

Engineering of Mammalian Cells - II

genetic circuits, optogenetics, cybergenetics, prosthetic gene networks, engineered cell



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Plant Energy Biology



Lecture outline

Synthetic tools for sophisticated control of cell activity, and their use and implementation in mammalian cells

- **Synthetic genetic circuits**

Artificial constructs for boolean logic that enable programmable genetic computation in mammalian cells

- **Optogenetics**

Light-based control of gene expression

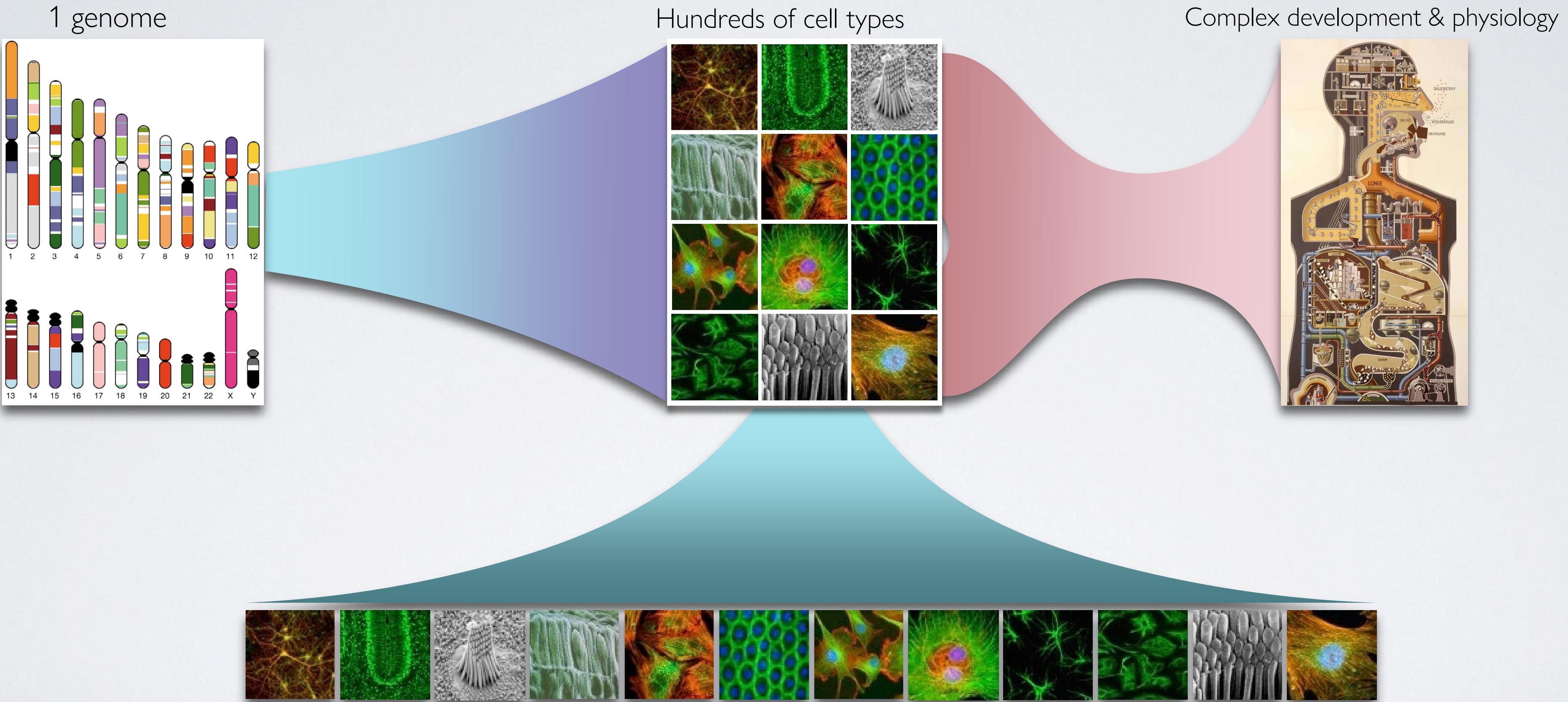
- **Cybergenetics**

Integrating computational control systems with engineered living systems

- **Prosthetic gene networks and cellular therapies**

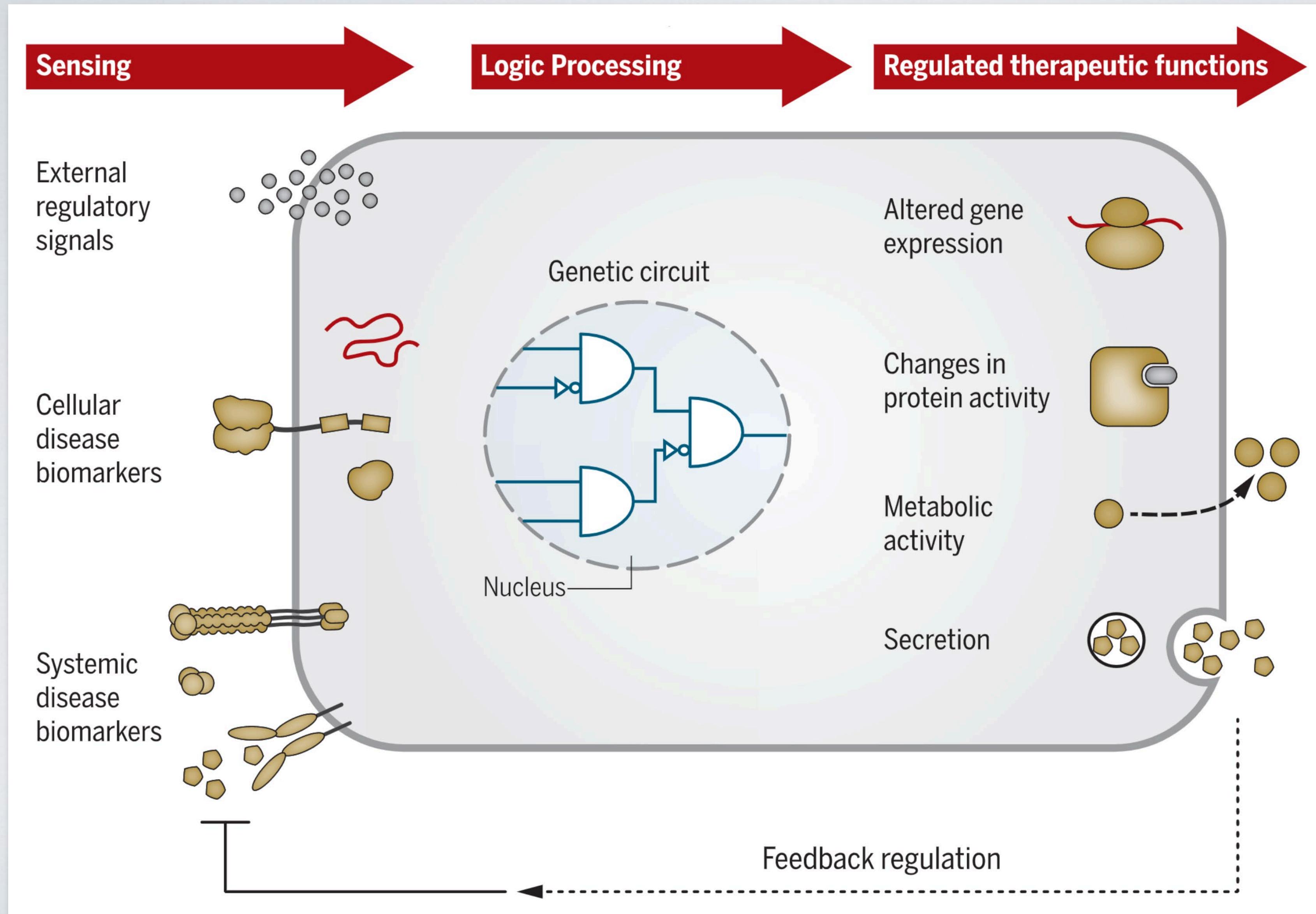
Engineering cells with sensing and responding circuits for advanced cellular therapeutics

Sophisticated manipulation of cellular programs to control functions



We need sophisticated tools to control cell activity

New therapeutics based on programmable gene and engineered cells



Addition of genetically encoded cellular functions

Regulation of dosage, timing, and localisation of therapeutic functions

May enable therapies that are safer and more effective than existing approaches (eg. duration, specificity)

