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Convection Driven Pull-Down Assays in Nanoliter Droplets using Scaffolded Aptamers

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Abstract

One of the great challenges in cellular studies is to develop a rapid and biocompatible analytical tool for single-cell analysis. We report a rapid, DNA nanostructure-supported aptamer pull-down (DNaPull) assay under convective flux in a glass capillary for analyzing contents of droplets with nano or picoliter volumes. We have demonstrated that the scaffolded aptamer can greatly improve the efficiency of target molecules' pull-down. The convective flux enables complete reaction in less than 5 min, which is an 18-fold improvement compared to purely diffusive flux (traditional model of stationary case). This established DNaPull assay can serve as a rapid and sensitive analytical platform for analyzing a variety of bioactive molecules, including small molecules (ATP, limit of detecton (LOD) of 1 μM), drug (cocaine, LOD of 1 μM), and biomarker (thrombin, LOD of 0.1 nM). Significantly, the designed microfluidic device compartmentalizes live cells into nanoliter sized droplets to present single-cell samples. As a proof of concept, we demonstrated that cellular molecules (ATP) from a discrete number of HNE1 cells (0, 1, 2, 3, 4, 5 cells) lysed inside nanoliter sized droplets can be analyzed using our DNaPull assay, in which the intracellular ATP level was estimated to be ~3.4 mM. Given the rapid assay feature and single-cell sample analysis ability, we believe that our analytical platform of convection driven DNaPull in glass capillary can provide a new paradigm in biosensor design and will be valuable for single cell analysis.

Introduction

Single-cell analysis has become a highly promising tool for cellular studies.[1-4] Microfluidic generation of spatially defined droplets with nano or picoliter volumes is extremely useful in biomedical devices for single-cell samples because of their comparable size with living cells and high-throughput assay capability.[5-10] Such devices equipped with a rapid and biocompatible analytical tool can provide a powerful and versatile approach for single-cell analysis.[11-15] A simple and direct way is combining microfluidic platform with pull-down assay. In the classical pull-down assay, the target molecule (bait) is captured on an immobilized affinity-specific ligand, bringing along its binding partners (prey) from cell lysate.[16-18] The identity of the prey is usually determined using western blotting or mass spectrometry. A key advantage of pull-down assay in microfluidics is that the analyte can be directly visualized from nano or picoliter droplet under fluorescent microscope, bypassing further laborious sample pre-preparation steps necessary for western blotting or mass spectometry. Pull-down assay is similar in methodology to ELISA, both of which are powerful analytical techniques, except that the antigen/antibody interaction is replaced by some other affinity system. It is thus highly desirable to devise an efficient biomolecule-recognition interface for pull-down assay inside microfluidic channel.

DNA nanotechnology has attracted intense interest because it enables the functionalization of macroscopic surfaces with atomic spatial precision and versatile functionality,[19-21] thus offering a highly promising approach for the design and construction of an intelligent interface for efficient pull-down assay. In particular, three-dimensional (3-D) DNA tetrahedron architectures, possessing mechanical

rigidity and structural stability, have proven to be excellent candidates to immobilize biomolecules on macroscopic surfaces.[22-28] Taking advantage of the steady structure and consistently favourable orientation of DNA tetrahedron bridges, the capture biomolecules can be directly immobilized on the surface with specific orientation and well-defined spacing.[28] Many recent advances have demonstrated that DNA tetrahedron-decorated gold surfaces exhibit superior biomolecular recognition, and thus hold great promise to develop high-performance bioassay platforms.[26,28,29] Despite the progress, their practical applications in fluorescent microarrays are still hindered by the complicated fabrication process, together with the high cost and strong fluorescence quenching effect of gold substrates.[30]

Here we developed a rapid DNA nanostructure scaffold-supported aptamer pull-down (DNaPull) assay in nano or picoliter droplet, for applications in single-cell analysis. In this design, we employed DNA aptamers to ensure highly selective recognition of target molecules and immobilized them onto the inner surfaces of glass capillary via a rigid and spatially isolated 3D DNA nanostructure bridge. A bubble-mediated shuttle reaction[31] was introduced to improve the rate performance of DNaPull assay by automatic sample delivery and convective transport with pressure-driven fluid flow. An array of DNaPull-based sensors inside glass capillary can serve as a rapid and highly sensitive multiplex assay to simultaneously detect various bioactive molecules in nanoliter droplet, including small molecule (ATP), biomarker (thrombin), and drug (cocaine). Importantly, cellular molecules (ATP) can be analysed using our DNaPull assay by compartmentalizing cells (1~5 cells) into

nanoliter sized droplets.

