ACS Partner Journal

CHEM & BIO ENGINEERING

pubs.acs.org/ChemBioEng

Review

Advances in Cell Separation: Harnessing DNA Nanomaterials for High-Specificity Recognition and Isolation

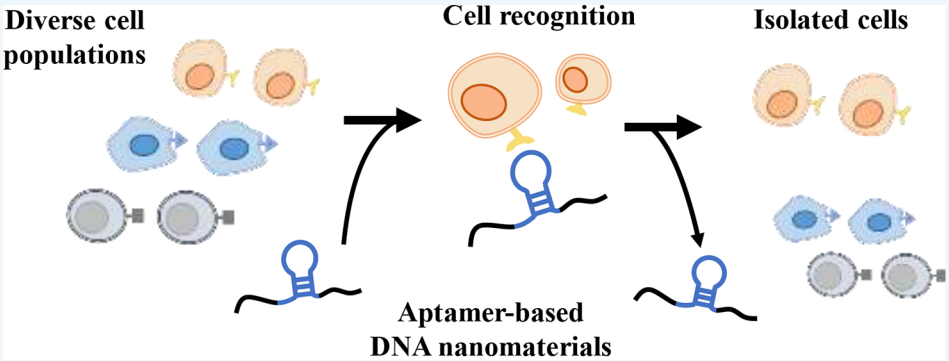
Published as part of Chem & Bio Engineering special issue "Advanced Functional Hydrogels".

Huimin Bao, Yao Yao, Wenqi Tang, and Dayong Yang*

Cite This: *Chem Bio Eng.* 2025, 2, 171−181

Read Online

ACCESS | Metrics & More | Article Recommendations



ABSTRACT: Advancements in cell separation are essential for understanding cellular phenotypes and functions, with implications for both research and therapeutic applications. This review examines the evolution of cell separation techniques, categorizing them into physical and affinity-based methods, with a primary focus on the latter due to its high specificity. Among affinity techniques, DNA nanomaterials have emerged as powerful tools for biomolecular recognition owing to their unique properties and diverse range of nanostructures. We discuss various DNA nanomaterials, including linear aptamers, multivalent DNA constructs, DNA origami, and DNA hydrogels and their roles in cell recognition and separation. Each section highlights the distinctive characteristics of these DNA nanostructures, providing examples from recent studies that demonstrate their applications in cell isolation and release. We also compare the four DNA nanomaterials, outlining their individual contributions and identifying the remaining challenges and opportunities for further development. We conclude that DNA nanotechnology holds great promise as a transformative solution for cell separation, particularly in the context of personalized therapeutics.

KEYWORDS: *DNA nanotechnology, cell recognition, cell separation, aptamer, DNA hydrogel*

1. INTRODUCTION

Cell separation aims to sort cells into distinct, monodisperse populations with high purity and viability to explore their potential functions in living entities.[1,2] Progress in cell separation technology is crucial for advancing our understanding of cellular heterogeneity and the development of precision medicine. For instance, isolating circulating tumor cells (CTCs) from blood samples can provide insights into the progression of metastatic disease and treatment decisions.[3] Moreover, therapeutic applications require large-scale supplies of high-purity stem cells, and advances in stem cell isolation technology will expedite their use in regenerative medicine.[4] Overall, this technology underpins many discoveries in cell biology and further enables research in clinical applications.

Cell separation techniques depend on the specific characteristics of the target cells, physical properties, or features of the cell surface markers. They can be categorized into two main types: physical property- and immunoaffinity-based separation strategies (Figure 1). Physical property-based separation capitalizes on differences in cell density, size, surface charge, hydrophobicity, adherence tendencies, and light scatter. These techniques are label-free methods that are frequently used in laboratories and routinely employed in clinical settings. Density-based techniques, such as centrifugation or ultracentrifugation, are conventional separation methods.[2,5] Inertial microfluidics exploits the inertial forces acting on cells with microchannels to

Received: December 19, 2024
Revised: January 22, 2025
Accepted: January 27, 2025
Published: January 31, 2025

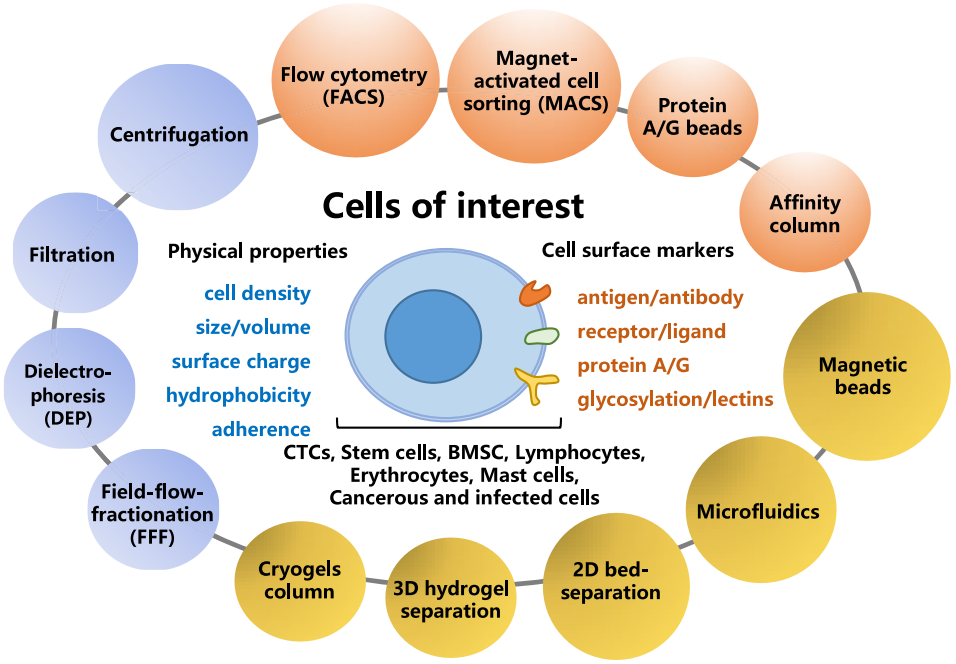


Figure 1. Overview of cell separation technologies. Physical property-based techniques are marked by blue circles, immunoaffinity-based techniques by orange circles, and integrated techniques by yellow circles.

Table 1. Summary of the Affinity Method Used in Cell Separation

Affinity method	Affinity interaction	Characteristics
Antibody-based	The interaction between the antibody and target protein	High selectivity by antibodies' binding
Ligand-based	A broad class of ligand ranging from proteins to small molecules and target receptor	Synthetic antigens used for cell purification
Protein A/G-based	Protein A and Protein G, two antibody-binding proteins	Protein A/G binding is less impacted by steric hindrance than binding to immobilized antibodies directly
Lectin-based	Lectins recognize specific carbohydrate sequences of glycoprotein on cell surface markers	Lectins enable highly specific cell fractionation as they target post-translational modifications
Aptamer-based	The interaction between the aptamer and target protein	DNA aptamers have comparable specificity and little batch-to-batch variation

separate cells based on size.[6,7] Dielectrophoresis utilizes the differences in the dielectric properties of cells to achieve separation in a nonuniform electric field.[8,9] Immunoaffinity-based separation relies on distinct cell surface markers, such as antigens, antibodies, receptors, ligands, or special glycosylation patterns, offering highly selective isolation.[10,11] In most instances, immunoaffinity is predicated on the specific recognition that occurs between antigens and antibodies. Magnetic-activated cell sorting (MACS)[12] and fluorescence-activated cell sorting (FACS)[13] are exemplary techniques widely used in clinical diagnosis and cell sorting. For example, MACS utilizes magnetic nanoparticles coated with specific antibodies to target and separate cells of interest, affording simplicity and high yield in the isolation of various immune cell populations.

Owing to its high target specificity, affinity-based separation has been increasingly integrated with many other separation techniques. The typical affinity method used in cell separation is summarized in Table 1. Based on specific surface antigens of CTCs, researchers have successfully implemented antibodies in microfluidic devices for the isolation of CTCs.[2,14] The combination of MACS and microfluidic devices further augments the efficiency and throughput of cell separation processes.[15] The integration of affinity-based techniques with dielectrophoresis permits the sorting of multiple types of bacterial cells in a single pass.[16] However, the application of antibody-based approaches is hampered by the antibody accessibility. First, the range of highly specific antibodies used at our disposal is quite limited. Second, the intrinsic immunogenicity of in vitro-synthesized antibodies could potentially hinder their therapeutic applications. Third, the prohibitive costs and intricate processes involved in the production and purification of antibodies can significantly impede their widespread adoption, particularly in settings in which resources are constrained. In field biorecognition, affinity-based separations have shifted from protein and antibody to synthetic ligands. Aptamers offer comparable binding affinity and specificity without the complexities associated with biosynthesis.[17] Furthermore, they can be synthesized at a cost-effective rate, ensuring consistency without batch-to-batch variability. As such, aptamers have emerged as promising alternatives to antibodies, catering to the growing demand for universal high-throughput cell separation techniques.

