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RESEARCH PAPER

An aptamer-based microfluidic device for thermally controlled affinity extraction

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Abstract We present a microfluidic device for specific extraction and thermally activated release of analytes using nucleic acid aptamers. The device primarily consists of a microchamber that is packed with aptamer-functionalized microbeads as a stationary phase, and integrated with a micro heater and temperature sensor. We demonstrate the device operation by performing the extraction of a metabolic analyte, adenosine monophosphate coupled with thiazole orange (TO-AMP), with high selectivity to an RNA aptamer. Controlled release of TO-AMP from the aptamer surface is then conducted at low temperatures using on-chip thermal activation. This allows isocratic analyte elution, which eliminates the use of potentially harsh reagents, and enables efficient regeneration of the aptamer surfaces when device reusability is desired.

Keywords Affinity binding · Isocratic elution · Microfluidics · Purification · Solid-phase extraction · Specificity · Thermal release

1 Introduction

Presently, there is strong interest in developing miniaturized bioanalytical systems that integrate various biochemical analysis components on a single chip. Sample preparation, which involves extracting, purifying, and amplifying relevant analytes before introduction into a subsequent quantitative analysis procedure, is critically important to such systems. For example, sample cleaning is often vital to the successful analysis and detection of a target compound, either for desalting in separation systems (Kim et al. 2004; Oleschuk et al. 2000) or toxicity measurements in environmental monitoring systems (Karube and Normura 2004). This need is especially crucial in systems where impurity and non-target analytes can degrade subsequent analyses, and is in general addressed by a procedure to extract and purify the target analytes from an impure mixture.

Solid-phase extraction (SPE) is one of the most commonly used techniques for analyte extraction and purification in biochemical analysis (Majors 2001). In this method, an analyte of interest, contained in a liquid medium, is introduced to a solid phase, typically microbeads coated with a thin layer of a functional material (Simpson 2000). Via interaction with the coating, the analyte is retained by the solid phase, while impurities and non-target compounds remain in the liquid phase and can hence be removed by rinsing. Next, a reagent (such as an organic solvent) is generally used to disrupt the interaction between the solid phase and the analyte, thereby eluting the analyte for further analysis.

There has been much effort in implementing SPE on microfluidic platforms (Oleschuk et al. 2000; Buchholz et al. 2001; Ramsey and Collins 2005; Broyles et al. 2003; Ekstrom et al. 2002, 2005; Huber et al. 2003; Doherty et al. 2004; Yu et al. 2001; Wang et al. 2002; Yang et al. 2005; Hatch et al. 2006; Tempels et al. 2004, 2006). To reversibly retain molecules, these systems typically rely on physisorption interaction between the target analyte and device surfaces, such as reversed-phase (Buchholz et al. 2001; Ramsey and Collins 2005; Broyles et al. 2003; Ekstrom

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et al. 2002, 2005; Huber et al. 2003), ion-exchange (Oleschuk et al. 2000; Doherty et al. 2004; Yu et al. 2001; Wang et al. 2002; Yang et al. 2005), and steric hindrance (Hatch et al. 2006; Tempels et al. 2004, 2006) methods. In this fashion, analytes adhere to the solid-phase through weak intermolecular forces. For instance, Ramsey and Collins (2005) use a glass microchip packed with reversed-phase octadecyl-derived beads to capture fluorescent molecules with similar polarity to the surface. The retention is reversed by the addition of a polar solvent, which changes the surface polarity of the support to release the bound analyte. Ion-exchange support media, such as the methacrylate based gels employed by Yu et al. (2001), rely on adjusting charged molecular sites in the retention media to interact with analytes. In this format, strong pH reagents are generally introduced to subsequently release the molecules of interest. Chen et al. (2006) demonstrate a microfluidic platform for charge-based extraction of DNA using guanidine modified channels. Elution is achieved by a change in potassium iodide salt concentration to promote electrostatic repulsion of the analyte from the solid-phase. Although not as heavily explored, size-exclusion polymeric gels, which retain biomolecules based on molecular weight, are also utilized. For example, Hatch et al. (2006) trap proteins below 10 kDa in a sodium dodecyl sulfate polyacrylamide gel membrane laser-formed in a glass microchannel. Using modulation of an applied electric field, the protein to membrane adherence reverses to elute the molecules for further analysis.

For unambiguous, sensitive detection of analytes, it is highly desirable and sometimes required, that the analyte extraction process be specific, i.e., only the analyte and ideally no impurities are retained by the solid-phase (Chen et al. 2006; Handley 1999; Zhang et al. 2005; D'Orazio 2003). Unfortunately, the microfluidic devices mentioned above do not possess this attribute. Based on the reversed-phase or ion-exchange SPE methods that are intrinsically unspecific, these devices would extract the target analyte as well as impurities that have similar interactions with the solid-phase. In addition, analyte elution in these devices is accomplished by using pH, salt-concentration or solvent gradients which could comprise the integrity of the analyte and may present other biocompatibility issues.

This paper intends to address both of these concerns by presenting an aptamer-based microfluidic SPE device. Based on the affinity interaction between an analyte and an aptamer molecule, highly specific extraction can be accomplished. As explored for the first time here, we utilize the temperature-dependent reversibility of the aptamer-analyte complex and hence can release the captured analyte from the solid phase with a moderate thermal stimulus, eliminating the use of potentially harsh reagents. Our device consists of a microchamber packed with aptamer-functionalized microbeads for analyte extraction/purification, a microheater and temperature sensor for thermally induced analyte release, and microchannels for the regulation of sample and buffer fluids. Using the metabolic hormone adenosine monophosphate (AMP) as a model analyte, the device has successfully demonstrated specific and thermally controlled SPE operation. Our systematic experiments included the specific extraction and purification of AMP at physiologically relevant concentrations. Furthermore, the captured AMP was released by increasing the surface temperature modestly above room temperature (32–38°C), shown to be more efficient than the standard competitive displacement technique. Moreover, thermal release of AMP also successfully regenerates the aptamer surface for continued reuse.

