Synthetic Biology Cellular Devices: Mammalian Cell Based Theranostic Systems

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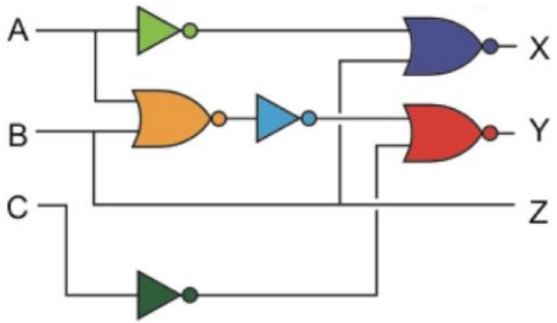

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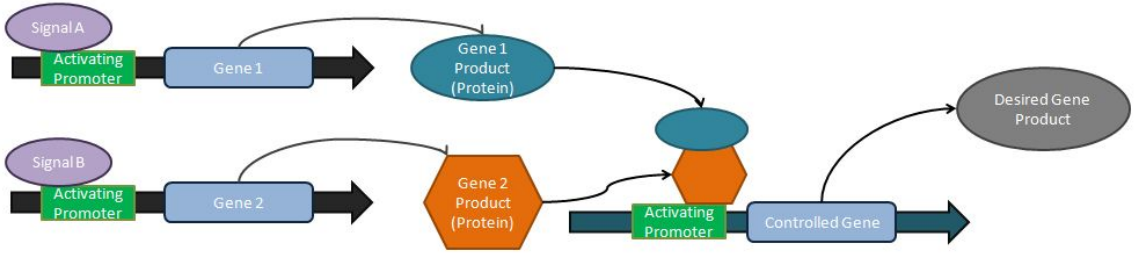
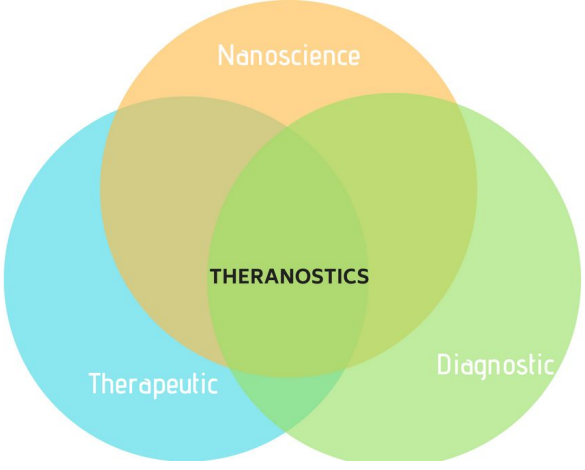
Introduction: Synthetic Biology & Gene Circuits

- What is Synthetic Biology?
  - Redesigning organisms for useful purposes
  - Engineering them to have new abilities
  - Used in agriculture, biofuels, plastics, food, health and life sciences
- Synthetic Biology Cellular Devices
  - Integrate human design control systems into biological environments



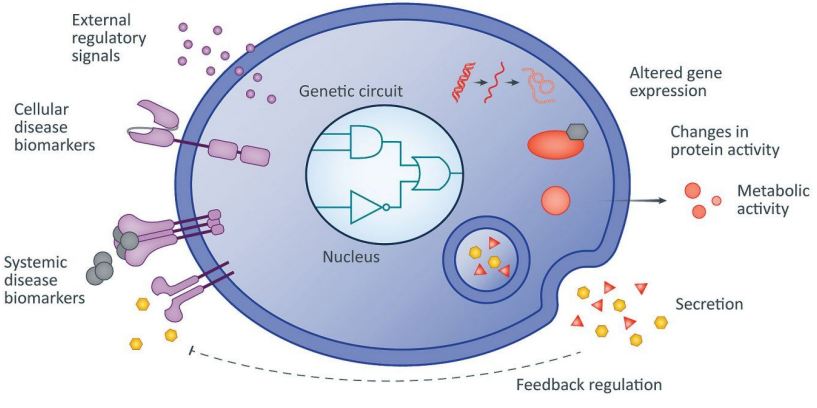
Introduction: What are Theranostic Devices?