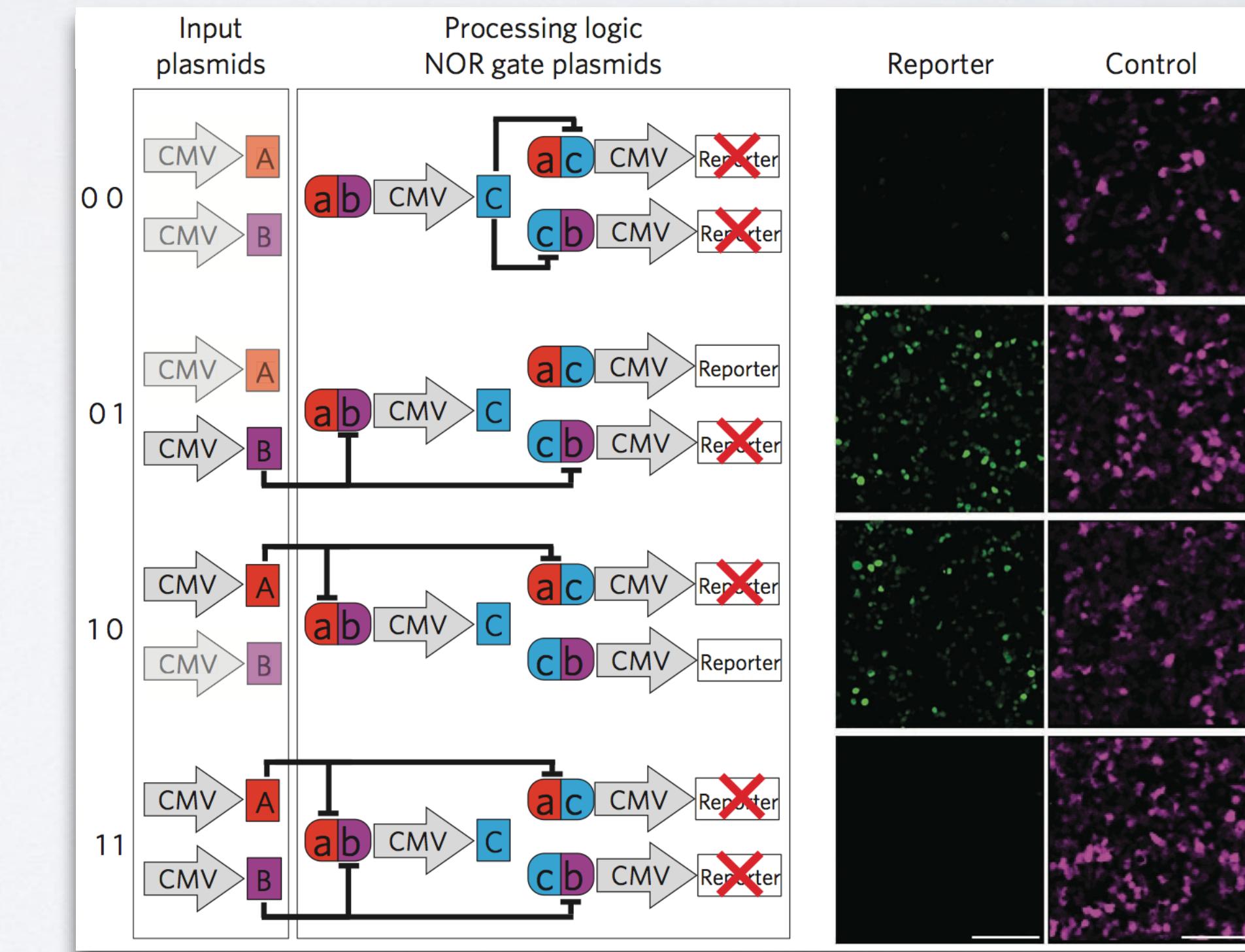
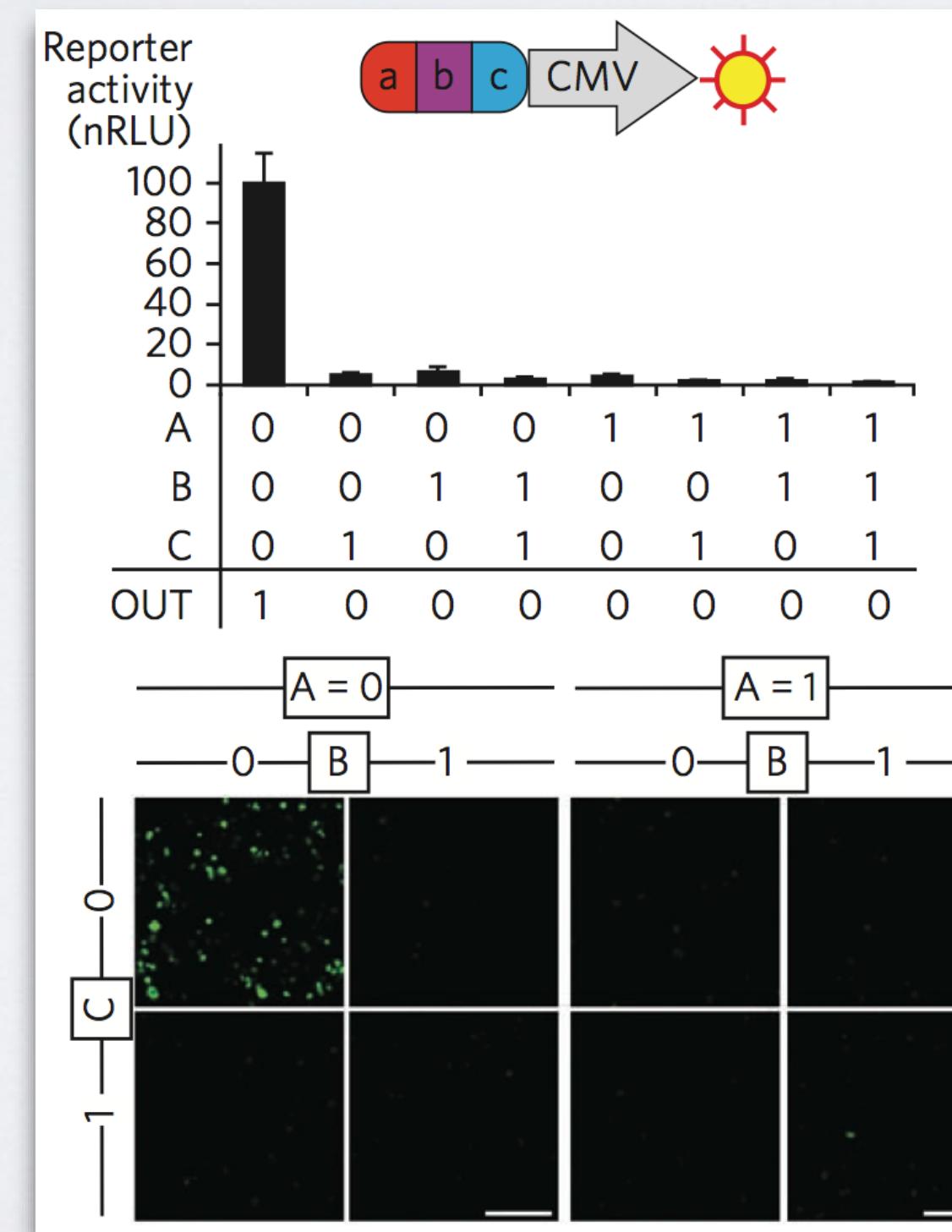
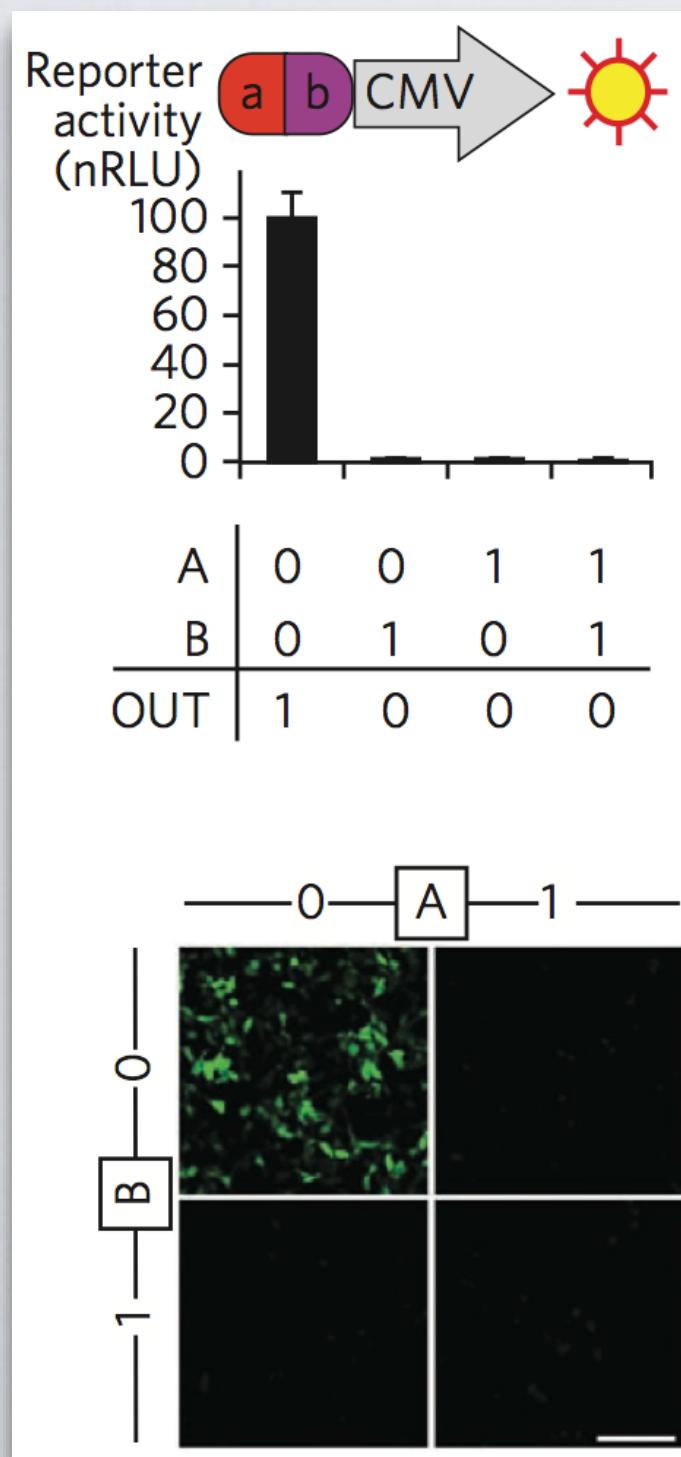
Mammalian synthetic gene logic circuits

Custom DBDs with transcriptional regulatory function now enables construction of logic circuits in mammalian cells

TALE-KRAB: TALE DBD linked to KRAB transcriptional repressor domain

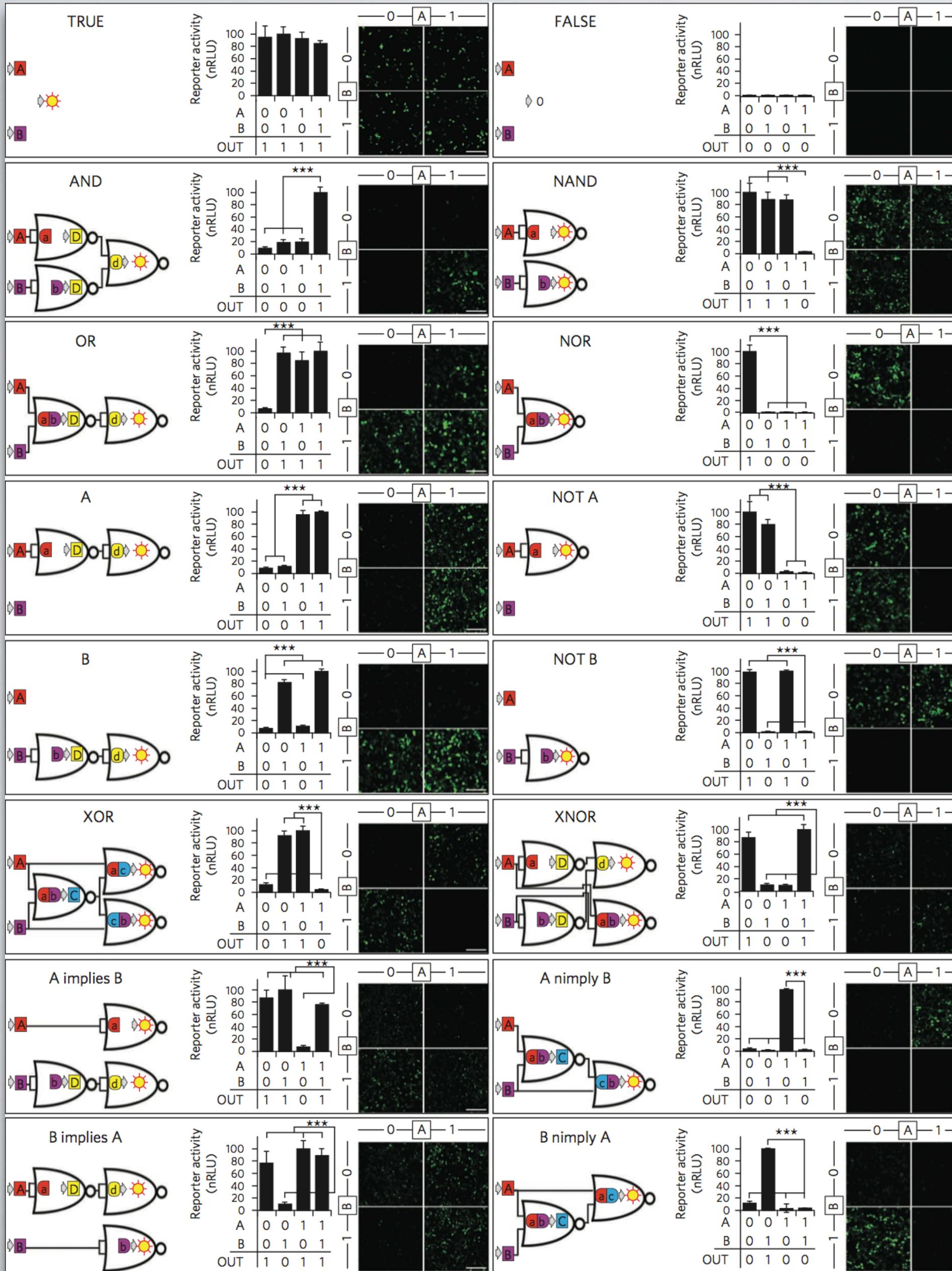
Cellular system: mammalian cells containing artificial gene construct comprised of different potential TALE-KRAB DNA binding sites (**a**, **b**, **c**) upstream of a promoter, linked to a gene encoding a fluorescent protein

Adding different combinations of TALE-KRAB constructs (input plasmids), each of which binds a different reporter domain (**a**, **b**, **c**), can affect the output of the synthetic NOR gate (**a** nor **b**) or XOR gate



INPUT	OUTPUT	
A	B	A XOR B
0	0	0
0	1	1
1	0	1
1	1	0

Mammalian synthetic gene logic circuits



Implementation of all 16 two-input Boolean logic functions constructed from combinations of designed TALE repressor-based NOR gates

Gaber et al., Designable DNA-binding domains enable construction of logic circuits in mammalian cells, Nat. Chem. Biol., 1–7 (2014)

Inputs and outputs could be linked to cellular processes

Cells containing artificial gene logic circuits could be used as biosensors, or could integrate complex environmental signals to react in programmed ways only under particular combinations of exposures

e.g. crop plant that activates particular stress responses when perceiving combinations of threats (drought, salinity)

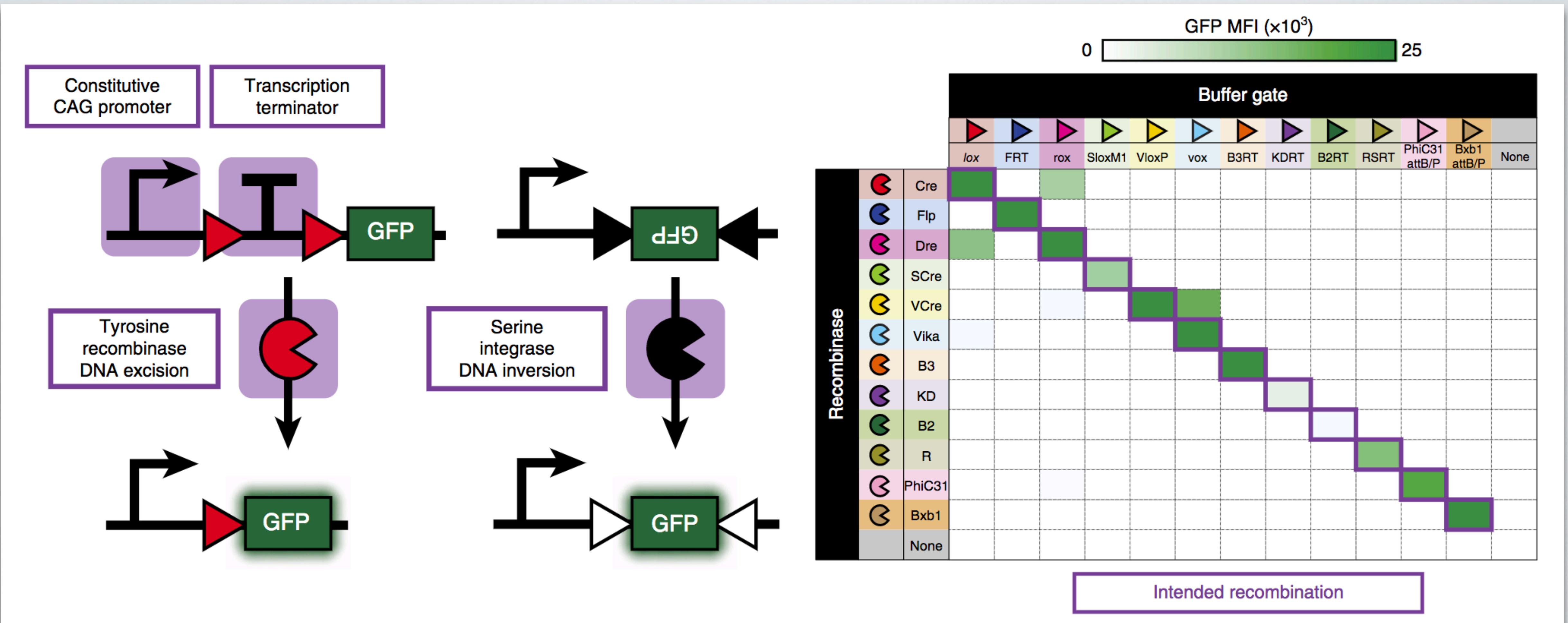
e.g. artificial mini-organ that produces a hormone/drug only when it detects particular metabolic environments within the body

