Cell Engineering Lecture 2:

Protein Circuit Design

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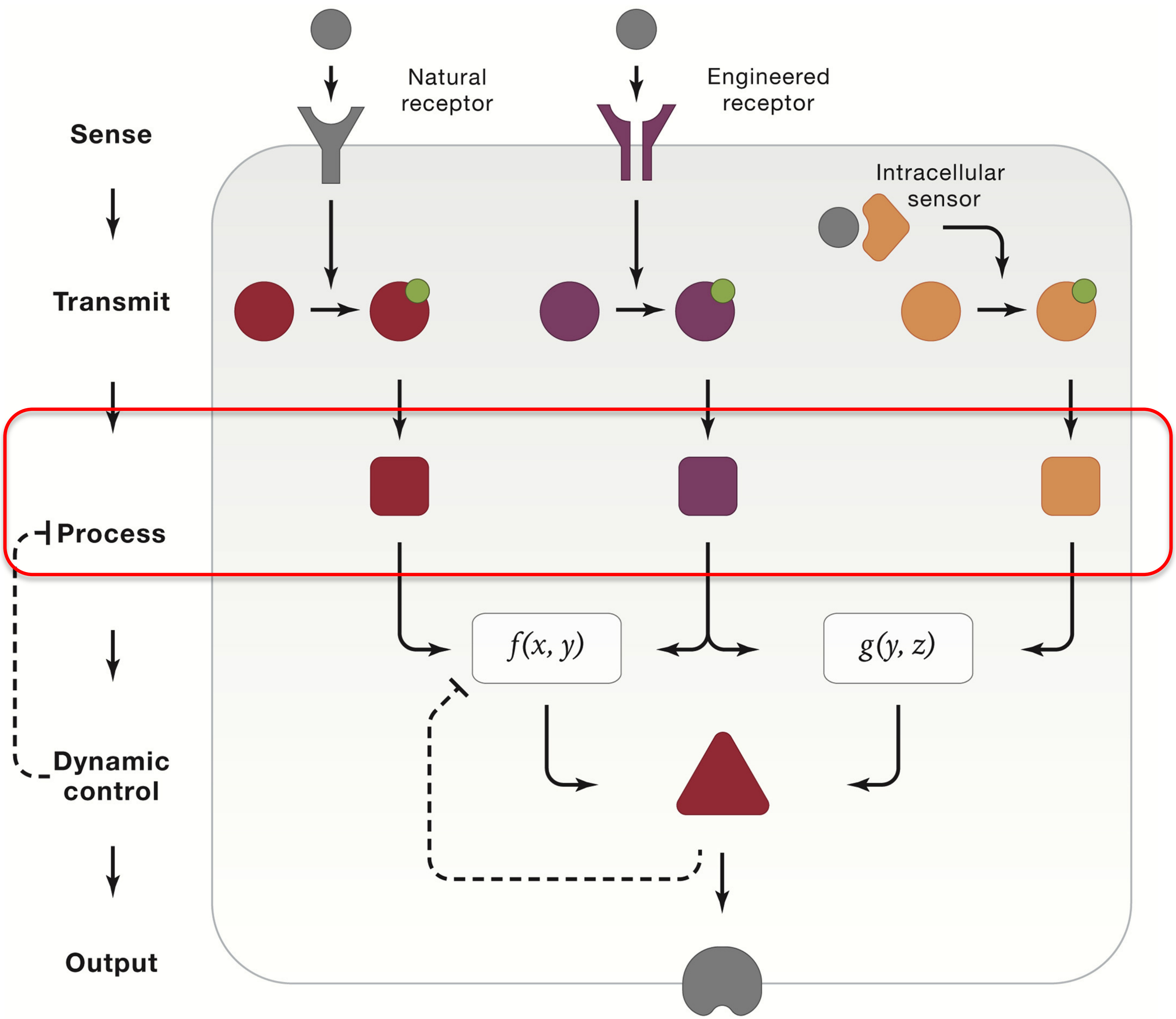
BIOENG-320

Generic protein circuit operational in a living cell



Generic protein circuit operational in a living cell

Signal processing operations enable powerful computational capabilities:

1. Logic
2. Amplification
3. Analog-to-digital conversion

through combination of orthogonalizability and composability principles

Protein-based processors can carry out logic operations

Logic is ubiquitous in cell signaling, allowing cells to selectively respond only under certain input combinations

How to engineer LOGIC into protein circuits?

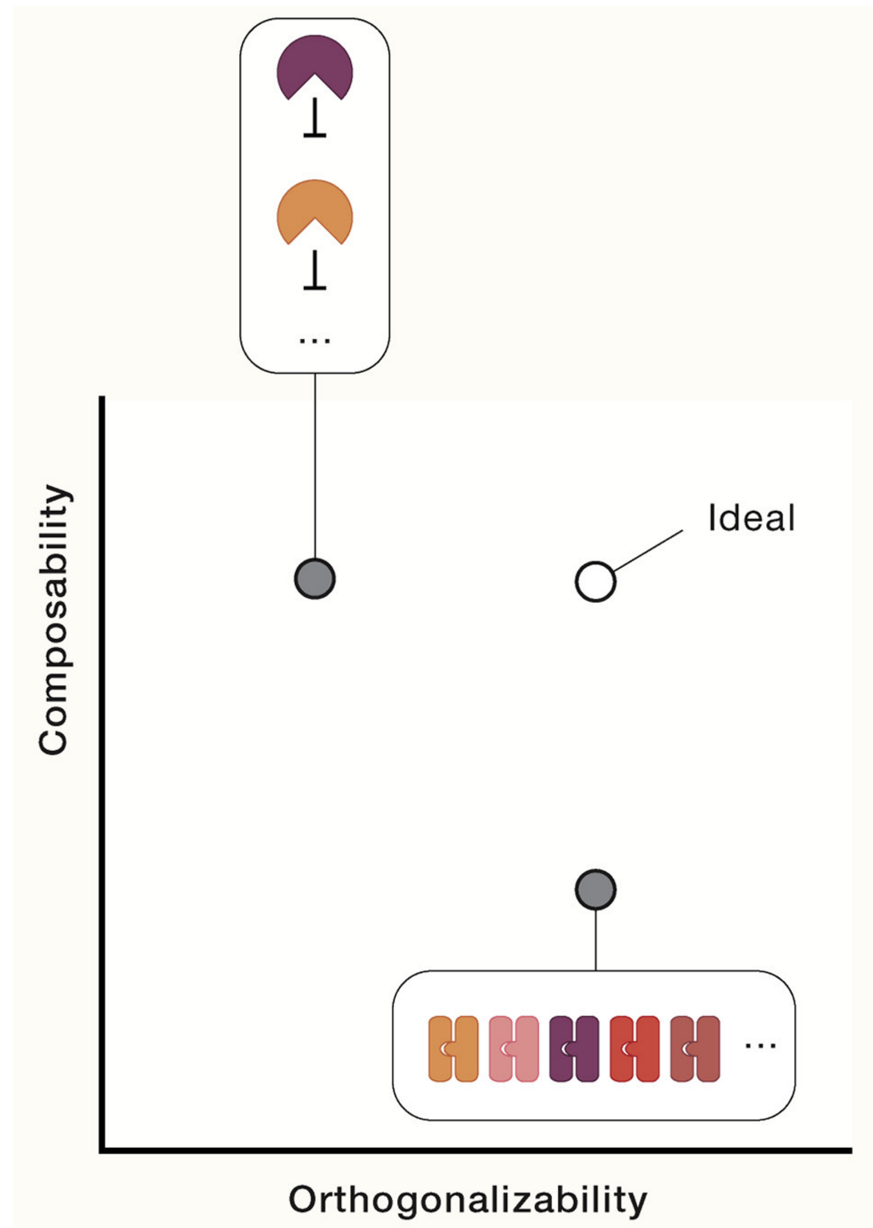
Principles for protease-based logic gates and circuits

Need of circuits that:

1. allow proteases to directly inhibit and activate each other, offering composability.

2. use of potentially unlimited *de-novo*-designed protein heterodimers, enabling orthogonalizability.