2. DNA NANOTECHNOLOGY

DNA, as a genetic material with sequence programmability, is built up with four different deoxynucleotide monomers. In the 1980s, the foundational principles for the synthesis of DNA nanostructures were first introduced, sparking a rapid advance-

https://doi.org/10.1021/cbe.4c00185
Chem Bio Eng. 2025, 2, 171−181

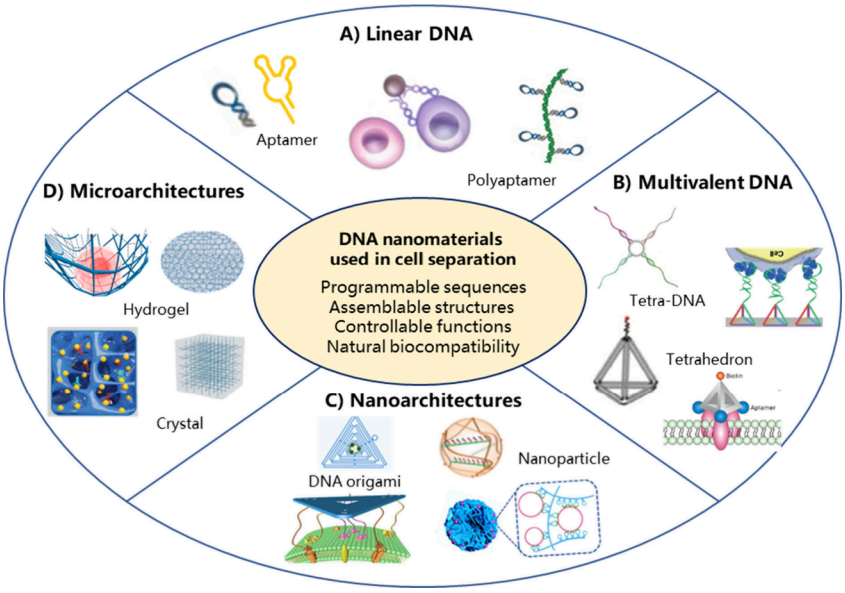


Figure 2. Structures of the DNA nanomaterials used for cell separation. (A) Linear DNA can be used as a single molecule or a polyaptamer. Reproduced with permission from ref 27. Copyright 2019 Springer Nature. Reproduced with permission from ref 31. Copyright 2020 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (B) Multivalent DNA assembled from linear DNA such as tetra-DNA and polyhedrons. Reproduced with permission from ref 37. Copyright 2020 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. Reproduced with permission from ref 40. Copyright 2020 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. Reproduced with permission from ref 38. Copyright 2020 Wiley-VCH GmbH. Reproduced with permission from ref 25. Copyright 2019 American Chemical Society. (C) Nanoarchitectures with complex topologies hybrid with functional moieties, such as DNA origami and nanoparticles. Reproduced with permission from ref 26. Copyright 2023 American Chemical Society. Reproduced with permission from ref 21. Copyright 2022 American Chemical Society. Reproduced with permission from ref 23. Copyright 2023 American Chemical Society. (D) Microarchitectures using DNA networks such as liquid hydrogels and crystals. Reproduced with permission from ref 59. Copyright 2021 American Chemical Society. Reproduced with permission from ref 22. Copyright 2023 Springer Nature. Reproduced with permission from ref 19. Copyright 2020 American Chemical Society.

ment in DNA nanotechnology.[18] The diversity of DNA and programmability of DNA structures can be achieved through precisely designed and freely defined compositions.[19−23] Over the following decades, DNA nanostructures have been applied in biomedical fields such as diagnosis, therapeutics, and tissue regeneration. Owing to the highly specific recognition of surface markers on individual cells, it has become a powerful tool for live cell separation.

DNA nanomaterials can be categorized into four groups, according to their size and function (Figure 2). In the linear form, single-stranded DNAs (ssDNAs) or RNAs, which form secondary structures through self-pairing, are recognized as functionalized aptamers. Aptamers possess a remarkable ability to target molecules, which is essential for marker recognition in target recognition and clinical diagnosis (Table 2).

Table 2. Common Aptamers and Their Binding Coefficient

Aptamer Name	Target proteins	K_d Value	ref.
Mucin 1-Apt	Mucin 1	10−100 nM	24
EpCAM-Apt	EpCAM	30 nM	25
HER2-Apt	HER2	20 nM	26
EGFR-Apt	EGFR	1−200 nM	26
A3t	CD8	Not provided	27

Second, multivalent DNA has been developed with diverse structures, such as tetra-DNA and polyhedrons. Each vertex of a tetrahedron can be designed to carry a type of oligonucleotide. Multivalent structures have been explored for their potential to interact with various agents, each of which has unique functions. Third, DNA structures can be further assembled into 2D and 3D structures with complex topologies. The domain of DNA nanostructures has been significantly expanded to include various DNA origami and nanoparticles. These structures can integrate many functional molecules and dramatically expand their applications in immune recognition and drug delivery. Fourth, the domain of DNA structures has been significantly expanded to the microscale to form liquid or crystal hydrogels. DNA hydrogels, a type of biopolymer, are constructed via Watson−Crick base-pairing of ultralong ssDNA or liquid-phase crystallization and dense packing of double-stranded DNAs (dsDNAs). These hydrogels exhibit excellent monodispersity and provide a favorable microenvironment, making them particularly advantageous for isolating living cells.

3.1. Linear DNA. DNA aptamers are single-stranded oligonucleotides with a peculiar sequence that can specifically and efficiently bind to various target molecules, such as proteins, small molecules, and cells.[28] Compared to antibodies with the same specific recognition capabilities, DNA aptamers exhibit a number of distinct advantages, including small molecular weight, comparable specificity, high stability, facile chemical modification, and little batch-to-batch variation.[29] Given these characteristics, DNA aptamers present a more promising alternative for affinity-based techniques than traditional methods. In recent years, DNA aptamers have been widely used for cell separation. A reversible aptamer selection technology was reported for the isolation of label-free $CD8^+$ T cell.[27] The A3t aptamer generated through a modified cell-SELEX procedure was immobilized on immunomagnetic Anti-Biotin Microbeads. $CD8^+$ T cells were isolated using immobilized aptamers and released by adding a reversal agent that disrupted the secondary structure of the aptamer (Figure