EXPERIMENTAL SECTION:

Chemicals and Materials. The glass capillary with a 200 μm inner diameter was purchased from the Shanghai Xinpeng Co., Ltd (Shanghai, P. R. China). The syringe pump (LSP02-1B) was purchased from the Baoding Longer Precision Pump Co., Ltd (Baoding, Hebei, P. R. China). The CCD cameras (TK-C9200EC for capturing videos and a high performance cooled CCD, Alta U4000, for data collection) were purchased from the JVC (China) Investment Co., Ltd (Shanghai, P. R. China) and Apogee Instruments Inc. (Roseville, CA, US), respectively. A green solid state laser (MXL-III-532, 532 nm, 50 mW) was purchased from the Changchun New Industries Optoelectronics Technology Co., Ltd (Changchun, Jilin, P. R. China). A narrow band pass interference filter (JSL600-25, 600±5 nm, diameter of 25 mm) was purchased from the Zolix Instruments Co., Ltd (Beijing, P. R. China). The luciferin-luciferase-based ATP luminescence assay kit was purchased from Beyotime Biotechnology (China). Human α-thrombin, ATP disodium, GTP disodium, CTP disodium were purchased from HeFei BoMei Biotechnology Co., Ltd. (Hefei, China). UTP trisodium was purchased from Shanghai yuanye Bio-Technology Co., Ltd. (Shanghai, China). ADP disodium, AMP disodium, 3-aminopropyltriethoxysilane (APTES), 25% glutaraldehyde and Tris (hydroxymethyle)aminomethane were purchased from Sigma-Aldrich. 100 mL 10 mM phosphate buffer (PB) (pH 7.4) solution was prepared by mixing 81 mL 10 mM Na_2HPO_4 aqueous solution with 19

mL 10 mM NaH_2PO_4 aqueous solution. All Oligonucleotides were synthesized and purified by Sangon Biotech Shanghai CO. (Shanghai, China), and DNA sequences and their labeling are shown in Table S1.

Synthesis of DNA nanostructure scaffold-supported aptamer pull-down (DNaPull) Probes. The DNaPull probes were formed based on the previous literature.[30] The four oligonucleotides were mixed stoichiometrically and dissolved in 1×TAE/Mg^{2+} buffer (40 mM Tris-HCl, 1 mM EDTA, 3 mM Na^+, 12.5 mM Mg^{2+}, pH 8.0) with a final concentration of 10 μM. The mixture was heated to 95 °C for 2 min and then was cooled to 4 °C within 30 s.

Sample Assay. The detection of ATP, cocaine, and thrombin was carried out in the sandwich format inside glass capillary. A bubble-mediated shuttle reaction was introduced, which the droplets are shuttled back and forth along the glass copaillary, which sweeped over DNaPull probes inside the glass capillary. Specifically, for the ATP assay, four strands, DNaPull-A-ATP, DNaPull-B, DNaPull-C, DNaPull-D were annealed to form DNaPull-ATP, which was then used to fabricate microarrays inside glass capillary. Briefly, 10 μM DNaPull-ATP was dissolved in the immobilization buffer (1.0 M NaCl+0.15 M $NaHCO_3$), and the alcohol (butanol, pentanol and hexanol) acted as the organic carrier fluid. After the probe droplet array was generated along the glass capillary, the glass capillary was then incubated at room temperature for overnight for immobilizing the probe in the droplets onto the inner wall of the