3. A combination of these two schemes should result in an ideal system with full scalability.

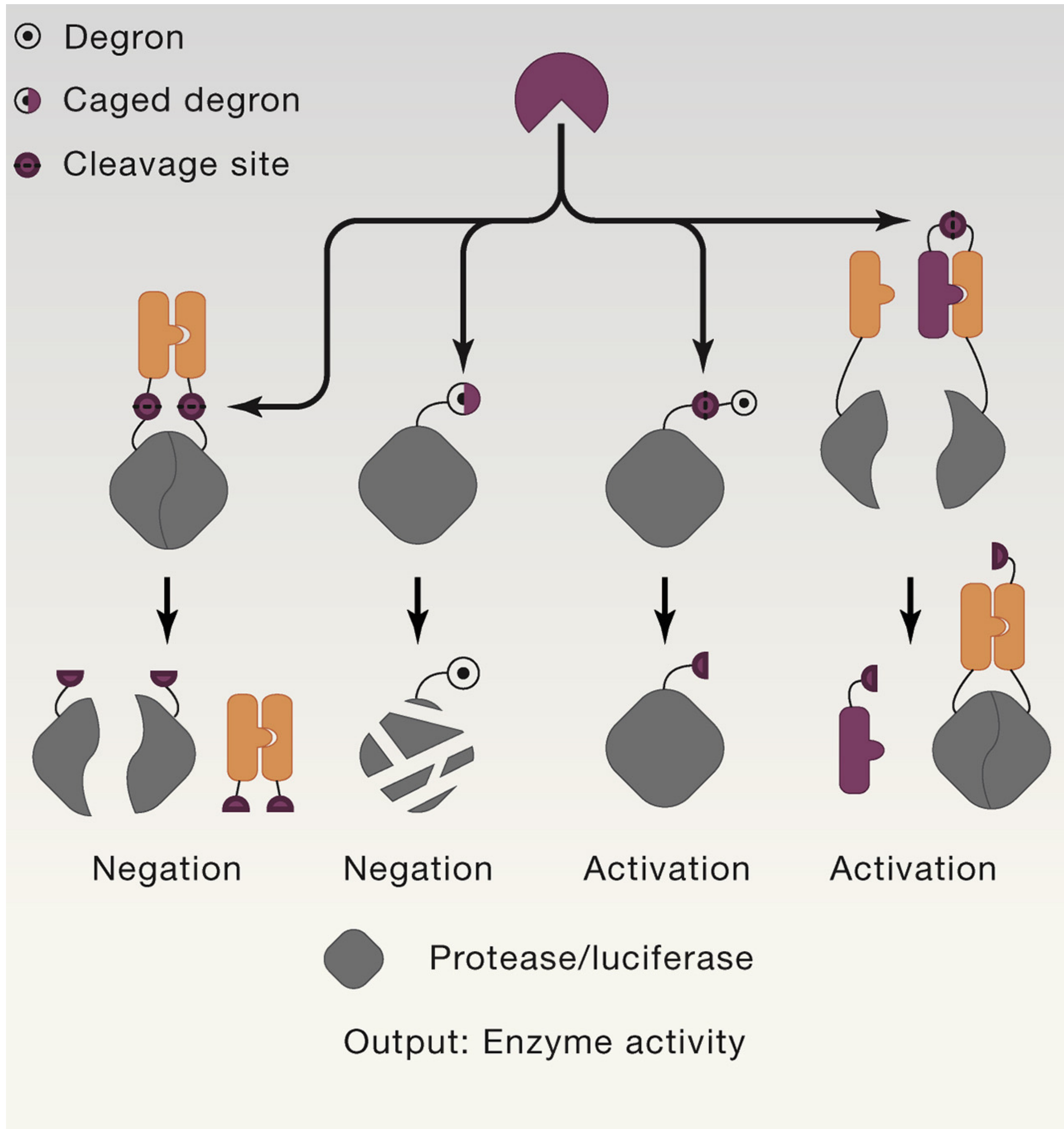


Examples of protease-based logic gates and circuits

Composability

CHOMP
circuits of hacked
orthogonal
modular proteases

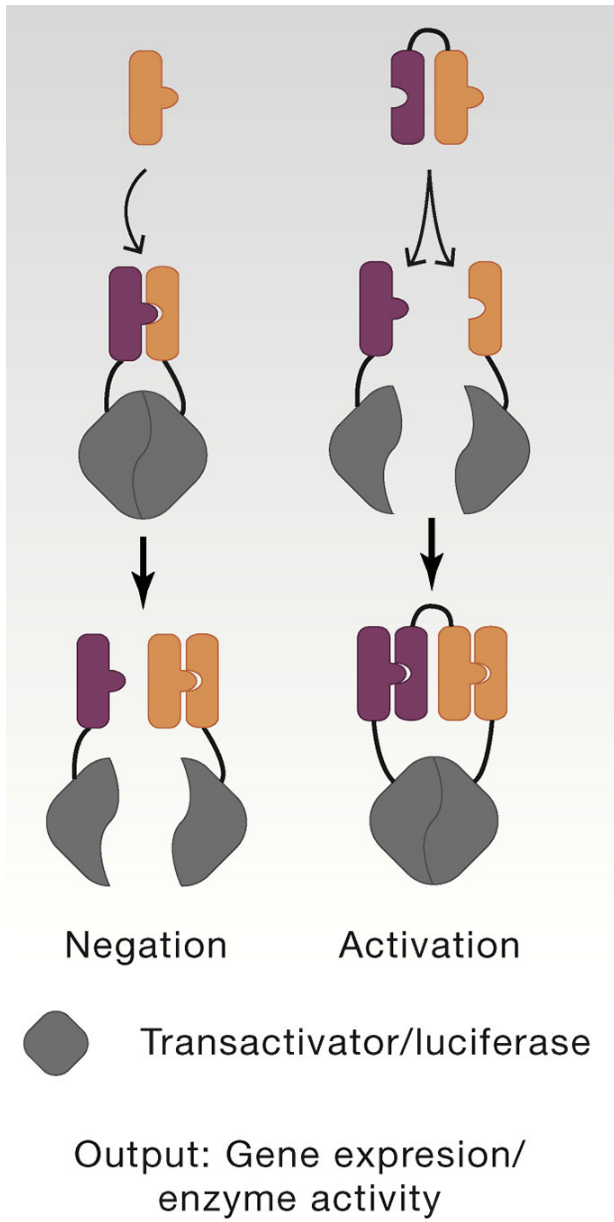
SPOC
split-protease-
cleavable
orthogonal-
coiled coil-based



Examples of protease-based logic gates and circuits

Orthogonalizability

CIPHR
(cooperatively
inducible protein
heterodimer)



Protein-based processors carry out analog-to-digital conversions

What is analog-to-digital conversion and why is it important in living systems?

Protein-based processors carry out analog-to-digital conversions

What is analog-to-digital conversion?

In electronics, an analog-to-digital converter (ADC, A/D, or A-to-D):

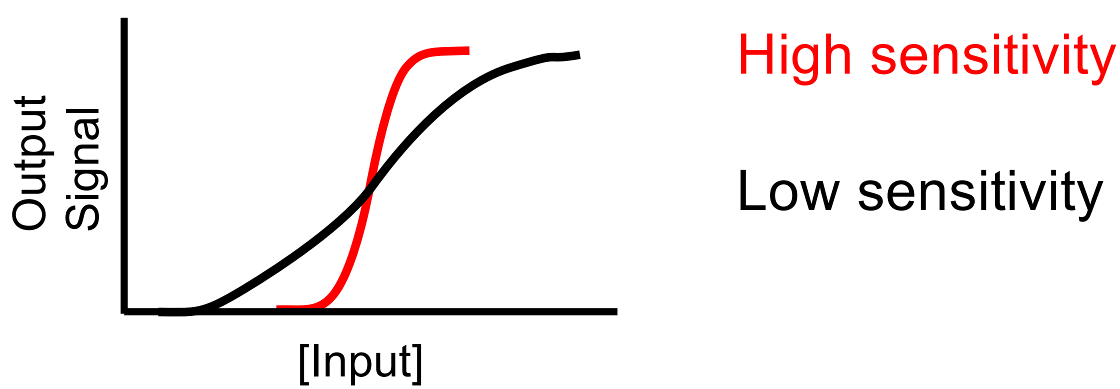
Conversion of a continuous analog signal (sound picked up by a microphone or light entering a digital camera) into a digital signal, discrete quantized value.

Protein-based processors carry out analog-to-digital conversions

Why is it important in living systems?

Ultrasensitive responses convert analog input signals to digital all-or-none outputs => background noise suppression, accurate detection of molecular targets, dynamic behaviors such as oscillation and multistability.