- Theranostic Devices
  - Theranostic = Diagnostic + Therapeutic
  - Maximize therapeutic effects while minimizing undesired side effects [3]
  - Personalized to an individual's microenvironment



Introduction: Mammalian Cell Based Theranostics

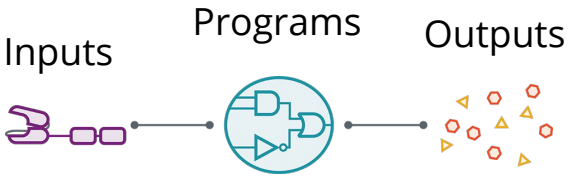
- Why do we need them?
  - Allow us to develop **programmable** and more **sophisticated** therapies
  - ‘Biofactories’ - produce medicine within the body for **targeted** delivery
  - Engineered mammalian cells have a much wider spectrum of diseases that can be treated because various receptors can be used as disease biomarkers
- What are they?

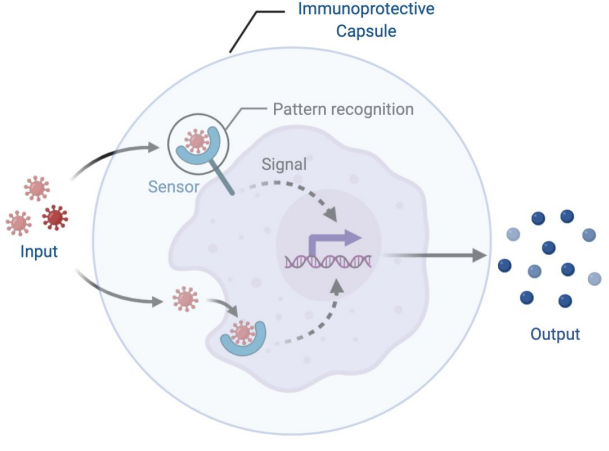


Developing a Model System: How does it work?

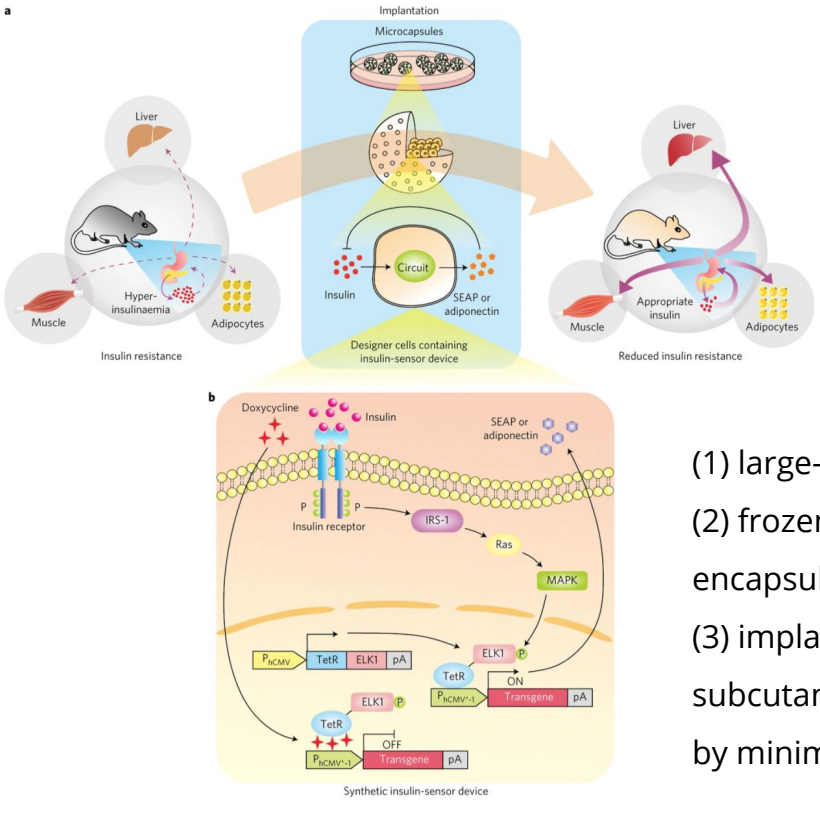
Every cell based theranostic has three main components:

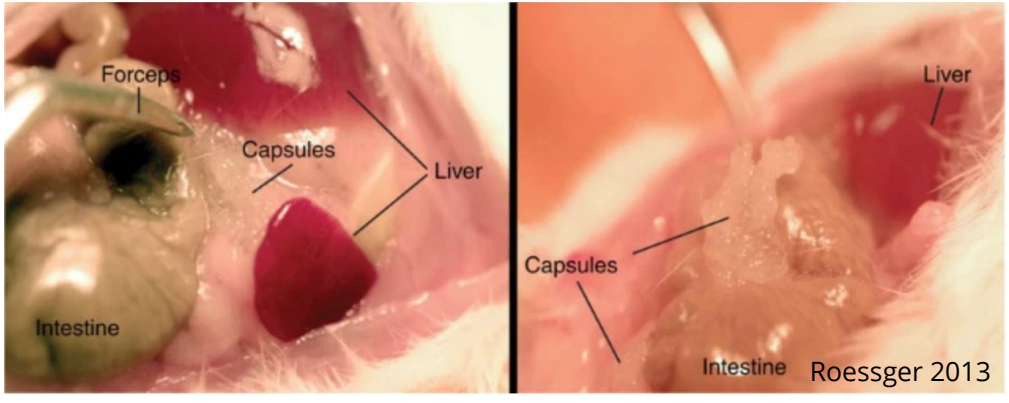
- Software
  - Closed loop control (what are the inputs, outputs, and control logic?)
- Hardware
  - What cells are we using, do we need to engineer special receptors?
- Accessories
  - Do we need to encapsulate the cells in biomaterials to promote survival?
  - How do we deliver the theranostic?





Developing a Model System: How is it deployed?





(1) large-scale manufacturing of patient-specific designer cells

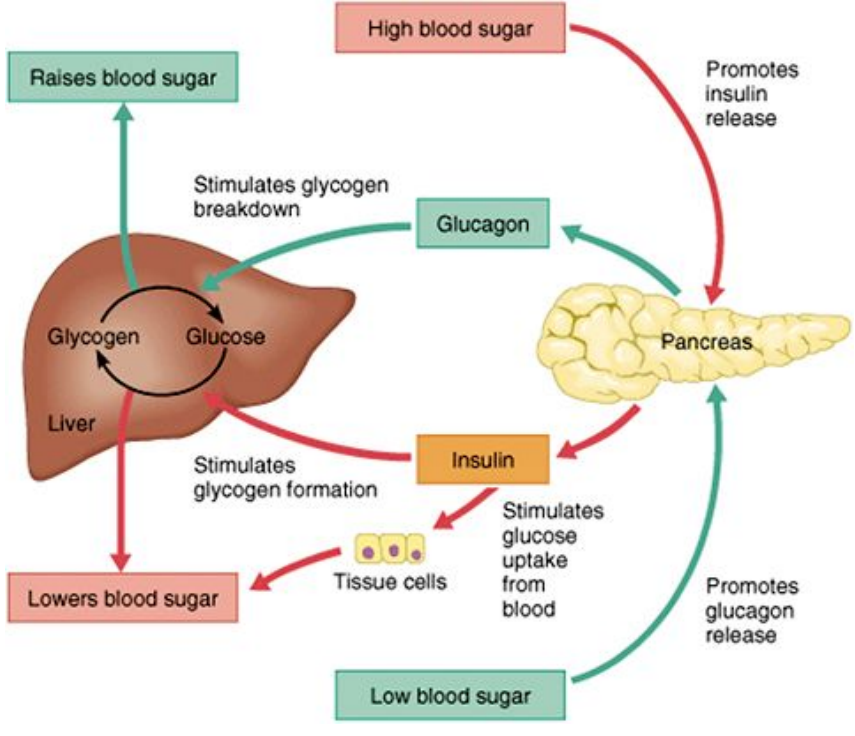
(2) frozen storage of the designer cells, either before or after encapsulation inside a vascularizing immunoprotective container

(3) implantation of the encapsulated designer cells, preferably subcutaneously where they can easily be replaced at regular intervals by minimal ambulant intervention in the event of fibrosis.[1]

