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論 文 要 約

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According to the Watson-Crick base pairing rule, DNA molecules can self-assemble to form well-defined secondary and higher-order structures. This property makes DNA a predictable and controllable material that can be used to construct various structures, including newly discovered DNA droplets. To date, molecular robots using DNA have great potential for biological applications. However, the molecular robots currently constructed in the four components of structure, sensor, computer, and actuator, have not yet been realized at the system level, combining multiple functions into cell-sized structures. 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, including sensing, computing, diagnostics, and therapy. Here, I used DNA droplets as the body of the molecular computer and constructed two DNA droplet-based molecular computers: a DNA droplet computer by combining functional nucleic acid molecules and DNA computation for disease diagnosis, a macrophage-like molecular robot 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[1]. DNA-based assembly relies on the Watson−Crick base pairing principle to hybridize two single strands of DNA with complementary sequences and form double-strand DNA molecules, thus making DNA-based materials highly predictable and programmable. The programmability enables the creation of DNA droplets with different patterns and controls the droplet behaviors, such as selective fusion and fission. These phase-transition and fusion properties of DNA droplets are excellent features for forming cellular-scale structures and diverse actuations in constructing cellular-scale molecular robots with multiple functions. DNA droplets have been used to create phase-separated Janus droplets[1,2] and form capsule-like structures[3] by exploiting the sequence recognition capability of DNA droplets that fuse with the identical sticky end sequence but not with droplets having orthogonal sequences. Using the membraneless property of DNA droplets, mimic intracellular communication based on molecular transport between DNA droplets in a water-in-oil environment was achieved[4]. 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 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[5]. Experimentally results demonstrate that upon reaction with miRNAs, the DNA droplet computer was phase-separated into three DNA droplets and can perform miRNA pattern recognition represented by the above logical formula for breast 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, 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—the azobenzene molecule switch from *trans* form to *cis* form under UV irradiation. The *trans*-azobenzene is planar and, therefore, $\boldsymbol{\pi}$-$\boldsymbol{\pi}$ stacking facilitates DNA sequence bonding, whereas the *cis*-azobenzene is nonplanar, thus destabilizing the DNA sequence stability. The instability of the DNA sequence allows the state of the molecular robot 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.

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