https://doi.org/10.1021/cbe.4c00185
Chem Bio Eng. 2025, 2, 171−181

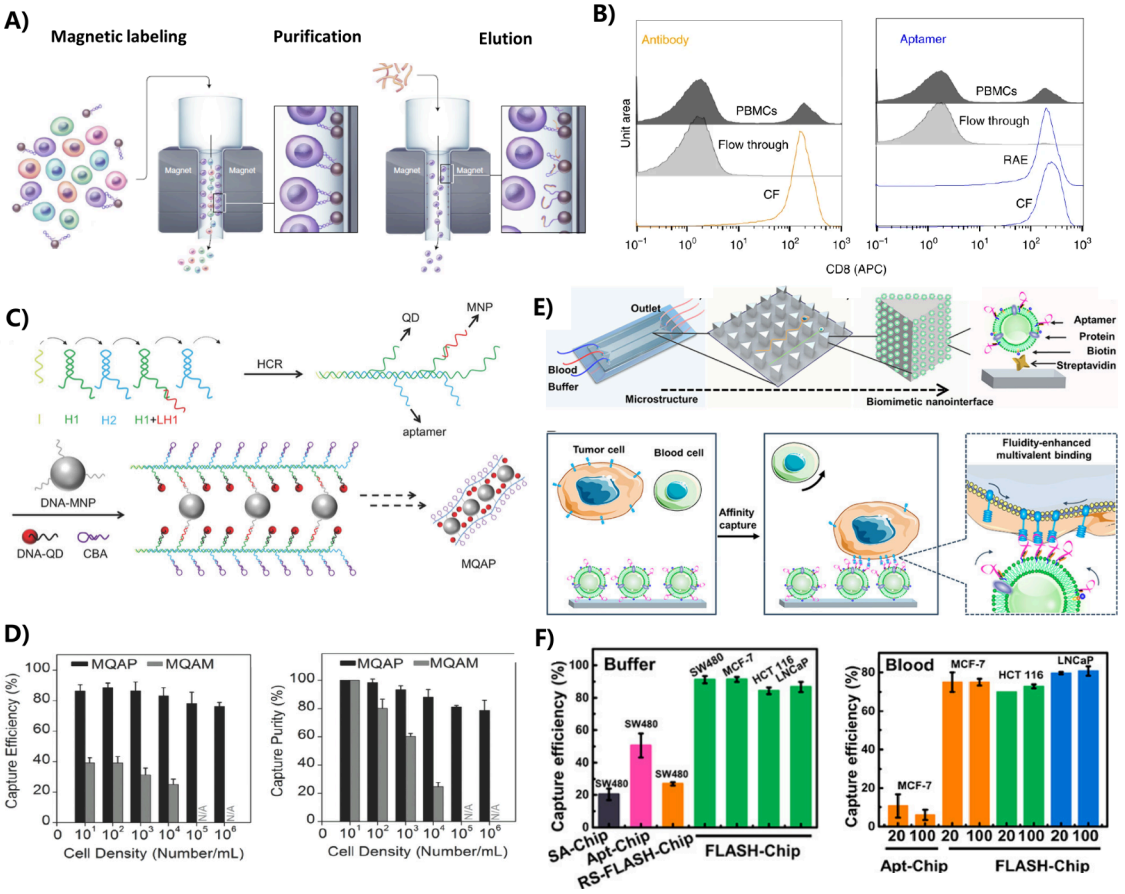


Figure 3. Aptamer-based techniques for cell separation. (A) Workflow of the traceless selection of CD8$^+$ T cells through the A3t aptamer. (B) Comparison of CD8 expression in the different fractions of standard antibody-based isolation (left) and traceless aptamer-based isolation (right) by flow cytometry. Reproduced with permission from ref 27. Copyright 2019 Springer Nature. (C) Construction of MQAPs by hybridization chain reaction (HCR), quantum dot-aptamer (DNA-QD), and coassembly with magnetic nanoparticles (MNPs) for magnetic isolation of CTCs. (D) The capture efficiency and capture purity of CEM cells by MQAPs and MQAMs are measured for different concentrations of background cells spiked in. Reproduced with permission from ref 30. Copyright 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (E) The working principle of FLASH-Chip by fabricating biomimetic nanointerface modified with biotin, protein, and aptamer. (F) The capture efficiency of the FLASH-Chip and Apt-Chip in buffer and blood samples. Reproduced with permission from ref 36. Copyright 2020 American Chemical Society.

3A). To assess their ability to isolate CD8$^+$ T cells, PBMCs were incubated with magnetic beads and subsequently applied to a column under a magnetic field. Near-complete depletion of CD8$^+$ cells from the flow-through fraction was achieved using aptamer-loaded microbeads, with results comparable to those obtained using a widely used antibody-based approach (Figure 3B). This aptamer-based selection approach enabled highly efficient, label-free, and cost-effective isolation of T cells while maintaining reasonable yields and purity. Furthermore, a magnetically separable nanodevice was developed by integrating mucin 1 aptamer with miRNA-responsive probes. The nanodevice realized the efficient capture and subtype identification of heterogeneous CTCs.[24]

DNA assembly strategies, such as hybridization chain reaction (HCR)[30,31] and rolling circle amplification (RCA),[32,33] have been exploited to construct multi-DNA aptamers, enhancing their functional performance compared to their monomeric counterparts. DNA-templated magnetic nanoparticle quantum dot (QD)-aptamer copolymers (MQAPs) were developed, which assembled magnetic nanoparticles (MNPs), QDs, and aptamers via HCR (Figure 3C).[30] In contrast to MQAMs containing monovalent aptamers, MQAPs captured CTCs with efficiency and purity close to 80% even in complex backgrounds (Figure 3D). MQAPs improve the magnetic response and binding selectivity of target cells via the amplification of the aptamers. In additionally, a general and simple strategy for CTCs capture was proposed by constructing a multivalent dual-aptamer-tethered RCA network.[33] The long DNA strands modified with dual-aptamers exhibited remarkable flexibility, which enabled reversible CTCs capture and release via DNA-triggered strand displacement and ensured efficient cell capture with less cell destruction. Microfluidic devices have been engineered for cancer detection, with aptamers attached to the substrate surface.[34,35] A fluidic aptamer-functionalized nanointerface microfluidic chip, named FLASH-Chip, was developed for tumor cell separation.[36] The workflow of fabricating FLASH-Chip mainly included the biotinylation of the interface and the modification with aptamer (Figure 3E). This dynamic biomimetic nanointerface captured colorectal cancer cells SW480 with an efficiency as high as 91.2%, while monovalent aptamer-modified chips (Apt-chip) only achieved an efficiency of 50.5%. Moreover, FLASH-Chip has extensive applicability to distinguish tumor cells from the breast, colon, and prostate by capturing MCF-7, HCT 116, and LNCaP cells. The capture efficiencies were between 84.3% and 91.3% (Figure 3F). This example demonstrates that aptamers can be widely integrated

https://doi.org/10.1021/cbe.4c00185
Chem Bio Eng. 2025, 2, 171−181

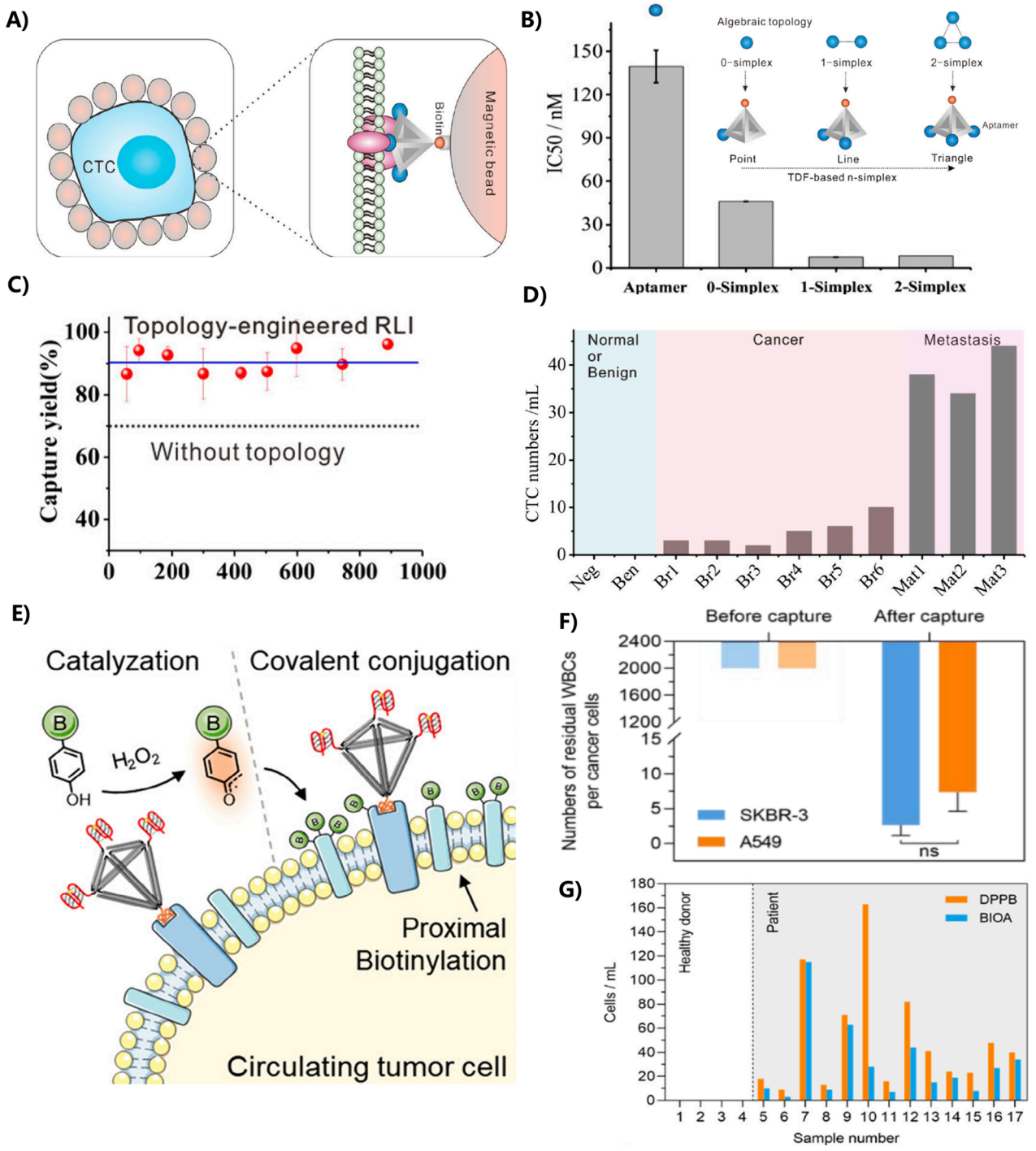


Figure 4. DNA framework-based techniques used for cell separation. (A) Scheme of CTCs capture with tetrahedral DNA framework and isolation with magnetic beads. (B) Experimental analysis of recruitment binding between n-simplexes and EpCAM using a competition assay. (C) CTCs capture yield using the 2-simplex from buffer spiked with various MCF-7 cells with and without topology-engineered receptor−ligand interactions. (D) Quantification of CTCs numbers captured from breast cancer patients, healthy controls, and patients with benign tumors. Reproduced with permission from ref 25. Copyright 2019 American Chemical Society. (E) A proximal protein biotinylation strategy employing DNA-framework-based DNAzymes binding to the surface of CTCs and biotinylating membrane proteins in situ for CTCs separation. (F) The comparison of the number of residual WBCs before and after cell enrichment by DPPB from the initial composition of 2000 WBCs per cancer cell. (G) The comparison of enriched CTCs cell numbers was via two different enrichment strategies from healthy donors and patients with cancer. Reproduced with permission from ref 41. Copyright 2022 American Chemical Society.

with versatile techniques to expand the scope of applications for cell separation.