2 Principle and design

This section describes the principle and design of our microfluidic device. We first describe the thermally controlled aptamer-based affinity extraction approach, and then present the device design and operation.

2.1 Affinity binding and aptamers

Affinity binding is a chemisorption process which results in high strength bonds between ligand and receptor molecules (D'Orazio 2003). Unlike physisorption, it involves a greater number of intermolecular forces and activation energies acting between receptors and their specific target molecules. The specific nature of these interactions results from the two participating compounds being ideally suited to one another both electrostatically and geometrically (Meyer 1994). Hence, affinity binding can enable highly selective target detection and analysis. Affinity-based extraction has traditionally included antigen and antibody, lectin and carbohydrate, and enzyme and inhibitor systems. However in the past decade, novel oligonucleotide aptamers have been receiving increasing attention (Bunka and Stockley 2006; Collett et al. 2005; Tombelli et al. 2005; You et al. 2003).

Aptamers are a special class of high-affinity molecules derived from ribonucleic (RNA) or deoxyribonucleic (DNA) acids. An aptamer can be designed to bind to a target analyte with high specificity by way of precise stacking of moieties, specific hydrogen bonding, and folding upon binding to their target molecule (i.e., they can incorporate small molecules into their nucleic acid structure or integrate into the structure of larger molecules such as proteins; Hermann and Patel 2000). These innate binding mechanisms allow aptamers to distinguish between their target analytes and non-specific molecules by as

subtle variation as chirality, functional end group (e.g., methyl or hydroxyl), or isoform. When compared to more conventional high-affinity reagents such as antibodies or enzymes, aptamers present several attractive advantages. They are particularly attractive since they can be synthesized to produce high affinity toward virtually any target protein or molecule (Tuerk and Gold 1990). In addition, DNA and RNA aptamers are more stable at room temperature, easily synthesized and chemically modified at terminal sites as to facilitate attachment to stationary surfaces (Jayasena 1999; Langer et al. 1981; Cho et al. 2004). Moreover, as will be exploited in this paper, aptamers are thermally responsive in that the affinity complex can be reversed by using temperature variations.

2.2 Extraction by aptamer binding

The operational principle of our device is illustrated in Fig. 1. Initially, an aqueous solution of a sample, which is intermixed with non-target compounds, is introduced to a solid surface that is functionalized with an aptamer (Fig. 1a). At a suitable (e.g., room) temperature, the aptamer specifically captures the analyte from the liquid-phase while impurities are flushed from the system (Fig. 1b). As the temperature of the solid support is altered, conformational changes in the aptamer structure disrupt the analyte binding mechanisms and subsequently release the captured analyte into a pure liquid phase (Fig. 1c). Returning the temperature to the initial state allows the aptamer to revert to its initial functional structure, permitting reuse. Unlike conventional microfluidic SPE systems which employ competitive displacement or potentially harsh solvents for analyte release, thermally activated analyte release using aptamers can be more efficient and biocompatible since isocratic elution (i.e., elution using a single mobile phase with constant composition) in an aqueous solution is possible. However, thermally activated analyte release is still a novel concept not well explored to date. Hence by following the above scheme, a microfluidic platform for selective SPE, thermally induced release and regeneration, and detection of AMP can be constructed.

2.3 Microfluidic device design

For demonstration purposes, our device utilizes a model system in which AMP serves as a target and is recognized by an RNA aptamer derived for adenosine triphosphate (ATP-aptamer). The molecular structures of AMP and ATP-aptamer are shown in Fig. 2. In this system, AMP is captured by ATP-aptamer through an induced 11-base loop flanked by double-stranded RNA, which forms a well-suited binding pocket for the small molecule (DieckMann et al. 1996). To detect the resulting complex, an asymmetric cyanine (thiazole-orange) which increases its fluorescence quantum yield by orders of magnitude upon binding to nucleic acid, is covalently attached to AMP

