5[b]
5-HT_{2C}	>10,000[c]	22[b]	0.03[b]	4.8	4.1	48	32	3,500	6.0	0.9	4,700	132[b]
α_1	7,100[c]	26[b]	1.2[b]	6.8	44	10	2.7	8.1	3.9	2.6	17	10
α_2	1,600[c]	74[a]	1.2[b]	158	280	80	8	80	190	154	600	500
H_1	>10,000[d]	30[b]	1.0[b]	3.1	0.08	3.4	5.2	19	440	4.6	260	8
M_1	N/A	6,780[b]	8128[b]	1.4[b]	2.5[b]	>10,000[b]	>10,000[b]	120[b]	5,000	300[b]	>10,000[b]	1,500
M_2	N/A	3,510[b]	4.5[b]	204[b]	622[b]	>10,000[b]	>10,000[b]	630[b]	N/A	>3,000[b]	>10,000[b]	N/A
M_3	N/A	4,680[b]	4.67[b]	109[b]	126[b]	>10,000[b]	>10,000[b]	1,320[b]	2,692[b]	>1,300[b]	>10,000[b]	1,848[b]
M_4	N/A	1,520[b]	5.09[b]	27[b]	350[b]	>10,000[b]	>10,000[b]	660[b]	N/A	>1,600[b]	>10,000[b]	N/A

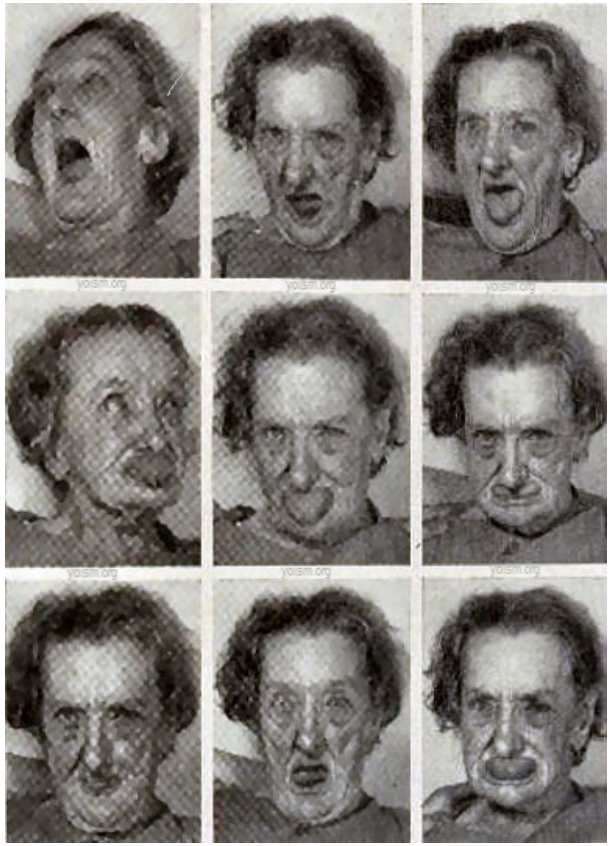
Notes: Adapted with permission from Correll CU, From receptor pharmacology to improved outcomes: individualizing the selection, dosing, and switching of antipsychotics, *Eur Psychiatry,* 2010;25(Suppl 2):S12–S21, Copyright © 2010, Elsevier Masson SAS. All rights reserved.[79] Data represented as the equilibrium constant (Ki; nM), ie, nanomolar amount of the antipsychotic needed to block 50% of the receptors in vitro. Therefore, a lower number denotes stronger receptor affinity and binding. [a]Partial agonism. [b]Data from cloned human brain receptors. [c]Data extracted from rat. [d]Data extracted from guinea pig.

Abbreviations: AMI, amisulpride; ARI, aripiprazole; ASE, asenapine; CLO, clozapine; HAL, haloperidol; OLA, olanzapine; PALI, paliperidone; PER, perphenazine; QUE, quetiapine; RIS, risperidone; SER, sertindole; ZIP, ziprasidone; N/A, not applicable.