aldehyde modified glass capillary. After being rinsed with immobilizing buffer and DI water, the glass capillary was chemical reduced using $NaBH_4$ solution (100 mg of $NaBH_4$ dissolved in 30 mL of 1 × PBS and 10 mL of 95% EtOH) for 45 min, and followed by a blocking step using 5% BSA in PBS (20 mM sodium phosphate, pH 7.5, 100 mM NaCl and 0.1 mM EDTA). The mixture containing ATP at variable concentrations (1 μM, 10 μM, 100 μM, 250 μM, 500 μM, and 1 mM) and ATP reporter-Cy3 (500 nM) was introduced into the glass capillary and incubated at 37 ℃ for 30 min in a humidity chamber. After being washed with washing buffer (twice with PBST (1*PBS buffer contains *0.1% Tween 20*) buffer and once with PBS buffer). In a typical experiment, we have characterized three replicate assays. For each sample replicate, the fluorescence image of each DNaPull sensor spots after capturing the target molecules was collected by the high performance cooled CCD camera with a 80×80 pixel region-of-interest and further analyzed with the MaxIm DL software. The fluorescence intensities were thus averaged over 2400 pixels and 3 sample replicates with error bars showing the standard deviation. Before use, we randomly selected 3 from the same batch of DNaPull-ATP assay functionalized glass capillaries and carried out the calibration in the presence of 1000 μM ATP. The system exhibited good reproducibility and batch homogeneity with standard deviation less than 5%. Likewise, for the cocaine assay, DNaPull-A-Cocaine (cocaine aptamer), DNaPull-B, DNaPull-C, and DNaPull-D were annealed to form DNaPull-Cocaine. 10 μM DNaPull-Cocaine was then used to fabricate microarrays inside glass capillary for the cocaine assay. The mixture containing cocaine at variable concentrations(1 μM, 10

μM, 50 μM, 100 μM, 200 μM, 500 μM, and 1 mM) and cocaine reporter-Cy3 (500 nM) was introduced into the glass capillary and incubated at 37 °C for 30 min in a humidity chamber, followed by the same procedure described in thrombin assay. The thrombin assay was conducted in a similar procedure except that thrombin at variable concentrations (100 pM, 1 nM, 10 nM, 100 nM, 250 nM, 500 nM, and 1 μM) was used.

Multiplex Detection in Serum. Three types of DNaPull probes (DNaPull -ATP, DNaPull -Cocaine and DNaPull -Thrombin) microarrays were sequentially immobilized along the glass capillaries using a droplet array generator. As schematically illustrated in Figure S2, the slotted vials which contained three types of DNaPull probes (DNaPull-ATP, DNaPull-Cocaine and DNaPull-Thrombin), immobilization buffer (1.0 M NaCl, 0.15 M $NaHCO_3$), and the organic carrier fluid, were arranged alternately on a stepping motor. As the stepping motor rotates, the tip of the aldehyde modified glass capillary sweeps through all the vials. The probe solution flows into the capillary sequentially with the drawing of the syringe pump, and then an array of probe droplets in the carrier is formed along the glass capillary automatically, followed by the same chemical reducing and blocking treatment as described for preparing ATP assay. Subsequently, serum sample 1 (50% serum, blank, row 1), serum sample 2 (250 μM ATP and 500 nM ATP reporter-Cy3 in 50% serum, row 2), serum sample 3 (100 μM cocaine and 500 nM cocaine reporter-Cy3 in 50% serum, row 3), serum sample 4 (100 nM human α-thrombin and 500 nM thrombin

reporter-Cy3 in 50% serum, row 4), serum sample 5 (250 μM ATP and 500 nM ATP reporter-Cy3, 100 μM cocaine, and 500 nM cocaine reporter-Cy3 in 50% serum, row 5), serum sample 6 (250 μM ATP , 500 nM ATP reporter-Cy3, 100 nM human α-thrombin, and 500 nM thrombin reporter-Cy3 in 50% serum, row 6), serum sample 7 (100 μM cocaine 500 nM cocaine reporter-Cy3, 100 nM human α-thrombin, and 500 nM thrombin reporter-Cy3 in 50% serum, row 7) and serum sample 8 (250 μM ATP , 500 nM ATP reporter-Cy3, 100 μM cocaine, 500 nM cocaine reporter-Cy3, 100 nM human α-thrombin, and 500 nM thrombin reporter-Cy3 in 50% serum, row 8). The mixture was introduced into tubular DNM sensor and incubated for 30 min at 37 °C. The washing buffer was then introduced to wash the capillary three times (twice with PBST buffer and once with PBS buffer).