In natural circuits: ultrasensitivity through cooperativity, stoichiometric inhibitors etc…



Protein-based processors carry out analog-to-digital conversions

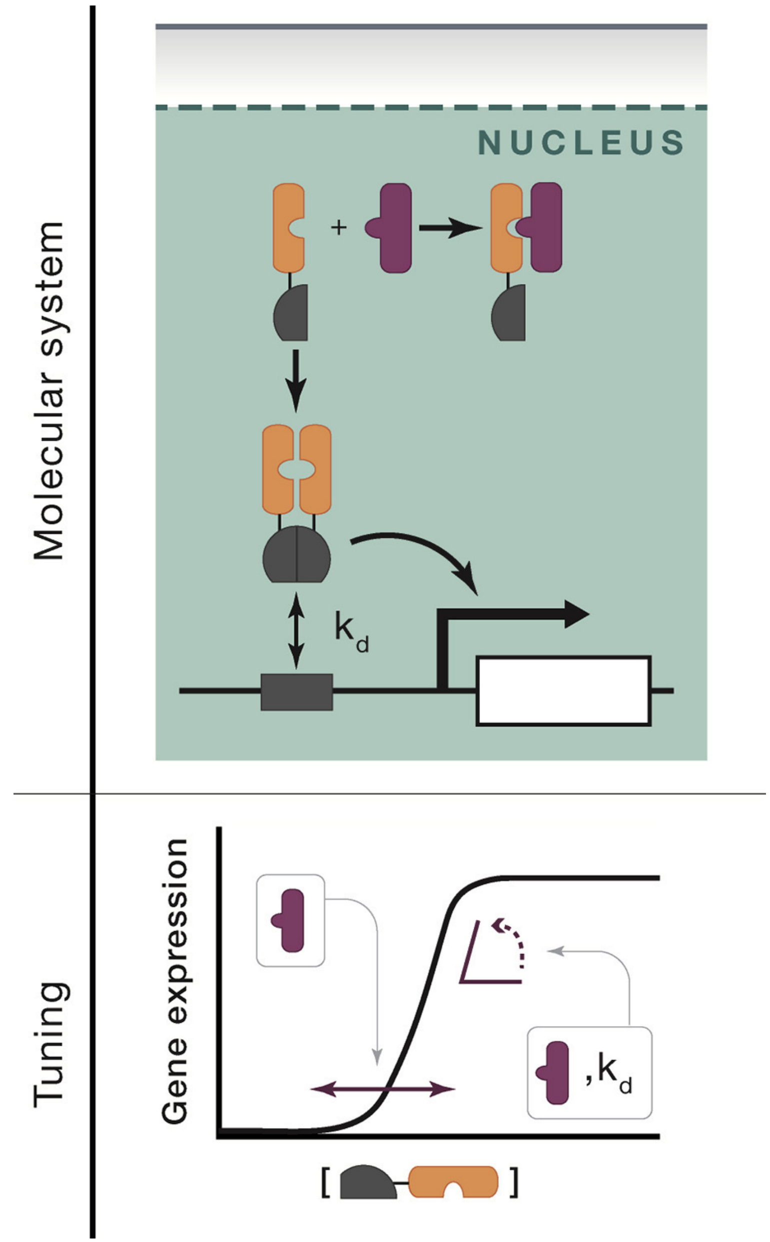
Molecular titration:

inhibitory molecule stoichiometrically binds to and inhibits a target

An analog-to-digital converter that makes use of intermolecular sequestration

Response threshold: f([inhibitor])

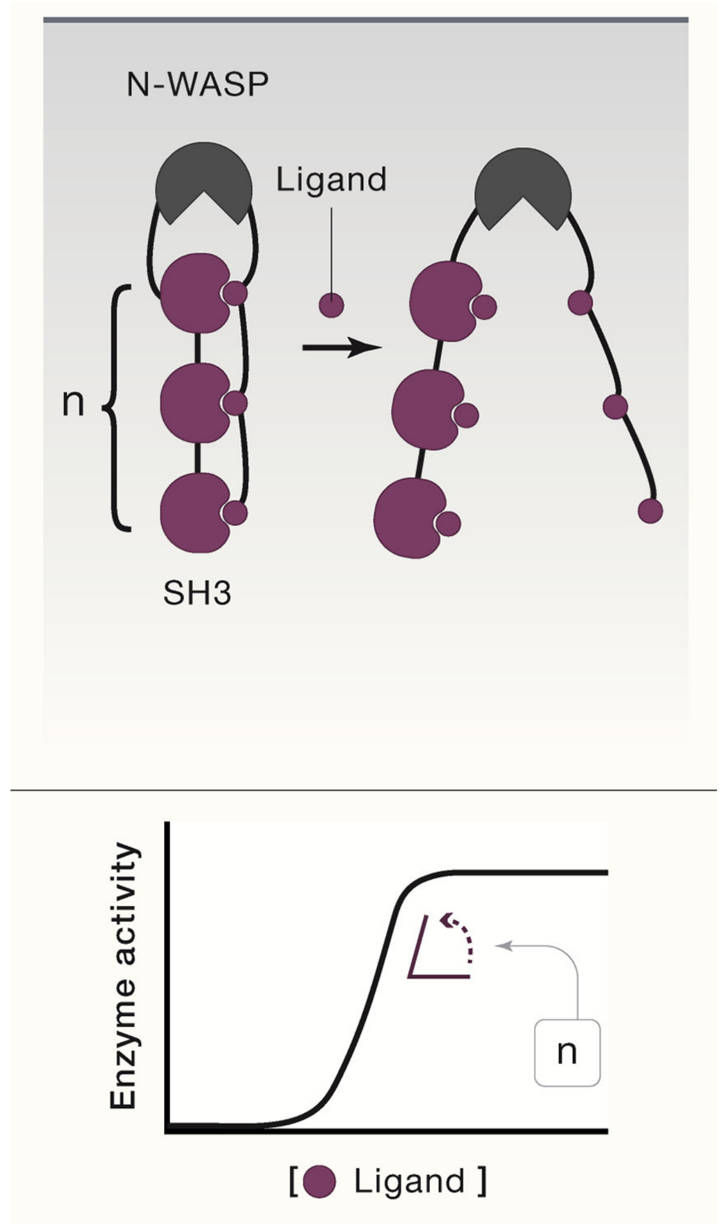
Response slope: f($Kd_{inhibitor}$ & Kd_{DNA})



Protein-based processors carry out analog-to-digital conversions

Intramolecular sequestration of the N-WASP domain by fused tandem repeats of SH3-ligand pairs results in **ultrasensitivity**

Response slope = f(the number of SH3-ligand interactions).



Quiz: synthetic versus natural signal processing systems

	natural	synthetic
orthogonal		
promiscuous		
signal outcome		
complex signal processing operations		

Quiz: synthetic versus natural signal processing systems

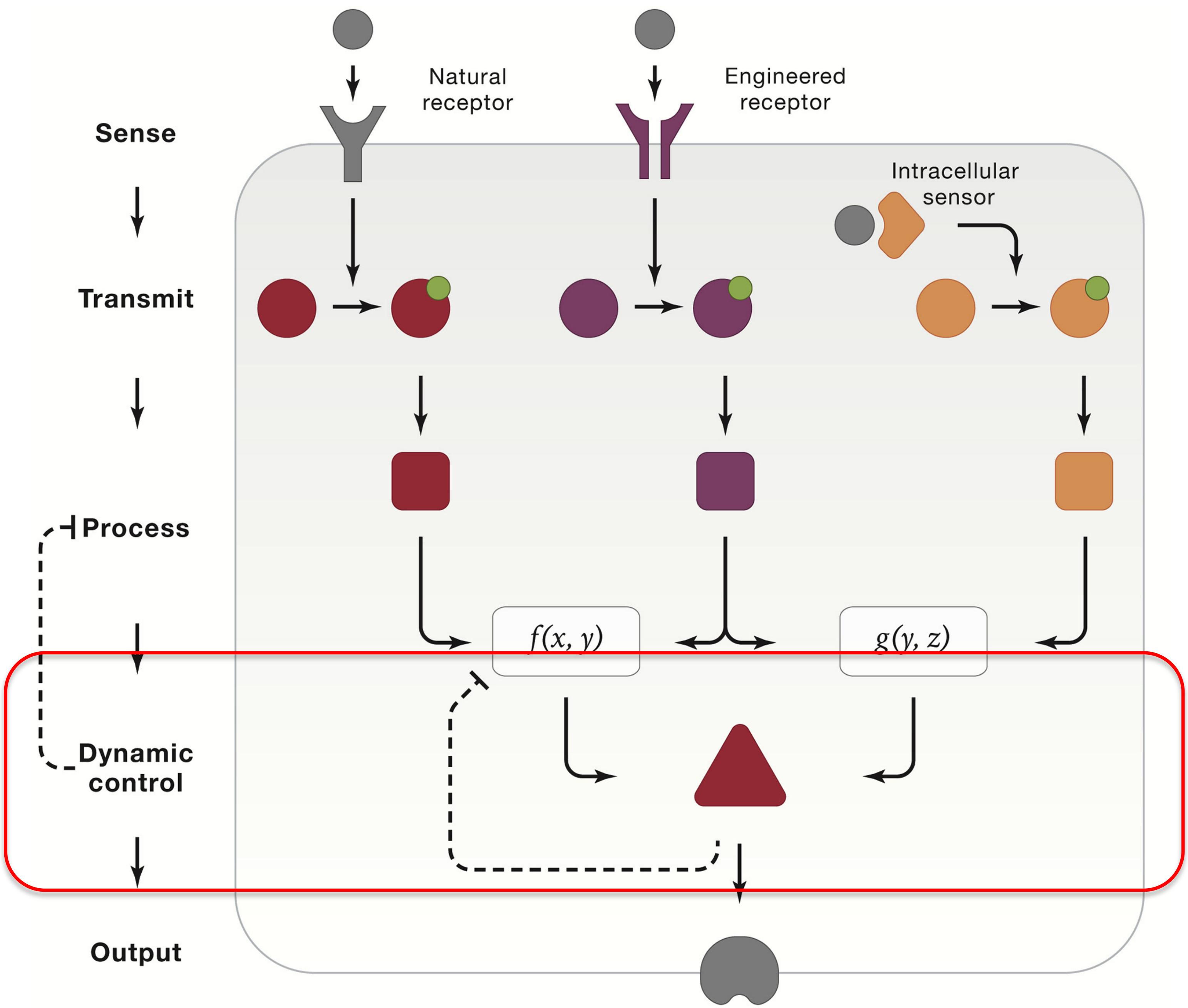
Synthetic signal processing schemes so far: signal is encoded in the concentration of **a particular** protein species.