We can now design and introduce these artificial operations into living organisms

Using recombinases for building synthetic genetic regulatory circuits

Boolean logic and arithmetic through DNA excision (BLADE)

Weinberg *et al* 2017 *Nature Biotechnology* (Wilson Wong, Boston U)

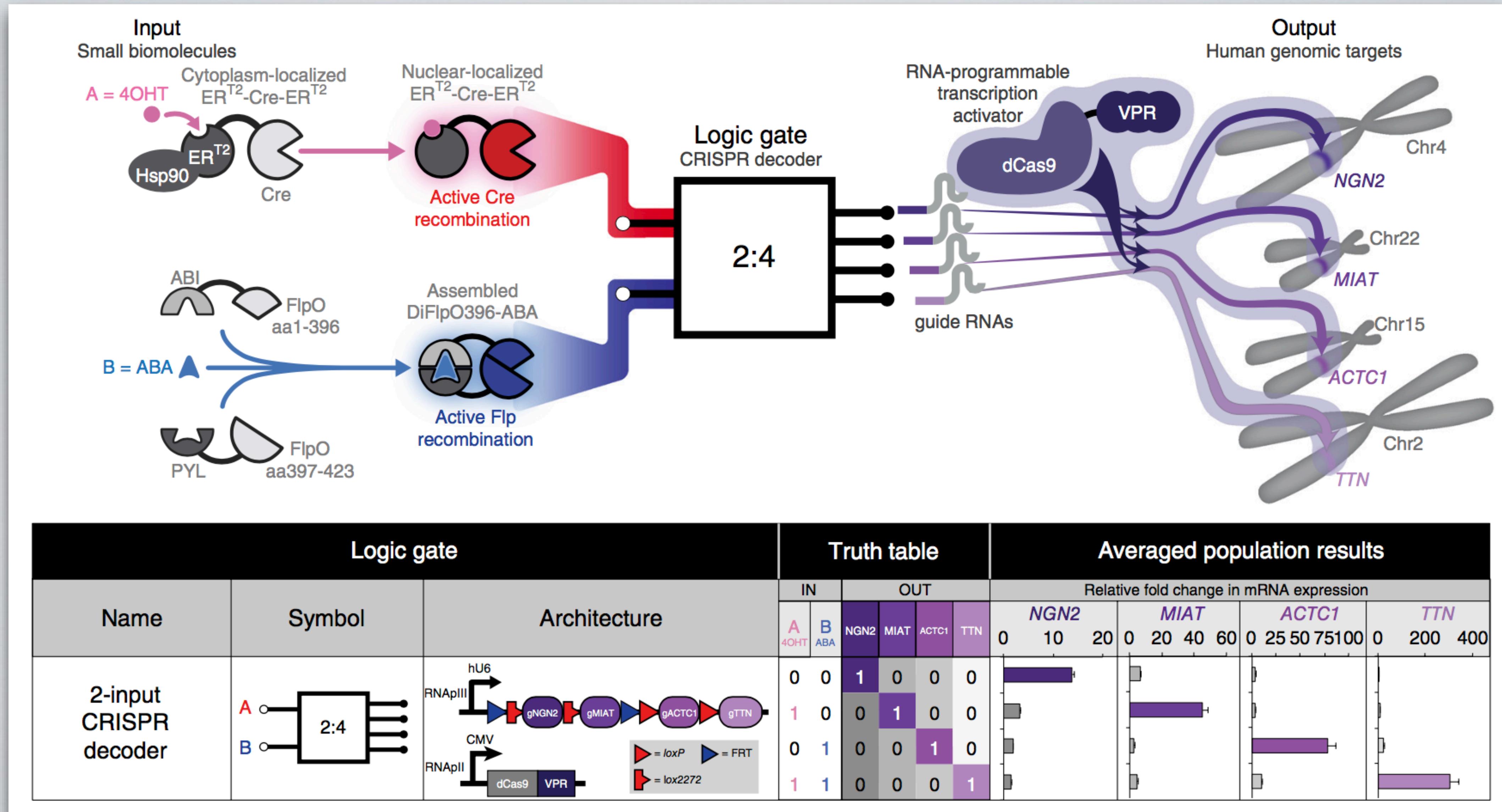


Robust **single fire** genetic circuits with multiple inputs and outputs for mammalian cells

Boolean logic and arithmetic through DNA excision (BLADE)

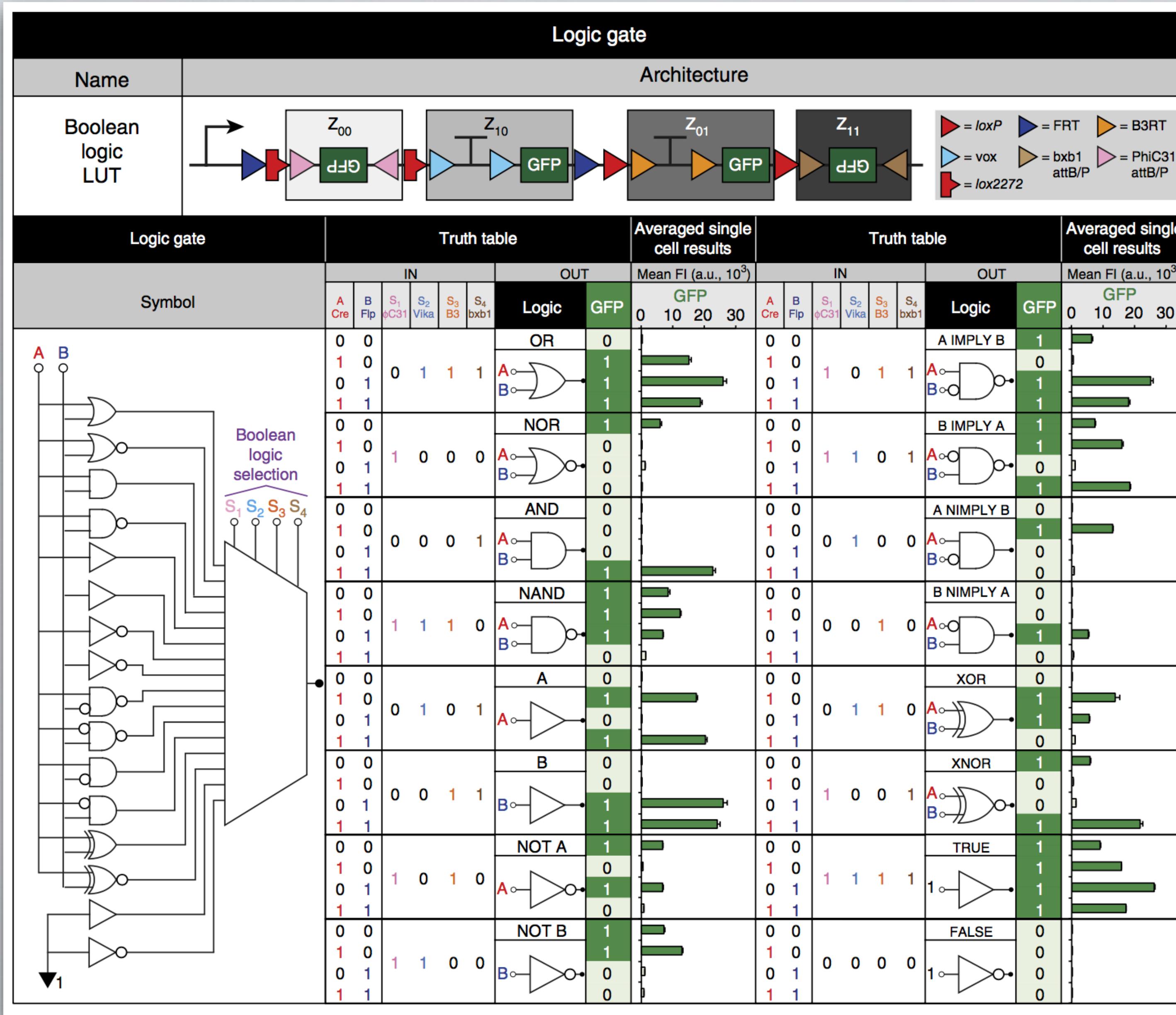
Robust ***single fire*** genetic circuits with multiple inputs and outputs for mammalian cells

Boolean logic and arithmetic through DNA excision (BLADE)



Decoder to control 4 different transcriptional outputs based on the 2 inputs

Boolean logic and arithmetic through DNA excision (BLADE)



All 16 two-input Boolean logic functions

Boolean logic and arithmetic through DNA excision (BLADE)

Logic gate		
Name	Symbol	Architecture
3-input BLADE template		

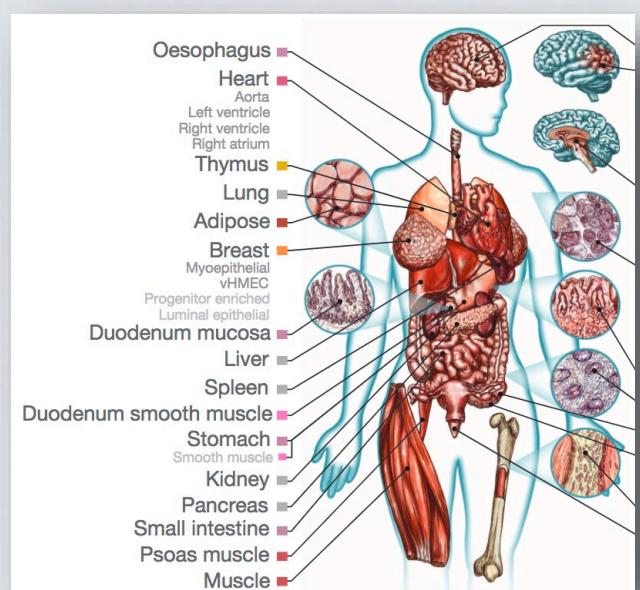
b

Logic gate		Truth table			Averaged single-cell results			
Name	Symbol	Architecture			IN	OUT	Mean FI (a.u., 10^3)	
		A Cre	B Flip	C VCre	DEC	P mCh	mCherry	GFP
Full adder			0 1 0 0 1 1 1 0 1 1	0 0 0 1 0 1 0 1 1 1	0 1 0 1 0 2 1 2 1 3	0 0 1 0 0 1 0 1 1 1	0 50 100 0 0 100 0 0 0 20 40	0 20 40 60
Full subtractor			0 1 0 0 1 1 1 0 1 1	0 0 0 1 0 -1 -1 -1 0 0	0 1 0 -1 1 	0 0 1 0 1 0 0 0 0 	0 20 0 0 0 0 0 0 0 0	0 20 0 0 0 0 0 0 0 0
Half adder - subtractor			0 1 0 0 1 1 1 0 1 1	0 0 0 1 0 2 0 -1 0 0	0 1 0 1 0 	0 0 1 0 1 2 1 1 0 	0 50 100 0 0 100 0 0 0 20 40	0 20 0 0 0 0 0 0 0 0

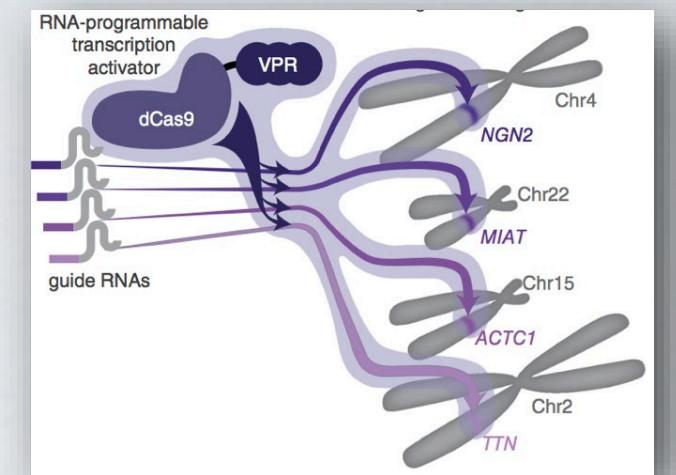
3-input arithmetic computational circuits
(addition, subtraction, both)

Mammalian synthetic genetic regulatory circuits

Detect and process specific desired cellular properties or states, to elicit a desired transcriptional or cellular outcome