3.2. Multivalent DNA. Due to the limitation of monovalent aptamers in improving capture efficiency and detection sensitivity, multibranched structures, such as branched DNA[37] and polyhedrons, have been utilized[25,38−41] for ultrasensitive isolation and detection of tumor cells. For example, tetra-DNA is designed to form four arms with different types of DNA sequences, in which multiple binding sites can enhance affinity and improve capture variability of CTCs isolation and detection.

https://doi.org/10.1021/cbe.4c00185
Chem Bio Eng. 2025, 2, 171−181

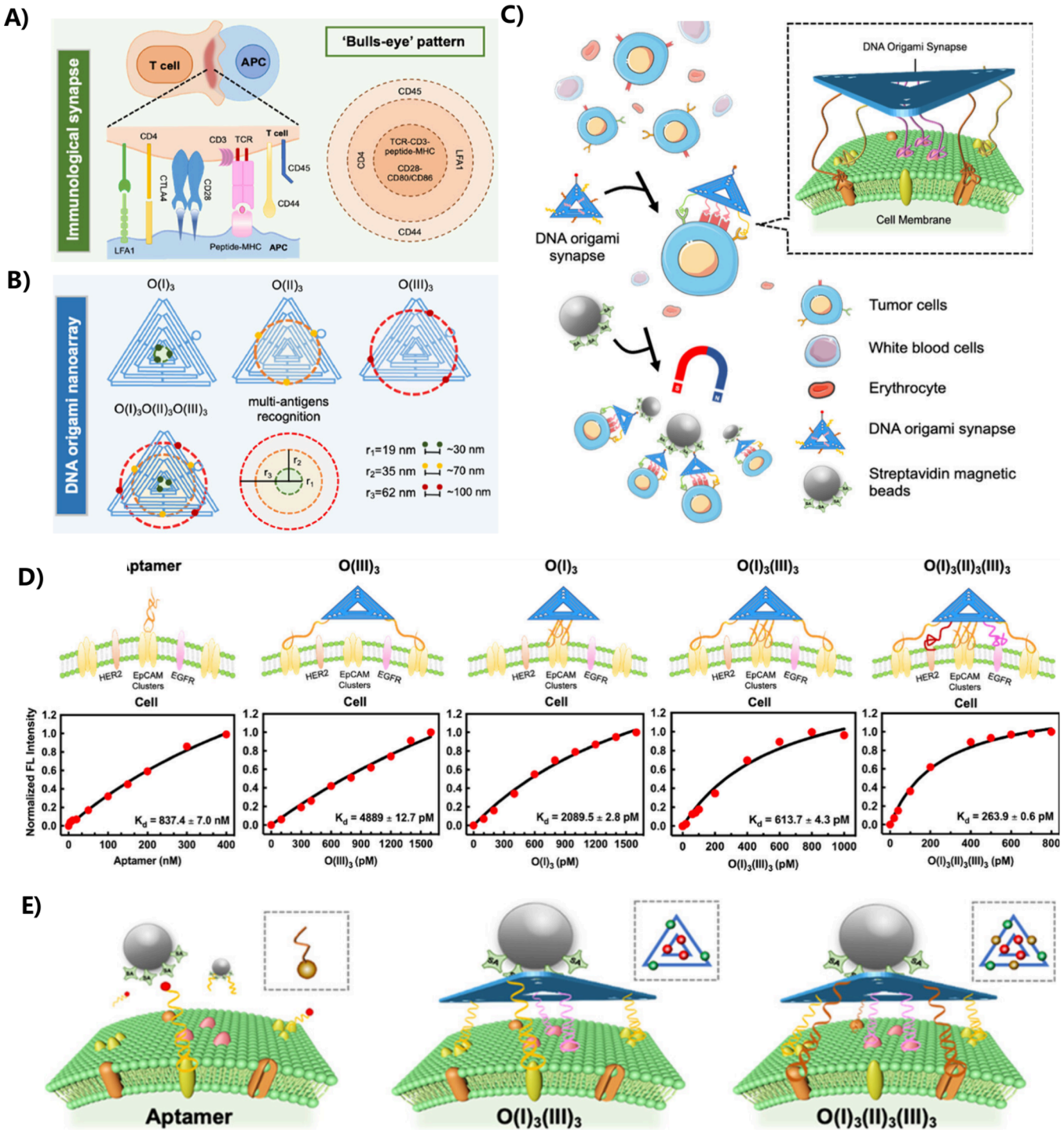


Figure 5. DNA-origami-based nanoarrays enhance cell binding affinity used for tumor cell separation. (A) A pattern of immunological synapse showing the composition and key ligands between T-cell and APC for accurate cell recognition. (B) Construction of modular nanoarrays with multiantigen recognition based on DNA triangle origami. (C) Schematic of the precise recognition and high-affinity binding of target cells captured using DNA nanoarrays. (D) Affinity assessment of the valency and interspacing of aptamers and auxiliary molecules when DNA nanoarrays bind to the target cells. (E) Biotinylated Apt-EpCAM, $O(I)_3O(III)_3$, and $O(I)_3O(II)_3O(III)_3$, using streptavidin magnetic beads for target cell capture. Reproduced with permission from ref 26. Copyright 2023 American Chemical Society.

The capture efficiency and purity for CTCs is improved as high as 97.63% and 96.96%, respectively.[37] DNA tetrahedra exhibit multivalent and topologically programmed properties. The four vertices can be conjugated with versatile ligands, and the presence of multiple ligands enhances the binding. The triangular plane of the tetrahedron contributed to the improved contact area and enhanced stability. The highly ordered nanointerfaces formed by DNA nanostructures provide a powerful means in physiological settings and serve as a platform for high-affinity cell capture.[25,39] Tetrahedral DNA framework (TDF) programming platforms were employed to mimic the topology of receptor−ligand interactions (RLIs) on the cell membrane,[25] providing a powerful means for the efficient isolation of CTCs in real clinical breast cancer patient samples

https://doi.org/10.1021/cbe.4c00185
Chem Bio Eng. 2025, 2, 171−181

(Figure 4A). TDF-based n-simplexes were engineered using 1−3 aptamers conjugated to their vertices. With an increasing number of aptamers within TDF (n-simplexes), binding affinity and cell membrane adhesion were remarkably enhanced (Figure 4B). A binding kinetics study demonstrated that rapid binding was achieved within 20 min using 2-simplex. Moreover, the capture yield of 2-simplex was 90.5%, which is significantly higher than that of the method based on a single aptamer (70.3%; Figure 4C). Additionally, clinical cases were examined, including nine cases of whole blood samples from breast cancer patients (three of them had metastasis), one healthy individual, and one benign individual serving as controls (Figure 4D). The results indicated a higher capture capability than that of aptamers without topological structures. All these studies demonstrate that tetrahedral DNA holds great potential for clinical sample analysis.

The innovative applications of TDFs involving the conjugation of multiple DNAzymes to vertices enable DNAzyme-Catalyzed Proximal Protein Biotinylation (DPPB) (<20 nm) and subsequent streptavidin separation (Figure 4E).[41] This DNA framework ensures the proper functioning of the aptamers and DNAzymes. Furthermore, the DNA framework allowed the quantitative display of DNAzymes (1, 2, or 3) to enhance the biotinylation efficiency. Taking advantage of the compact size of the DNA framework (~6 nm side length), the engineered DNAzyme was anticipated to maintain a small catalytic range for the specific labeling of CTCs, distinct from that of blood cells. In this strategy, the binding of CTCs to magnetic beads was synergistically strengthened by increasing the quantities of biotin and the ratio of covalent-to-noncovalent interactions on the cell surface. In comparison to magnetic capture via biotinylated-EpCAM aptamers, this strategy enabled more unbiased enrichment of CTCs with variable EpCAM expression. Using DPPB-based enrichment for CTCs, cancer cells were mixed with 2000-fold higher amounts of Jurkat cells. After magnetic separation, the ratio of Jurkat cells to SKBR-3 or A549 cells decreased to 2.6:1 and 7.4:1, respectively, implying an improvement in CTCs purity to 769 for high-EpCAM cells (270 for low-EpCAM cells) following DPPB-based enrichment (Figure 4F). CTCs in the peripheral blood of cancer patient samples were detected (ranging from 9 to 163 cells/mL), while no CTCs were detected in healthy donors (Figure 4G). Additionally, the integration of DNA frameworks with microfluidic and immunochip technology, owing to highly ordered nanointerfaces, was utilized for ultrasensitive profiling of CTCs.[42] Together, multiframework structures were successfully synthesized to recognize the signals of tumor-relevant circulating free DNA.[43] The aptamer on TDNs offering homogeneous orientation can effectively avoid interstrand entanglement and nonspecific interactions with the substrate interface, thereby enhancing the capture and release of CTCs from whole blood.[44] Tetrahedra play a pivotal role in augmenting the specificity and efficiency of CTCs capture, leveraging their unique structural attributes to achieve superior biomolecular interactions and cell isolation.