Results and discussion

The DNaPull probes featuring three amino groups and extended aptamer-sequences were synthesized by simply mixing four single-stranded DNA through self-assembly within 2 min.[24] These specifically designed DNA nanostructures were covalently bonded onto the surface inside glass capillary via the amine-aldehyde reaction (Figure 1a). For generating the droplet array inside glass capillary, different DNaPull probes were immobilized along the glass capillary to form one-dimensional array without employing sophisticated and expensive photolithography procedure[32]. As the sample (e.g., cell or tissue extracts) is applied into the glass capillary, the DNA nanostructure-supported aptamers selectively pull

down molecules of interest and their binding partner from the droplet, followed by washing away the unbound components. Captured molecules are then determined using fluorescence microscopy (Figure 1b).[33]

DNaPull performance in glass capillary was first evaluated by ATP analysis employing two split fragments of anti-ATP aptamer.[34] One fragment was appended to the rigid DNA nanostructure inside the glass capillary as the pull down element. The other was modified with a fluorescein (Cy3) tag to introduce signal. In the presence of ATP, the two parts of the aptamer were expected to form a sandwich structure; thus the fluorescein tag was pulled down to the surface and produced signal (Figure 1c). We challenged the DNaPull assay with a series of concentrations of ATP (1 μM to 1 mM). The fluorescent signal increased monotonically with the concentration of ATP (Figure 1d). A control experiment was carried out to confirm that the observed fluorescent change was only specific to the binding of ATP with the split aptamer. When three ATP analogues (1 mM each, CTP, GTP, UTP, ADP and AMP) were employed, the produced fluorescence was merely detectable (Figure 1e). The DNaPull assay exhibited a significantly improved performance (~3 fold) compared to the conventional ssDNA probe-based pull down assay (Figure 1b, f). We attribute this improvement to the highly rigid and orientated DNA nanostructures in glass capillary, which accommodates the pendant probe of DNA tetrahedron with highly ordered upright orientation to avoid entanglement and local aggregation that are often encountered by soft ssDNA probes.[30]

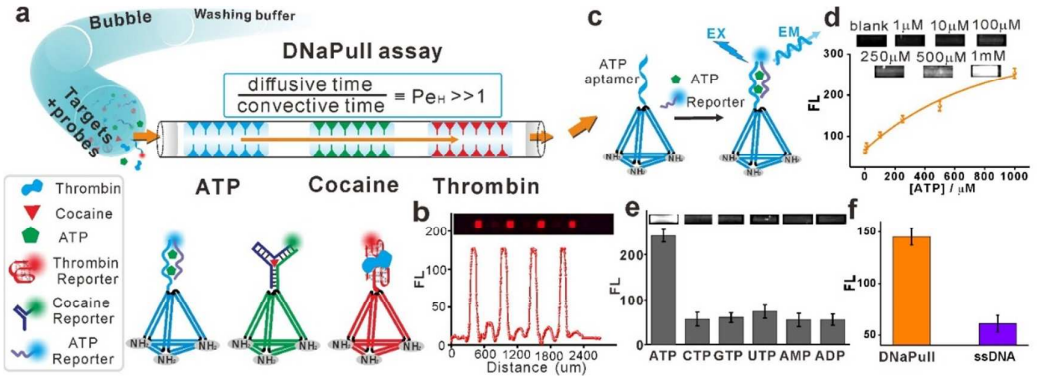

Figure 1. a) Construction of an array of DNaPull-based sensors inside glass capillary for the detection of ATP, cocaine and thrombin through a bubble-mediated shuttle reaction process. b) The spatially defined droplets with nanoliter in the glass capillary. c) Construction of a DNaPull based ATP sensor. d) The fluorescence intensities and corresponding fluorescent images (shown as inset) of DNaPull probes in the presence of ATP at 1, 10, 100, 250, 500, or 1000 μM. e) Selectivity of the DNaPull based ATP sensor over CTP, GTP, UTP, ADP and AMP (all at 1 mM). The corresponding fluorescent images are shown as inset. f) Comparison of DNaPull and ssDNA based sensor performance (with 500 μM ATP).