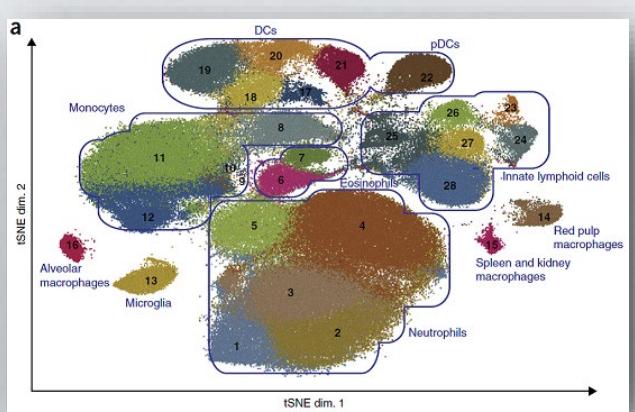
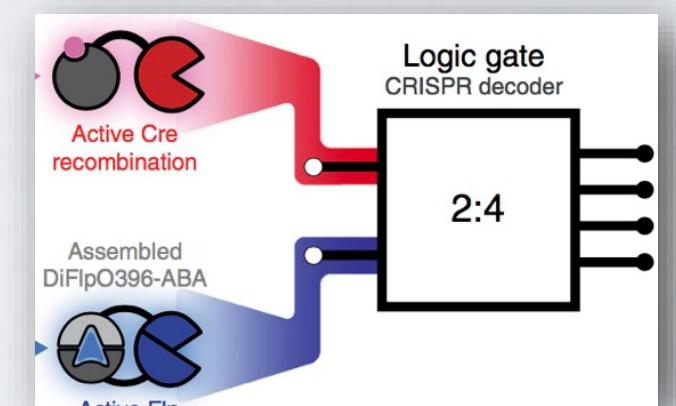


Customizable, flexible control of gene expression and cell state/activity



Improved spatiotemporal control of gene expression and cell activity

Circuits introduced into cells to integrate multiple signals to e.g. report specific cell/transcriptional states, trigger cell death, restrict cell state, induce transdifferentiation, monitor disease-relevant metabolites, produce therapeutic compound in response etc

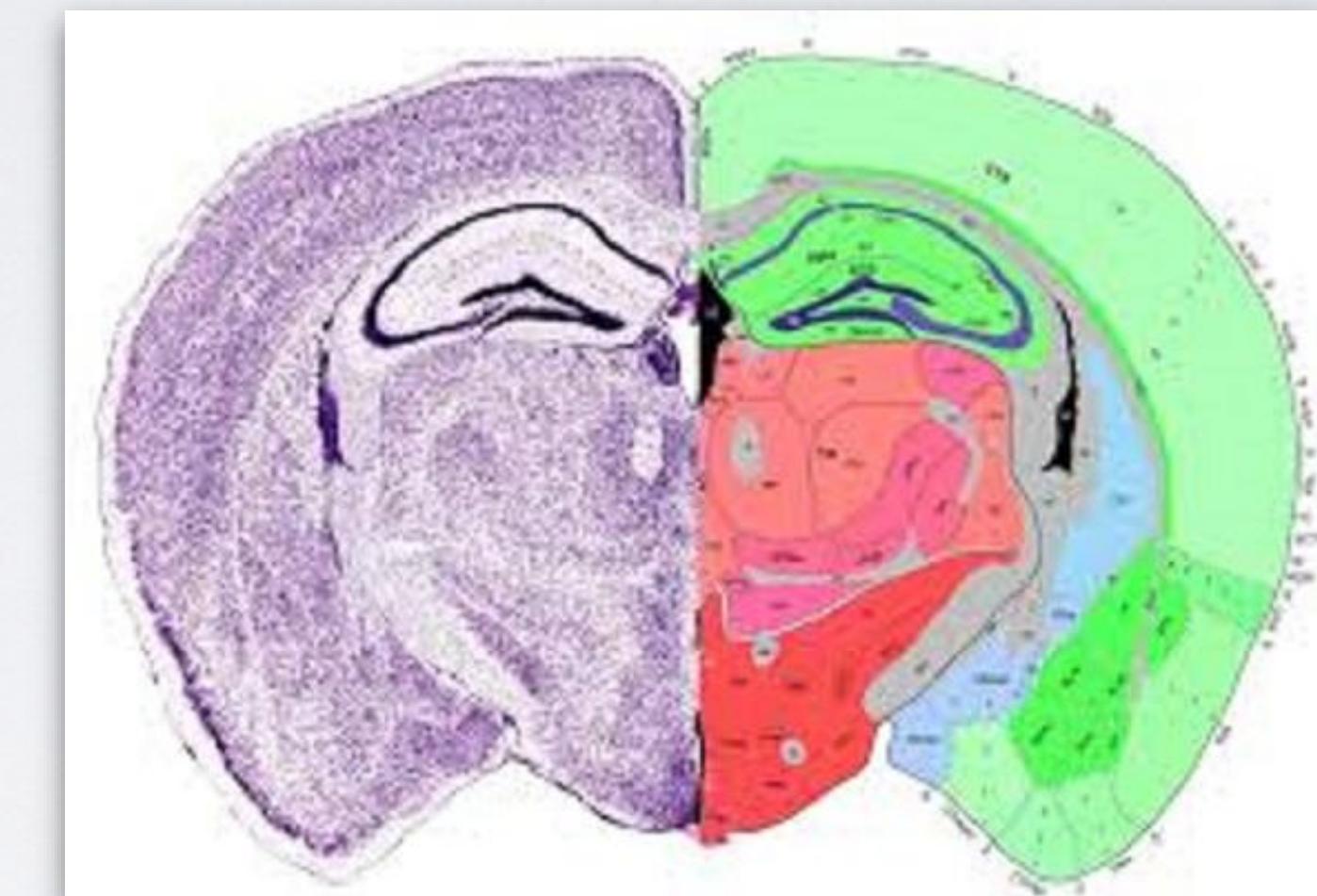


Information on input/output regulatory processes/elements identified from single cell RNA, chromatin etc profiling

Optogenetics - light-based control of gene expression

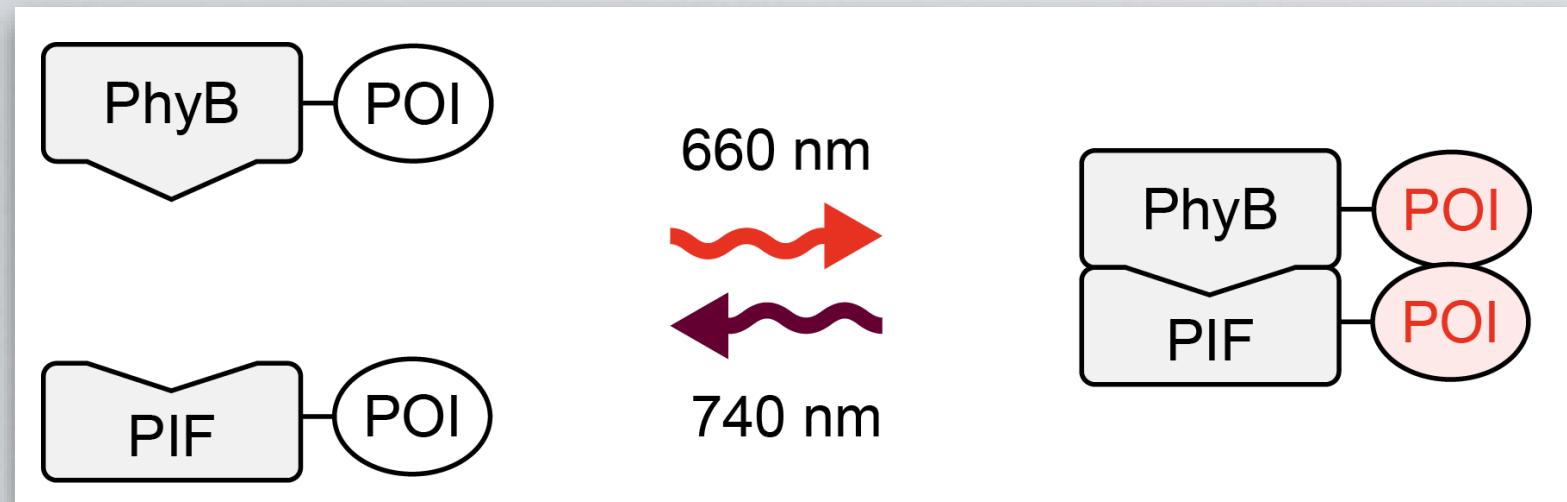
Manipulation of exogenous genes is a powerful technique for investigating and manipulating cellular functions

Many inducible systems can't achieve fine spatial and temporal control, making it challenging to control processes highly dependent on spatial localization, timing, or duration



Optogenetics - manipulation and use of natural photo-switching molecules to control gene expression

Optogenetics - light-based control of gene expression



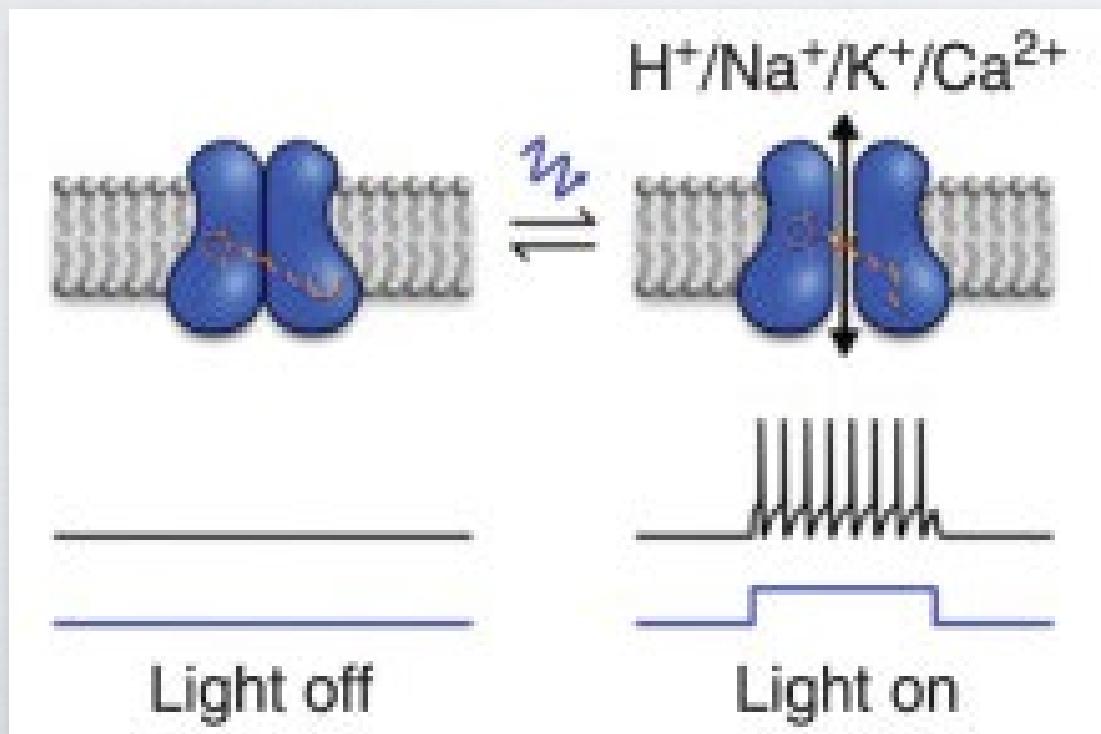
Engineered photo sensing proteins can undertake specific molecular functions in response to illumination with particular wavelengths of light

Rapid and precise response to specific wavelengths



Light is the catalyst, providing several advantages:

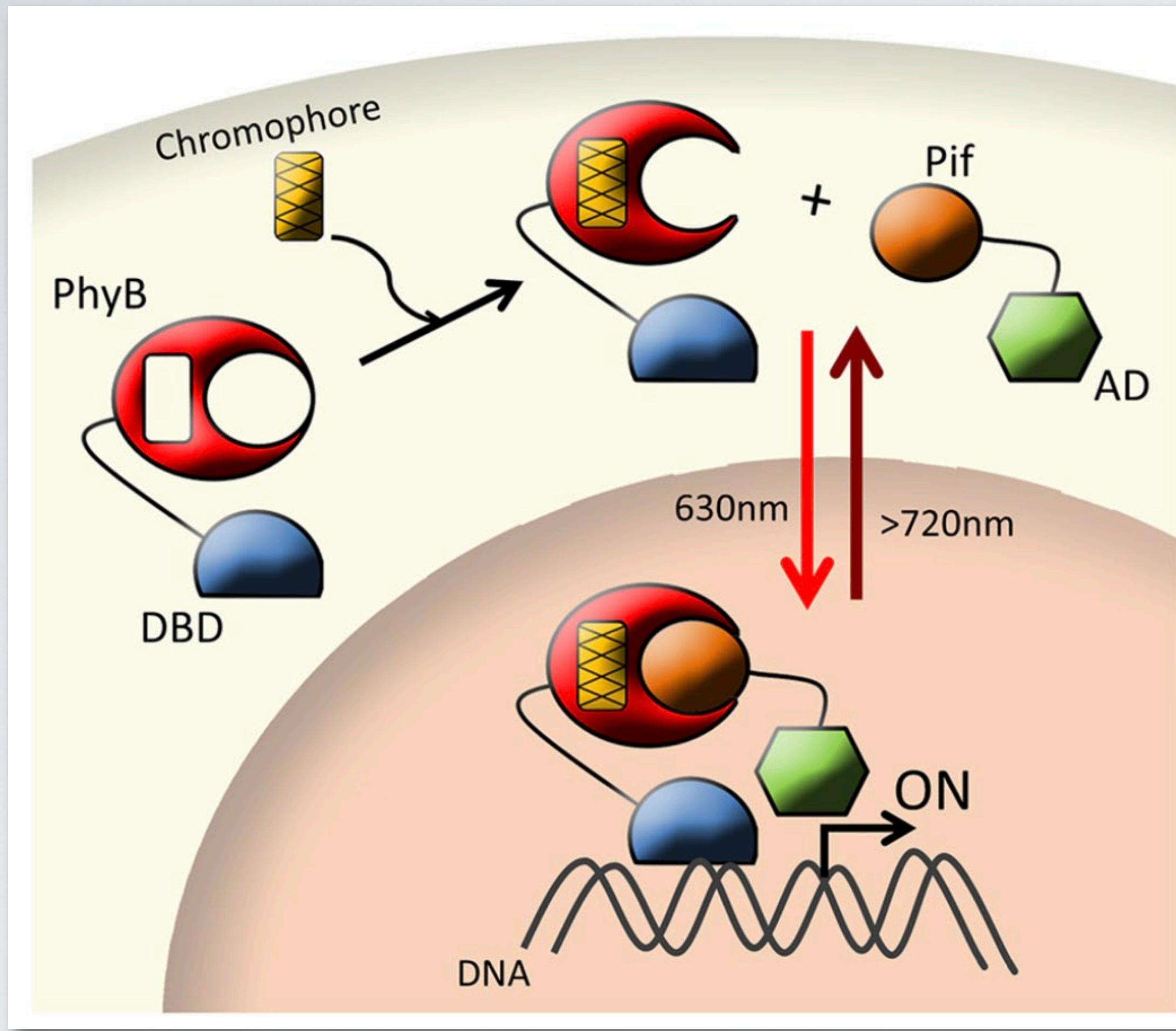
- stimulus is immediate
- highly controllable (spatially, duration)
- rapidly reversible
- little to no unexpected toxic side-effects



Channelrhodopsins
from the algae
Chlamydomonas
reinhardtii

light dependent channels for
manipulating neural networks

Optogenetics - PhyB-PIF system



DBD: DNA Binding Domain

AD: transcriptional Activation Domain

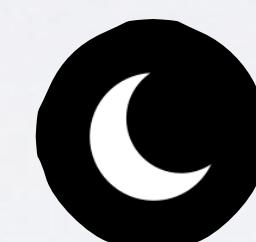
Pif: Phytochrome interacting factor

Red-light inducible PhyB-PIF system

Phytochromes (Phy): photoreceptors sensitive to red and far red light

Photoactive proteins found in bacteria, cyanobacteria, fungi and plants, involved in light-dependent functions including growth, development, seed germination, flowering, and circadian rhythm

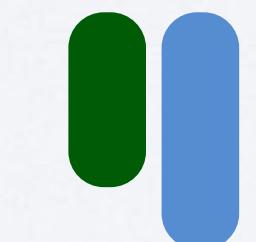
For photoswitching, a phytochrome (e.g. PhyB) needs to interact with a chromophore (e.g. phycocyanobilin - absorbs photon), which is endogenous to plants, but needs to be introduced in animal models



In the dark, phytochromes are in the inactive form (Pr)



Upon red light stimulation (~630 nm), Pr undergoes a conformational change turning into the active form (Pfr)



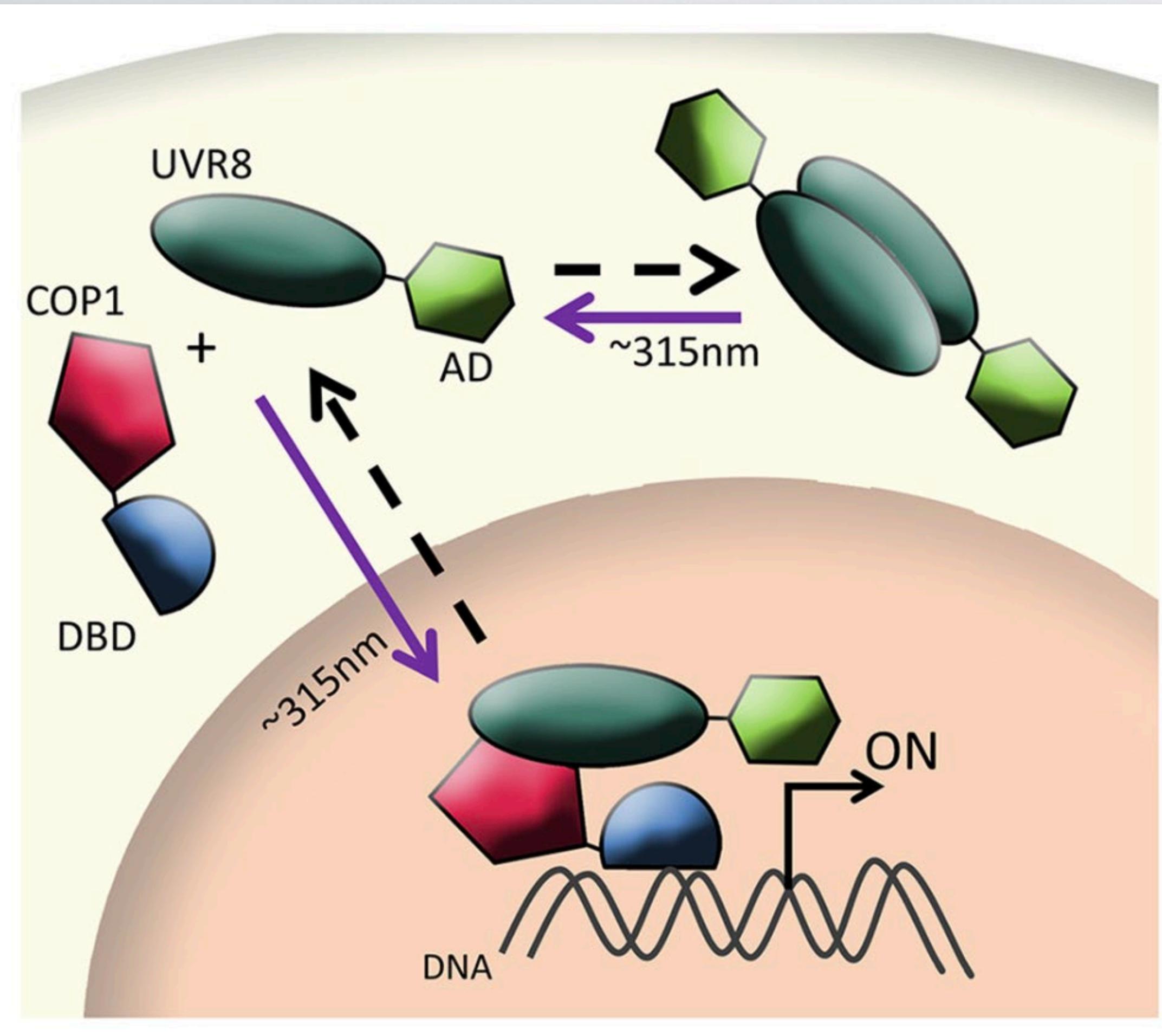
Pfr heterodimerizes with phytochrome-interacting factors

Heterodimer is highly stable - short red illumination pulse maintains active state for hours

Link PhyB to a DBD and PIF to an ADs for red light-induced activation of target gene

Under far-red light (720 nm) the PhyB-PIF complex dissociates, abrogating transcription

Optogenetics - UVR8-COP1 system



UV-dependent UVR8-COP1 system

In dark, UVR8 accumulates in the cytoplasm as homodimers

Under UV light irradiation, UVR8 monomerizes and binds to COP1

A transcription factor can be split into DBD and AD, and each linked to UVR8 or COP1

Upon UV light irradiation, the UVR8-COP1 interactions reconstitutes the functional components of the transcription factor and relocates to the nucleus, enabling targeted gene regulation

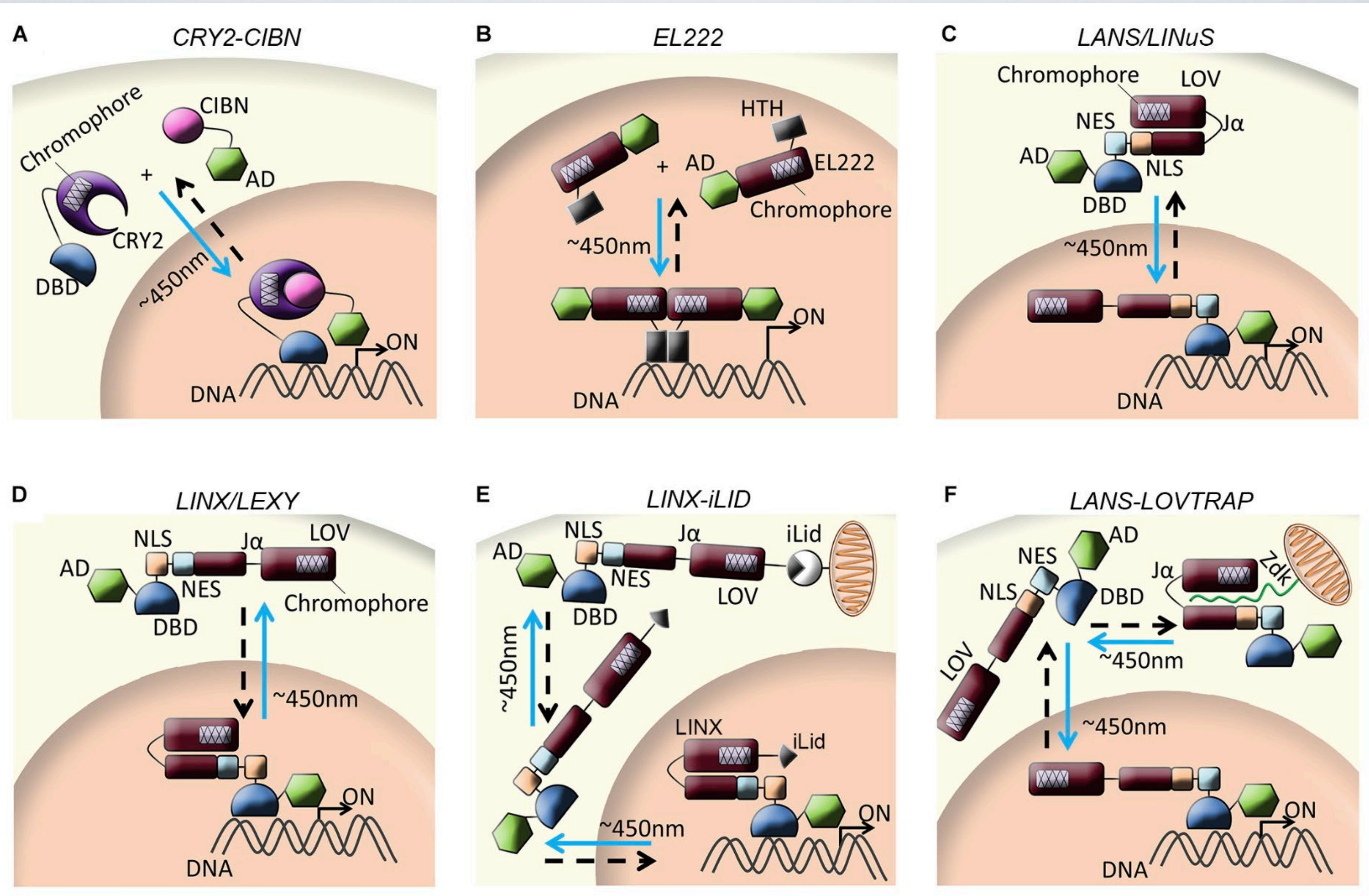
Different protein domains can be linked to UVR8 and COP1 to customise desired light-inducible functions

de Mena *et al* (2018). Bringing Light to Transcription: The Optogenetics Repertoire. *Front Genet.* 9: 518. 30450113