Antipsychotics as an example

Tardive dyskinesia



TD occasionally occurs after long-term treatment with typical APS.

Symptoms persist even after APS is discontinued.

Patients have involuntary twitches of facial muscles predominantly, but also hands and legs.

Thought to reflect dopamine receptor hypersensitivity – is also seen with L-dopa treatment in PD

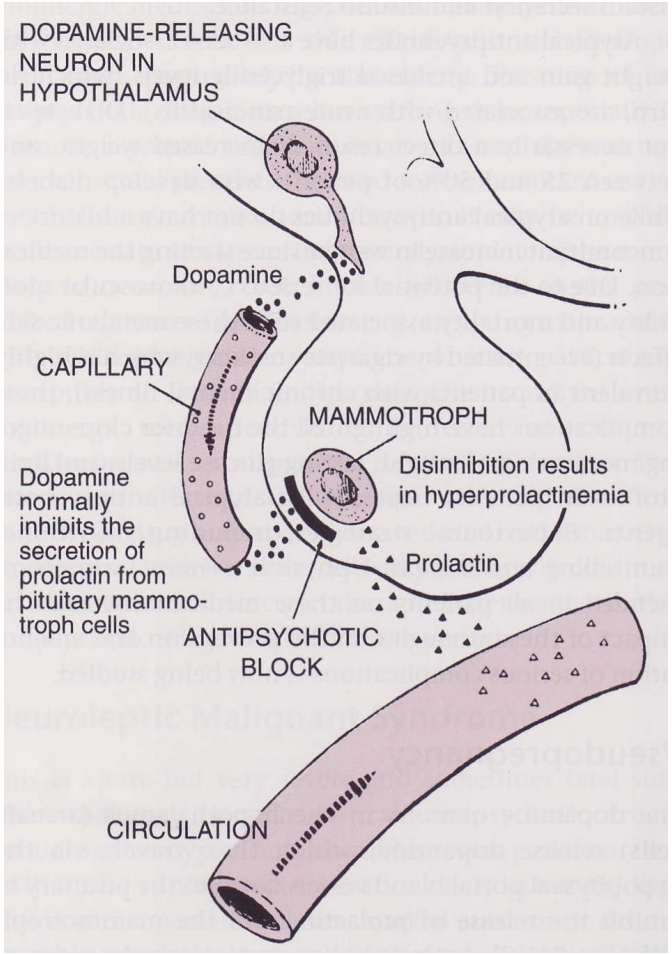
In both of these situations (PD and SZ), there are fluctuating states of high and low dopamine tone depending on drug PK.

Hyperprolactinemia – endocrine effects of APS

Dopamine neurons in the hypothalamus project to the pituitary and release dopamine onto mammotroph cells. This normally reduces the secretion of prolactin.

When D2 receptors are blocked, there is more prolactin that is secreted.

Prolactin secretion leads to production of milk & suppression of GnRH. This causes disruption of menstrual cycle in females and ED and infertility in males.



Expected Effects from a Drug's Blockade of Receptors

Receptor Type	Adverse Effects
Dopamine	Extrapyramidal symptoms, weight gain, endocrine effects, akathisia, tardive dyskinesia, increased prolactin, sexual or reproductive system dysfunction
Serotonin	Weight gain, diabetes, increased appetite
Histamine	Weight gain, diabetes, sedation
Muscarinic	Dry mouth, blurred vision, urinary retention, diabetes, memory problems, cognitive problems, tachycardia, hypertension
Adrenergic	Postural hypotension, dizziness, syncope

C. Correll. "Assessing and maximizing the safety and tolerability of antipsychotics used in the treatment of children and adolescents." *J Clin Psychiatry* 69, suppl. 4 (2008):26-36. Also see, C. Correll, "Antipsychotic use in children and adolescents." *J Am Acad Child Adolesc Psychiatry* 47 (2008):9-20.