Tip cells in angiogenesis: the role of selection and behavior

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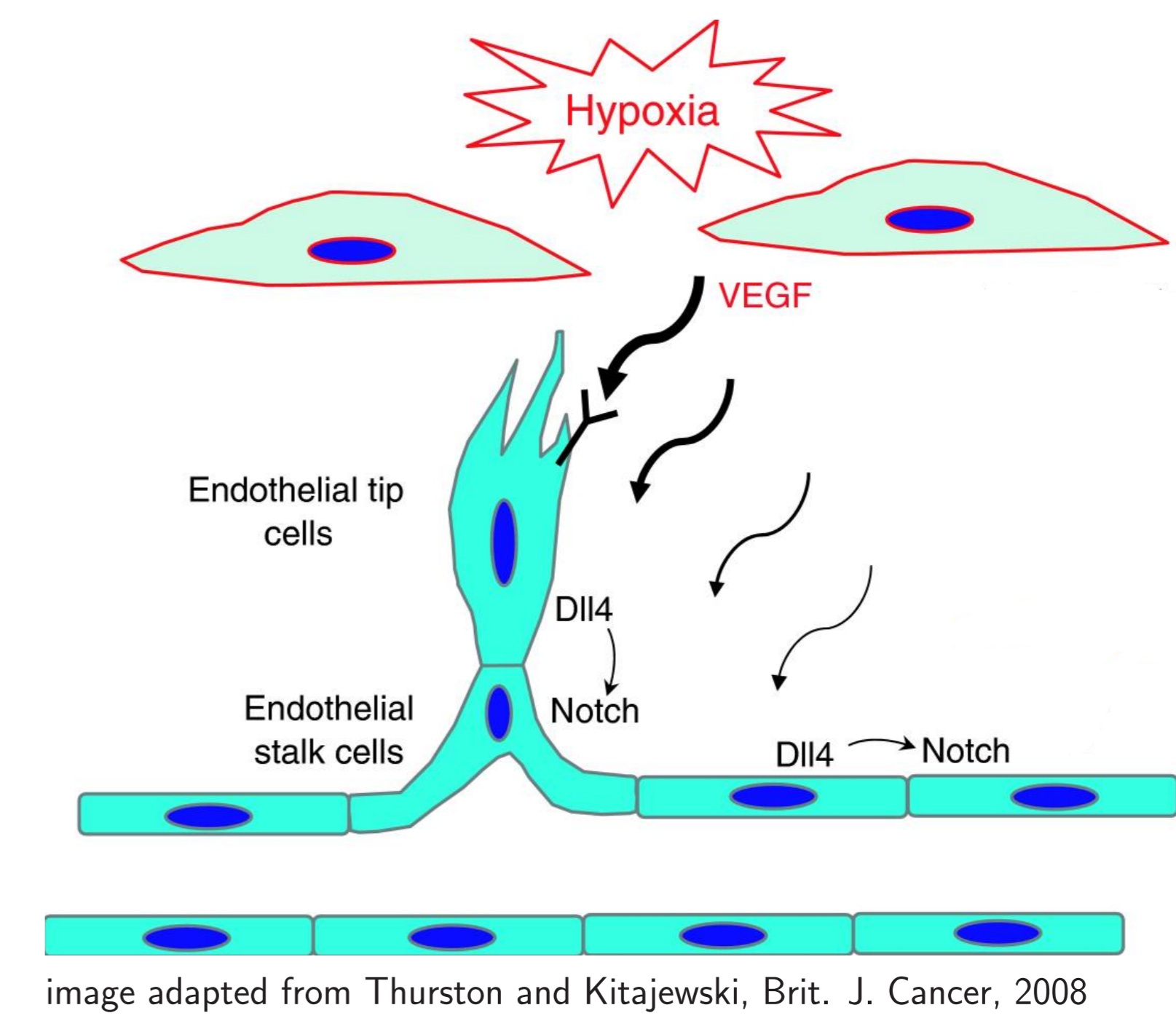
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Introduction

Sprouting angiogenesis is the formation of new blood vessels by splitting of a new vessel from an existing one. This process plays a vital role in pathological and physiological processes, including tumor growth, wound healing, and age-related macular degeneration. New sprouts are formed when a **tip cell** grows out of the vessel, followed by **stalk cells**. The tip cell is highly motile and extends a multitude of filopodia allowing the tip cell to explore its environment and sense chemoattractants secreted by the hypoxic tissue such as vascular endothelial growth factor (VEGF). Tip cell selection is regulated by Dll4–Notch lateral inhibition: a tip cell inhibits tip cell fate of its neighbors. This regulation is augmented by VEGF which can induce tip cell fate.