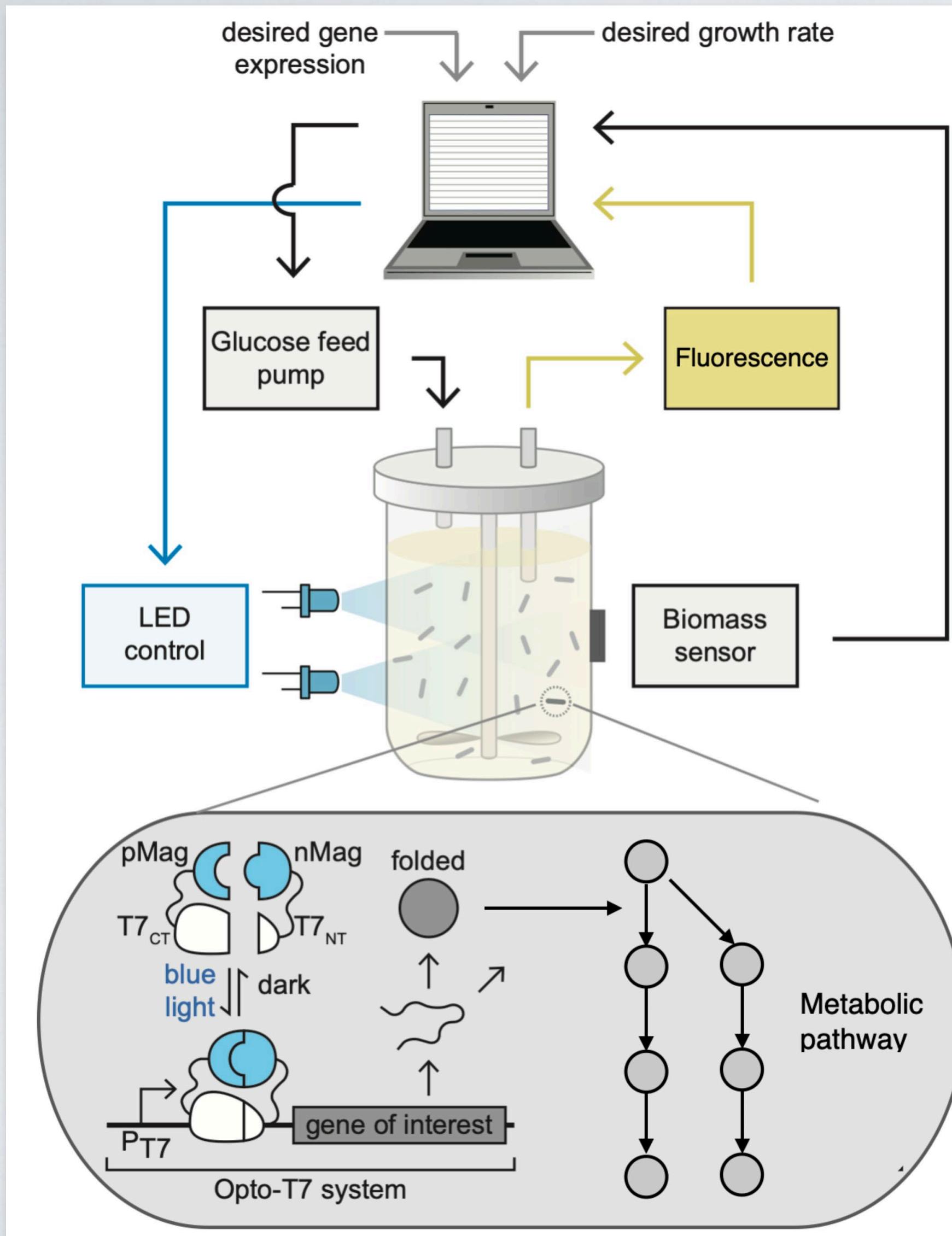
Proteins with unique functions that have evolved in diverse organisms can be used as “parts” for customisable new functions

Optogenetics - blue light-dependent strategies

de Mena et al (2018). Bringing Light to Transcription: The Optogenetics Repertoire. *Front Genet.* 9: 3045013



Cybergenetics



Interfacing electronic control systems with living organisms to manipulate them

Computer Control of Cell Populations (Khammash Lab, ETH Zurich)

Bioreactor containing cells that in the presence of blue light (optogenetic control) drive expression of a gene, for example involved in a desired cellular pathway

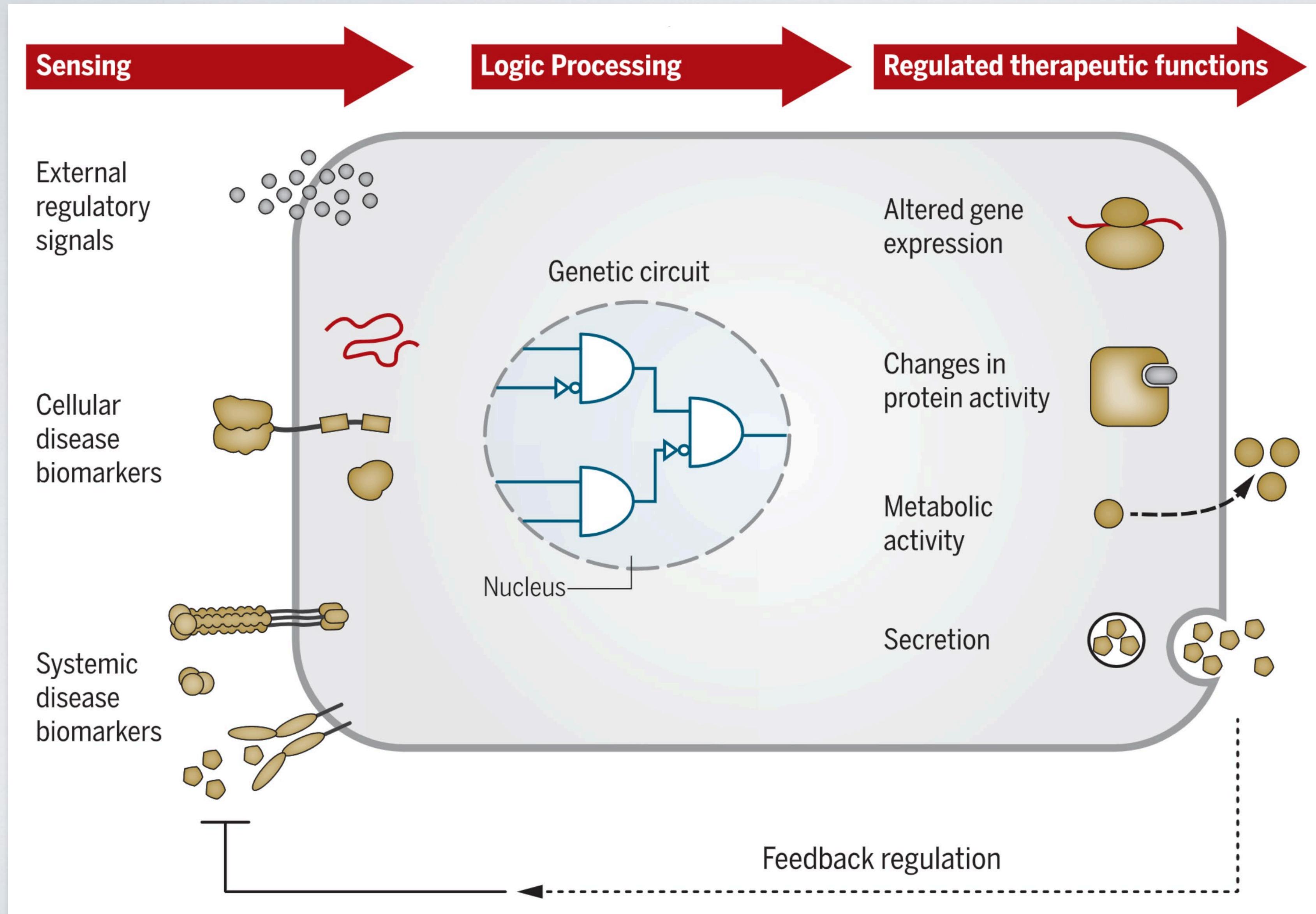
Sensors in bioreactor measure output of the pathway (e.g. protein concentration, by fluorescence), and feed this back into computer

Computer can then alter light delivered to the bioreactor to increase or decrease pathway activity

Computer can adjust nutrient supply to bioreactor to control cell proliferation

This allows system to control both cell growth rate and desired gene expression

New therapeutics based on programmable gene and engineered cells

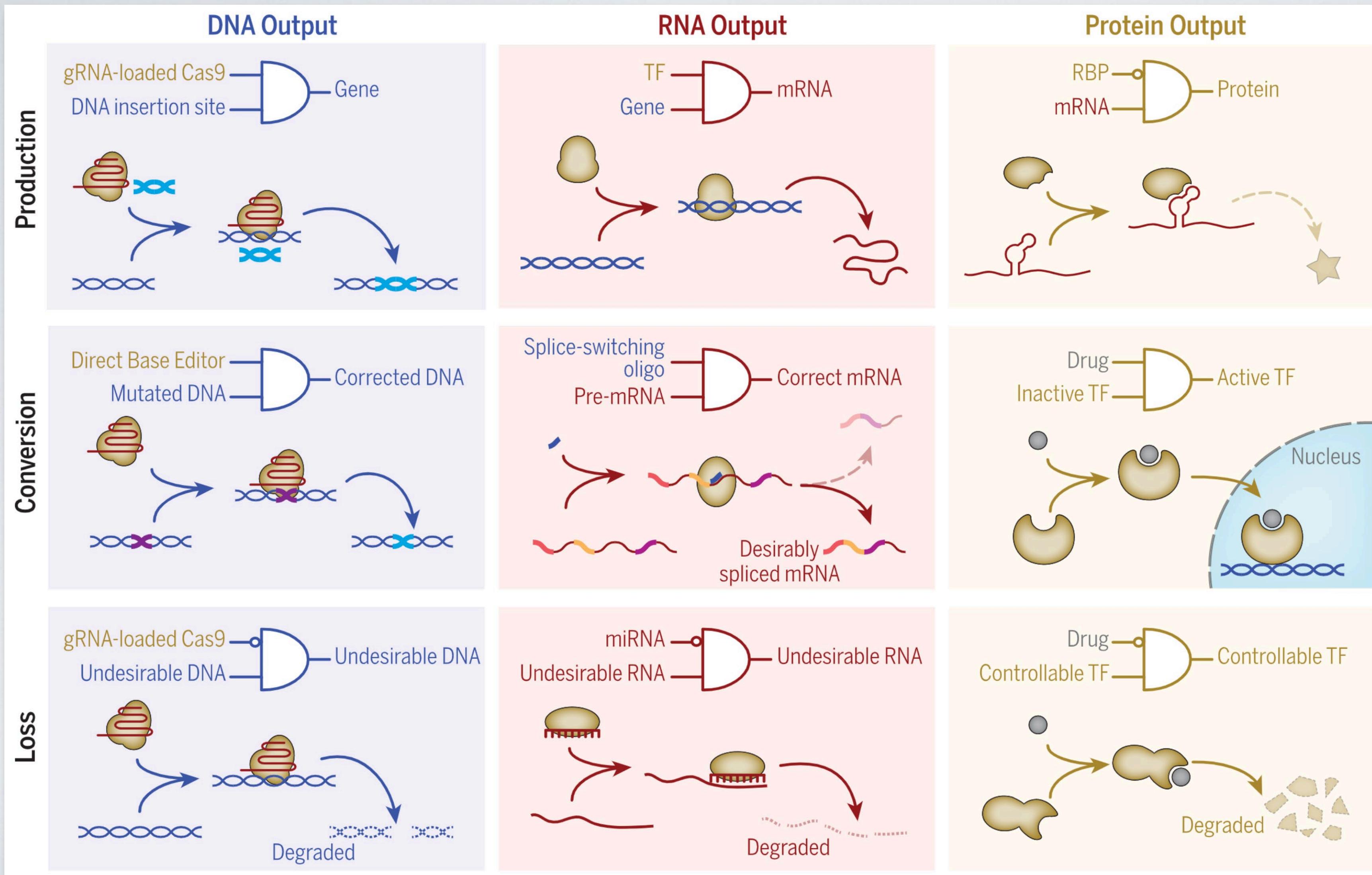


Addition of genetically encoded cellular functions

Regulation of dosage, timing, and localisation of therapeutic functions

May enable therapies that are safer and more effective than existing approaches (eg. duration, specificity)

Engineerable modules for control of DNA, RNA, and protein



A range of different regulatory modules can control **production**, **conversion**, or **loss** of desired **DNA**, **RNA**, or **proteins**

Prosthetic gene networks

Prosthetic gene networks (or circuits) are synthetic regulatory networks that are added to a host cell that have the ability to dynamically sense and respond to the environment to drive appropriate network activity (e.g. for a particular therapeutic effect)

