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# Emergent dynamics of cellular decision making in multi-node mutually repressive regulatory networks

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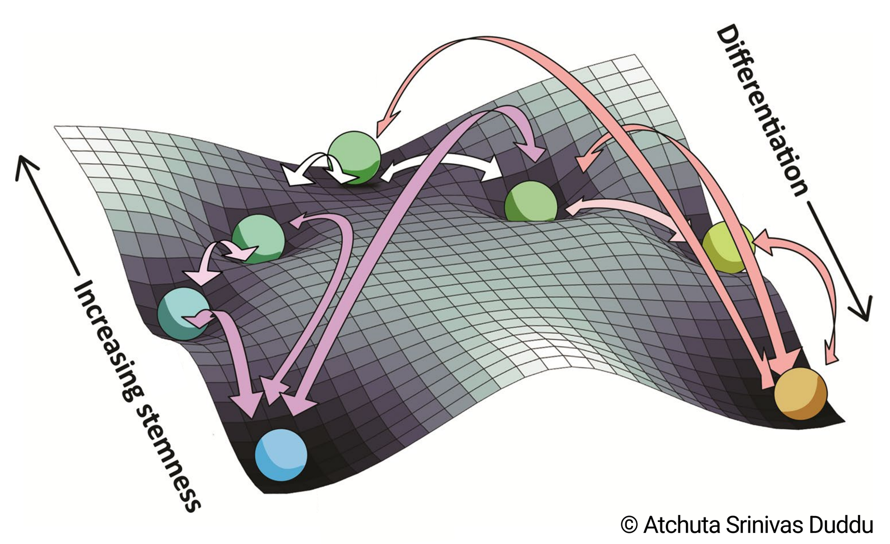
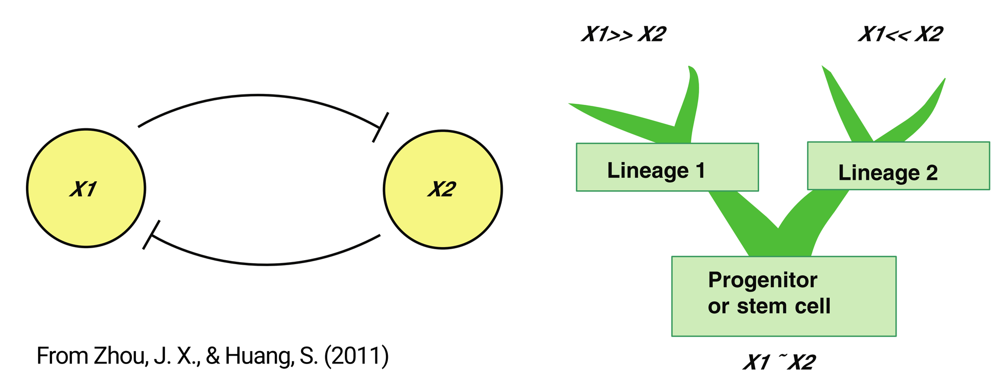
## Introduction

Cell fate determination ≡ Wadington landscape
Valley: Terminally differentiated cell fate
Gene regulatory networks regulate trajectories




Cell-fate choices between two possible states
Toggle Switch: Mutual inhibition b/w
2 cell-state specific transcription factors




Generalize to n-terminal states?

**Can Toggle-n networks capture differentiation into n-terminal states ?**

## Methods

Adjacency Matrix:

$$Adj_{ij} = \begin{cases} +1 & \text{if } i \text{ activates } j \\ -1 & \text{if } i \text{ inhibits } j \\ 0 & \text{if no interaction from } i \text{ to } j \end{cases} \qquad Adj_{ij}(Toggle) = \begin{cases} -1 & \text{if } i \neq j \\ 0 & \text{if } i = j \end{cases}$$

Asynchronous boolean simulation with Ising formalism

$$\mathbf{x} \in \{-1, +1\}^n \quad \text{Where, } x_i = -1 \implies Low; \; x_i = +1 \implies High$$