3.3. Nanoarchitectures. Upon assembling the aptamer into periodic DNA nanostructures, the origami tiles have significantly contributed to applications in cell recognition or molecular interaction studies.[26,45,46] DNA origami provides designed geometries and is fully addressable with nanometer precision, which makes it a robust platform for the precise organization of functional materials.[47−49] In particular, a triangular DNA origami framework with site-specifically anchored and spatially organized artificial epitopes enables us to reveal antibody−antigen interactions at the single-molecule level that will deepen our understanding of immunology recognition.[50] Inspired by natural intercellular interactions in T-cell immune synapses, the TCR-MHC peptide ternary structures are designed and surrounded by adhesion molecules.[51,52] The DNA nanojunctions with distinct sizes for precise control over the intermembrane spacing at the APC-T-cell interface were engineered.[53] Typical dissociation constants for a single aptamer−protein interactions in the range of K_d 1−100 nM are often not strong enough for specific applications. Triangle origami with interspaces of multivalent aptamers[26] was designed to achieve high-intensity RLIs to improve the binding affinity (Figure 5A). Considering the size and surface distribution of the membrane protein clusters, the pattern was designed with three concentric rings, and the interspaces were 30, 70, and 100 nm, respectively. The inner and outer rings were modified with EpCAM aptamers to target EpCAM clusters at different distances, and the middle ring was used to synergistically recognize the corresponding neighboring proteins HER2 and EGFR (Figure 5B). Due to the origami platform providing interspacing adjustment, spatial steric hindrance from the increased number of multiaptamers is avoided. As a result, the tumor cells can be effectively captured by DNA origami synapse and isolated by streptavidin magnetic beads (Figure 5C). This design covers most of the possibilities for the spatial distribution of target proteins on the membrane surface. The DNA nanoarrays binding with multiantigens were explored to recognize MCF-7 cells. The dissociation constant (K_d) was measured with flow cytometry, which indicated about 3000-fold higher binding affinity than that of the monovalent aptamer (Figure 5D). With the addition of auxiliary aptamers, the binding affinity was improved three times (O(I)3O(II)3O(III)3 over O(I)3O(III)3) indicating that the synergistic recognition of neighboring proteins HER2 and EGFR facilitated cell binding (Figure 5E). Additionally, the binding affinity may be related to the spatial distribution of most antigen clusters, and the relatively short spacing of EpCAM aptamers is favorable for binding EpCAM clusters. These results point out that the nanoarray will have stronger cell affinity when it properly matches the spatial pattern. Such nanoarrays are hopeful to provide new ideas for the accurate identification and efficient separation of cells. However, the rigid structure and large size of DNA origami usually stimulate cells and cause a significant immune response.[44] For example, researchers found that CpG-carrying DNA origami in spleen cells stimulated a stronger immune response than when equal amounts of CpG were delivered with Lipofectamine or without delivery agents. This result indicated that immunogenicity and biocompatibility must be considered when exploring the application prospects of DNA origami technology. Additionally, DNA origami can be used to create nanoscale devices that manipulate cell behavior, such as inducing cell adhesion or guiding cell migration.[51] These applications expand the range of DNA origami technology in cellular engineering, offering new tools for cell-based research and therapeutic development.

3.4. Microarchitectures. In comparison with nanoscale DNA origami, the DNA network can provide a favorable microscale structure and exhibit superior biocompatibility for cell separation and purification without inflicting harm on cells.[54,55] DNA hydrogels are three-dimensional network structures formed by DNA molecules via physical or chemical cross-linking.[56,57] For example, a DNA cryogel-based platform

https://doi.org/10.1021/cbe.4c00185
Chem Bio Eng. 2025, 2, 171−181

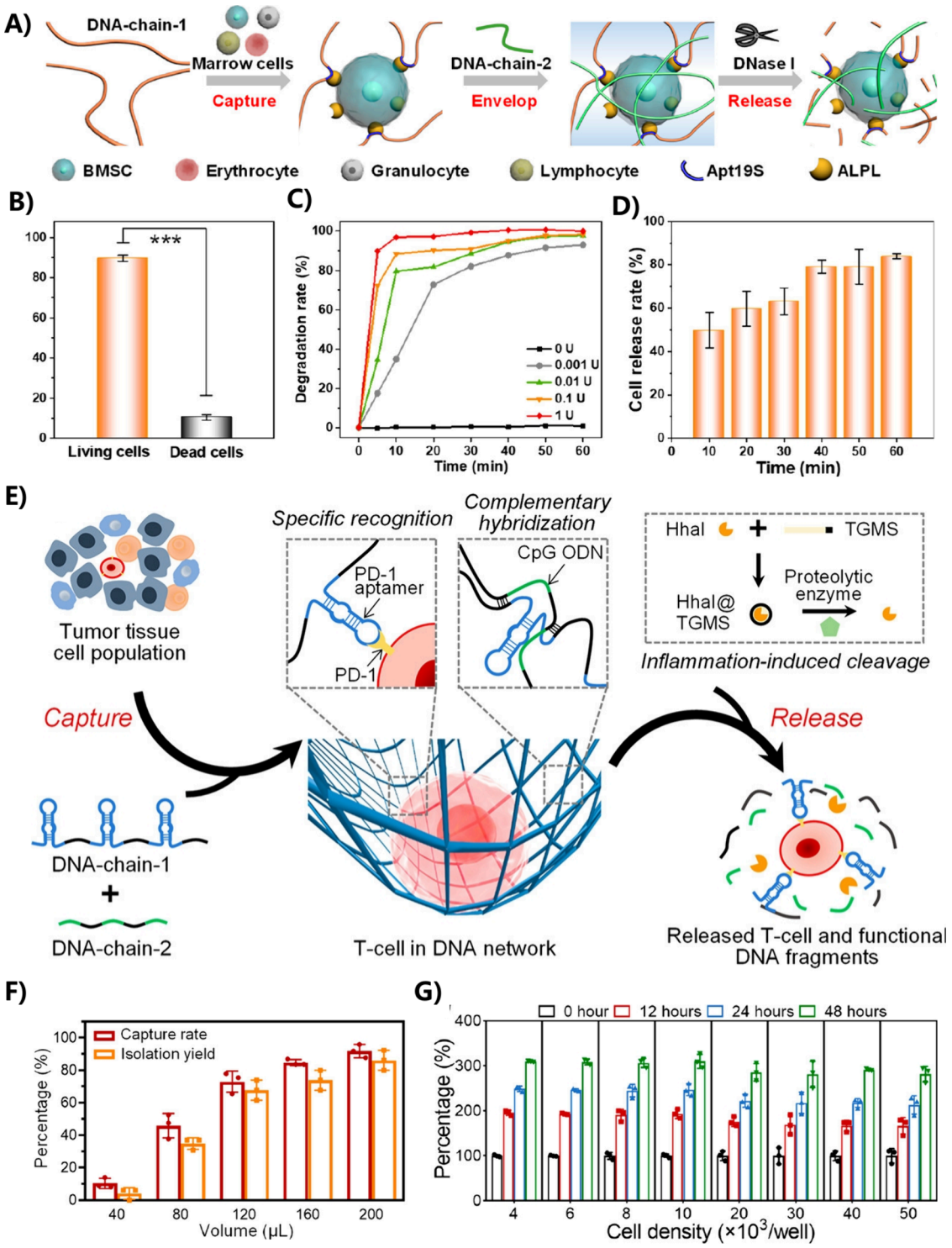


Figure 6. DNA hydrogels used for stem cell and tumor cell separation. (A) Workflow of the DNA network used for capturing, enveloping, and releasing BMSCs. (B) Cell viability determination by quantitative statistics of living and dead cells after hydrogel enrichment. (C) Degradation rate of the DNA network under different concentrations of DNase I. (D) Cell release rate monitored during degradation of the DNA network. Reproduced with permission from ref 58. Copyright 2020 American Chemical Society. (E) Construction of DNA network used for isolation of tumor-infiltrating T-cell. (F) Capture rate and isolation yield with an increase in the amount of long-strand DNA. (G) Proliferation statistics of tumor-infiltrating T cells captured by MTS at different cell densities and culture spans. Reproduced with permission from ref 59. Copyright 2021 American Chemical Society.

was constructed with an abundance of oriented and long macroporous channels in the gel matrix facilitating the migration of cells. The cryogel-based platform demonstrated a high cell capture rate of 83.3% under optimal conditions.[57] We developed a cell capture strategy based on a DNA hydrogel network, which accomplished the efficient capture, encapsulation, and enzyme-triggered release of bone marrow mesenchymal stem cells (BMSCs).[58] DNA-chain-1 contains the aptamer Apt19S sequence, which can selectively capture BMSCs via affinity interactions. DNA-chain-2 was used to construct a three-

https://doi.org/10.1021/cbe.4c00185
Chem Bio Eng. 2025, 2, 171−181

dimensional DNA network with DNA-chains-1 via partial complementarity to encapsulate cells. Subsequently, the captured BMSCs were released by DNAzymes (Figure 6A). The cell state of the released BMSCs was evaluated by microscopy, and the percentage of living cells was 89.6% (Figure 6B). This result suggested that the BMSCs released from the DNA network retained an excellent viability. Degradation of the DNA network can be controlled by DNase I concentration (Figure 6C) and 85% of the cells were released within 60 min (Figure 6D). The released cells with positive surface markers, CD29 and CD44, demonstrated that the DNA hydrogel retained the multipotential characteristics of the BMSCs.