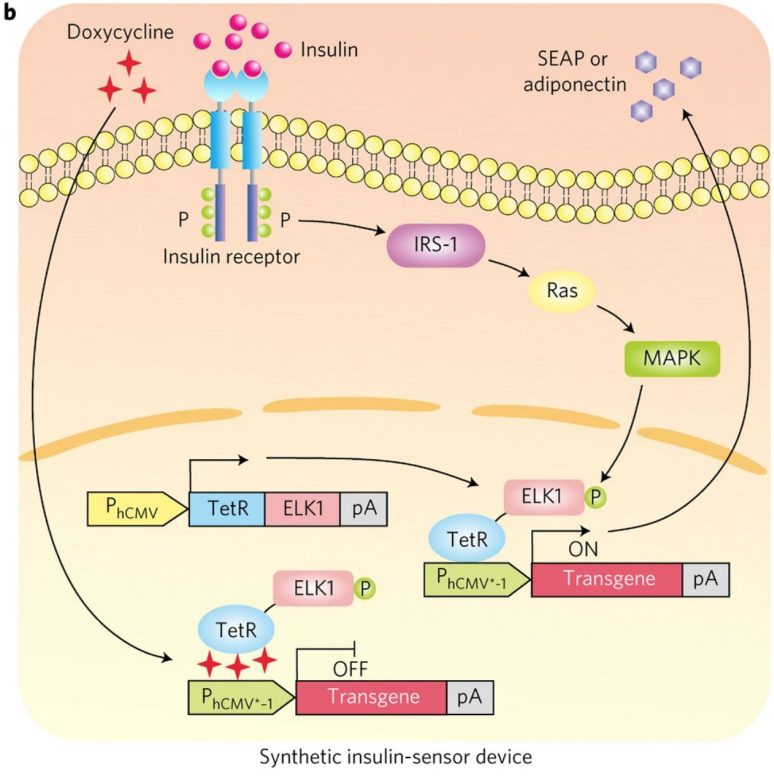
1. Ye 2016

Uses in Treating Metabolic Disease

- >1/10 Americans have diabetes & 1/3 have pre-diabetes -> $15.7 B per year
- Standard of care involves external insulin control: finger pricks, insulin injections, behavioral therapies
- No cure. Typical patient does **treatment for life**



“Self-adjusting synthetic gene circuit for correcting insulin resistance” Nature BME Ye et al.2016



Synthetic insulin-sensor device

Key Circuitry Features

- Synthetic TetR-ELK1 transcription factor and PhCMV*-1 promoter were designed and coordinated with native intracellular IRS-1–Ras–MAPK signalling cascade
- Transgene transcribes adiponectin, a hormone with insulin-sensitizing, anti-atherogenic, and anti-inflammatory effects
- Doxycycline (antibiotic) acts as a ‘kill switch’
- Circuit was expressed in CHO, HeLa, hMSC, hMSC-TERT, and HEK-293 cells with varying induction (all could successfully discriminate hi-Insulin vs lo-Insulin).