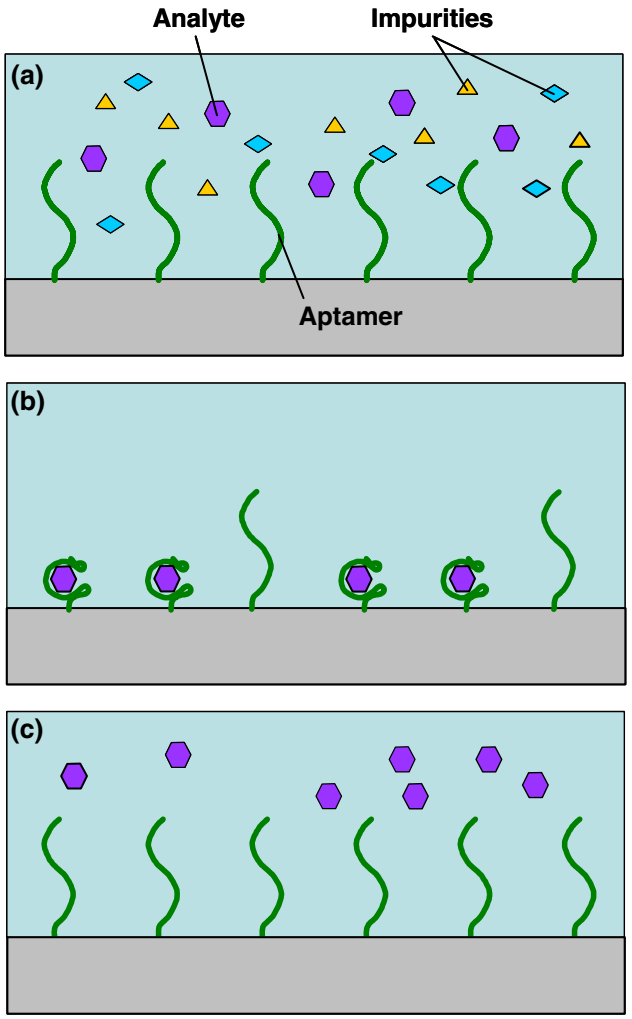

Fig. 1 Principle of aptamer-based microfluidic extraction. **a** A mixture of target and impurities introduced to a specific aptamer on a surface at a certain temperature. **b** Rinsing out impurities and purifying the analyte. **c** Release of the target molecule at a suitably different temperature

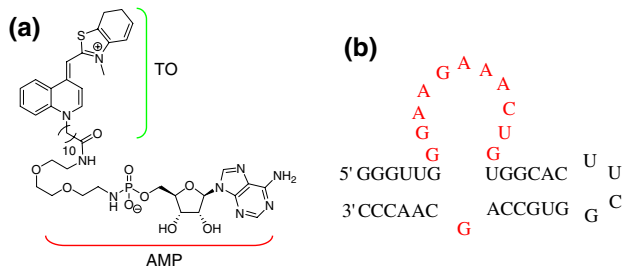

Fig. 2 **a** Molecular structure of TO-AMP. **b** Chemical structure of bio-ATP-40-1 aptamer

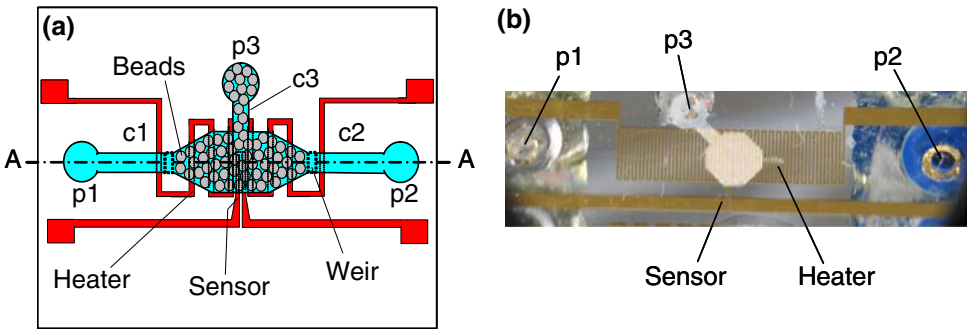

Fig. 3 **a** A schematic of the microfluidic SPE device. c1 and c2 respectively introduce and remove solution to and from the chamber, inside which beads packed via c3. **b** A photograph of a packaged device

(TO-AMP). Therefore, the device can be interfaced to an inverted fluorescence microscope system to signal the capture of TO-AMP by ATP-aptamer.

A schematic, along with a photograph, of the device is shown in Fig. 3, in which the channels are numbered for reference. The device consists of a tapered microchamber (8.7 mm × 3 mm × 140 μm) with structural weirs to retain a microbead support matrix on which the aptamer is immobilized. The taper for the chamber is designed in order to minimize dead volume and bubble formation in the device. An integrated Cr/Au resistive heater (500 Ω) and temperature sensor (27.5 Ω, with a temperature coefficient of resistance of 2.65×10^{-3}/°C) are used in conjunction with off-chip programming to control temperature and thus, vary thermal stimulation. A serpentine heater design is chosen to provide adequate heating for the entire chamber, while the use of a single temperature sensor allows detection of the temperature near the center of the chamber. Lastly, microfluidic channels are used to deliver samples and buffer from the inlet port to the microchamber while transferring waste materials to the outlet. Channels c1 and c2 both measure 5.1 mm × 400 μm × 40 μm. Channel c3 (1 mm × 400 μm × 140 μm) is used to pack the support matrix. The ports p1 and p2 are each 2 mm in diameter and 140 μm thick. Hence, the chamber has an effective volume of ~3.1 μl with the tapers taken into consideration, whereas the microfluidic system volume (on-chip) is ~3.6 μl.

It is important to monitor the pressure used to drive fluids through the device during its operation. Since the bead-packed extraction chamber produces the largest flow resistance, a modified *Poiseuille* equation is used to determine its pressure drop and hence, the working pressure of the system (Darby 2001):

$$\Delta p = \frac{150\eta u(1-\varepsilon)^2 L}{d_0^2 \varepsilon^2} \quad (1)$$

Here, η, u, ε, L, and d_0 represent the dynamic viscosity of water, average pumping velocity, void fraction, channel length of the packed bead chamber, and bead diameter, respectively.