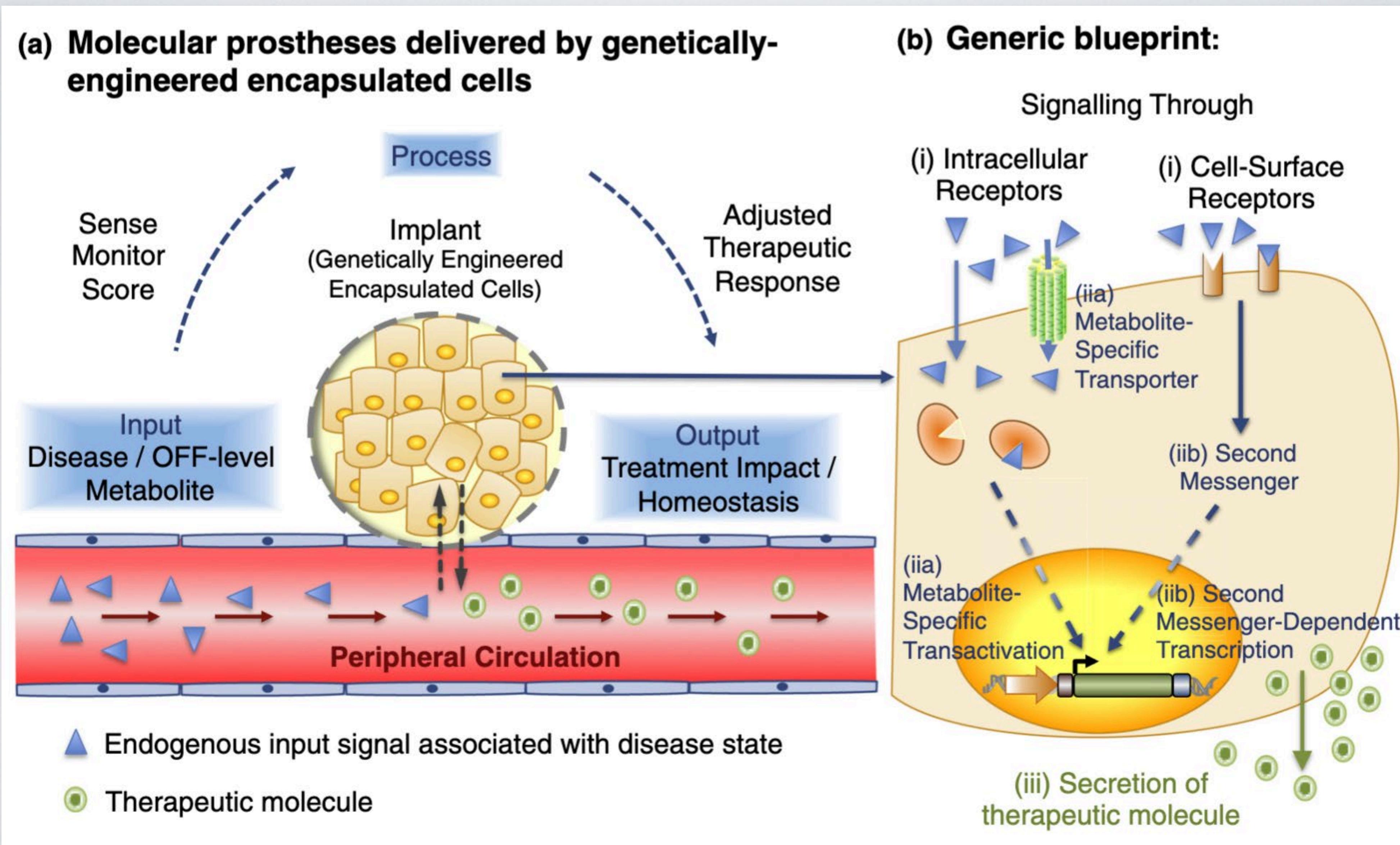
Characteristics prosthetic gene networks for delivery of therapeutic responses include:

Operate in engineered cells that are implanted / delivered

Continually sense and respond to metabolic / chemical environment

Use feedback loops to automatically adjust therapeutic response in a self-regulated manner

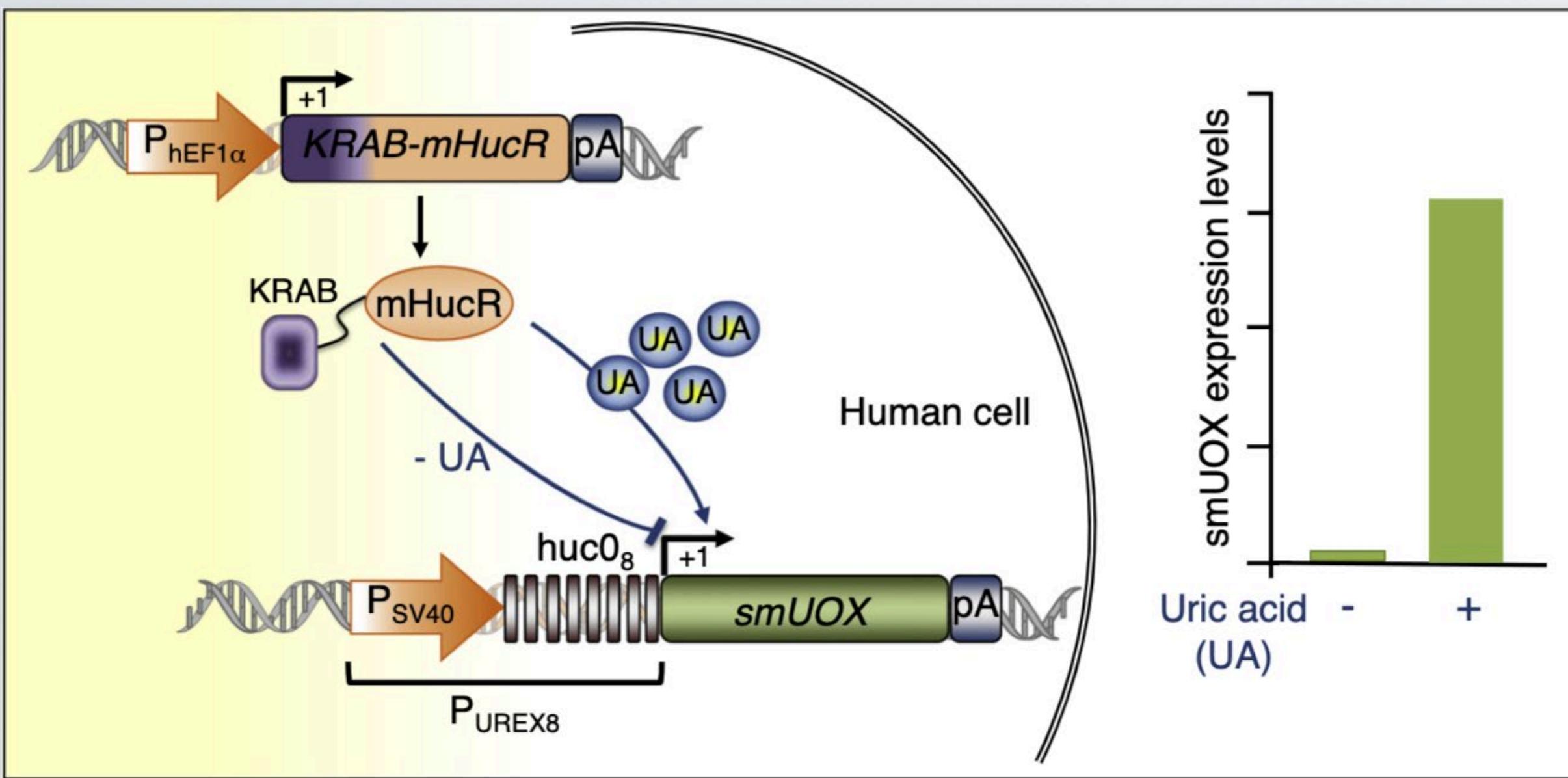
Encapsulated engineered cells as devices to monitor and deliver therapeutics



Prosthetic gene network for treatment of hyperuricemia

First example of an autonomous closed loop prosthetic gene network

Kemmer et al (2010) Self-sufficient control of urate homeostasis in mice by a synthetic circuit. Nat. Biotechnol. 28



Hyperuricemia - disruption of normal uric acid homeostasis

Therapeutic transgene: *Aspergillus flavus* urate oxidase (*smUOX*)

smUOX transgene was put under control of a chimeric sensor protein: mHucR repressor protein linked to the KRAB repressor

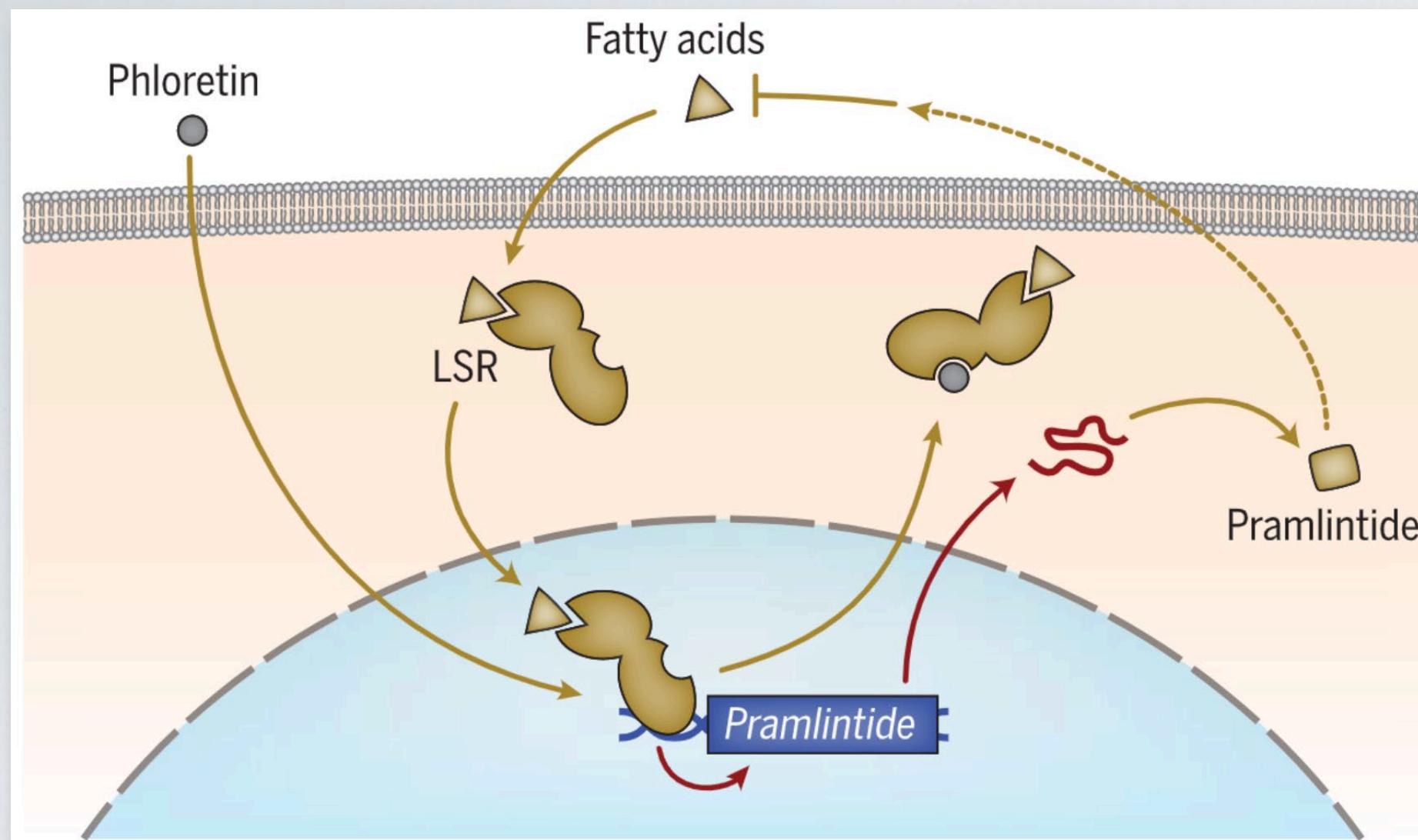
When uric acid is absent, KRAB-mHucR binds to its cognate binding sequence to repress *smUOX* expression

But, binding of uric acid to mHucR causes dissociation of KRAB-mHucR from the *smUOX* promoter, causing expression of *smUOX* that then processes any uric acid present

Implantation of cells containing this prosthetic network into mice with hyperuricemia resulted in return of uric acid homeostasis by *smUOX*, clearance of uric acid crystals in the kidneys, and normal bloodstream rate levels —> engineered cellular therapy