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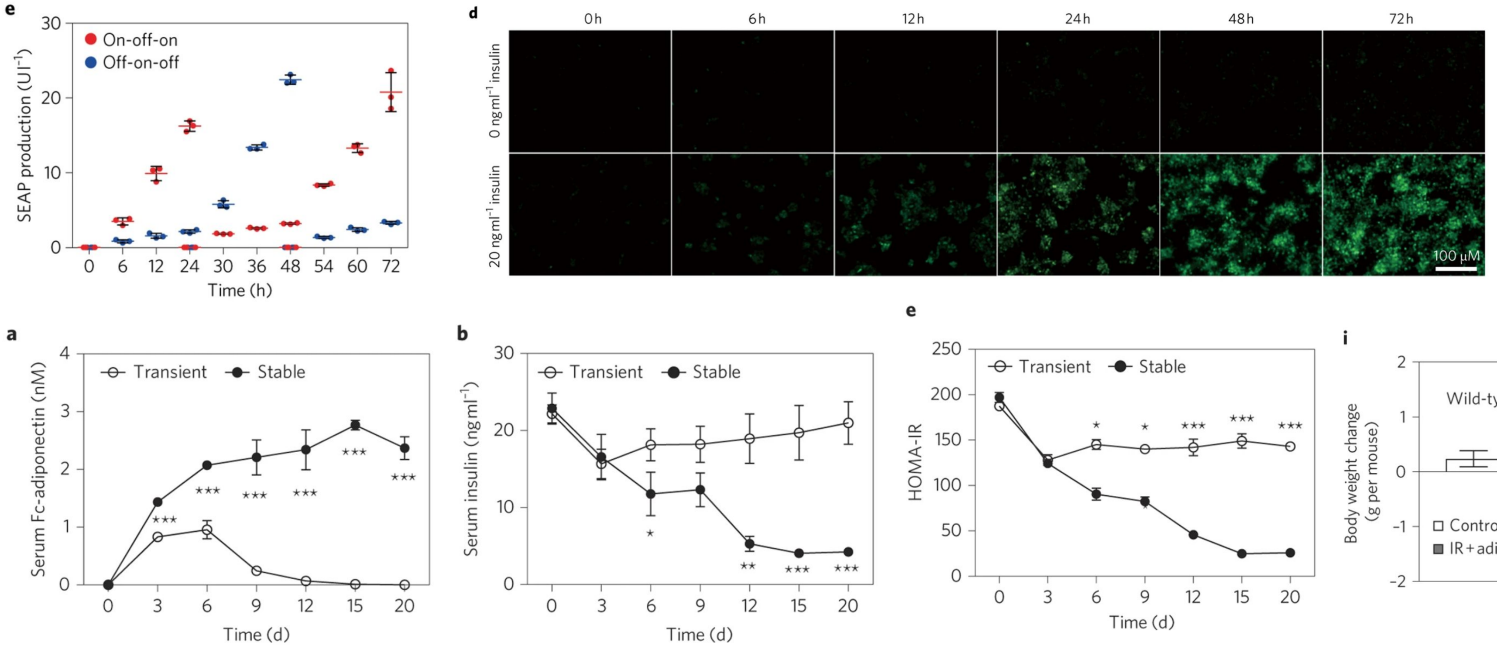
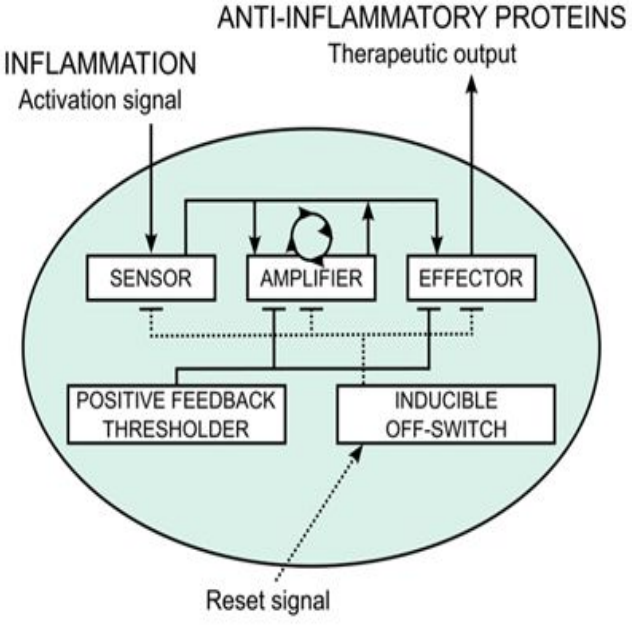
- Theranostics potentially offer **long-term automated treatment**



Uses in Treating Inflammatory Disease

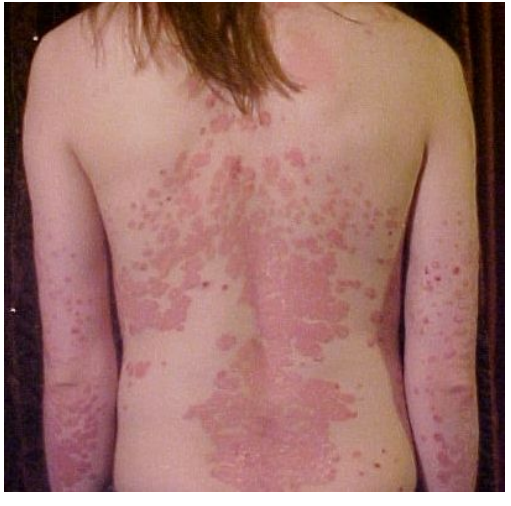
- Inflammation is body's immune response to pathogens
  - Deregulated inflammation lead to autoimmune diseases
    - Inflammatory bowel disease, rheumatoid arthritis, psoriasis
- Current treatments suppress immune system, weakening our body's defenses
  - Need theranostic to detect and treat flare ups only
- Proposed gene circuit involves: sensor, amplifier, thresholder, and effector



“Implantable synthetic cytokine converter cells with AND-gate logic treat experimental psoriasis” Schuker et al.2016

Background

- Psoriasis is chronic inflammatory disease affecting the skin
  - Marked by flare ups of tumor necrosis factor (TNF) and interleukin 22 (IL22)
- Clinical trials using immunomodulatory cytokines IL4 and IL10 showed rapid patient improvement
  - Half lives of these cytokines are short
  - Daily injections decrease patient compliance