3 Experimental

3.1 Materials and instrumentation

A biotinylated ATP-aptamer was acquired through Integrated DNA Technologies. TO-AMP was synthesized in-house while triphosphates of adenosine, cytidine, uridine, and guanosine (ATP, CTP, UTP, and GTP) were purchased through Sigma-Aldrich. An aqueous buffer solution (pH 7.4) was prepared with a mixture of purified water (sterile RNase/Protease-free water, Fisher), Tris–HCl (20 mM), NaCl (140 mM), KCl (5 mM), and $MgCl_2$ (5 mM) and subsequently used in all experiments. UltraLink streptavidin coated agarose beads (50–80 μm in diameter) were acquired from Pierce and where used to immobilize ATP-aptamer using a biotin-streptavidin link. Microfabrication materials, including SU-8 2025 and 2100 (MicroChem), *Sylgard 184* polydimethylsiloxane (PDMS), Torr Seal epoxy, and microscope grade glass slides (25 mm × 75 mm), were purchased from MicroChem, Dow Corning, Varian, and Fisher, respectively. A mercury vapor induced epi-fluorescence microscope (Nikon Eclipse TE300) coupled with a CCD camera (Q-Imaging Retiga 2000R) was used for detection. Device temperature control was performed using the integrated resistive heating and sensing elements connected to a DC power supply and a proportional-integral-derivative (PID) controlled LabVIEW program. Fluid flow in the device was driven by a New Era model NE-1000 syringe pump.

3.2 Microfluidic device fabrication

The microchip SPE device was fabricated from PDMS and bonded on glass slides using standard soft lithography techniques (Fig. 4). The process began with fabrication of an SU-8 mold on silicon wafers. Patterning of SU-8 2025 and 2100 was performed consecutively to define channels c1 and c2 (40 μm thick), and the microchamber and channel c3 (140 μm thick). A PDMS pre-polymer solution was mixed with a volume ratio of 10:1 and distributed on the mold. The pre-polymer was degassed by vacuum

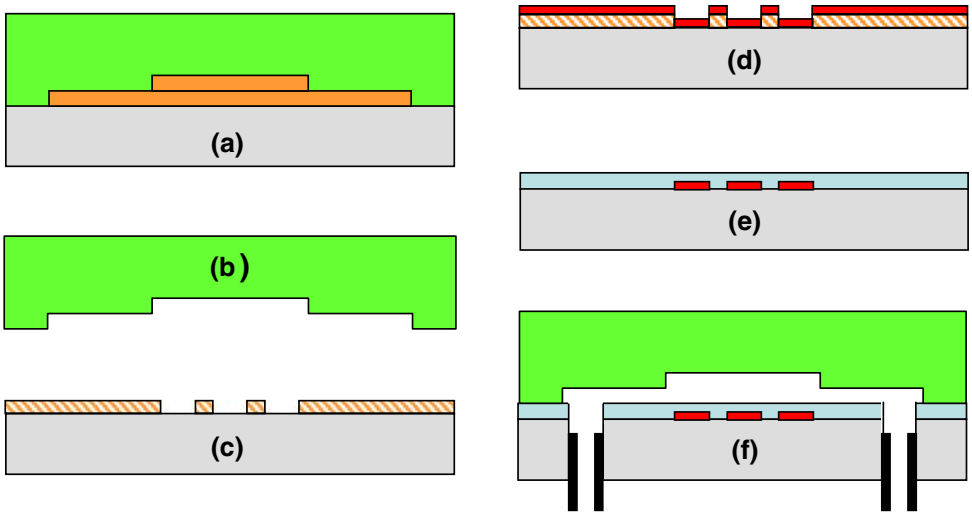

Fig. 4 A simplified device process flow referring to cross section A-A in Fig. 3. **a** PDMS channel fabrication. **b** PDMS channel demolding. **c** Resist patterning for metal deposition. **d** Metal deposition and lift-off patterning. **e** SiO_2 deposition for metal passivation. **f** Bonding and packaging

(30 min) and semi-cured (70°C, 50 min). In parallel, glass substrates were diced (25 mm × 30 mm) and drilled to create the access ports (p1–p3). The glass substrates were then cleaned using Piranha. Subsequently, Cr/Au (5/100 nm) thin films were deposited and patterned on the substrates via thermal evaporation followed by lift-off in resist stripper overnight. The metal films were then passivated with SiO_2 using plasma-enhanced chemical vapor deposition, realizing the microheater and temperature sensor. The PDMS sheet was removed from the SU-8 mold, aligned and bonded to the glass slides following O_2 plasma treatment of the bonding interface. Permanent bonding and curing of PDMS to the substrate was performed by heating the sandwiched glass and PDMS layers (25 min at 85°C). Packaging was accomplished by inserting a combination of silica capillary tubing (0.6 mm ID, 0.7 mm OD) segments along with polyvinyl chloride tubing (0.6 mm ID, 3.18 mm OD) through the drilled access ports. The connection interfaces were finally sealed with epoxy.

3.3 Testing procedures

Using a nominal, maximum sample flow rate of 10 µl/min (used for all sample washing and injection procedures), we analyzed multiple chamber dimensions to determine each correlating working pressure using Eq. (1). A suitable extraction chamber design was chosen to produce a pressure of approximately 6.6 kPa which was within the maximum allowed working pressure range (0–10 kPa) of the syringe pump used in our experiments. The entire microfluidic system was thoroughly flushed with the buffer solution for 5 min (similar for subsequent rinses in the experiment). Sample solutions in varying concentrations of TO-AMP, AMP, CTP, UTP, GTP, and ATP-aptamer were prepared using the appropriate mass weights of the respective compound and buffer solution. Manual pressure was used to pack the beads from channel c3 via port p3 into the chamber. Subsequently, channel c3 was sealed permanently. The extraction chamber and channels were washed through c1 and an ATP-aptamer solution (3 µl, 20 µM) was injected and allowed to incubate (20 min) in the chamber. The extraction chamber was washed again and a baseline fluorescence signal was taken. Specifically, a 10× objective was focused at the center of the extraction chamber and the resulting CCD image recorded. Then, using the Q-Capture Pro software, an 8-bit RGB average was obtained over the entire recorded fluorescence image. Subsequently, the green integer value is extracted from the RGB average and used to determine the fluorescence signal intensity.