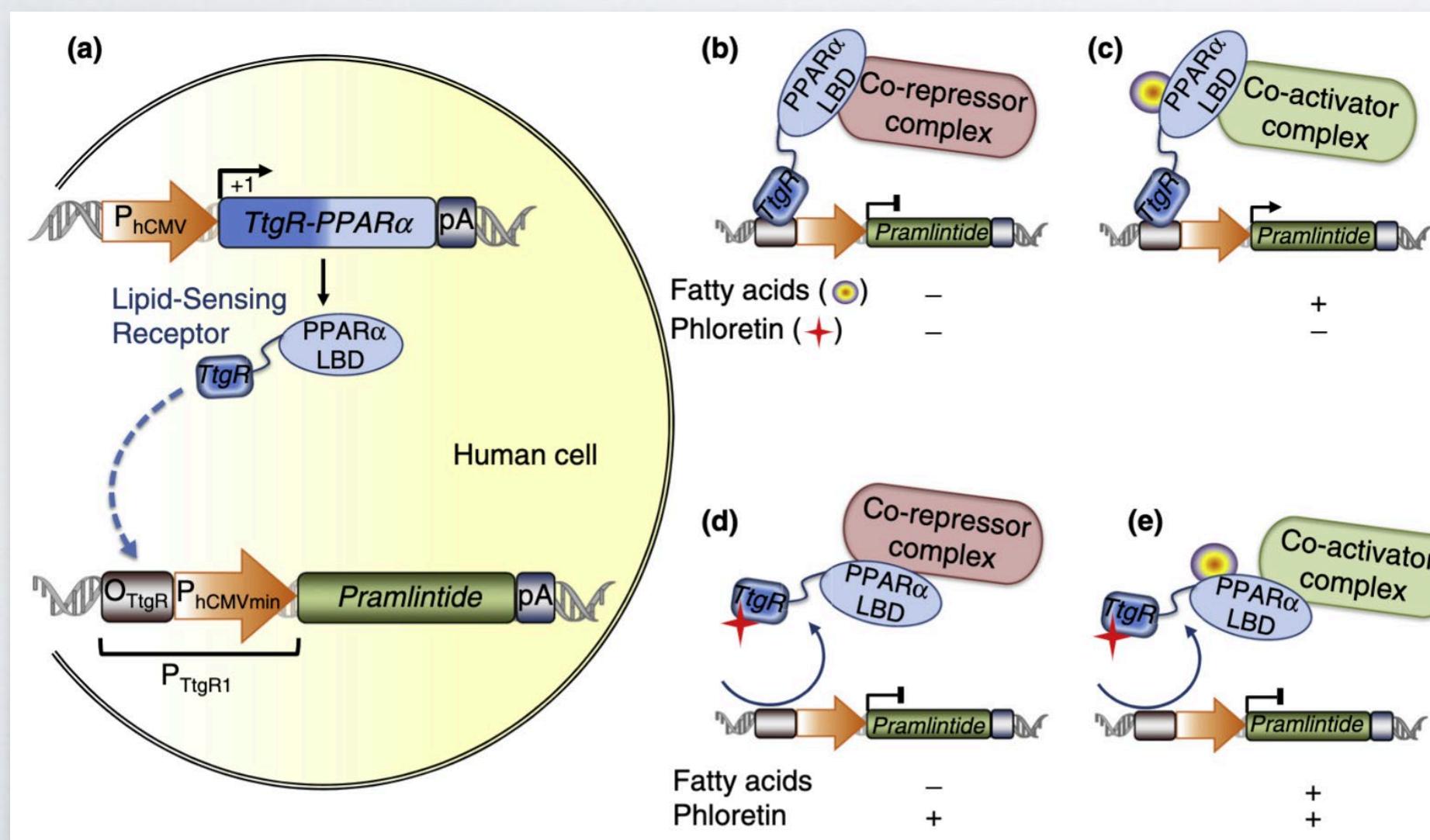
Heng et al (2015). Prosthetic gene networks as an alternative to standard pharmacotherapies for metabolic disorders. *Curr Opin Biotechnol.* 35

A prosthetic gene circuit for regulating satiety to treat diet-induced obesity



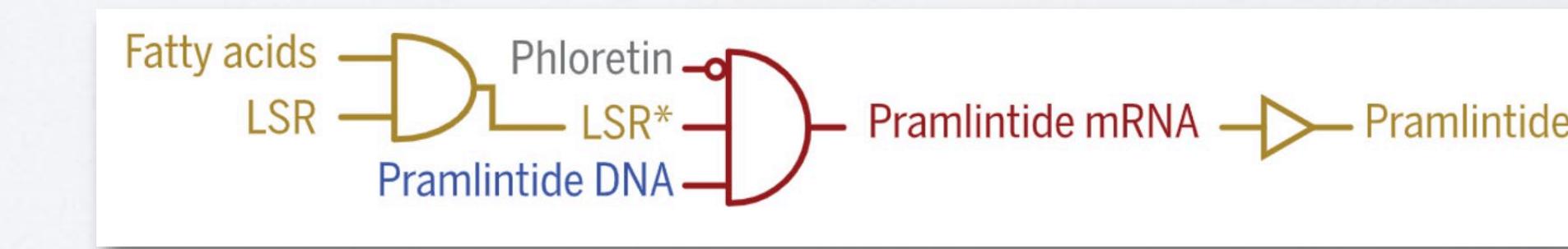
Kitada T et al (2018). Programming gene and engineered-cell therapies with synthetic biology. *Science*. 359(6376)

Gene circuits that can sense and respond to surrounding cues (e.g. disease biomarker) through feedback loops have the capability to activate (e.g. to produce a therapeutic) at the appropriate time, duration, and intensity



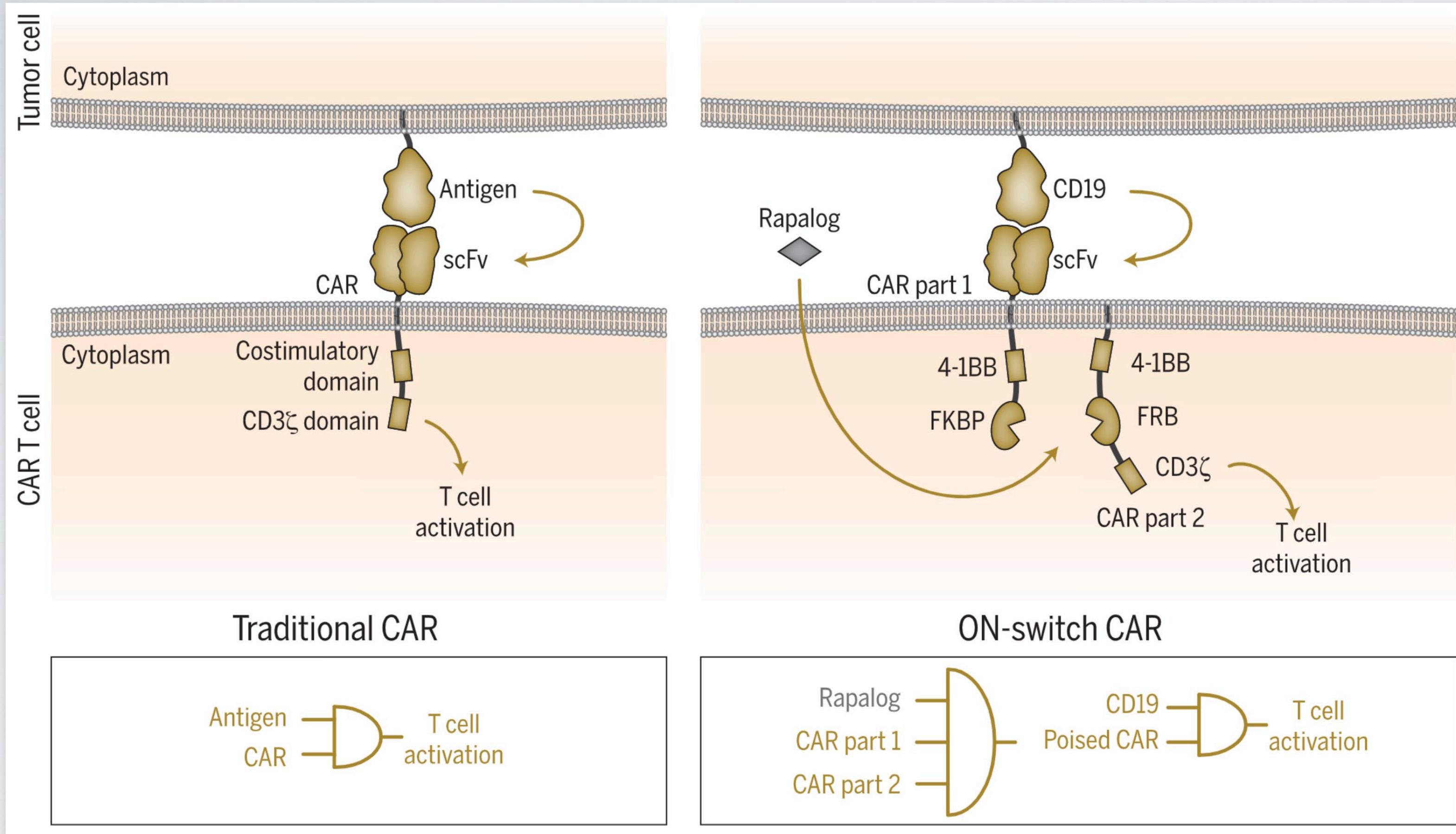
Heng et al (2015). Prosthetic gene networks as an alternative to standard pharmacotherapies for metabolic disorders. *Curr Opin Biotechnol*. 35

Rössger et al (2013): made a Lipid-Sensing Receptor (LSR) prosthetic gene circuit, introduced it into human cells encapsulated in a structure, and implanted these cells in mice to treat diet-induced obesity



Prosthetic gene circuits have now been created to regulate thyroid hormones, blood pH, blood pressure, blood glucose, insulin, and more

Small molecule regulation of engineered cell therapies: CAR-T cells



Chimeric Antigen Receptor T (CAR-T) cells

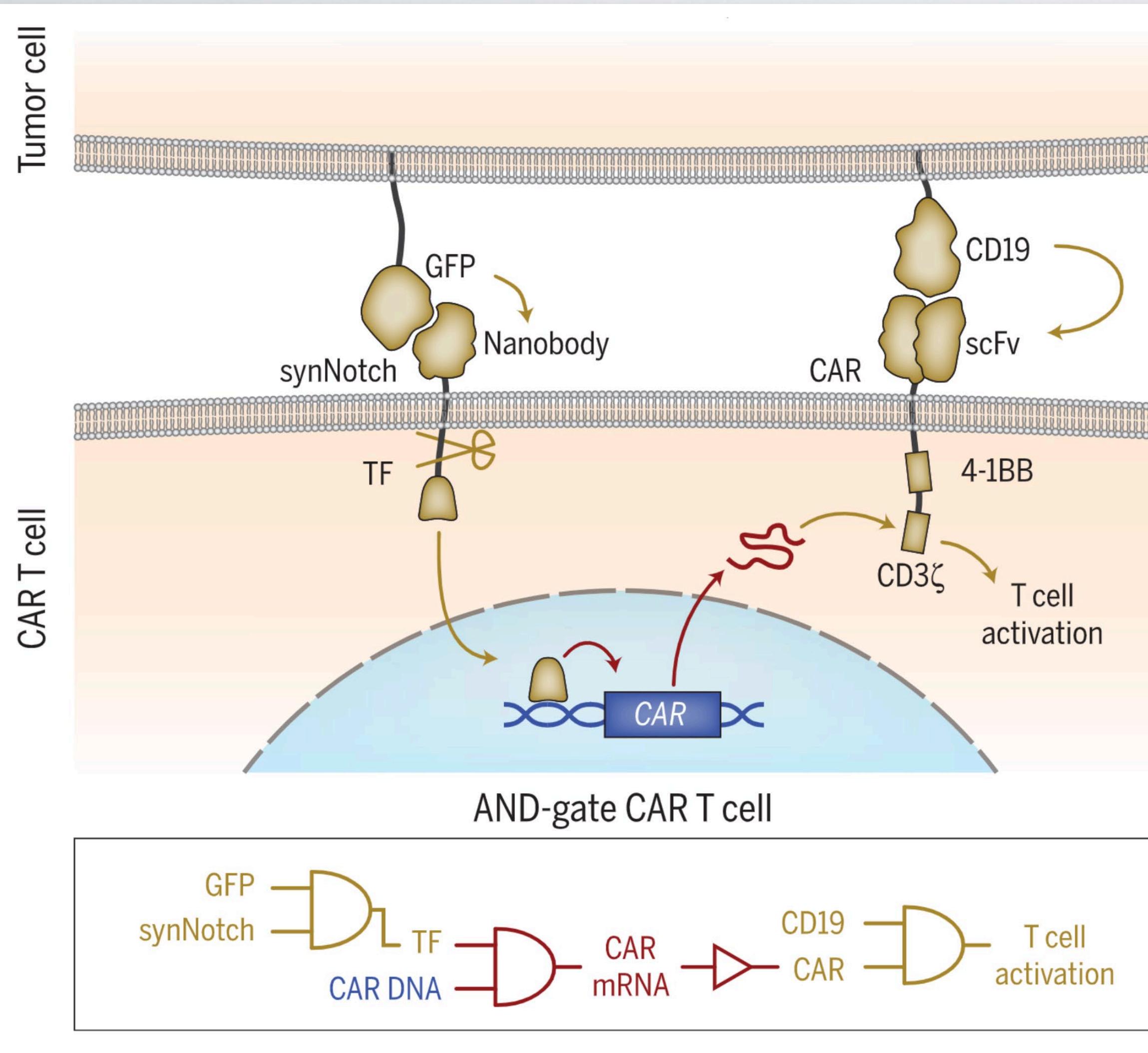
Patient's T cells harvested, genetically modified to express a chimeric antigen receptor that will recognise an antigen on a tumour cell (which will trigger T cell activation), expanded, and reinfused into the patient

In some cases, excessive CAR-T cell activation in the patient can cause severe toxicity that can be fatal

External control (activation) of CAR-T cell activation has been achieved by adding an ON switch, whereby a small molecule mediated protein heterodimerization of a split-CAR is required for T cell activation

Kitada T et al (2018). Programming gene and engineered-cell therapies with synthetic biology. *Science*. 359(6376)

Adding logical operations to CAR-T cells for improved specificity



Tumor cell
Antigen expressed by the tumour cell is rarely solely expressed in the tumour, but is also expressed in normal cells, sometimes leading to non-specific T-cell activation in tissues besides the tumour

CAR T cell
AND-gate CAR-T cells require recognition of two different antigens for T-cell activation (one by synNotch synthetic receptor, the other by CAR), leading to highly specific activation

Highly flexible system because both the synNotch and CAR can be engineered with any ligand binding domain

Platform for highly specific targeted cellular therapies

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