We **aim** to understand the role of tip cells and dynamic tip cell selection. For this we study a **cell-based** computational model of angiogenesis to which with add predefined or dynamically selected tip cells. Furthermore, we develop a more elaborate tip cell selection model that allows us to link expression levels of for example Dll4 and Notch

image adapted from Thurston and Kitajewski, Brit. J. Cancer, 2008

Tip cell behavior

To model sprouting and subsequent network formation we used a cell based angiogenesis model (Merks, PLoS Comput. Biol. 2008). In this model cells form vascular networks due to contact inhibited chemotaxis towards an autocrine source. We have added tip cells to this model (yellow), all remaining cells are stalk cells (red). Tip cells differ from stalk cells by their increased matrix adhesion. This could be related to the abundance of filopodia observed in tip cells. The images below show that this property suffices for the migration of tip cells towards the sprout endings. These tip cells were assigned at the initiation of the model; they are **static**.

time = 400   time = 800   time = 1200

Movie
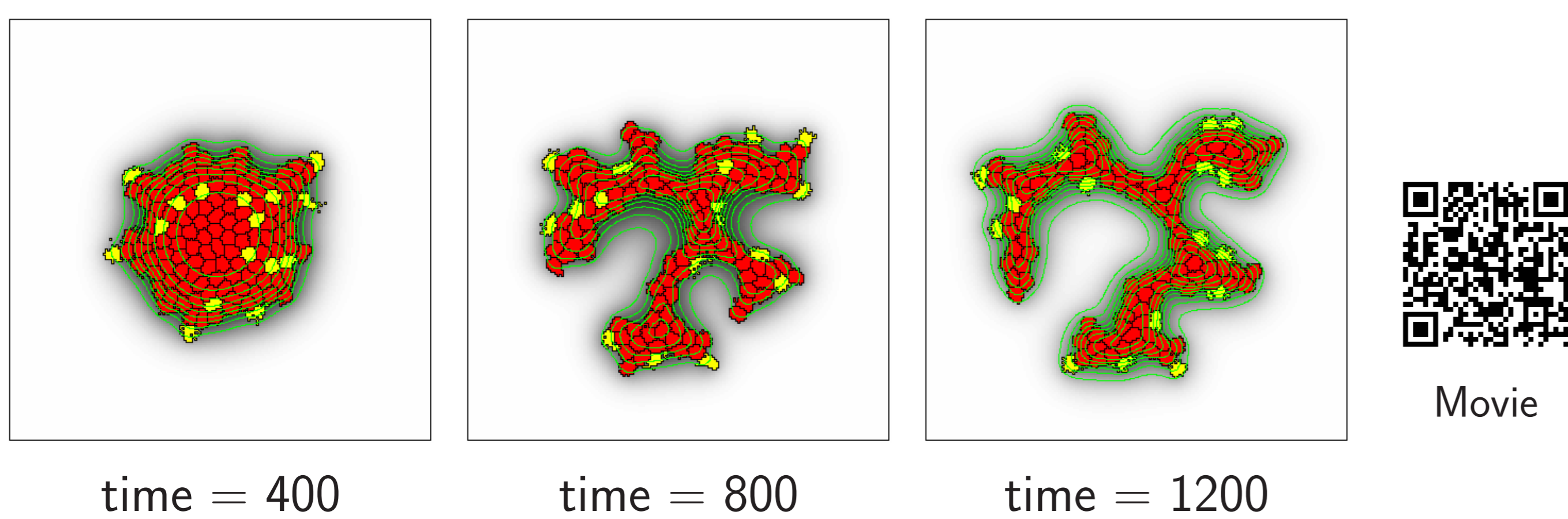
The proportion of tip and stalk cells determines the morphology of the formed pattern. A small amount of tip cells results in the most highly branched structures. When the amount of tip cells increases past the optimum, the patterns become less branched as they do without tip cells. The proportion of tip and stalk cells also determines the dynamics of branching. High amounts of tip cells destabilize the network, which delays network formation.
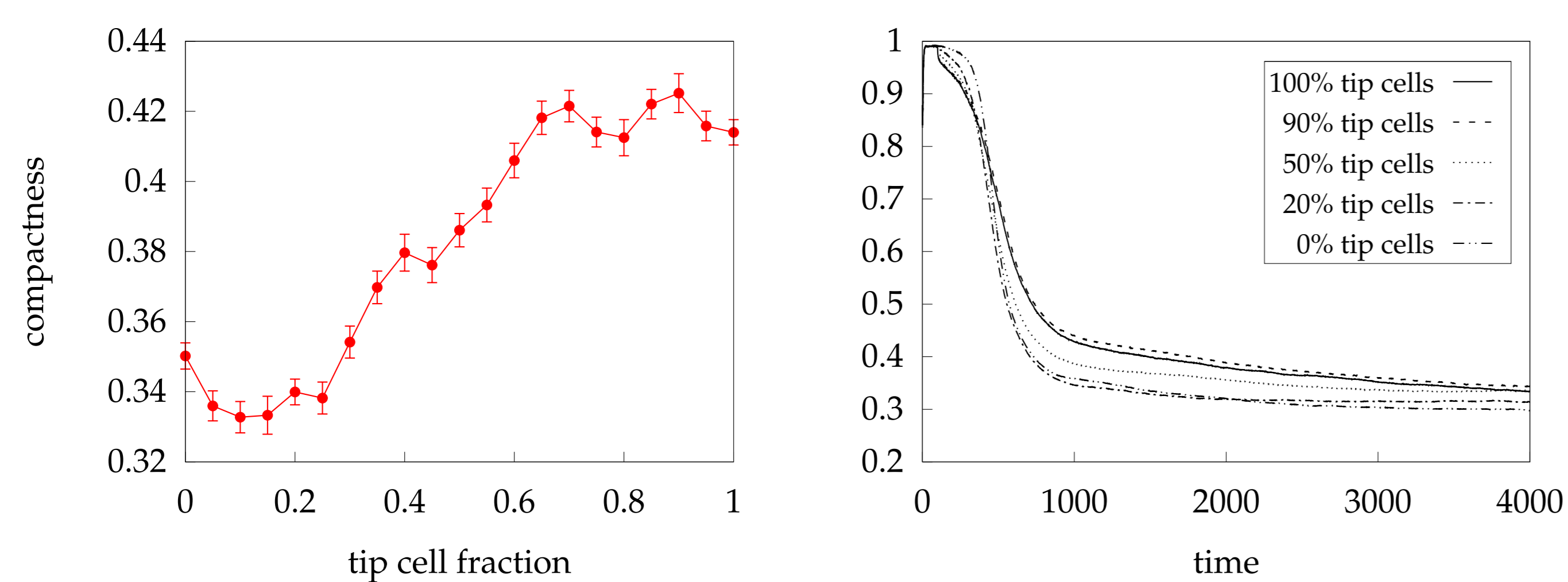

