UTILIZING LOGIC-GATED DNA STRAND DISPLACEMENT TO INDUCE CANCER PRODRUG ACTIVATION

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Highly selective cancer therapeutics with minimal off target effects that preserve patient quality of life are in high demand. Towards creating more targeted therapies, advances in bioinformatics and systems biology reinforce that cancer is an extremely complex disease, and multiple biomarkers must be considered to exclusively identify cancer cells. Furthermore, unique multi-input cancer signatures can vary between cancer subtypes, individual patients, and even over time within an individual. To address this, we have developed a flexible platform based on synthetic nucleic acid computation for a "smart drug" that can match the complex and dynamic nature of cancer. Harnessing the powerful properties of toehold-mediated strand displacement, our technology has the ability to control protein assembly through input-triggered nucleic acid circuits. By conjugating DNA to fluorescent proteins or a split version of yeast cytosine deaminase, a cancer prodrug activating enzyme, we can use nucleic acid computation to control protein behavior for sensing or therapeutic applications, respectively. We first created multi-input logic-gated circuits controlled by various cancer-specific miRNA inputs. Realizing the limitations that low input concentrations inside cells could place on our protein-DNA devices, we utilized catalytic hairpin assembly (CHA) for signal amplification. Next, we integrated both Boolean-logic and amplification architectures into a programmable and streamlined design. Moving beyond an in vitro demonstration, we executed our protein-DNA computation device in various cancer cells lines that endogenously express cancer-specific RNA. To our knowledge, this is the first time protein assembly/activity controlled by strand displacement computation has been executed inside live mammalian cells, a significant step in realizing the power of nucleic acid nanotechnology for future applications.