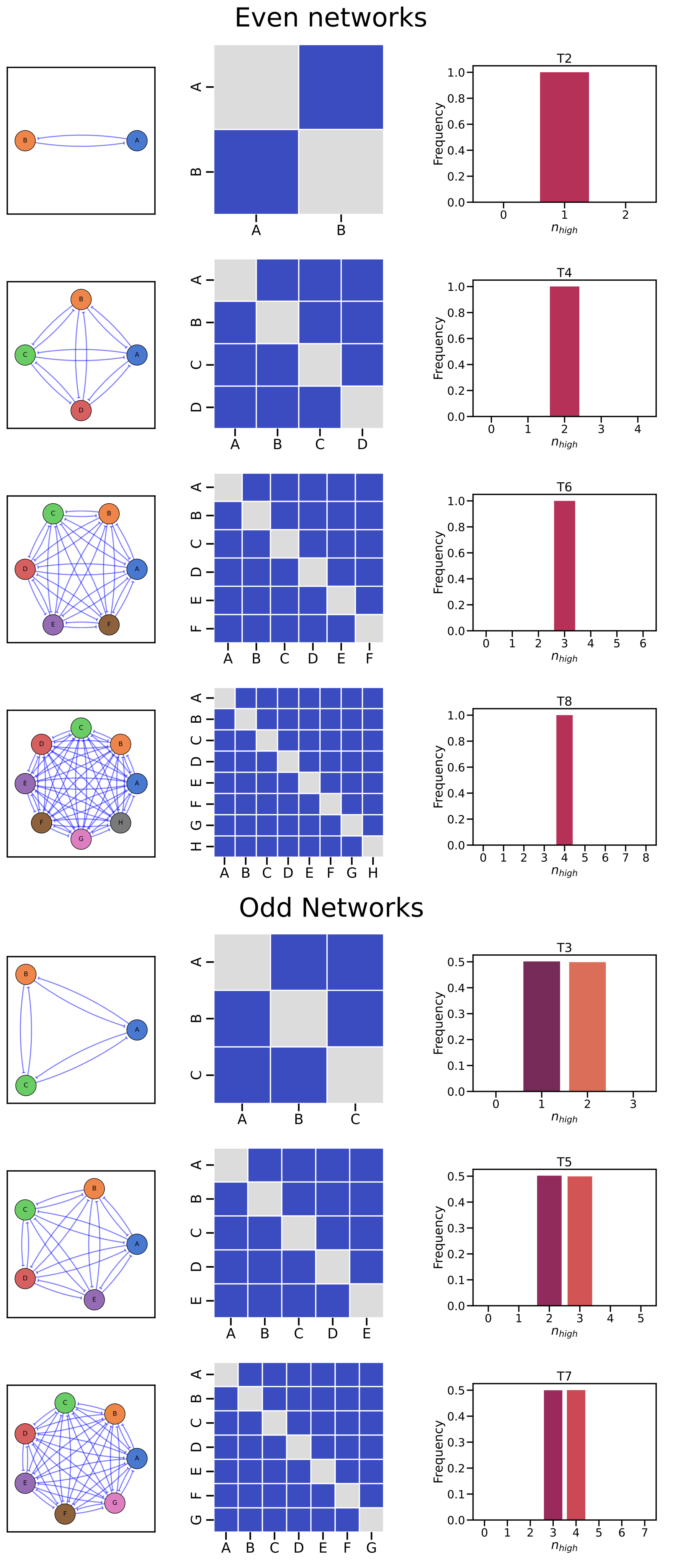
Choose random node k

$$x_j(t+1) = \begin{cases} +1 & \text{if } \sum_i x_i(t) \cdot Adj_{ij} > 0 \;\&\; j = k \\ -1 & \text{if } \sum_i x_i(t) \cdot Adj_{ij} < 0 \;\&\; j = k \\ x_j(t) & \text{otherwise} \end{cases}$$

## Toggle-n networks allow the cell to bifurcate into precursor lineages

Network | Adjacency | Steady State Freq.