4 Results and discussion

4.1 Solid-phase extraction of TO-AMP

The ability for an SPE device to capture analytes in a wide range of concentrations is important for its versatility in sample preparation. To demonstrate aptamer-based capture of a specific analyte, solutions of TO-AMP at five different concentrations (0.0001, 0.001, 0.1, 1, and 10 µM) were injected into the extraction chamber from channel c1. Fluorescence light generation, as a result of TO-AMP/ATP-aptamer binding, becomes stable after an experimentally predetermined time (∼5 min). Hence, for each sample introduction, fluorescence yield was quantified after an initial 5 min incubation time. Following the extraction of analytes, the chamber was washed with buffer to rid all non-specific compounds, un-reacted molecules, and impurities. Results are presented in Fig. 5. It can be seen that below 100 pM, no measurable signal above the

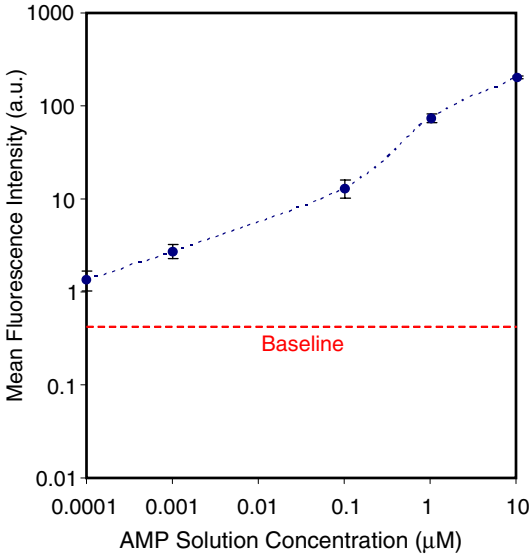

Fig. 5 Solid-phase extraction of TO-AMP samples at varying concentrations. A sample was injected into and incubated in the extraction chamber. Fluorescence emission of TO in the aptamer-captured TO-AMP occurred at 530 nm when excited at 480 nm. Mean fluorescence intensity was obtained by averaging the measured fluorescence emission over a consistent chamber area

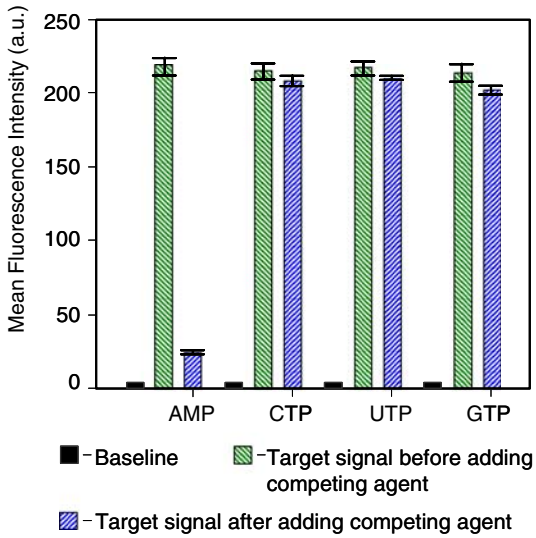

Fig. 6 Selectivity of ATP-aptamer to TO-AMP molecules compared to non-target compounds (CTP, UTP, and GTP). A TO-AMP sample was injected into the chamber, producing an initial high-intensity fluorescence signal. Subsequently, a non-target analyte was introduced and fluorescence acquired again. Reduction in signal was proportional to the level of non-specific binding

background is detected. Concentrations at and above 100 pM, however, are readily detectable for this device at a 3:1 signal-to-noise ratio. Additionally, for single concentrations, an S-shaped relationship can be observed between the mean fluorescence intensity and AMP concentration, which appears to be a dose-responsive characteristic (Calabrese 1994). By using analysis based on experimental noise for this system, we calculated the detection of AMP on the order of 1 pM is possible with an improved optical system.

4.2 Specificity of ATP-aptamer to TO-AMP

Analyte specificity is another important characteristic for efficient SPE. We tested the specificity of ATP-aptamer to TO-AMP by subjecting the aptamer phase to competitive binding experiments with non-target compounds (CTP, UTP, and GTP; Fig. 6), which differ in structure to AMP by only two phosphate groups. For a control experiment, 10 μM of free AMP was also tested as the competitive agent. A solution of 10 μM TO-AMP was extracted by the aptamer phase and the observed fluorescence signal was recorded. This is immediately followed with an injection of a non-target analyte solution at equal concentration (10 μM) and measurement of the subsequent reduction in signal. To reduce the effect of fluorescence photo-bleaching, the shutter to the mercury lamp was closed for the time period between the two intensity measurements. As expected in the control experiment, the addition of unlabeled AMP caused immediate fluorescence quenching (the signal is reduced by ~90%). However, there was very little fluorescence reduction (~10%) after the addition of any non-target analyte. If affinity of the aptamer to AMP were low, then competitive dissociation would decrease the TO-AMP by a significant fraction. The experimental data above indicates that this is not the case. Therefore, it is possible to achieve a 10:1 ratio of specific to non-specific binding in our device, despite the close resemblance of AMP to CTP, UTP, and GTP. This analyte recognition specificity is considered excellent according to commonly used criteria (Mulvaney et al. 2007).