Natural (especially mammalian) sensing systems: use of **promiscuous (many-to-many) interaction**s between sets of ligand and receptor variants to selectively respond to **complex** combinations of their **inputs**.

Computational approaches indicate that **competition** to form a variety of protein **complexes with different activities** can perform **complex signal processing operations**.

Can these principles be adapted to enable synthetic circuits with similar functions?

Generic protein circuit operational in a living cell



Generic protein circuit operational in a living cell

Dynamic control systems allow:

1. robust adaptation to the environment

2. oscillations and time-based regulation

3. the basis for cellular memory, etc…

Dynamic control

Many biological circuits have the following dual properties:

1. maintain constant output level across a broad dynamic range of steady-state input concentrations,

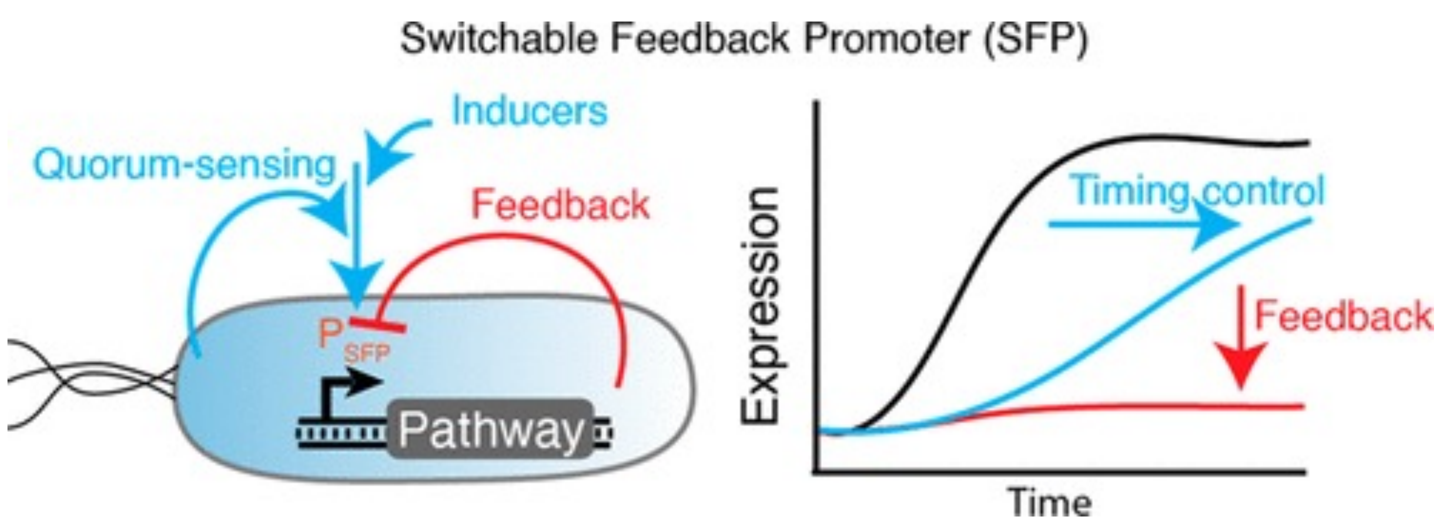
2. while also responding transiently to input perturbations.

Can you define one key circuit mechanism enabling such control?

Dynamic control

Can you define one key circuit mechanism enabling such control?

incorporation of feedback and feedforward loops



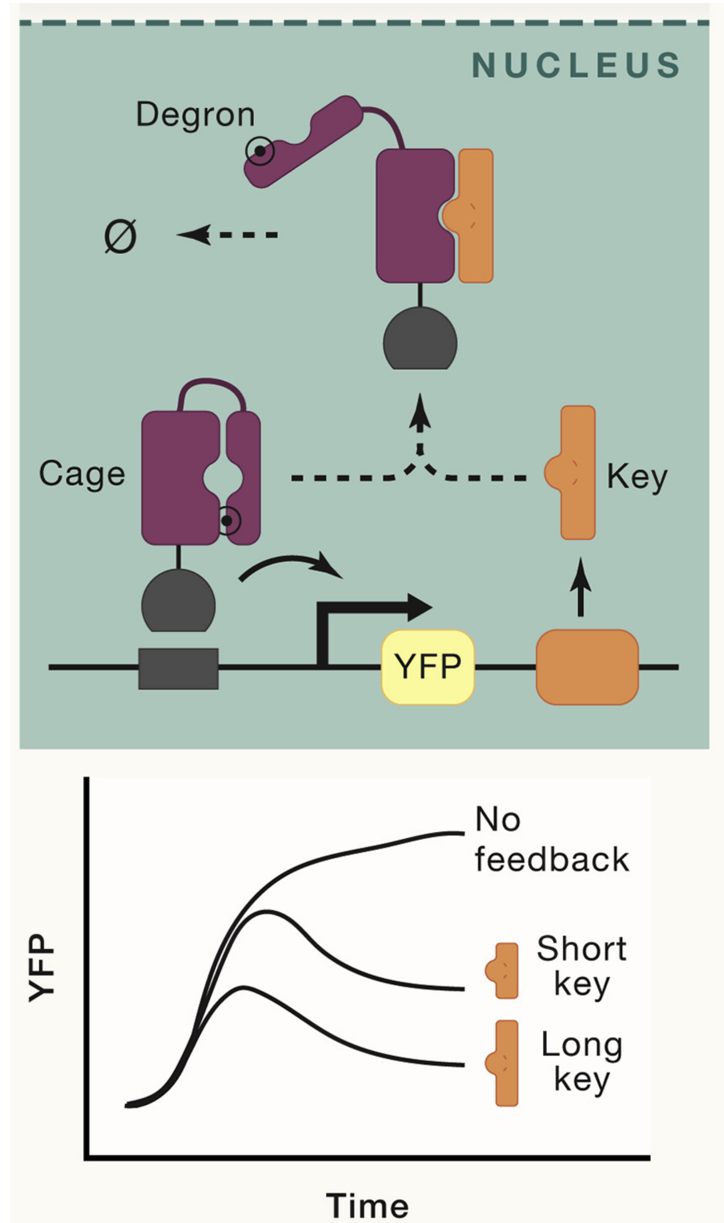
Protein-based dynamic control systems enable negative feedback control

A negative-feedback system based on LOCKR

Transactivator fused to the cage activates expression of a YFP reporter and the key

Cage-key binding => degradation of the cage-transactivator fusion & reduced YFP expression

Negative feedback strength = f(key length) ~ binding affinity to the cage



Dynamic control: Oscillation

Can you define two key periodic biological processes?