Predefined tip cells move towards the tip of the sprouts and do increase branching. Yet, these static tip cells cannot start new branches. Therefore, we implemented a **discrete** tip cell selection model, based on Dll4–Notch lateral inhibition. Cells with high levels of Dll4 become tip cells, while cells with low levels of Dll4 become stalk cells. Below, some snapshots of a simulation with this model is shown. Clearly, dynamic tip cell selection helps the formation of networks.


time = 500   time = 1000   time = 2000

Movie
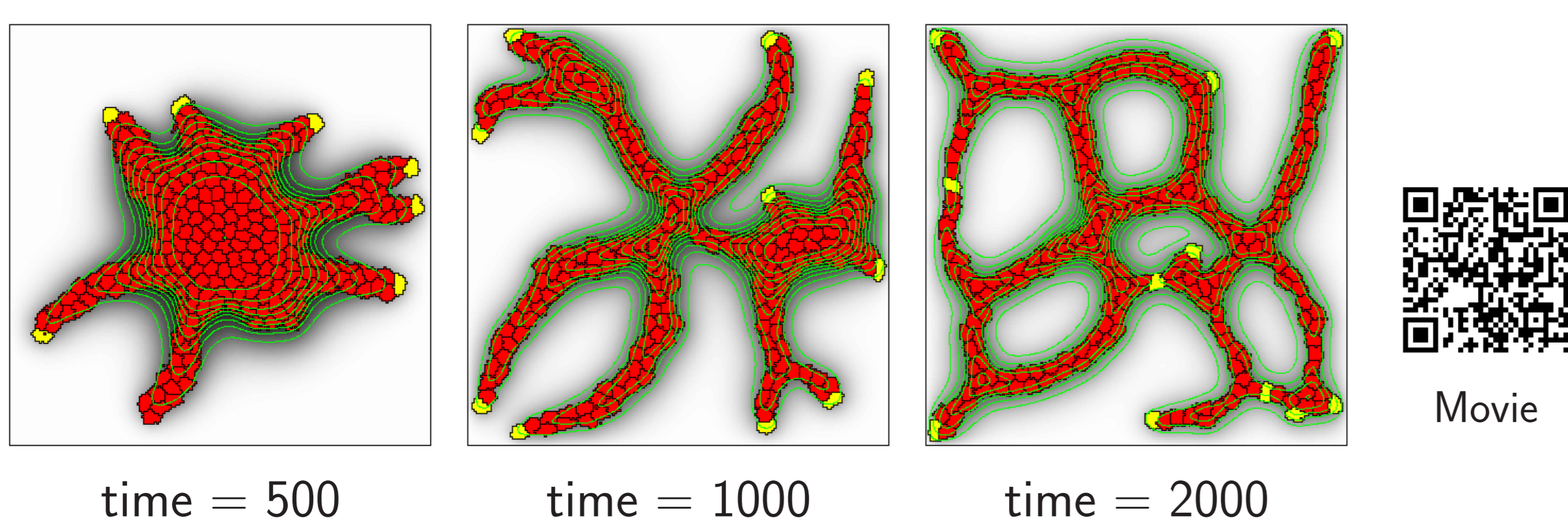
Tip cell selection

Tip cells are selected dynamically during angiogenesis. This process is regulated by **VEGF** and **Dll4–Notch signaling**. VEGF induces high levels of Dll4, which is typical for tip cells. Dll4 activates Notch in neighboring cells which, via Notch intra-cellular domain (NICD), inhibits both the VEGF receptor VEGFR-2 and Dll4.
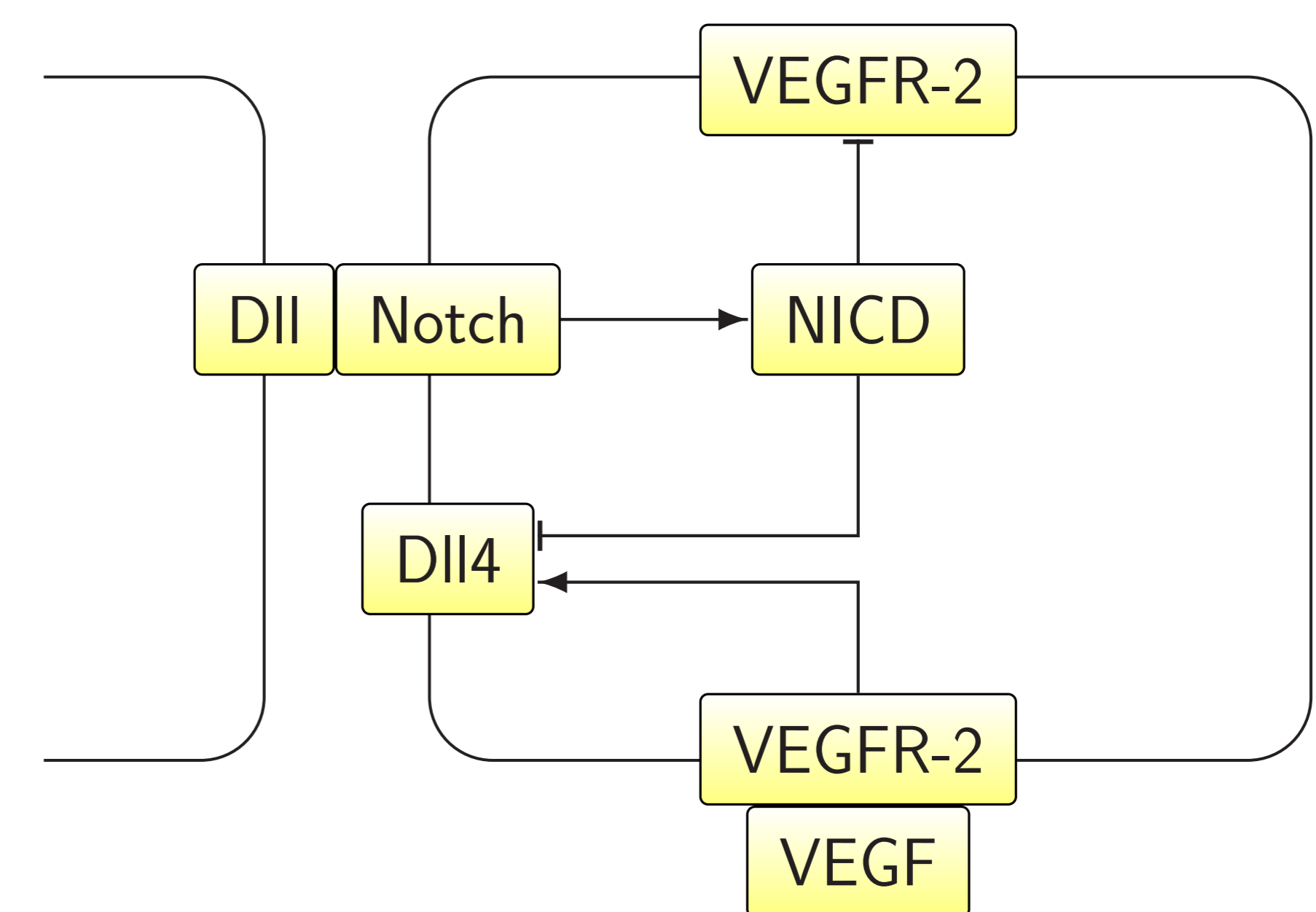
Considering both VEGF signaling and Dll4–Notch signaling enables tip cells to be induced by hypoxic tissue that secretes VEGF. That is, VEGF induces a tip cell and Dll4–Notch signaling prevents neighboring cells from becoming tip cells. An explicit representation of NICD and VEGFR-2 enables us to link cell properties directly to **cell behavior** such as adhesion, motility and