Immunotherapy based on tumor-infiltrating T cells has made breakthrough progress in clinical trials. The isolation of high-purity and high-activity T-cells remains a major challenge in clinical therapy. We used a DNA hydrogel to successfully capture and separate tumor-infiltrating T cells.[59] First, we constructed a DNA network containing aptamers, complementary sequences, and restriction enzyme sequences. These functional modules were used to recognize and capture T cells, construct the network, and respond to cutting the DNA network to release T cells (Figure 6E). The capture rate of T-cells reached 91.8%, and the cell isolation yield was 86.2% as the volume of the long-chain DNA solution increased to 200 μL (Figure 6F). The proliferation ability of the captured T-cells was tested by comparison with that of the control group, and the cell proliferation percentage reached 280% at 48 h (Figure 6G). These results demonstrate the superior proliferation ability of the captured T-cells, thereby confirming that the DNA network-based approach ensures high viability in T-cell isolation. To enhance their stability in complex biological environments and to prevent degradation, we developed a DNA/poly-L-lysine (PLL) hydrogel for 3D cell culture, which extended the shelf life of the hydrogel.[60] In addition to integrating the aptamer sequence into the DNA network, more powerful functions for the synergistic treatment of tumors can be endowed with the hydrogel by designing DNA sequences and integrating multivalent medical functional units.[61,62] DNA networks characterized by their microscale environment, expansive contact volume, and enhanced stability possess unique advantages that render them particularly effective for translating DNA hydrogels into therapeutic settings.

4. SUMMARY AND FUTURE PERSPECTIVES

Cell separation technologies have advanced significantly to meet the growing demands in cell biology and biomedical applications. Aptamer-based affinity separation, in particular, offers highly pure cell populations and effectively targets diverse surface markers on cells. This review highlights the unique characteristics of DNA nanomaterials and examines their interactions with target cells during the separation process. Beyond their superior biocompatibility and programmable sequences, DNA nanomaterials provide versatile nanostructures for precise cell recognition and isolation, positioning them as powerful tools in biomedicine and biotechnology.

The merits of various DNA nanostructures are summarized in Table 3. Aptamers, as synthetic alternatives to antibodies, enable highly specific cell capture, which can be easily reversed by competitive DNA sequence binding. DNA tetrahedra, with their rigid structures and defined dimensions, offer mechanical strength and stability, making them reliable scaffolds for membrane-anchored capture and release. These structures have been widely used in delivering functional nucleic acids, such as aptamers and siRNAs, due to their precise orientation. DNA origami, with its customizable geometries and spatial precision, has advanced biological recognition applications. However, the potential of exogenous DNA to trigger immune responses remains a challenge for its in vivo use. DNA hydrogels, with their large dispersion and macroporous structures, show great promise for large-scale cell processing, particularly by maintaining the high bioactivity of isolated cells through hydrogel encapsulation.

Table 3. Characteristics and Functions of Typical DNA Nanostructures Used in Cell Separation and Biomedical Application

Typical DNA nanostructure	Characteristics	Enabled cell separation
Aptamer	ssDNA or RNA, 20−100 nucleotides	i) Universally available through Systematic Evolution of Ligands by Exponential Enrichment (SELEX); ii) Affordable by in vitro synthesis; iii) High specificity for cell capture; iv) Released by competition with DNA sequence; v) Superior biocompatibility.
Tetrahedron	each edge, ∼20 base pairs; tetrahedron frame 5−8 nm	i) Controllable multivalent for variable ligands; ii) Rigid frame providing geometric stability; iii) Fixed orientation for highly efficient capture and release; iv) Good penetration for targeting subcellular organelle.
DNA origami	edge length of triangle, ∼120 nm	i) Possesses designable geometries; ii) Offers spatial addressability; iii) Serves as a platform for receptor and ligand interactions; iv) Potential immune responses.
DNA hydrogel	ultralong DNA chains, >20,000 nucleotides; 3D network with micropores	i) Ensures high cell viability; ii) Enables direct isolation by hydrogel network; iii) Allows high-throughput for cell capture; iv) Release through disruption of DNA network by nuclease; v) Superior biocompatibility.

However, DNA may have a lower affinity and specificity compared to some antibodies, and their in vivo stability can be a concern due to susceptibility to nucleases. Additional challenges include higher specificity and sensitivity when DNA nanomaterials are applied to separate diverse cell populations or heterogeneous clinical samples. First, the specificity of cell separation mainly relies on the recognition of aptamers to cell surface markers. DNA origami serves as a designable platform for various receptor and ligand interactions, which can significantly enhance the recognition specificity for cell separation. Second, the sensitivity of cell separation is decided by binding capacity (K_d) between aptamer and cell markers and release efficiency. The designable sequence of aptamers and competitive DNA offers broad prospects.

Advances in computational modeling will further enhance DNA nanotechnology's application in cell recognition, improving target binding and affinity with cell surface markers. Optimizing the balance between cell capture and elution will ensure high recovery rates and maintain the bioactivity of isolated cells. As a promising technology, DNA nanomaterials will continue to drive the separation of specific cell populations,

https://doi.org/10.1021/cbe.4c00185
Chem Bio Eng. 2025, 2, 171−181

thereby expanding the exploration and therapeutic applications of functional cells in medicine.

AUTHOR INFORMATION

Corresponding Author

Dayong Yang − *Department of Chemistry, State Key Laboratory of Molecular Engineering of Polymers, Shanghai Key Laboratory of Molecular Catalysis and Innovative Materials, College of Chemistry and Materials, Fudan University, Shanghai 200438, P.R. China; Bioinformatics Center of AMMS, Beijing 100850, P.R. China;* orcid.org/0000-0002-2634-9281; Email: dayongyang@fudan.edu.cn

Authors

Huimin Bao − *Department of Chemistry, State Key Laboratory of Molecular Engineering of Polymers, Shanghai Key Laboratory of Molecular Catalysis and Innovative Materials, College of Chemistry and Materials, Fudan University, Shanghai 200438, P.R. China*

Yao Yao − *Department of Chemistry, State Key Laboratory of Molecular Engineering of Polymers, Shanghai Key Laboratory of Molecular Catalysis and Innovative Materials, College of Chemistry and Materials, Fudan University, Shanghai 200438, P.R. China*

Wenqi Tang − *Department of Chemistry, State Key Laboratory of Molecular Engineering of Polymers, Shanghai Key Laboratory of Molecular Catalysis and Innovative Materials, College of Chemistry and Materials, Fudan University, Shanghai 200438, P.R. China*

Complete contact information is available at: https://pubs.acs.org/10.1021/cbe.4c00185

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (Grant No: 22225505). Dayong Yang thanks Fudan University Ruiqing Education Funding.

REFERENCES

(1) Witek, M. A.; Freed, I. M.; Soper, S. A. Cell Separations and Sorting. *Anal. Chem.* **2020**, *92* (1), 105−131.

(2) Zhu, B.; Murthy, S. K. Stem Cell Separation Technologies. *Curr. Opin. Chem. Eng.* **2013**, *2* (1), 3−7.

(3) Nagrath, S.; Sequist, L. V.; Maheswaran, S.; Bell, D. W.; Irimia, D.; Ulkus, L.; Smith, M. R.; Kwak, E. L.; Digumarthy, S.; Muzikansky, A.; Ryan, P.; Balis, U. J.; Tompkins, R. G.; Haber, D. A.; Toner, M. Isolation of Rare Circulating Tumour Cells in Cancer Patients by Microchip Technology. *Nature* **2007**, *450* (7173), 1235−1238.

(4) Halme, D. G.; Kessler, D. A. FDA Regulation of Stem-cell-based Therapies. *N. Engl. J. Med.* **2006**, *355* (16), 1730−1735.

(5) Sims, N. R.; Anderson, M. F. Isolation of Mitochondria from Rat Brain Using Percoll Density Gradient Centrifugation. *Nat. Protoc.* **2008**, *3* (7), 1228−1239.

(6) Wu, Z.; Willing, B.; Bjerketorp, J.; Jansson, J. K.; Hjort, K. Soft Inertial Microfluidics for High Throughput Separation of Bacteria from Human Blood Cells. *Lab Chip* **2009**, *9* (9), 1193−1199.