Even networks




## Theorem

*Ising model update for network Tn for any n ≥ 2, applied to any initial condition will converge to a steady state with k high states, where*

$$k = \begin{cases} \frac{n}{2} & n \text{ is even} \\ \frac{n-1}{2} \text{ or } \frac{n+1}{2} & n \text{ is odd} \end{cases}$$

**Proof**

Let A denote the set of nodes with $x_i = +1$ and B denote the set of nodes with $x_i = -1$. And, $|A| = k$ and $|B| = s$, with $k + s = n$

$$H = -\sum_{ij} Adj_{ij} x_i x_j$$

$$H = \sum_{i\in A, j\in A, i\neq j} x_i x_j + \sum_{i\in B, j\in B, i\neq j} x_i x_j + \sum_{i\in A, j\in B} x_i x_j + \sum_{i\in B, j\in A} x_i x_j$$

$$H = k(k-1) + s(s-1) - 2ks$$

Consider the case when a node flips from B → A.
So, $k' = k + 1$ and $s' = s - 1$.

$$H' = (k+1)k + (s-1)(s-2) - 2(k+1)(s-1)$$

$$\Delta H^+ = H' - H$$

$$\Delta H^+ = 4(k - s + 1)$$

By symmetry, $\Delta H^- = 4(s - k + 1)$

We discuss identity of lowest energy states. If the state is in the lowest energy configuration, we must have both $\Delta H^+ \geq 0$ and $\Delta H^- \geq 0$

$$\frac{n-1}{2} \leq k \leq \frac{n+1}{2}.$$

## Most Monotone Boolean Functions (MBFs) support n/2 high steady states

**Definition 1.** A function $f_i : B^k \to B$ is a negative monotone Boolean function with respect to input $x_j$ if $b_1 \prec b_2$ implies $f(b_1) \geq f(b_2)$, for all pairs $b_1 \prec b_2 \in B^k$ which only differ in j-th component and $b^j_1 < b^j_2 \in B$

**Approach**

- Construct all MBFs for Tn
  $f_i : B^{n-1} \to B$
- Count $u^n$ where $f_i(b) = 0$
- Count $v^n$ where $f_i(b) = 0$
- For steady state (1,0,0):
  $f_1(0,0) = 1$, $f_2(1,0) = 0$, $f_3(1,0) = 0$
- Calculate no. of MBFs supporting state
  $\phi_1^3 = v^3(0,0) \cdot u^3(1,0) \cdot u^3(1,0)$