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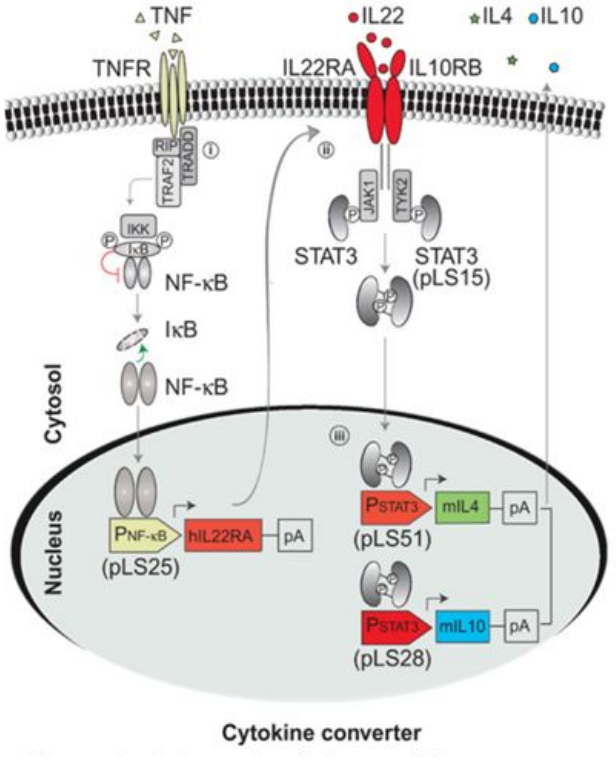
Cytokine Converter: Key Circuitry Features

- Connect TNF receptor (TNFR) signaling to promoter controlling expression of human IL22 receptor α (hIL22RA)
- IL22 activated hIL22RA to produce a cascade connected to synthetic promoter driving IL4 and IL10 expression
- Create AND-gate: IL4 and IL10 produced only when TNF and IL22 (marker of psoriatic flare) are present



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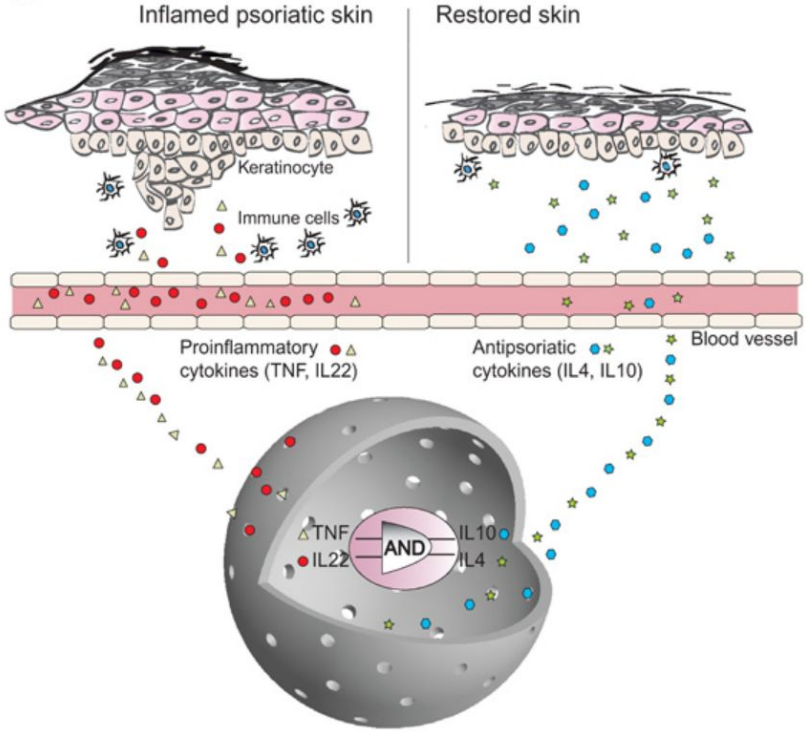