4.3 Thermally activated release of TO-AMP

In addition to specific extraction, it is critically important for captured analytes to be properly released for subsequent analysis. This is accomplished in our device by thermally activated release and isocratic elution of analytes from aptamer-functionalized surfaces. To demonstrate this, a 10 μM TO-AMP solution was first extracted. The captured TO-AMP was then released by heating the surface to an elevated device temperature (Fig. 7a), or for comparison, by a competitive binding method (Fig. 7b). After extraction of TO-AMP on the aptamer surface, a high intensity fluorescence signal was initially obtained. The temperature on-chip was increased to a predetermined setpoint and held for 2 min. We repeated the experiment for several setpoint temperatures (30–50°C). A sharp decrease (93%) in signal

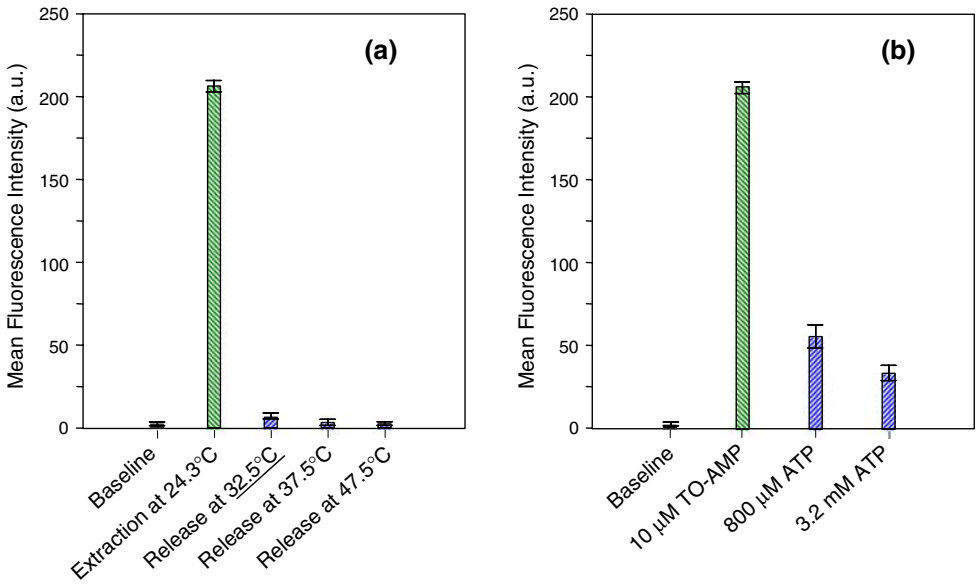

Fig. 7 **a** Thermally induced release of captured TO-AMP. Extraction of TO-AMP from solution occurred at room temperature. Subsequently, the temperature was raised incrementally. Notice the sharp decrease in signal at 32.5°C which continues to vanish as the temperature increases, implying release of the TO-AMP from the ATP-aptamer. **b** In comparison, release by competitive binding of ATP at high concentrations is considerably less effective

intensity occurred at 32.5°C. As the temperature was further increased, the analyte release became more thorough. At 47.5°C, the post-release fluorescence was only about 1.5–2.0× the baseline, indicating nearly complete release of the captured TO-AMP. The accuracy of our temperature measurements was approximately 0.07°C, which was determined from estimates of the resistance measurement accuracy (0.005 Ω) and temperature to resistance sensitivity (13.9°C/Ω) of the device and testing instruments. We also estimated that photobleaching due to UV irradiation of the fluorophore for 1 min, the duration used in the experiment, would have caused a reduction in the fluorescence signal by less than 15%. Thus, the drastic fluorescence reduction as shown in Fig. 7a can be attributed to the release of TO-AMP from the aptamer-functionalized surface. This establishes the capability of our device for thermally activated release and isocratic elution of the captured target analyte. The use of only modest temperatures is significant, as they will generally not compromise the viability of thermally sensitive analyte biomolecules.

Results from thermally activated analyte release were then compared with those from the commonly used conventional method of competitive release, where a complementary ATP DNA strand (ATP-analyte) was introduced to displace the captured TO-AMP from the aptamer. A 10 μM TO-AMP solution was extracted and released using consecutive injections of ATP, which also has high affinity to the aptamer, at high concentrations (800 μM and 3.2 mM, Fig. 7b). It can be observed that the injection of 800 μM ATP caused the fluorescence intensity to decrease by 75%. A subsequent injection of 3.2 mM ATP led to an additional 10% decrease in fluorescence, but the remaining fluorescence was still much higher than the baseline. This final competitive dose was very high in concentration (320× more than the initial TO-AMP concentration), but still there was 33.5% of TO-AMP left on the aptamer phase. This is nearly 5× more than what remained after a modest increase in temperature to 32.5°C. Thus, it follows that the thermal approach allows the captured analyte to be released with considerably more efficacy than the competitive method, while also affording a much more simplified operational procedure. In this way, more consistent reuse of the device is possible since thermally induced release allows full regeneration of the aptamer-functionalized bead surfaces.

4.4 Microfluidic device regeneration

The capability of the thermal method for highly effective release of captured analyte molecules, coupled with the reversibility of aptamer-analyte binding, allows the regeneration of the aptamer-functionalized surface and hence renders the device reusable. To demonstrate this, the device was tested with consecutive extraction-release cycles. In the first cycle, a TO-AMP (10 μM) sample was injected and extracted by the aptamer and subsequently released using thermal activation at 40°C. Upon cooling of the extraction chamber to room temperature, a second operation cycle was performed, in which another TO-AMP sample was introduced for extraction, and followed by thermally activated release at 40°C. This was continued until five complete cycles were performed. As can be seen in Fig. 8, the fluorescence signals resulting from TO-AMP extraction and release in all subsequent cycles were comparable to those obtained in the first cycle. This indicates that the thermal stimulation did not affect the functionality of the aptamer molecules and successfully allowed device regeneration.

