Programming DNA-Based Biomolecular Reaction Networks on Cancer Cell Membranes

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Nodes:
- There are two types of nodes in the network that are indicated by two different colors.
- Each node has a reaction module (a DNA hairpin (HP)) and an addressing module (a single DNA strand containing a DNA aptamer), where a DNA aptamer (the curved part) is a DNA sequence that can be rationally designed and recognize a particular cell membrane receptor which can range from small molecules to proteins.32–35
- The reaction module and addressing module are connected by DNA hybridization between two complementary domains.

Operation:
- Each node targets a designated cancer cell membrane receptor via aptamer-receptor binding.
- When operating the reaction network, we first mix the nodes with the cancer cells in a reaction buffer.
- If both targeted receptors exist on the membrane, both nodes will be localized on the membrane by aptamer-receptor binding.
- We then filter out the free nodes in the buffer to exclude potential non-localized reactions.

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(a) 2-layer linear cascade reaction network by our architecture:

(b) DNA strand displacement reactions in the 2-layer linear cascade:
• The initiator starts the cascade reaction between node A and node B.
• The output of node B reacts with the reporter complex to tag the cell by a fluorophore.
• Note that reaction networks that are more complex than linear cascades can be built using the same strategy.

(c) Abstraction of the 2-layer linear cascade in (b).
• In the abstraction, we use the name of the aptamer of a node to denote the node.
• An arrow to a node indicates
• its input and an arrow from a node indicates its output.

A 2-layer linear cascade reaction network:
- There are two types of nodes in the network that are indicated by two different colors
- Each node is a DNA hairpin connected with a DNA aptamer, where a DNA aptamer is a DNA strand that can be rationally designed and recognize a particular cell membrane receptor which can range from small molecules to proteins using a DNA aptamer via aptamer-receptor binding.

Operating the reaction network:
- We first mix the nodes with the cancer cells in a reaction buffer.
- IF both targeted receptors exist on the membrane, both nodes will be localized on the membrane by aptamer-receptor binding.
- We then filter out the free nodes in the buffer to exclude potential non-localized reactions.
  - By introducing the initiator strands, the 2-layer linear cascade reaction is started.
  - First, the initiator opens up the red hairpin by DNA strand displacement.
  - Then, the output strand from the red hairpin opens up the blue hairpin.
  - The output strand from the blue hairpin can hybridize with a reporter DNA strand (conjugated with a fluorophore), such that the cancer cells are labeled by the fluorophore and can be recognized by flow cytometry.

(b) Group “S”: cancer cells labeled by fluorophore via a reaction network on the membrane. Group “N”: cancer cells of the same type without fluorophore. Note that the density of each node on the cell membrane is determined by the density of the corresponding receptor, and the nodes can move on the cell membrane because of the mobility of the receptors.
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Node design and an example of 2-layer linear cascade:

(a) Node design:
- A node has two modules: a reaction module (black) and an addressing module (red).
- The two modules are connected by the DNA hybridization between A2 and A2*, where A2* is the reverse complement of A2.
- The addressing module has an aptamer for targeting a particular cell membrane receptor.
- The initiator reacts with the reaction module (a DNA hairpin) to produce an output.
- The output has the same domain motif as the initiator, which makes it possible to cascade such nodes into reaction networks.

(b) Abstraction of the 2-layer linear cascade.
- We use the name of the aptamer of a node to denote the node.
- An arrow to a node indicates its input and an arrow from a node indicates its output.

(c) An example reaction network which is a 2-layer linear cascade.
- The initiator starts the cascade reaction between node A and node B.
- The output of node B reacts with the reporter complex to tag the cell by a fluorophore.
- Note that reaction networks that are more complex than linear cascades can be built using the same logic.

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Linear cascades on cancer cell lines: CCRF-CEM and Ramos:

(a) Flow cytometry result of a single repeat for testing 2-layer (top) and 3-layer (bottom) cascades on cancer cell lines CCRF-CEM.

- For example, using the 2-layer cascade, the cell population treated by the cascade has much stronger fluorescence intensity than the cell population without any treatment.

- We get the geometric means of fluorescence intensity for both populations, and calculate the ratio between the two geometric means (blue population over red population) to get a signal-to-background ratio (SBR).

- We repeated each experiment 3 times to get three SBRs and get the statistics in (b) (left), and it is the same for all reaction networks demonstrated in this paper. Note that the horizontal axis is fluorescence intensity (log-scale) and the vertical axis is cell count.

(b) Statistics of SBRs for linear cascades on CCRF-CEM from three repeats for each case. The abstractions at the bottom indicate the targets of each cascade.

(c) Flow cytometry result of a single repeat for testing 2-layer (top) and 3-layer (bottom) cascades on Ramos.

(d) Statistics of SBRs for linear cascades on Ramos from 3 repeats of each case.

* Tianqi Song, Shalin Shah, Hieu Bui, Sudhanshu Garg, Abeer Eshra, Ming Yang, and John Reif, Programming DNA-Based Biomolecular Reaction Networks on Cancer Cell Membranes, Journal of the American Chemical Society (JACS), Vol. 141, No. 42, pp. 16539-16543. (Oct 2019). [https://doi.org/10.1021/jacs.9b05598](https://doi.org/10.1021/jacs.9b05598)
Explaining our 2-Layer and 3-layer Cascades:

- The cell populations treated by the 2-layer and 3-layer cascades have much stronger fluorescence intensity than the cell population without any treatment.

- We calculated the geometric mean of fluorescence intensity for both populations, and calculate the ratio between the two geometric means (green population over red population) to get a signal-to-background ratio (SBR).

- We repeated these experiments 3 times to get three SBRs and the statistics in (e).
- We used the same experimental techniques for all reaction networks demonstrated.
- Note that the horizontal axis is fluorescence intensity (log-scale) and the vertical axis is cell count.

(C) Flow cytometry result of a single repeat of testing 2-layer and 3-layer cascades on CCRF-CEM.

(D) Flow cytometry result of a single repeat for testing 2-layer (top) and 3-layer (bottom) cascades on Ramos.

(E) Statistics of SBRs for linear cascades and control experiments.

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