Rapid sensing capability is a crucial requirement for the successful development of single-step point-of-care diagnostics. Since the transportation of target molecules to the sensing interface is of equal importance in influencing the binding kinetics as the chemical reaction itself,[35] we therefore examined the effect of convection in our DNaPull assay in glass capillary (Figure 2a). We started with the simplest scenario, in which target diffused through solution and binds immediately upon encountering the sensor surface. Theoretical studies were conducted on the binding reaction process inside glass capillary under different conditions using a

computational fluid dynamic simulation. Target solution flows with a constant velocity (υ) through a glass capillary with the inner diameter D (=200 μm) and the inner wall of which contains a sensing surface of length L (=200 μm) in the flow direction. The sensing surface was functionalized with DNaPull probes, with a surface density of 10 μM/m^2. The effect of purely diffusive flux was first evaluated with a setup velocity (υ) of 0 mm/s and a target concentration at 1 μM under a series of reaction times, including 1 s, 20 s, 180 s, 540 s, and 1620 s. Figure 2b shows the behavior of this ideal DNaPull assay. As targets are pulled down at the sensing interface, a depleted zone is formed (20 s), which starts relatively flat and then grows radially (between 20 s and 540 s) until its thickness range spans comparable to the length of the sensing surface L (540 s). It then extends into the entire glass capillary (1620 s). The simulation results suggest that the complete reaction time for all probes at the tubular sensing interface was 1620 s. Moreover, our simulation study suggests that the reaction could be greatly accelerated by adding convective transport, which the target move along with a fluid flow. For instance, at a target solution concentration of 1 μM, the reaction was completed within 30 s under a flow velocity of 10 mm/s, which was shortened approximately 54-folds (30 s vs. 1620 s) compared to the case of purely diffusive flux (i.e., flow velocity of 0 mm/s). The completed reaction time in glass capillary of DNaPull assay decreased monotonically (360 s to 30 s) as the flow velocity increased from 0.01 mm/s to 10 mm/s (Figure. 2c), showing that the reaction time is highly dependent on flow velocity of the target solution. In this scenario, target would be forced to diffuse into the depleted zone of the sensing interface under

constant continuous flow, therefore the reaction time was determined by fluid velocity. More importantly, our simulation studies here are in agreement with the experimental results observed with 1 mM ATP assay. We found that it took over 90 min to complete ATP pull-down reaction under purely diffusive flux (Figure. 2d). By adapting pressure-driven flow with volumetric flow rate 5 μL/min inside DNaPull probes functionalized glass capillary, we could achieve complete pull down of target molecules within 4 min (Figure. 2e).