Dynamic control: Oscillation

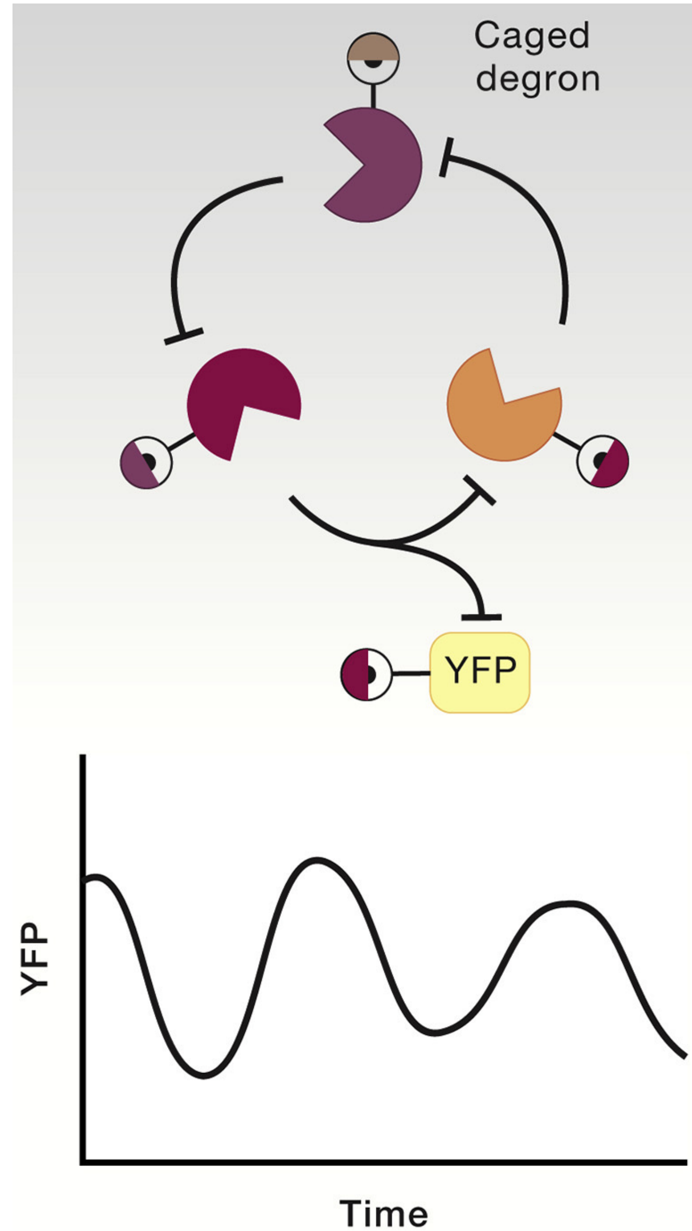
Can you define two key periodic biological processes?

the cell cycle and circadian clock

Periodic processes sequentially advancing the cell from one stage or phase to the next before restarting again from the beginning

Protein-based dynamic control systems enable oscillation

Three proteases that inhibit each other by exposing caged degrons lead to oscillatory behavior in bacteria



Dynamic control: Memory

Memory allows cells to **alter** their **behavior** depending on their **own individual history**.

=> *use protein-based circuits to **store** and **read** out information encoded in the states of proteins or DNA, providing a foundation for protein-based memory*

Can you define one mechanism for information storage in natural cells other than the DNA itself?

Dynamic control: Memory

Can you define one mechanism for information storage in natural cells other than the DNA itself?

chromatin-based epigenetic memory system:

The chromatin can **store** information in **DNA or histone modifications**

In natural epigenetic systems, **chromatin regulators** actively **propagate** these modifications, ensuring their **stable mitotic inheritance**

Protein-based dynamic control systems enable multistability and memory

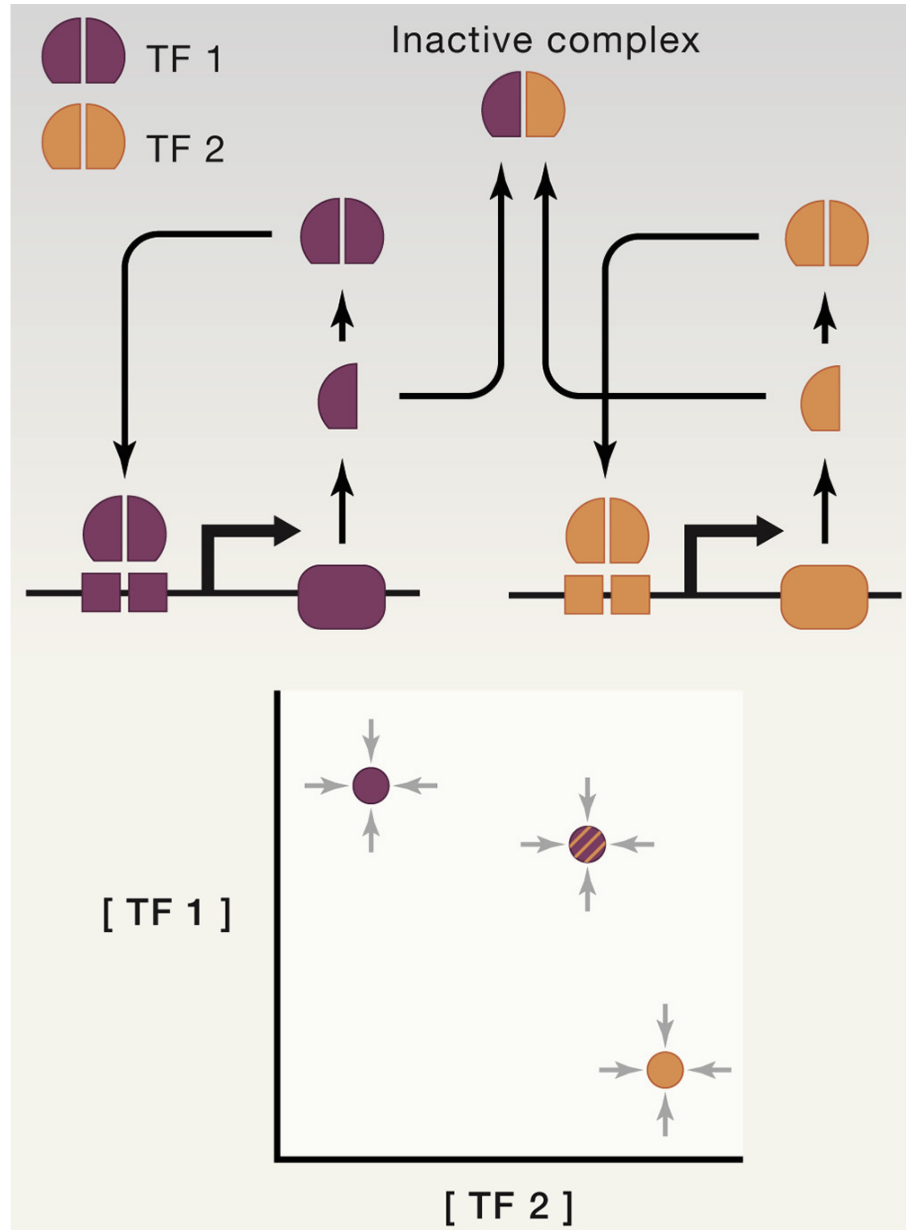
Engineered transcription factors:

1. **positively** autoregulate their own expression as **homodimers**

2. **inhibit** one another's activity through **heterodimerization**

=> **multiple stable states** with different levels of TF expression, e.g. 3 TFs => 7 distinct states stable for weeks

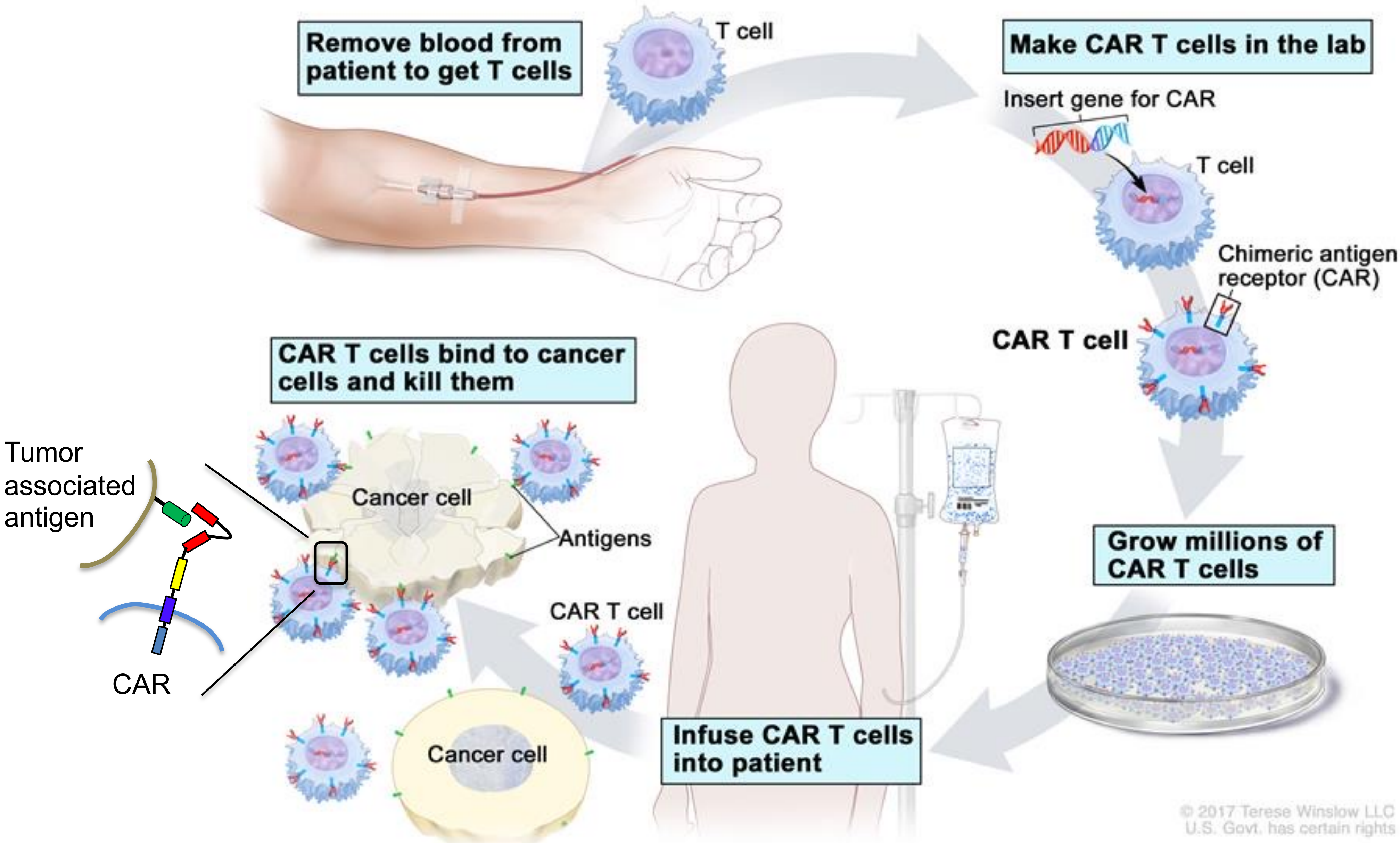
=> ***scalable*** *architecture,* ***exponentially increasing*** *numbers of cell states with additional engineered TFs*