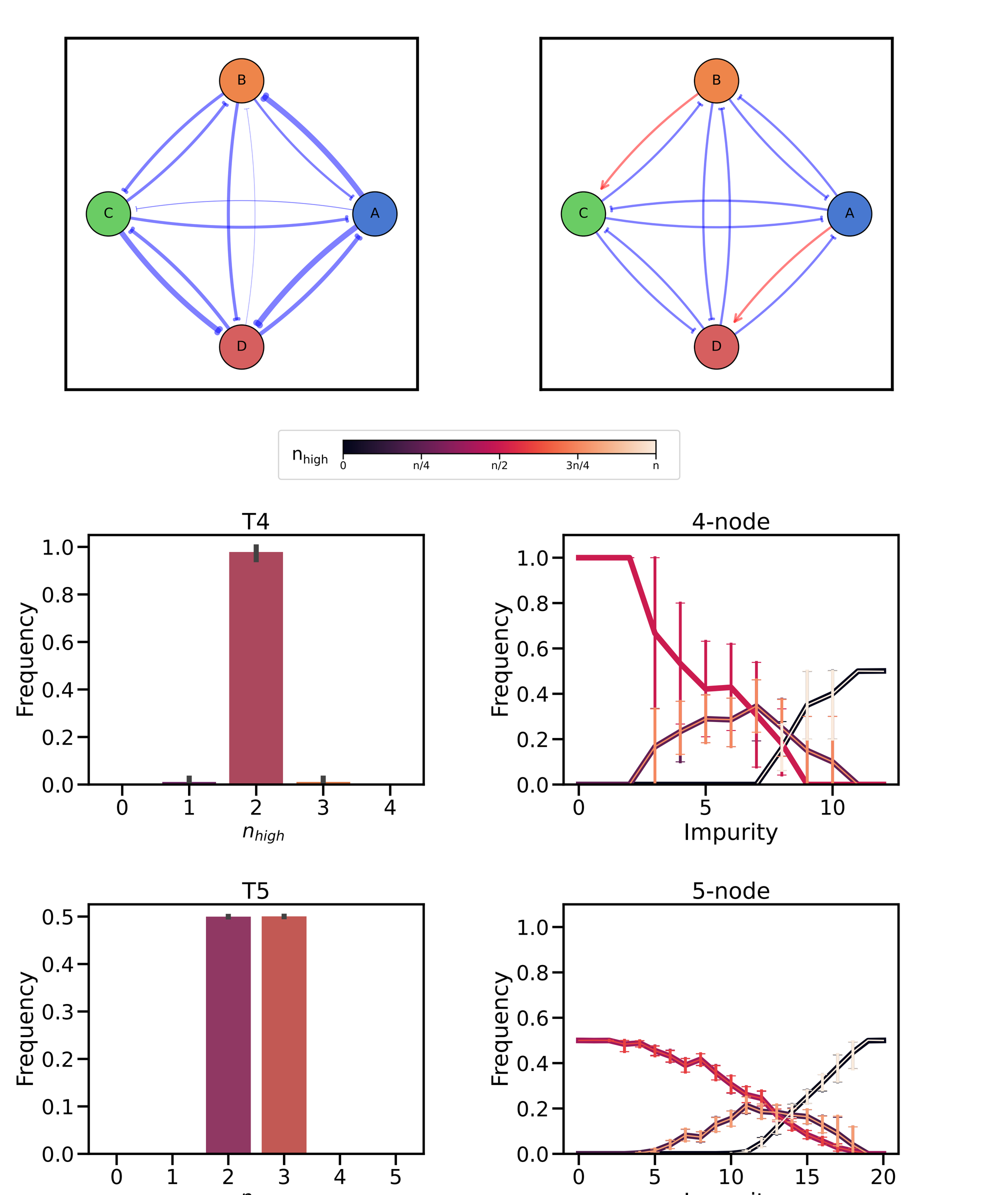
| Inputs | | Outputs | | | | | | |
|---|---|---|---|---|---|---|---|---|
| X | Y | **0** | ¬∨ | ¬X | ¬Y | ¬∧ | **1** |
| 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 |
| 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |

| Input in $B^{n-1}$ | $u^n$ | $v^n$ |
|---|---|---|
| $n = 3$ | | |
| (0,0) | 1 | 5 |
| (1,0) | 3 | 3 |
| (1,1) | 5 | 1 |