(7) McFaul, S. M.; Lin, B. K.; Ma, H. Cell Separation Based on Size and Deformability Using Microfluidic Funnel Ratchets. *Lab Chip* **2012**, *12* (13), 2369−2376.

(8) Hughes, M. P. Strategies for Dielectrophoretic Separation in Laboratory-on-a-chip Systems. *Electrophoresis* **2002**, *23* (16), 2569−2582.

(9) Yildizhan, Y.; Erdem, N.; Islam, M.; Martinez-Duarte, R.; Elitas, M. Dielectrophoretic Separation of Live and Dead Monocytes Using 3D Carbon-Electrodes. *Sensors* **2017**, *17* (11), 2691−2704.

(10) Putnam, D. D.; Namasivayam, V.; Burns, M. A. Cell Affinity Separations Using Magnetically Stabilized Fluidized Beds-Erythrocyte Subpopulation Fractionation Utilizing a Lectin-magnetite Support. *Biotechnol. Bioeng.* **2003**, *81* (6), 650−665.

(11) Rawal, S.; Yang, Y.-P.; Cote, R.; Agarwal, A. Identification and Quantitation of Circulating Tumor Cells. *Annu. Rev. Anal. Chem.* **2017**, *10*, 321−343.

(12) Gruetzkau, A.; Radbruch, A. Small But Mighty: How the MACS®-Technology Based on Nanosized Superparamagnetic Particles has Helped to Analyze the Immune System Within the Last 20 Years. *Cytometry Part A* **2010**, *77A* (7), 643−647.

(13) Doi, D.; Samata, B.; Katsukawa, M.; Kikuchi, T.; Morizane, A.; Ono, Y.; Sekiguchi, K.; Nakagawa, M.; Parmar, M.; Takahashi, J. Isolation of Human Induced Pluripotent Stem Cell-Derived Dopaminergic Progenitors by Cell Sorting for Successful Transplantation. *Stem Cell Rep.* **2014**, *2* (3), 337−350.

(14) Shen, Q.; Xu, L.; Zhao, L.; Wu, D.; Fan, Y.; Zhou, Y.; OuYang, W.-H.; Xu, X.; Zhang, Z.; Song, M.; Lee, T.; Garcia, M. A.; Xiong, B.; Hou, S.; Tseng, H.-R.; Fang, X. Specific Capture and Release of Circulating Tumor Cells Using Aptamer-Modified Nanosubstrates. *Adv. Mater.* **2013**, *25* (16), 2368−2373.

(15) Pei, H.; Li, L.; Han, Z.; Wang, Y.; Tang, B. Recent Advances in Microfluidic Technologies for Circulating Tumor Cells: Enrichment, Single-Cell Analysis, and Liquid Biopsy for Clinical Applications. *Lab Chip* **2020**, *20* (21), 3854−3875.

(16) Kim, U.; Soh, H. T. Simultaneous Sorting of Multiple Bacterial Targets Using Integrated Dielectrophoretic-Magnetic Activated Cell Sorter. *Lab Chip* **2009**, *9* (16), 2313−2318.

(17) Xu, Y.; Phillips, J. A.; Yan, J.; Li, Q.; Fan, Z. H.; Tan, W. Aptamer-Based Microfluidic Device for Enrichment, Sorting, and Detection of Multiple Cancer Cells. *Anal. Chem.* **2009**, *81* (17), 7436−7442.

(18) Seeman, N. C. DNA Nanotechnology: Novel DNA Constructions. *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 225−248.

(19) Dong, Y.; Yao, C.; Zhu, Y.; Yang, L.; Luo, D.; Yang, D. DNA Functional Materials Assembled from Branched DNA: Design, Synthesis, and Applications. *Chem. Rev.* **2020**, *120* (17), 9420−9481.

(20) Seeman, N. C.; Sleiman, H. F. DNA Nanotechnology. *Nat. Rev. Mater.* **2018**, *3* (1), 1−23.

(21) Yao, C.; Xu, Y.; Hu, P.; Ou, J.; Yang, D. Gene-like Precise Construction of Functional DNA Materials. *Acc. Mater. Res.* **2022**, *3* (1), 42−53.

(22) Singh, N. K.; Wang, Y.; Wen, C.; Davis, B.; Wang, X.; Lee, K.; Wang, Y. High-Affinity One-Step Aptamer Selection Using a Non-fouling Porous Hydrogel. *Nat. Biotechnol.* **2024**, *42* (8), 1224−1231.

(23) Ren, Y.; Ge, K.; Lu, W.; Xie, X.; Lu, Y.; Wang, M.; Yao, B. Multivalent DNA Flowers for High-Performance Isolation, Detection, and Release of Tumor-Derived Extracellular Vesicles. *ACS Appl. Mater. Interfaces* **2023**, *15* (48), 55358−55368.

(24) Zhang, Q.; Xu, S.; Sun, Y.; Tan, T.; Zheng, J.; Zhou, Y.; Yu, S.; Zhang, J.; Zhu, J. A Detachable Magnetic Nanodevice for the Efficient Capture and Subtype Identification of Circulating Tumor Cells. *Adv. Funct. Mater.* **2024**, *34* (36), No. 2401333.

(25) Li, M.; Ding, H.; Lin, M.; Yin, F.; Song, L.; Mao, X.; Li, F.; Ge, Z.; Wang, L.; Zuo, X.; Ma, Y.; Fan, C. DNA Framework-Programmed Cell Capture via Topology-Engineered Receptor-Ligand Interactions. *J. Am. Chem. Soc.* **2019**, *141* (47), 18910−18915.

(26) Mao, M.; Lin, Z.; Chen, L.; Zou, Z.; Zhang, J.; Dou, Q.; Wu, J.; Chen, J.; Wu, M.; Niu, L.; Fan, C.; Zhang, Y. Modular DNA-Origami-Based Nanoarrays Enhance Cell Binding Affinity through the "Lock-and-Key" Interaction. *J. Am. Chem. Soc.* **2023**, *145* (9), 5447−5455.

(27) Kacherovsky, N.; Cardle, I. I.; Cheng, E. L.; Yu, J. L.; Baldwin, M. L.; Salipante, S. J.; Jensen, M. C.; Pun, S. H. Traceless Aptamer-Mediated Isolation of $CD8^+$ T Cells for Chimeric Antigen Receptor T-Cell Therapy. *Nat. Biomed. Eng.* **2019**, *3* (10), 783−795.