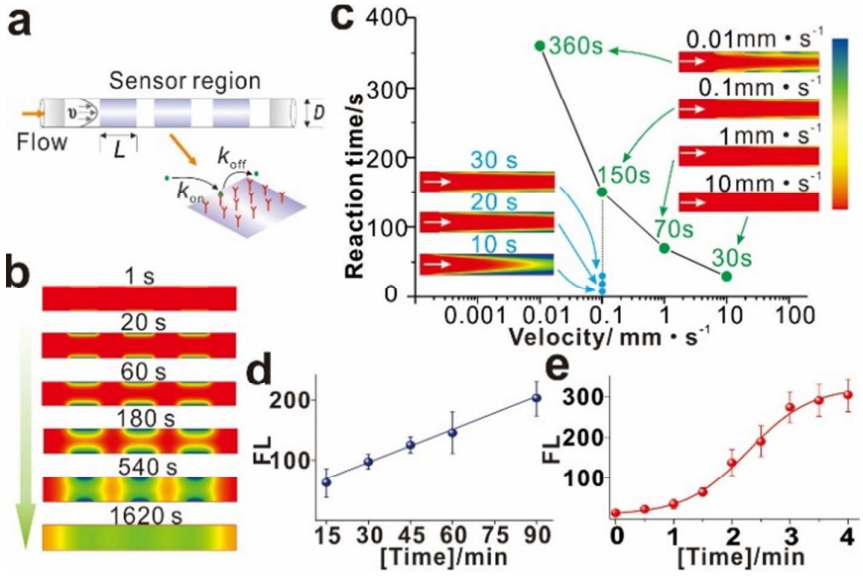

Figure 2. Theoretical and experimental studies on the bubble-mediated shuttle reaction process inside glass capillary. a) Schematic of the model system. The key simulation parameters were based on our sensing system: glass capillary of inner diameter D (=200 μm) and a tubular sensing surface of length L (=200 μm), the diffusion coefficient of the target solution was 10-11 m^2/s. b) The effect of purely diffusive flux (with 0 mm/s flow velocity) to the tubular sensing interface (with 1 μM target solution). c) The effect of flow velocity (0.01, 0.1, 1, 10 mm/s) on the

completed reaction time in glass capillary of DNaPull assay. The target solution was pressure driven. Experimental comparison of reaction time between d) purely diffusive flux and e) convective flux at a flow rate of 5 μL/min (both with 1 mM ATP).

Utilizing the similar theory of ATP sensing, this DNaPull assay in glass capillary could also be extended to a versatile platform for the quantitative detection of a broad range of biomolecules with high sensitivity. For instance, by incorporating an anti-cocaine aptamer (Figure 3a),[36,37] a DNaPull assay for cocaine detection was developed. The fluorescent intensities exhibited a non-linear dependence on the cocaine concentration in the range of 1 μM to 1 mM, with a detection limit of 1 μM (Figure 3b). Control experiments revealed that analogue molecules of cocaine (benzoylecgonine (BE) and methylecgoine (ME)) only produced very weak responses, demonstrating the high selectivity owing to the intrinsic specificity and affinity properties of DNA aptamers (Figure S3). This DNaPull based cocaine sensor was further challenged with a variety of cocaine-containing media that were critical to their practical applications (Figure S4). This DNaPull assay showed excellent performance in tainted samples, such as 10% or 50% serum, soda and sucrose. Furthermore, a similar strategy was used to construct a DNaPull assay for thrombin detection (Figure. 3c). We note that the fluorescence signal intensity increased gradually as the thrombin concentration was increased from 0.1 nM to 1 μM and showed a limit of detection of 0.1 nM (Figure. 3d). It is possible to optimize the

sensor performance for chemical and biomolecular detection. We investigated the concentration of Cy3-labled reporter and note that the fluorescence signals were dependent on the concentration of Cy3-labled reporter and the optimum concentration was determined to be 500 nM for the detection of thrombin, cocaine, and ATP (Figure S5).

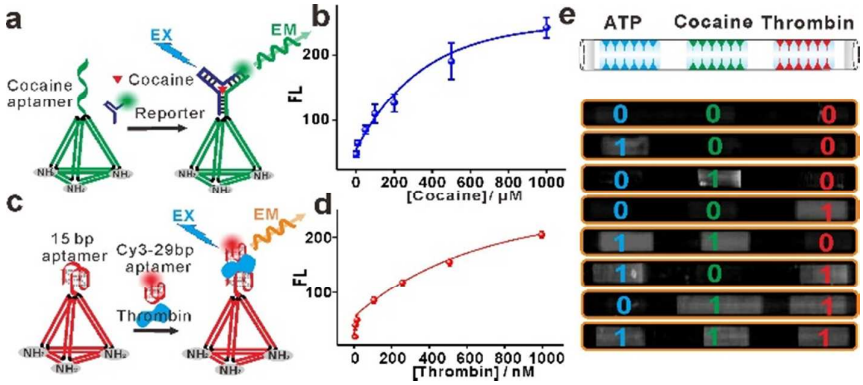

Figure 3. a) Construction of a DNaPull based cocaine sensor. b) The fluorescence intensities of DNaPull probes in the presence of cocaine at 1, 10, 50, 100, 200, 500, or 1000 μM. c) Construction of a DNaPull based thrombin sensor. d) The fluorescence intensities of DNaPull probes in the presence of thrombin at 0.1, 1, 10, 100, 250, 500, or 1000 nM. e) Top: scheme of an array of DNaPull-based sensors inside glass capillary for the simultaneous detection of ATP, cocaine, and thrombin. Bottom: Fluorescent images of DNaPull assay recorded from various samples containing different combinations of ATP, cocaine, and thrombin in 50% serum.

