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（博士課程）
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論 文 要 旨

THESIS SUMMARY

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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

DNA molecules self-assemble and form well-defined secondary or higher-order structures according to the Watson-Crick base pairing rule. This characteristic allows us to use DNA as a predictable and programmable material to construct various structures, including the newly discovered DNA droplet. Various dynamic DNA devices with different functionalities have been built, among which DNA-based molecular robots have great potential for biological applications. However, in terms of four essential components (structure, sensor, computer, actuator), the molecular robots currently constructed have not yet been realized at the system level, combining multiple functions into a cell-scale structure. This study aims to achieve DNA-based system-level complexity by combining compartments with different functions to build cell-scale molecular robots that can implement diverse functions such as sensing, computing, diagnostics, and therapy. Here, a DNA droplet computer was constructed by combining functional nucleic acid molecules and DNA computation for disease diagnosis; a macrophage-like molecular robot was constructed by combining functional molecules aptamer and azobenzene for the recognition and incorporation of cancer cells.

DNA droplet computers for cancer diagnosis:

The DNA droplet is a coacervation of sequence-designed DNA molecules; it forms in a specific temperature range between high-temperature dispersed nanostructures and low-temperature nanostructure-assembled hydrogels. DNA droplets have been used to create phase-separated Janus droplets and capsule patterning and mimic intracellular signal transduction based on molecular transport between DNA droplets. However, information processing control through molecular logic computation or biomedical applications in DNA droplets needs to be reached for more practicality. Here, I demonstrate a computational DNA droplet that can recognize specific combinations of tumor marker microRNAs (miRNAs) as molecular inputs and output the results of DNA logic operations through physical DNA droplet phase separation, which achieves the fusion of biosensing, molecular logic computation, and biomedical application in DNA droplets.

I designed Y-shaped DNA nanostructures from three single-stranded DNA molecules to generate DNA droplets. Each single-stranded DNA molecule of the Y-shaped DNA nanostructures has an eight-nucleotide self-complementary sequence called sticky-end to enable the Y-shaped DNA nanostructures to self-assemble into a micrometer-sized DNA droplet. The DNA droplet was used as the body of the DNA droplet computer. A mixed DNA droplet consisting of three Y-shaped DNA nanostructures with orthogonal sticky-end sequences and two linker DNAs to cross-bridge the orthogonal DNA nanostructures was

proposed. Within each linker, I designed two input receptors, which are complementary to miRNA inputs. By the hybridization of miRNAs with the linkers, the cross-bridging ability of the linker DNA is lost, causing the phase-separation of the mixed DNA droplet into three colors of DNA droplets, resulting in executing a miRNA pattern recognition described by a logical expression ((miRNA-1 ∧ miRNA-2) ∧ (miRNA-3 ∧ ¬miRNA-4)) (where ∧ indicates AND, ¬ indicates NOT). The logical operation was used as a model for breast cancer detection. Experimentally results demonstrate that the DNA droplet computer recognizes the above specific pattern of chemically synthesized miRNA sequences for cancer diagnosis.

Macrophage-like molecular robot for targeting and incorporating cancer cells:

I further extended the functionality to develop a macrophage-like molecular robot for cancer cell recognition and incorporation, aiming to be available for future cancer therapeutics. Given the excellent programmability, biocompatibility, controlled phase transitions, size control, and ease of preparation, there is growing interest in applying DNA-based structures as drug carriers and delivery systems. However, DNA-based delivery systems to date require strategies to address the challenge of low cellular uptake efficiency. Here, inspired by immune cells called macrophages, I developed macrophage-like molecular robots that can recognize and incorporate targeted cancer cells and accomplish wrapping the cells by altering the mobility of the molecular robots under UV irradiation.

I designed six-branched DNA nanostructures formed from six single-stranded DNA molecules. Each single-stranded DNA molecule of the six-branched DNA nanostructures has the same sticky end to enable the nanostructures to self-assemble into a micrometer-sized DNA droplet. Aptamer modifications were applied to two of the six single-stranded DNA strands to recognize the antigen molecules on the surface of cancer cells. The fluidity altering is achieved by azobenzene modification into the sticky ends—the azobenzene molecule switch from *trans* form to *cis* form under UV irradiation. The *trans*-azobenzene is planar and, therefore, suitable for the sticky-end sequence base-paring, whereas the *cis*-azobenzene is nonplanar, thus destabilizing the sticky-end sequence stability. The instability of the sticky-end sequence allows the state of the molecular computer from to go a solid gel state to a soft droplet state with fluidity. Experimentally results demonstrate that the macrophage-like molecular robot recognizes and binds to the target cancer cells; encapsulates the target cells by changing the state of the molecular robot from gel to droplet under UV irradiation.

In conclusion, I demonstrate two DNA droplet-based molecular computers: a DNA droplet computer that can recognize specific combinations of tumor marker miRNAs as molecular inputs and output the results of DNA logic operations through physical DNA droplet phase separation, which achieves the fusion of biosensing, molecular logic computation, and disease diagnosis in DNA droplets; a macrophage-like molecular robot that can recognize tumor cells, trigger changes in the state of DNA nanostructures, and effectively encapsulate tumor cells, which provides a new concept of a molecular computer that can perform tasks without relying on cellular uptake. In the future, we intend to build a molecular robot that integrates sensing, computing, diagnosis, and therapy and can act as a "mini doctor" inside the body.