Applications of synthetic protein circuits

Engineered cell-based therapies

Adoptive CAR T cell therapy



(NIH NCI)

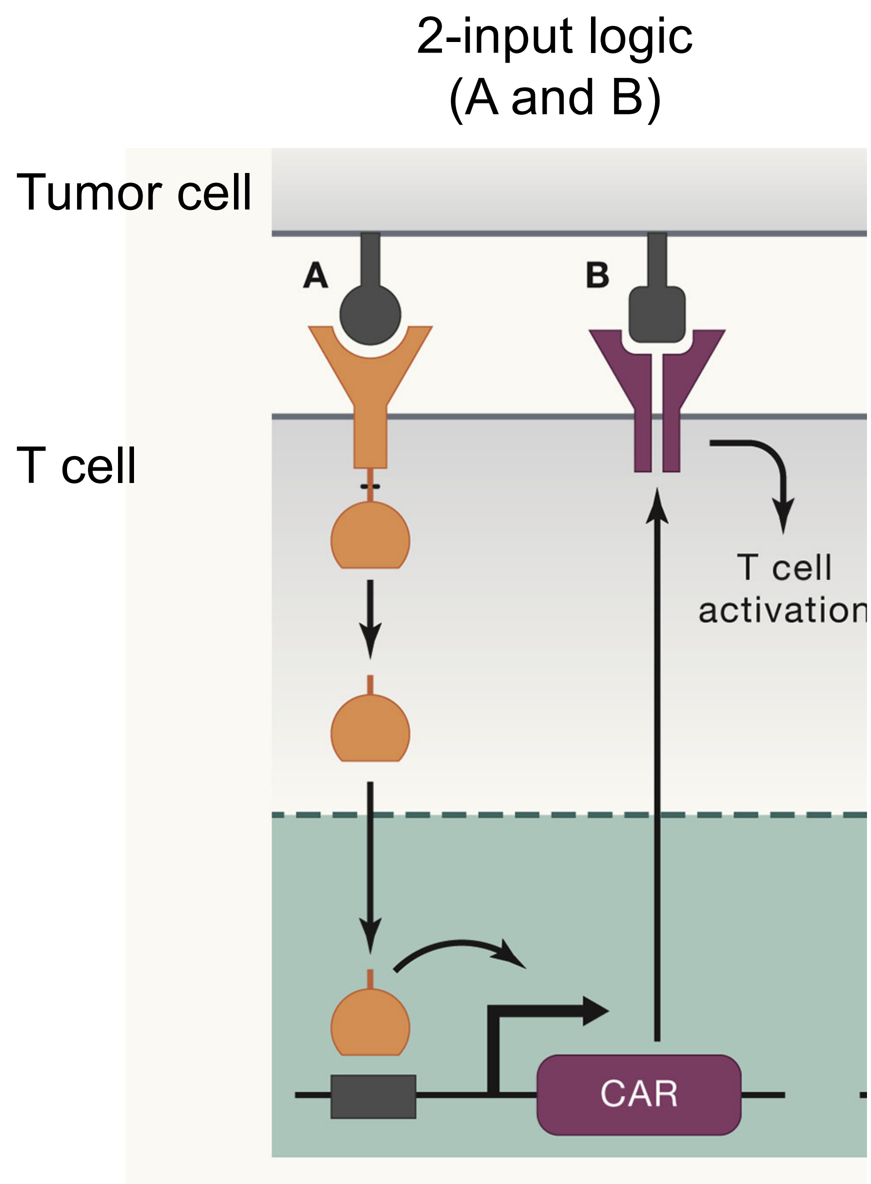
Current limitations of adoptive CAR T cell therapy

1. Lack of **tumor specificity** (many tumor associated antigens are also found at the surface of normal cells)

2. Lack of **sustained responses** in immunosuppressive environment

Protein circuits can provide useful capabilities for cell-based therapeutics

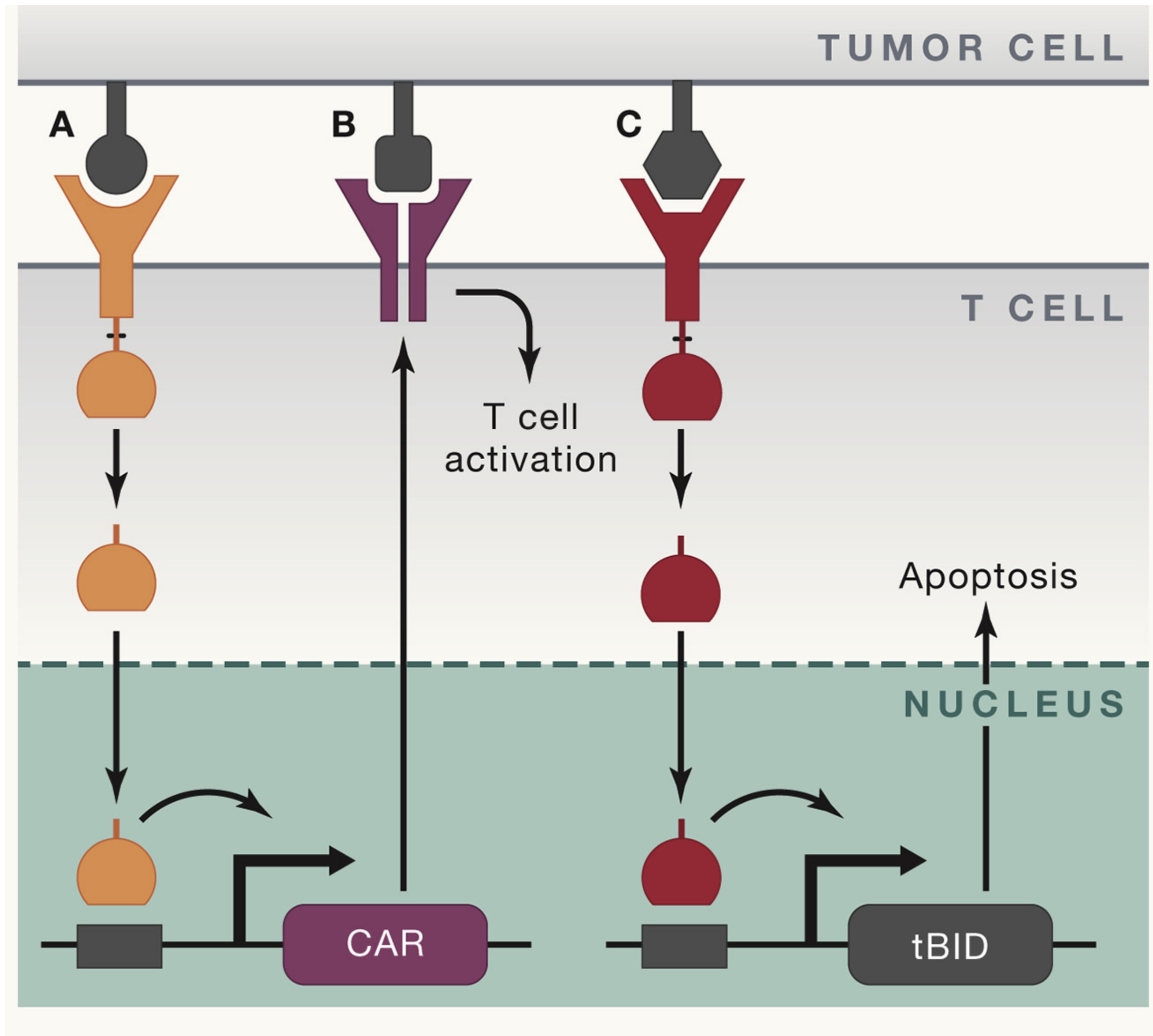
In the SynNotch "(A AND B)" logic gate, antigen A activates a SynNotch receptor, causing expression of a chimeric antigen receptor (CAR), which recognizes the second antigen B and triggers the T cell response.