chemotaxis. This replaces the discrete tip cell fate (as discussed in the left column) with a **transient phenotype**. We have implemented the tip cell selection network with a system of ordinary differential equations (ODEs), explicitly representing the levels of Dll4, NICD and VEGFR-2. We tested this model on a row of static cells to validate if the expected expression patterns could be reproduced.
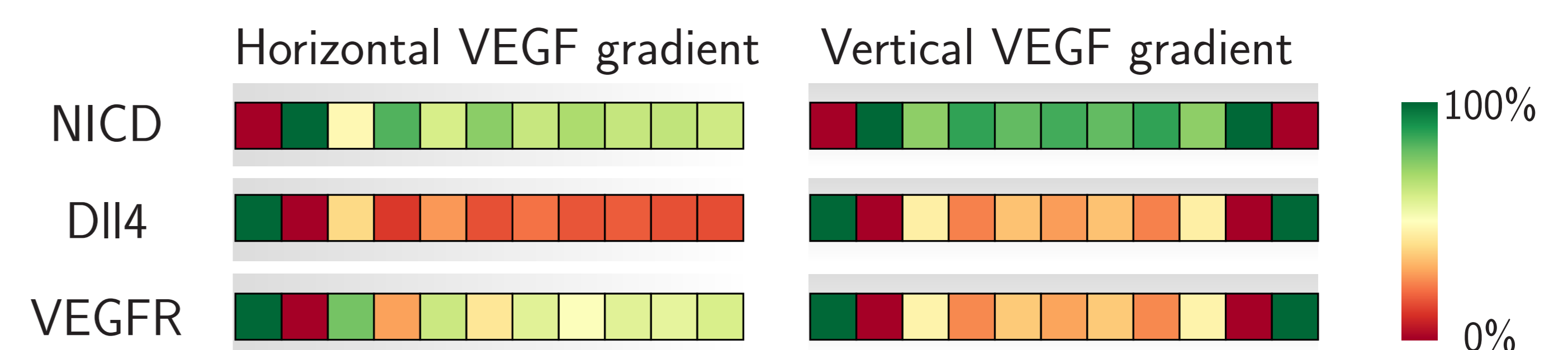
In a **horizontal VEGF gradient**, the first cell of a sprout has the highest Dll4 and VEGFR-2 levels, as expected in a tip cell. The cells after the tip alternate between more stalk and tip cell phenotypes. In a dynamic sprout, we expect that the tip cell-like cells further in the sprout are able to form new branches. When we rotate the field to a **vertical VEGF gradient**, tip and stalk cell phenotypes alternate. Note that the differences diminish towards the middle of the sprout. This is because these cells are less contact inhibited due to the absence of a second neighbor.

In the future, we will join this tip cell selection model with a cell based angiogenesis model, where the levels of VEGFR-2 and Dll4 are directly linked to cell properties. This will enable us to study the role of tip cells in sprouting and subsequent network formation.

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