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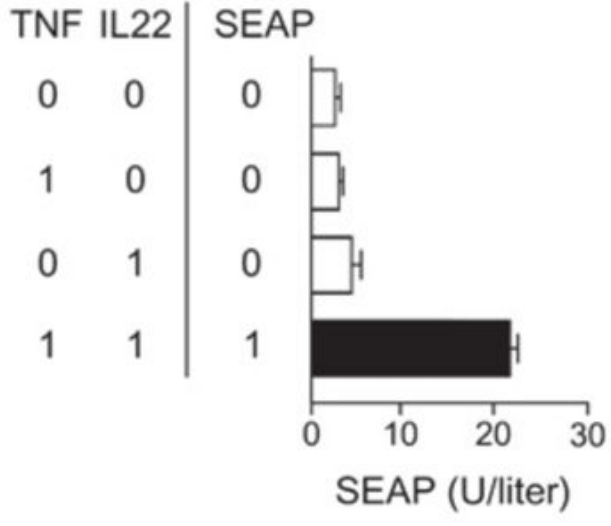
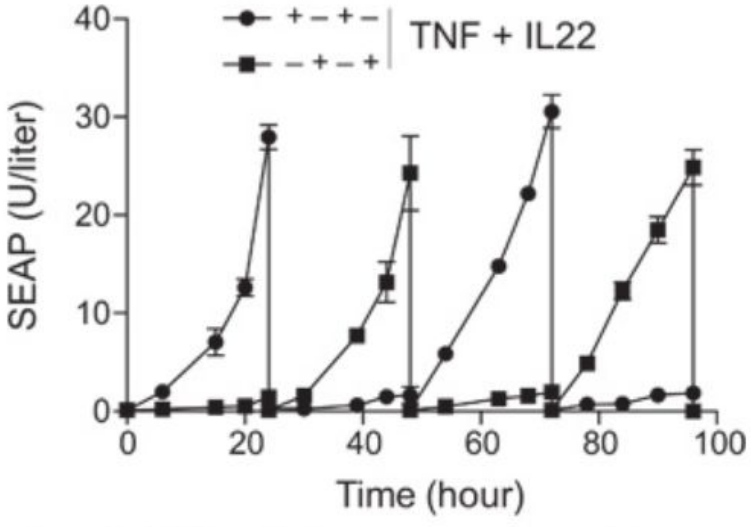
AND-gate Efficacy

- AND-gate shown to only produce output when both TNF and IL22 were present
- AND-gate expression is reversible and can be turned on and off





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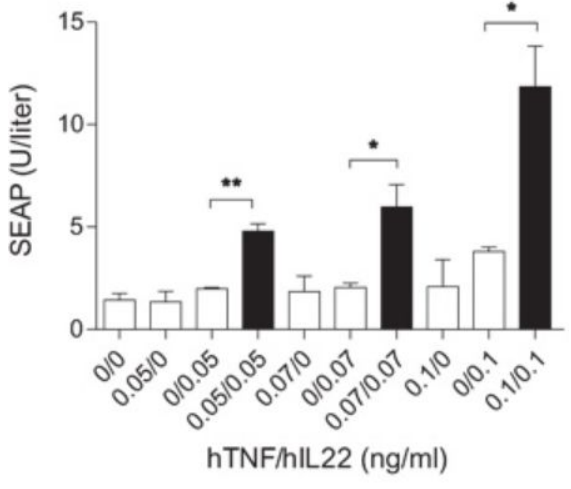
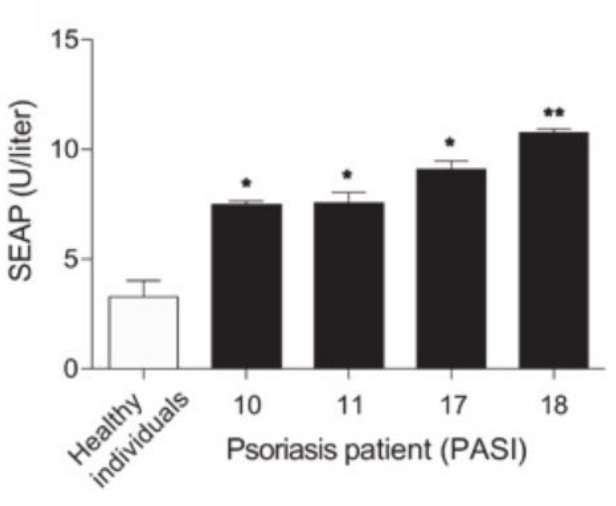
Physiological Effects and Results

- Synthetic cell device implemented in mouse models, prevented formation of psoriasis-like plaques and attenuated established psoriasis-like plaques
- AND-gate successfully detected TNF and IL22 in human patient blood samples