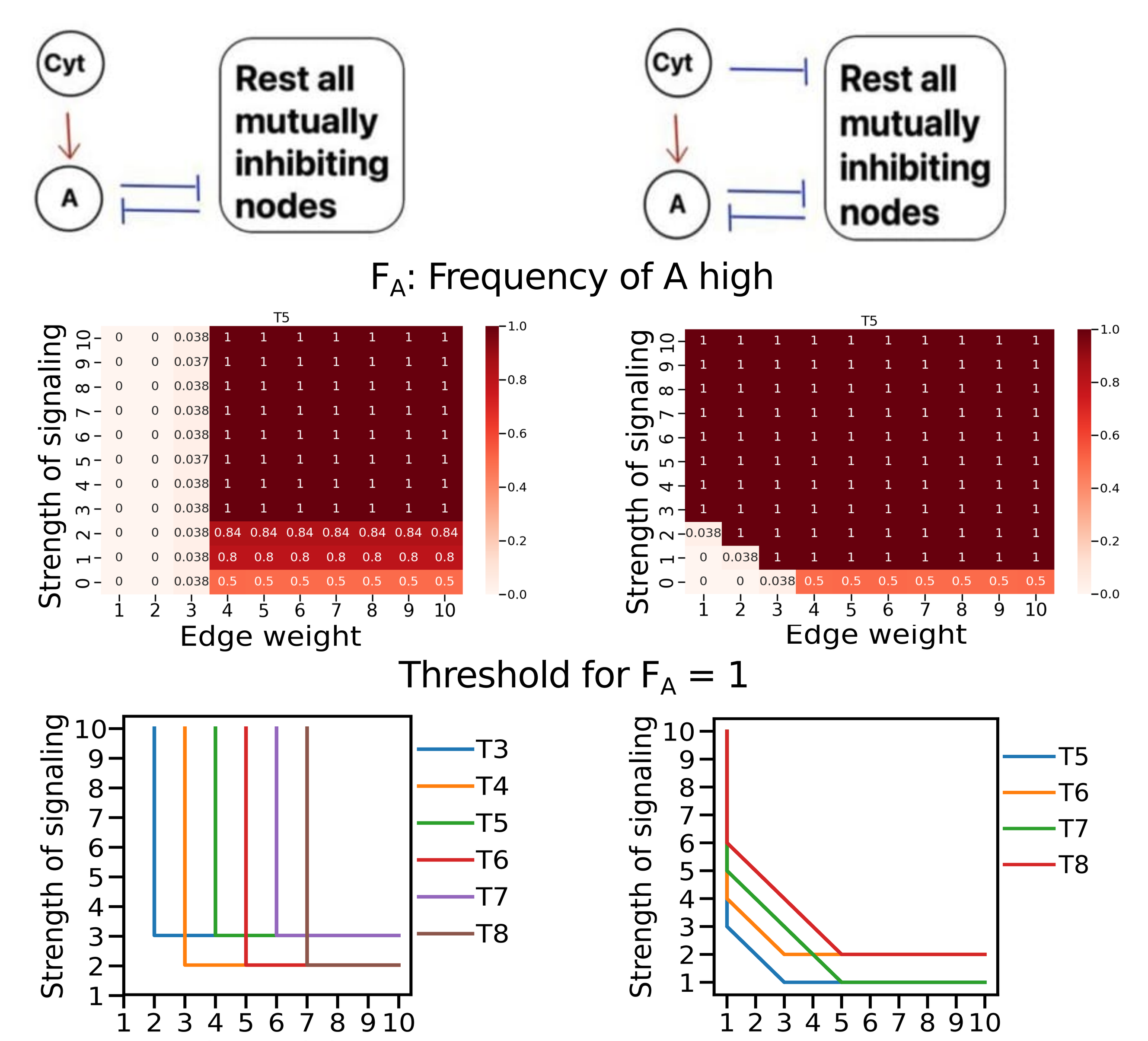
$\phi_k^n$

T2 : (1, 4, 1)
T3 : (1, 45, 45, 1)
T4 : (1, 4104, 38416, 4104, 1)
T5 : $(1, (20^4)(167), (84^3)(148^2), (84^3)(148^2), (20^4)(167), 1)$
T6 : $(1, (168^5)(7580), (2008^4)(7413^2), (5573^6), (2008^4)(7413^2), (168^5)(7580), 1)$

## Steady state patterns are robust to edge perturbations




## Synergy of epigenetic reprogramming & cytokine signaling required for terminal fate




## Conclusion

- Toggle-n networks cannot give terminally differentiated states
- n/2 factors co-expressed → bifurcating process to progenitors
- Random noise peturbations → traits maintained
- Acting in asymmetric or biased manner → Differentiation to A high

## Future Directions

- Unequal edge weights formal mathematical analysis
- Finding topologies that can give rise to single high states

## References

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