Having evaluated the DNaPull assay performance with a number of representative biomolecules, we further investigated whether such system could

function as multiplex detection platform in complex matrices. We designed a linear array of DNaPull assay by immobilizing three types of DNaPull probes (DNaPull-ATP, DNaPull-Cocaine, and DNaPull-Thrombin) onto inner wall of glass capillary in sequential order (Figure 3e), and challenged such system with a series of test samples by dissolving different combinations of ATP, cocaine, and thrombin in 50% serum. The multiplex assay can then be directly read from the microscopic fluorescent images. The addition of a type of biomolecule is defined as the "1" state, and the "0" state corresponds to no addition of biomolecules. Therefore, the output was "000" when neither biomolecule was introduced, corresponding to a blank control (Figure 3e, Row 1). The addition of either 250 μM ATP (Figure 3e, Row 2, "100"), 100 μM cocaine (Figure 3e, Row 3, "010"), or 100 nM thrombin (Figure 3e, Row 4, "001"), caused the fluorescence variations in corresponding to the DNaPull probes, which could be defined as the references. Based on the above results, it is thus possible to use this DNaPull assay to realize multiplex detection. For instance, when both ATP and cocaine were added simultaneously, the output became "110" (Figure 3e, Row 5). Similar results can also be obtained when both ATP and thrombin (Figure 3e, Row 6, "101"), or both cocaine and thrombin (Figure 3e, Row 7 "011"), were introduced into the glass capillary. Finally, the addition of all three types of biomolecules resulted in the increase of fluorescence intensities in all microarray regions, leading to a "111" state (Figure 3e, Row 8). Taken together, these multiplex assay studies clearly indicated that our DNaPull assay could potentially function as a rapid and sensitive point-of-test device for multiplex in vitro bioassays and clinical diagnostics.

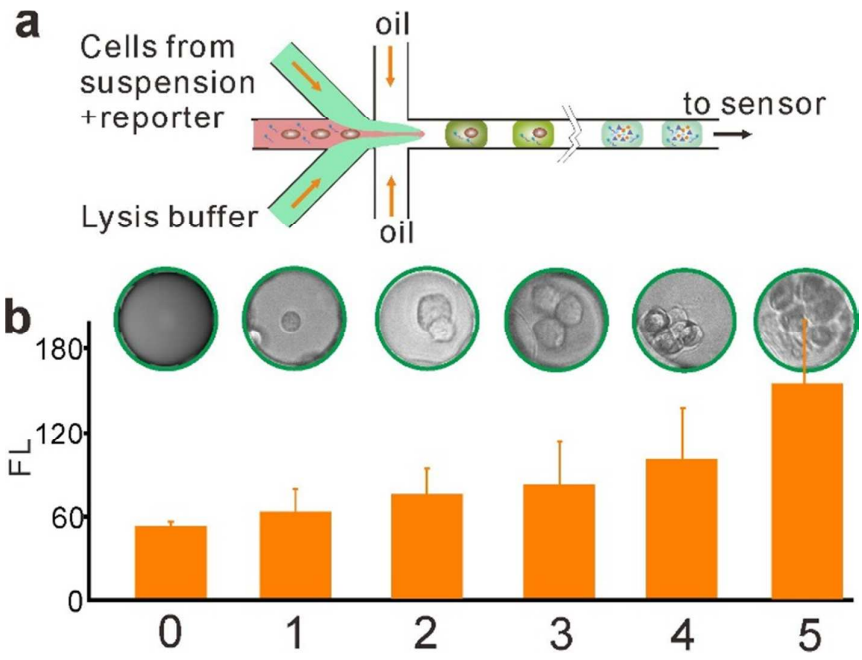

Figure 4. a) Schematic illustrating the sample preparation for the cellular ATP assay with nanoliter sized droplet encapsulation and lysis. b) Measurement of cellular ATP levels for HNE1 cells (0, 1, 2, 3, 4, 5 cells from left to right) encapsulated inside nanoliter sized droplets using DNaPull assay.