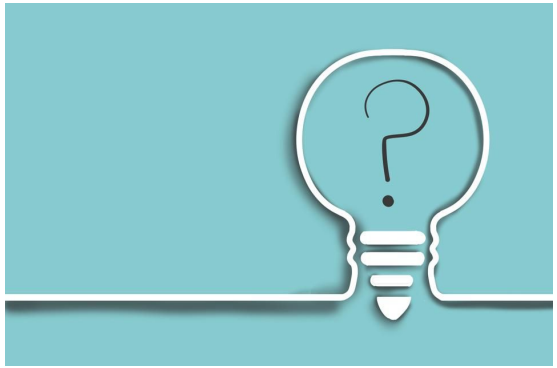


Limitations

Delivery	Sustained delivery of cells to a target is an issue. Transcriptional control is too slow.
Safety	Engineered cells need to be guarded against unexpected failure (out of control circuit) or mutation.
Reliability	Delivered cells must sustain and remain reliable through the lifecycle of the cell and also through proliferations.
Complexity	Complexity of mammalian gene networks, difficulty of multiple gene manipulation

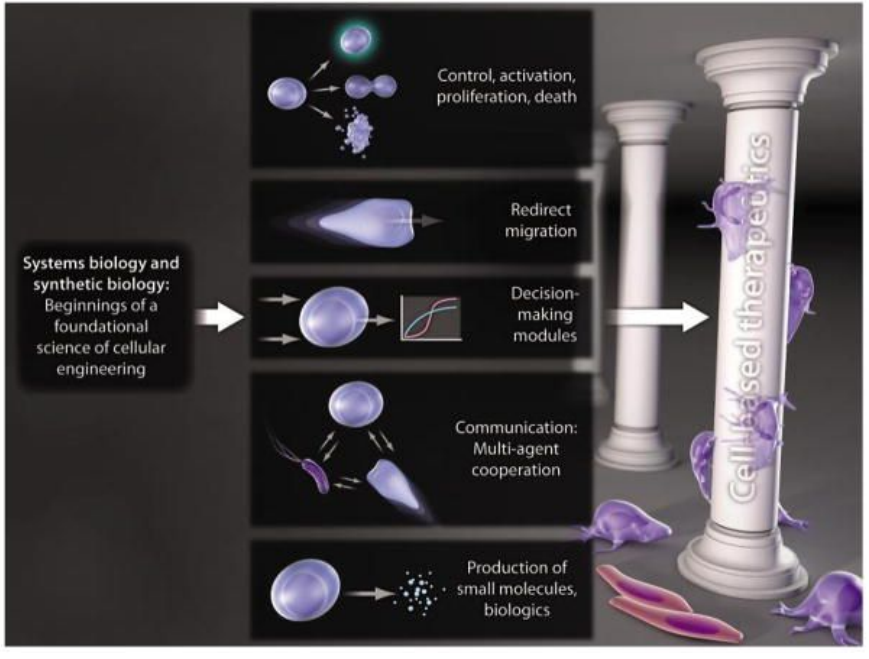
Ethical Concerns

- Will therapeutic cells be reliable and predictable with respect to their proliferative capacities, localization, behaviors, and mechanisms of action?
- Will engineered regulatory circuits be robust enough to remain in control of a cell even if it mutates in the host?
- What are the long-term effects of cell therapies?



Future Directions

- Increasing efficiency of cancer theranostics
- Complex decision making capacity → Process multiple disease input and provide multiple therapeutic output
- Sophisticated automated theranostics



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Summary

- Introduced synthetic biology, gene circuits, and mammalian cell theranostics
- Described current research methods and development of such technologies
- Demonstrated efficacy of such a strategy in both metabolic and inflammatory disease settings
- Outlined limitations, ethical considerations, and future direction of these systems as therapeutics

Conclusions

- Synthetic biology–based gene circuits are uniquely suited for the treatment of diseases with complex dynamics because they can autonomously couple the detection of disease biomarkers with the production of therapeutic proteins in situ
- Sophisticated genetic devices can be assembled to reprogram mammalian cell activities using tools from synthetic biology
- Self-sufficient synthetic gene circuits could become of clinical relevance in the not-too-distant future

Clinical Translation

- Cell Therapy Market was estimated at $7.75 billion in 2019 and is expected to hit $48.11 billion by 2027, registering a CAGR of 25.6% from 2020 to 2027[1]
- Senti Biosciences: Founded by Tim Lu, Jim Collins, and others (MIT) and advised by Martin Fussenegger (Basel) is bringing gene circuit cellular devices to market via a combination of in-house and pharma partnership approaches (founded in 2016, with $53 M in VC funding[2])
- Immediate applications in next-gen CAR-T (SBIR - Synthetic Biology Gene Circuits for Cancer Therapy), stem cell replacement therapies, organoids

Cell based theranostics are at the cusp of clinical translation

1. Allied Market Research 2. Crunchbase

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