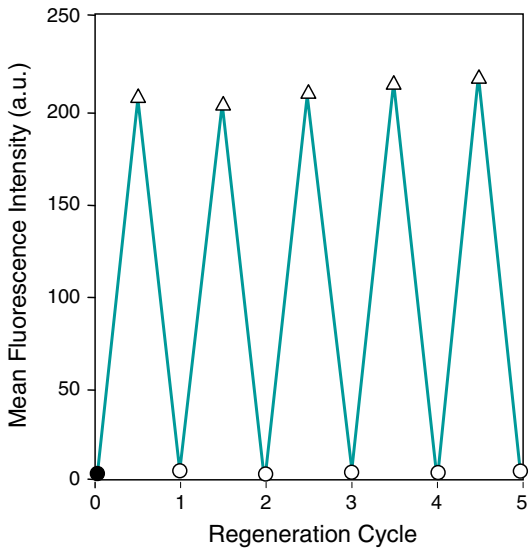

Fig. 8 Thermal release allows the aptamer surface to be regenerated for multiple extraction-release cycles. After an extraction of TO-AMP at 25°C, a high intensity signal was obtained. This was followed by a release of the captured TO-AMP at 40°C, yielding a low fluorescence intensity. This process was repeated for four additional cycles. (*Solid circle* baseline, *open triangle* extraction, *open circle* release)

Finally, it is interesting to consider the effects of higher release temperatures on the integrity of the aptamer-functionalized surfaces. It is known that the streptavidin-biotin link, which is used for aptamer immobilization in the device, denatures at ~80°C (Holmberg et al. 2005). Thus, we tested the device with three elevated release temperatures, 75, 85, and 95°C following a TO-AMP sample extraction (referred to as the 1st extraction) at room temperature (Table 1, series A, B, and C, respectively). It can be observed that when TO-AMP was released at 75°C in series A, the subsequent extraction (the 2nd extraction) yielded fluorescence intensity comparable to that from the pre-release extraction. On the other hand, in series B and C, 2nd extraction fluorescence signals were significantly lower following release at further elevated temperatures (85 and 95°C, respectively). This led us to conclude that most aptamer molecules had separated from the microbeads because of streptavidin–biotin denaturation. Thus, it is important to operate the device at temperatures below the denaturation temperature of the streptavidin–biotin binding. Fortunately, this requirement is readily satisfied by the modest release temperatures demonstrated above.

Table 1 Degradation of the streptavidin-biotin link, as shown by the mean fluorescence intensity for three test series

Fluorescence	Test series		
	A	B	C
Baseline (a.u.)	0.542	0.412	0.462
1st Extraction (a.u.)	219	215	209
Regeneration (a.u.)	0.853	0.713	0.977
2nd Extraction (a.u.)	210	38.5	11.1

In each series, the first extraction at 25°C was followed by thermal regeneration at a differing temperature (series A: 75°C; series B: 85°C; series C: 95°C), and then by the second extraction at 25°C. Fluorescence was measured following each experimental step as well as for the baseline

5 Conclusions

As miniaturized bioanalytical processes are increasingly dependent on the quality of the particular sample, the need for efficient sample preparation becomes ever more critical. We address this need by utilizing thermally responsive affinity aptamer molecules for specific extraction and release of analytes. This method offers distinct advantages over existing SPE systems, including selective analyte extraction, efficient analyte release and device regeneration at low temperatures, and simplistic design and fabrication. Also, the operation of the device entirely in an aqueous medium eliminates the need for potentially harsh solvents or reagents. The practical relevance of this method is established by interfacing analyte extraction with a standard fluorescence detection system. We have implemented this method on a device for selective extraction of TO-AMP at appropriate physiological concentrations, followed with thermally induced release of TO-AMP at a modest temperature above room (~32–38°C), which is generally safe for both thermally sensitive analytes and the aptamer matrix. Interestingly, we showed that thermal release was nearly 5× more effective at releasing the captured TO-AMP than a competitive displacement approach, while offering improved operational simplicity. Additionally, we demonstrated the reusability of the device by performing repeated capture and release cycles of TO-AMP with excellent consistency. Further studies will involve systems that use other practically important aptamer-analyte systems and are integrated with detection methods beyond fluorescence microscopy.

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References

Broyles BS, Jacobson SC, Ramsey JM (2003) Sample filtration, concentration, and separation integrated on microfluidic devices. Anal Chem 75:2761–2767

Buchholz BA, Doherty EAS, Albarghouthi MN, Bogdan FM, Zahn JM, Barron AE (2001) Microchannel DNA sequencing matrices