It is critically important to evaluate the single-cell analysis applicability of this DNaPull assay by challenging the sensor with cellular ATP from finite cells. We designed a microfluidic device to compartmentalize cells into nanoliter sized droplets for analysis of all of their ATP. One flow contains a cell suspension with reporters, and the other flow contains a lysis buffer. Once following droplet formed, the cell is lysed and released its ATP, which then be detected by the DNaPull assay in the glass capillary (Figure. 4a). As shown in Figure. 4b, a series of different numbers of HNE1 cells (0, 1, 2, 3, 4, 5 cells from left to right) were encapsulated inside single nanoliter

sized droplet. After lysing, the droplets contained HNE1 cells produced higher fluorescence intensity than that of the empty droplet (contained zero cells), which indicated the successful capture of the released cellular ATP. We noted that the fluorescence intensity increased gradually as the numbers of cells encapsulated in the droplet was increased from zero to five. In our preliminary experiment, we could quantify ATP concentration from ~5 cells obtained by encapsulating in nanoliter sized droplets compared to the ~5000 cells usually required for ATP bioluminometric assays kits.[38] The intracellular ATP level was estimated to be ~3.4 mM using DNaPull assay, which was comparable to the analysis (~2.6 mM) conducted using commercial ATP bioluminescent somatic cell assay kit.[39] Combining this with single-molecule fluorescence microscopy,[16,40-42] this method can be used to analyze and quantify cellular molecules at single-cell level.

Conclusion

We developed a rapid DNA nanostructure scaffold-supported aptamer pull-down (DNaPull) assay under convective flux in glass capillary, towards the goal of single-cell analysis. This system provides several unprecedented advantages that make them a promising sensor system for the rapid pull-down assay of nanoliter sized droplets. First, the DNA nanostructure scaffolded aptamer can greatly improve the biomolecular recognition capability owing to their specific orientation and well-defined spacing for efficient pull-down various bioactive molecules. Second, the DNaPull reaction could be greatly accelerated by adding convective transport in glass

capillary, since the target is forced to diffuse into the depleted zone of surface under constant continuous flow. The convective flux enables complete reaction in less than 5 min (that is an 18-fold improvement compared to the static case, 5 min vs. 90 min), thus allowing for a rapid pull-down assay. Third, by constructing a series of biomolecule-responsive DNaPull based sensors inside glass capillary, it can serve as a convenient and sensitive platform for analyzing nano or picoliter sized droplets, which could present single-cell samples. Lastly, it is envisioned that our method can analyze and quantify cellular molecules at single-cell level. Given the rapid assay feature and single-cell sample analysis ability, we believe that our analytical platform of convection driven DNaPull in glass capillary can provide a new paradigm in biosensor design and significantly further the field of single cell analysis.

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SUPPORTING INFORMATION.

Supporting material on DNA sequences, Immobilization of DNaPull probes in glass capillary, setup of the Laser Induced Fluorescence Detection (LIFD)platform and Cell culture and sample preparation for the ATP assay, is available free of charge via the Internet at http://pubs.acs.org.

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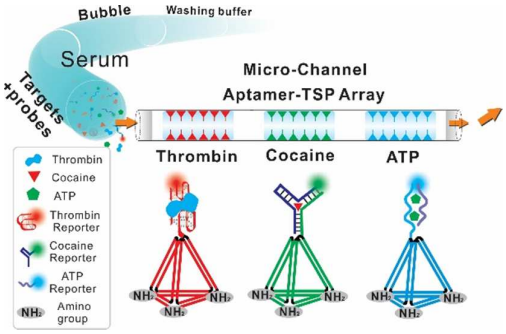
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