Protein circuits can provide useful capabilities for cell-based therapeutics

3-input logic
(A and B) not C

In the SynNotch "(A AND B) NOT C" logic gate, Recognition of a third antigen, C, by an additional SynNotch, leads to expression of truncated BID (tBID) to trigger apoptosis



Engineering advanced logic and distributed computing in human CAR immune cells

Cho, J.H., et al. *Nat Commun* **12,** 792 (2021)

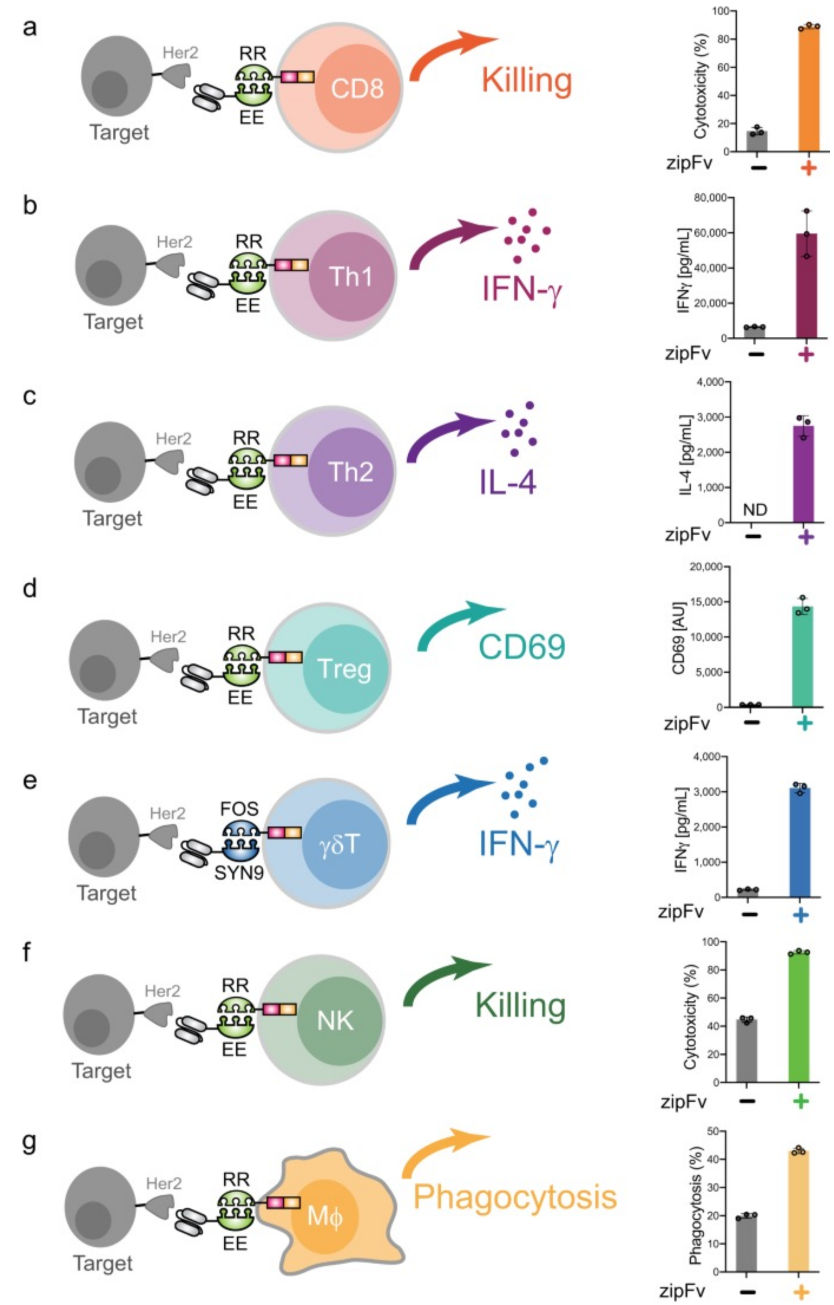
SUPRA CARs can activate diverse adaptive and innate immune cell types

Split, universal, and programmable (SUPRA) CAR system to improve specificity and controllability.

The SUPRA CAR system composition:

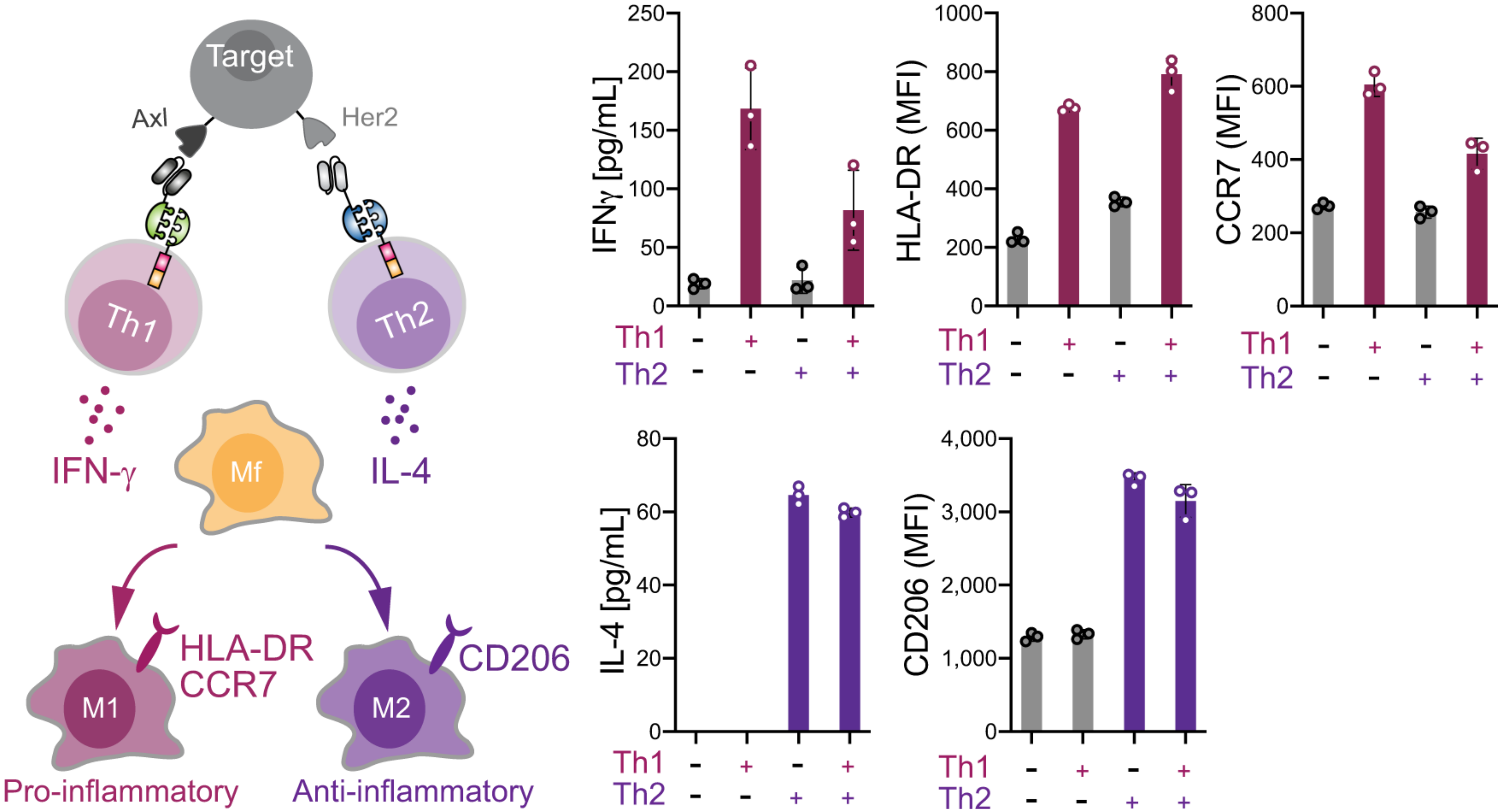
1. **soluble antigen-binding portion,** zipFv