with a thermally controlled "Viscosity Switch". Anal Chem 73:157–164
Bunka DHJ, Stockley PG (2006) Aptamers come of age—at last. Nat Rev Microbiol 4:588–596
Calabrese EJ (1994) Biological effects of low level exposures dose–response relationships. In: Research on the biological effects of low level exposure (BELLE) to chemical agents and radioactivity. CRC Press, Crystal City, pp 320
Chen X, Cui DF, Liu CC (2006) High purity DNA extraction with a SPE microfluidic chip using Ki as the binding salt. Chin Chem Lett 17:1101–1104
Cho S, Lee SH, Chung WJ, Kim YK, Lee YS, Kim BG (2004) Microbead-based affinity chromatography chip using RNA aptamer modified with photocleavable linker. Electrophoresis 25:3730–3739
Collett J, Cho E, Ellington A (2005) Production and processing of aptamer microarrays. Methods 37:4–15
Darby R (2001) Chemical engineering fluid mechanics. Marcel Dekker, New York
D'Orazio P (2003) Biosensors in clinical chemistry. Clin Chim Acta 334:41–69
DieckMann T, Suzuki E, Nakamura GK, Feigon J (1996) Solution structure of an ATP-binding RNA aptamer reveals a novel fold. RNA 2:628–640
Doherty EAS, Kan C-W, Paegel BM, Yeung SHI, Cao S, Mathies RA, Barron AE (2004) Sparsely cross-linked "Nanogel" matrixes as fluid, mechanically stabilized polymer networks for high-throughput microchannel DNA sequencing. Anal Chem 76:5249–5256
Ekstrom S, Malmstrom J, Wallman L, Lofgren M, Nilsson J, Laurell T, Marko-Varga G (2002) On-chip microextraction for proteomic sample preparation of in-gel digests. Proteomics 2:413–421
Ekstrom S, Wallman L, Hok D, Marko-Varga G, Laurell T (2005) Miniaturized solid-phase extraction and sample preparation for MALDI-MS using a microfabricated integrated selective enrichment target. J Proteome Res 5:1071–1081
Handley AJ (1999) Extraction methods in organic analysis, vol 2, 1st edn edn. CRC Press LLC, Boca Raton
Hatch AV, Herr AE, Throckmorton DJ, Brennan JS, Singh AK (2006) Integrated preconcentration SDS-page of proteins in microchips using photopatterned cross-linked polyacrylamide gels. Anal Chem 78:4976–4984
Hermann T, Patel DJ (2000) Adaptive recognition by nucleic acid aptamers. Science 287:820–825
Holmberg A, Blomstergren A, Nord O, Lukacs M, Lundeberg J, Uhlen M (2005) The biotin–streptavidin interaction can be reversibly broken using water at elevated temperatures. Electrophoresis 26:501–510
Huber DL, Manginell RP, Samara MA, Kim B-I, Bunker BC (2003) Programmed adsorption and release of proteins in a microfluidic device. Science 301:352–354
Jayasena S (1999) Aptamers: an emerging class of molecules that rival antibodies in diagnostics. Clin Chem 45:1628–1650
Karube I, Normura Y (2004) Enzyme sensors for environmental analysis. J Mol Catal B Enzym 10:177–187
Kim S, Ulz ME, Nguyen T, Li CM, Sato T, Tycko B, Ju J (2004) Thirtyfold mulitplex genotyping of the P53 gene using solid phase caturable dideoxynucleotides and mass spectrometry. Genomics 83:924–931
Langer P, Waldrop A, Ward D (1981) Enzymatic synthesis of biotin-labeled polynucleotides: novel nucleic acid affinity probes. Proc Natl Acad Sci USA 78:6633–6637
Majors R (2001) New directions for concentration sensitivity enhancement in CE and microchip technology. LC GC North Am 19:14–30
Meyer V (1994) Practical high-performance liquid chromatography. Wiley, Chichester
Mulvaney SP, Cole CL, Kniller MD, Malito M, Tamanaha CR, Rife JC, Stanton MW, Whitman LJ (2007) Rapid, femtomolar bioassays in complex matrices combining microfluidics and magnetoelectronics. Biosens Bioelectron 23:191–200
Oleschuk R, Shultz-Lockyear L, Ning Y, Harrison D (2000) Trapping of bead-based reagents within microfluidic systems: on-chip solid-phase extraction and electrochromatography. Anal Chem 72:585–590
Ramsey J, Collins D (2005) Integrated microfluidic device for solid-phase extraction coupled to micellar electrokinetic chromatography separation. Anal Chem 77:6664–6670
Simpson NJK (2000) Solid-phase extraction, 1st edn edn. Marcel Dekker, New York
Tempels FWA, Teeuwsen J, Kyriakou IK, Theodoridis G, Underberg WJM, Somsen GW, de Jong GJ (2004) Chromatographic preconcentration coupled on-line to capillary electrophoresis via a tee-split interface. J Chromatogr A 1053:263–268
Tempels FWA, Wiese G, Underberg WJM, Somsen GW, de Jong GJ (2006) On-line coupling of size exclusion chromatography and capillary electrophoresis via solid-phase extraction and a tee-split interface. J Chromatogr B 839:30–35
Tombelli S, Minunni M, Mascini M (2005) Analytical applications of aptamers. Biosens Bioelectron 20:2424–2434
Tuerk C, Gold L (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 249:505–510
Wang PC, Gao J, Lee C (2002) High-resolution chiral separation using microfluidics-based membrane chromatography. J. Chromatogr. A 942, pp. 115–122, 2002
Yang Y, Li C, Lee KH, Craighead HG (2005) Coupling on-chip solid-phase extraction to electrospray mass spectrometry through integrated electrospray tip. Electrophoresis 26:3622–3630
You KM, Lee SH, Im A, Lee SB (2003) Aptamers as functional nucleic acids: in vitro selection and biotechnological applications. Biotechnol Bioprocess Eng 8:64–75
Yu C, Davey M, Svec F, Frechet J (2001) Monolithic porous polymer for on-chip solid-phase extraction and preconcentration prepared by photoinitiated in situ polymerization within a microfluidic device. Anal Chem 73:5088–5096
Zhang Y, Zhu W, Wang B, Yu L, Ding J (2005) Postfabrication encapsulation of model protein drugs in a negatively thermosensitive hydrogel. J Pharm Sci 94:1676–1684