(28) Zhou, J.; Rossi, J. Aptamers as Targeted Therapeutics: Current Potential and Challenges. *Nat. Rev. Drug. Discovery* **2017**, *16* (3), 181−202.
(29) Rothlisberger, P.; Hollenstein, M. Aptamer Chemistry. *Adv. Drug Delivery Rev.* **2018**, *134*, 3−21.
(30) Li, Z.; Wang, G.; Shen, Y.; Guo, N.; Ma, N. DNA-Templated Magnetic Nanoparticle-Quantum Dot Polymers for Ultrasensitive Capture and Detection of Circulating Tumor Cells. *Adv. Funct. Mater.* **2018**, *28* (14), No. 1707152.
(31) Shi, P.; Wang, X.; Davis, B.; Coyne, J.; Dong, C.; Reynolds, J.; Wang, Y. In Situ Synthesis of an Aptamer-Based Polyvalent Antibody Mimic on the Cell Surface for Enhanced Interactions between Immune and Cancer Cells. *Angew. Chem., Int. Ed.* **2020**, *59* (29), 11892−11897.
(32) Zhao, W.; Cui, C. H.; Bose, S.; Guo, D.; Shen, C.; Wong, W. P.; Halvorsen, K.; Farokhzad, O. C.; Teo, G. S. L.; Phillips, J. A.; Dorfman, D. M.; Karnik, R.; Karp, J. M. Bioinspired Multivalent DNA Network for Capture and Release of Cells. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (48), 19626−19631.
(33) Lin, Y.; Jiang, L.; Huang, Y.; Yang, Y.; He, Y.; Lu, C.; Yang, H. DNA-Mediated Reversible Capture and Release of Circulating Tumor Cells with a Multivalent Dual-Specific Aptamer Coating Network. *Chem. Commun.* **2019**, *55* (37), 5387−5390.
(34) Reinholt, S. J.; Craighead, H. G. Microfluidic Device for Aptamer-Based Cancer Cell Capture and Genetic Mutation Detection. *Anal. Chem.* **2018**, *90* (4), 2601−2608.
(35) Song, Y.; Shi, Y.; Huang, M.; Wang, W.; Wang, Y.; Cheng, J.; Lei, Z.; Zhu, Z.; Yang, C. Bioinspired Engineering of a Multivalent Aptamer-Functionalized Nanointerface to Enhance the Capture and Release of Circulating Tumor Cells. *Angew. Chem., Int. Ed.* **2019**, *58* (8), 2236−2240.
(36) Wu, L.; Ding, H.; Qu, X.; Shi, X.; Yang, J.; Huang, M.; Zhang, J.; Zhang, H.; Song, J.; Zhu, L.; Song, Y.; Ma, Y.; Yang, C. Fluidic Multivalent Membrane Nanointerface Enables Synergetic Enrichment of Circulating Tumor Cells with High Efficiency and Viability. *J. Am. Chem. Soc.* **2020**, *142* (10), 4800−4806.
(37) Ding, C.; Zhang, C.; Cheng, S.; Xian, Y. Multivalent Aptamer Functionalized Ag2S Nanodots/Hybrid Cell Membrane-Coated Magnetic Nanobioprobe for the Ultrasensitive Isolation and Detection of Circulating Tumor Cells. *Adv. Funct. Mater.* **2020**, *30* (16), No. 1909781.
(38) Qin, W.; Chen, L.; Wang, Z.; Li, Q.; Fan, C.; Wu, M.; Zhang, Y. Bioinspired DNA Nanointerface with Anisotropic Aptamers for Accurate Capture of Circulating Tumor Cells. *Adv. Sci.* **2020**, *7* (19), No. 230647.
(39) Chen, Y.; Lin, M.; Ye, D.; Wang, S.; Zuo, X.; Li, M. Functionalized Tetrahedral DNA Frameworks for the Capture of Circulating Tumor Cells. *Nat. Protoc.* **2024**, *19* (4), 985−1014.
(40) Zhang, J.; Lin, B.; Wu, L.; Huang, M.; Li, X.; Zhang, H.; Song, J.; Wang, W.; Zhao, G.; Song, Y.; Yang, C. DNA Nanolithography Enables a Highly Ordered Recognition Interface in a Microfluidic Chip for the Efficient Capture and Release of Circulating Tumor Cells. *Angew. Chem., Int. Ed.* **2020**, *59* (33), 14115−14119.
(41) Chen, L.; Luo, S.; Ge, Z.; Fan, C.; Yang, Y.; Li, Q.; Zhang, Y. Unbiased Enrichment of Circulating Tumor Cells Via DNAzyme-Catalyzed Proximal Protein Biotinylation. *Nano Lett.* **2022**, *22* (4), 1618−1625.
(42) Ahmed, M. G.; Abate, M. F.; Song, Y.; Zhu, Z.; Yan, F.; Xu, Y.; Wang, X.; Li, Q.; Yang, C. Isolation, Detection, and Antigen-Based Profiling of Circulating Tumor Cells Using a Size-Dictated Immunocapture Chip. *Angew. Chem., Int. Ed.* **2017**, *56* (36), 10681−10685.
(43) Liu, Q.; Ge, Z. L.; Mao, X. H.; Zhou, G. B.; Zuo, X. L.; Shen, J. W.; Shi, J. Y.; Li, J.; Wang, L. H.; Chen, X. Q.; Fan, C. H. Valency-Controlled Framework Nucleic Acid Signal Amplifiers. *Angew. Chem., Int. Ed.* **2018**, *57* (24), 7131−7135.
(44) Pei, H.; Zuo, X.; Zhu, D.; Huang, Q.; Fan, C. Functional DNA Nanostructures for Theranostic Applications. *Acc. Chem. Res.* **2014**, *47* (2), 550−559.
(45) Douglas, S. M.; Dietz, H.; Liedl, T.; Hoegberg, B.; Graf, F.; Shih, W. M. Self-Assembly of DNA into Nanoscale Three-Dimensional Shapes. *Nature* **2009**, *459* (7245), 414−418.
(46) Douglas, S. M.; Marblestone, A. H.; Teerapittayanon, S.; Vazquez, A.; Church, G. M.; Shih, W. M. Rapid Prototyping of 3D DNA-Origami Shapes with CaDNAno. *Nucleic Acids Res.* **2009**, *37* (15), 5001−5006.
(47) Kretzmann, J. A. A.; Liedl, A.; Monferrer, A.; Mykhailiuk, V.; Beerkens, S.; Dietz, H. Gene-Encoding DNA Origami for Mammalian Cell Expression. *Nat. Commun.* **2023**, *14* (1), 1017−1027.
(48) Wang, P.; Meyer, T. A.; Pan, V.; Dutta, P. K.; Ke, Y. The Beauty and Utility of DNA Origami. *Chem.* **2017**, *2* (3), 359−382.
(49) Praetorius, F.; Kick, B.; Behler, K. L.; Honemann, M. N.; Weuster-Botz, D.; Dietz, H. Biotechnological Mass Production of DNA Origami. *Nature* **2017**, *552* (7683), 84−87.
(50) Hong, F.; Zhang, F.; Liu, Y.; Yan, H. DNA Origami: Scaffolds for Creating Higher Order Structures. *Chem. Rev.* **2017**, *117* (20), 12584−12640.
(51) Akbari, E.; Mollica, M. Y.; Lucas, C. R.; Bushman, S. M.; Patton, R. A.; Shahhosseini, M.; Song, J. W.; Castro, C. E. Engineering Cell Surface Function with DNA Origami. *Adv. Mater.* **2017**, *29* (46), No. 1703632.
(52) Zhao, S.; Tian, R.; Wu, J.; Liu, S.; Wang, Y.; Wen, M.; Shang, Y.; Liu, Q.; Li, Y.; Guo, Y.; Wang, Z.; Wang, T.; Zhao, Y.; Zhao, H.; Cao, H.; Su, Y.; Sun, J.; Jiang, Q.; Ding, B. A DNA Origami-Based Aptamer Nanoarray for Potent and Reversible Anticoagulation in Hemodialysis. *Nat. Commun.* **2021**, *12* (1), 358−368.
(53) Du, Y.; Lyu, Y.; Lin, J.; Ma, C.; Zhang, Q.; Zhang, Y.; Qiu, L.; Tan, W. Membrane-Anchored DNA Nanojunctions Enable Closer Antigen-Presenting Cell-T-cell Contact in Elevated T-cell Receptor Triggering. *Nat. Nanotechnol.* **2023**, *18* (7), 818−827.
(54) Tang, J.; Jia, X.; Li, Q.; Cui, Z.; Liang, A.; Ke, B.; Yang, D.; Yao, C. A DNA-based Hydrogel for Exosome Separation and Biomedical Applications. *Proc. Natl. Acad. Sci. U. S. A.* **2023**, *120* (28), No. e2303822120.
(55) Song, P.; Ye, D.; Zuo, X.; Li, J.; Wang, J.; Liu, H.; Hwang, M. T.; Chao, J.; Su, S.; Wang, L.; Shi, J.; Wang, L.; Huang, W.; Lal, R.; Fan, C. DNA Hydrogel with Aptamer-Toehold-Based Recognition, Cloaking, and Decloaking of Circulating Tumor Cells for Live Cell Analysis. *Nano Lett.* **2017**, *17* (9), 5193−5198.
(56) Yao, C.; Zhang, R.; Tang, J.; Yang, D. Rolling Circle Amplification (RCA)-based DNA Hydrogel. *Nat. Protoc.* **2021**, *16* (12), 5460−5483.
(57) Mu, Y.; Wang, X.; Du, X.; He, P.-P.; Guo, W. DNA Cryogels with Anisotropic Mechanical and Responsive Properties for Specific Cell Capture and Release. *J. Am. Chem. Soc.* **2024**, *146* (9), 5998−6005.
(58) Yao, C.; Tang, H.; Wu, W.; Tang, J.; Guo, W.; Luo, D.; Yang, D. Double Rolling Circle Amplification Generates Physically Cross-Linked DNA Network for Stem Cell Fishing. *J. Am. Chem. Soc.* **2020**, *142* (7), 3422−3429.
(59) Yao, C.; Zhu, C.; Tang, J.; Ou, J.; Zhang, R.; Yang, D. T Lymphocyte-Captured DNA Network for Localized Immunotherapy. *J. Am. Chem. Soc.* **2021**, *143* (46), 19330−19340.
(60) Tang, J.; Wang, J.; Ou, J.; Cui, Z.; Yao, C.; Yang, D. A DNA/Poly-(L-lysine) Hydrogel with Long Shelf-Time for 3D Cell Culture. *Small Methods* **2024**, *8* (7), No. 2301236.
(61) Tang, J.; Ou, J.; Zhu, C.; Yao, C.; Yang, D. Flash Synthesis of DNA Hydrogel via Supramacromolecular Assembly of DNA Chains and Upconversion Nanoparticles for Cell Engineering. *Adv. Funct. Mater.* **2022**, *32* (12), No. 2107267.
(62) Yang, S.; Wu, J.; Wang, Z.; Cheng, Y.; Zhang, R.; Yao, C.; Yang, D. A Smart DNA Hydrogel Enables Synergistic Immunotherapy and Photodynamic Therapy of Melanoma. *Angew. Chem., Int. Ed.* **2024**, *63* (14), No. e202319073.

https://doi.org/10.1021/cbe.4c00185
Chem Bio Eng. 2025, 2, 171−181