2. universal **signal transduction receptor,** zipCAR, expressed on T cells



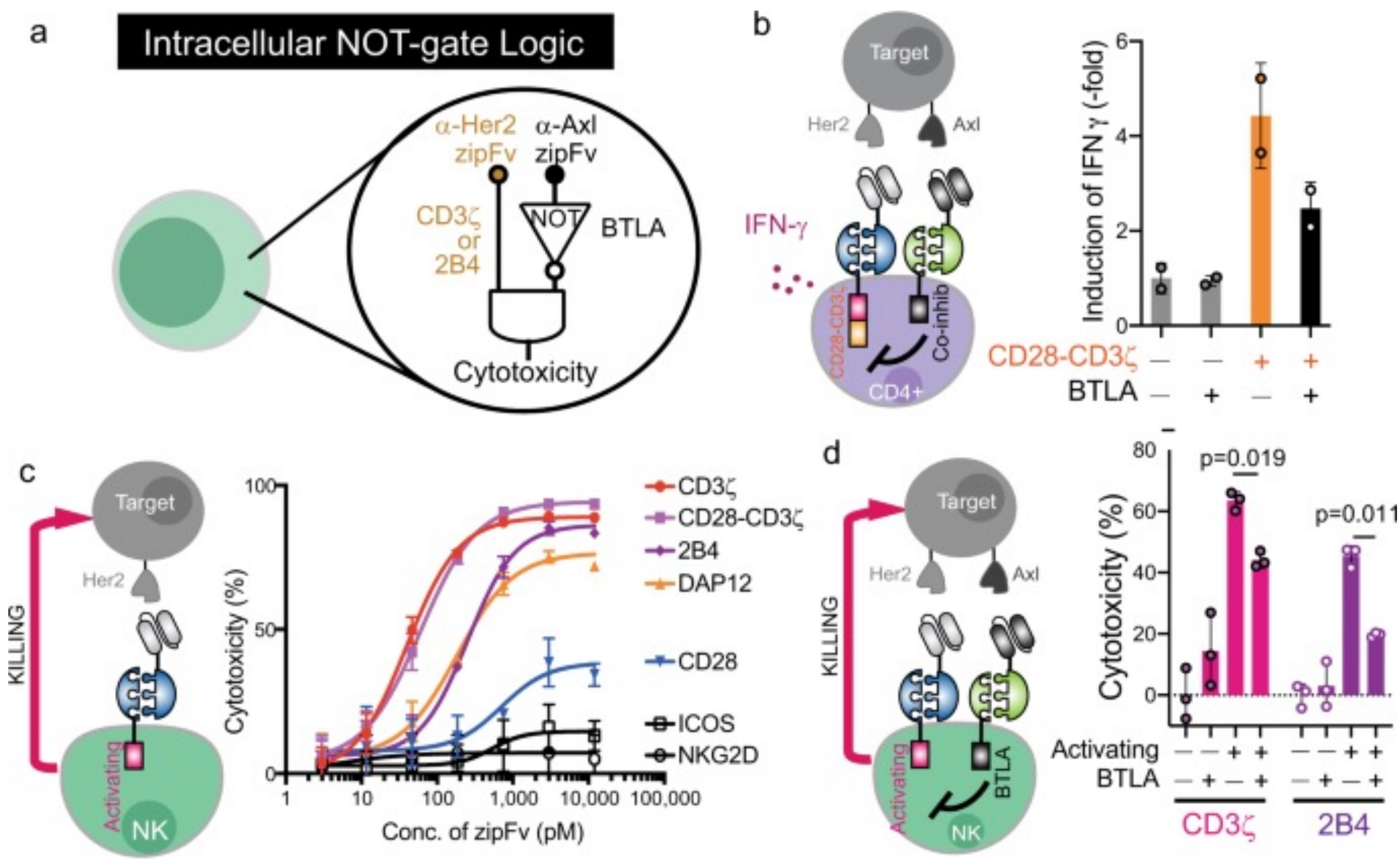
Engineering endogenous immune system with SUPRA CAR-expressing different T-cell subtypes

Controlling macrophage polarization by zipCAR-expressing Th1 and Th2 cells using RR zipCAR and FOS zipCAR

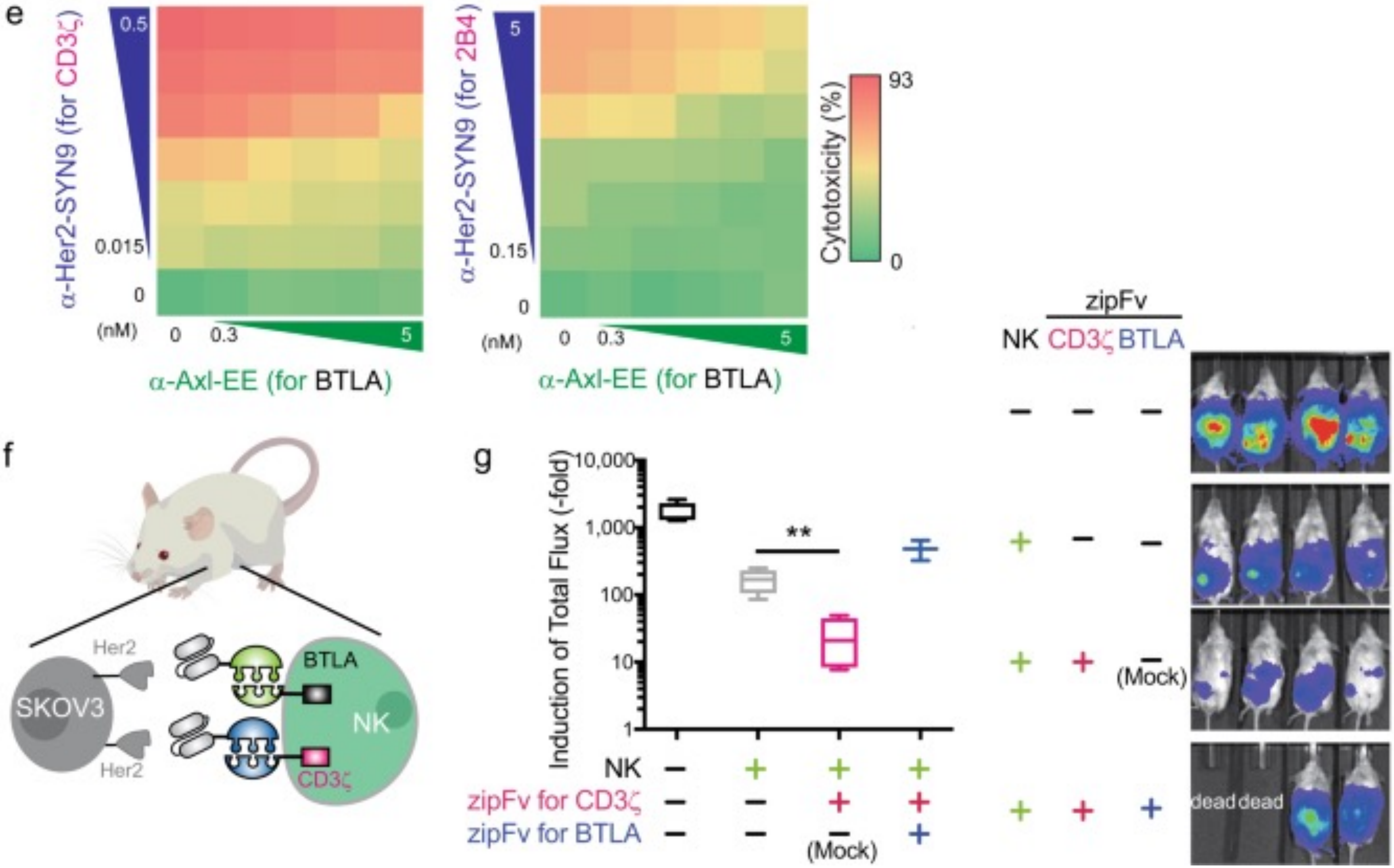


The intracellular NOT logic with BTLA in different cell types

T cells transduced with a FOS zipCAR that contains CD28 and CD3ζ signaling domains and a RR zipCAR with different inhibitory domains



The intracellular NOT logic with BTLA in different cell types



Take home messages

Protein circuits can be used to engineer advanced logic operations in mammalian cells, including sensing, signal transmission, processing and dynamic control

Direct therapeutic applications

Questions?