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Aptamer in Bioanalytical Applications

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With over 2,000 publications, including about 250 reviews, resulting from a *SciFinder* search in just a two year period (2009–2010), the field of aptamer research has continuously generated lots of interest in the scientific community. Aptamers, first reported by three groups independently in 1990,[1–3] are the artificial single-stranded DNA or RNA sequences (more recently, peptides) that fold into secondary and tertiary structures making them bind to certain targets with extremely high specificity. Owing to the high specific affinity of an aptamer to its target molecule (small molecules, proteins and even entire cells), it is thought to resemble chemical antibodies, with the dissociation constants ranging from nanomolar to picomolar level. Aptamers have a number of unique features which make them a more effective choice than antibodies. First, aptamers can be screened via *in vitro* process against a synthetic library, making it possible to target any molecules (from small inorganic ions to intact cells), overcoming the limit of having to use cell lines or animals, as is necessary for antibodies. Second, aptamers, once selected, can undergo subsequent amplification through polymerase chain reaction to produce a large quantity with high purity. Third, the simple chemical structure of aptamer makes it easily amendable to further modifications with functional groups according to different purposes. Finally, aptamers are much more stable than antibodies, making them suitable in applications requiring harsh conditions (e.g., high temperature or extreme pH).

The applications of aptamers remain very dynamic, with increasing explorations in the fields of biosensing, diagnostics and therapeutics (some aptamer-based applications are illustrated in Figure 1). There have been a numbers of excellent reviews in recent years with different emphases.[4–8] Herein, as the first review of aptamers on *Analytical Chemistry*, we attempt to cover major progresses in bioanalytical applications of aptamers in the past 2 years.

Selection

Aptamers are generally selected using the SELEX (systematic evolution of ligands by exponential enrichment) approach starting with a random library containing 10^{13}–10^{16} single-stranded DNA or RNA sequences. The library is incubated with a target of interest to initiate the first cycle of selection. This is typically followed by iterative cycles of absorption, recovery of bound DNA/RNA and amplification. Isolation of the bound DNA/RNA is the most critical step to ensure purity and selectivity. For example, the aptamer-target complex can be separated by filtration through nitrocellulose or by affinity chromatography from the unbound DNA/RNA sequences. A number of other separation techniques have been developed to increase the efficiency and throughput of aptamer isolation, including flow cytometry,[9] capillary electrophoresis (CE)[10], surface plasmon resonance (SPR)[11] and atomic force microscopy (AFM)[12–14]. The bound aptamers are then eluted and amplified by RT-PCR (for RNA libraries) or PCR (for DNA libraries) to generate new pools for the next selection cycle. A typical method for generating aptamers from

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random combinatorial libraries is often labor-intensive and time-consuming. An ideal aptamer selection procedure requires around 10–15 cycles and normally takes weeks to months to complete the whole selection process. A lot of efforts have been put forward to increase the speed and simplicity of production. The Krylov group[15] has developed non-equilibrium capillary electrophoresis of equilibrium mixtures (NECEEM) approach, which can screen aptamers with much fewer rounds of selection and can also determine accurate binding parameters. They further utilized NECEEM in the repetitive partitioning step to improve the affinity of a DNA library by more than 4 orders of magnitude.[16, 17] Very recently, the same group used the NECEEM method to screen aptamers against a target protein from a cell lysate without any prior purification of the target protein and subsequently utilize the developed aptamers for protein isolation from the same cell lysate.[18] This technique was demonstrated to be useful for the selection of aptamers for certain proteins that do not have intrinsic DNA-binding characteristics.[19]

CE-based separation methods have greatly accelerated the selection efficiencies due to the fast separation ability based on electrophoretic mobility of protein-aptamer complexes. However, the approach is limited to mainly proteins, because neutral species typically do not have the detectable electrophoretic mobility shift. Magnetic beads-based selection methods, where molecular targets can be first immobilized on a magnetic bead surface, show wider applications to any kind of molecule or species. However, the efficiency of magnetic selection techniques is significantly lower than CE. Soh group thus developed a platform that combines magnetic bead-assisted SELEX with microfluidics technology (M-SELEX; Figure 2) to take advantage of robust and efficient features of both separation approaches.[20] This procedure improves the aptamer selection by enabling highly stringent selection conditions through the use of very small amounts of target molecules. The separation of the magnetic bead is based on the magnetic field gradients generated by ferromagnetic patterns imbedded in the microfluidic channel. The M-SELEX could provide a highly efficient, low-cost, and automated system with high-throughput capabilities. In a follow-up work, the Soh group further improved the M-SELEX method using a disposable microfluidic chip to rapidly generate aptamers with high affinity and specificity.[21] In the experiment, picomolar amounts of target molecule bound to beads were recovered with high efficiency. The procedure was performed for both the positive selection toward targets and negative selection against BSA, which greatly increased the selection specificity. In the latest study, Soh and co-workers developed the Quantitative Selection of Aptamers through Sequencing (QSAS) method by combining M-SELEX with high-throughput DNA sequencing.[22] The QSAS method is capable of tracking a pool of individual sequences in the order of millions over multiple selection cycles. This offers the ability to selectively isolate aptamers without the need to reduce the original pool number.

A particularly interesting aptamer selection approach was introduced by Mi *et.al.*[23] who questioned whether *in vitro* SELEX procedure is effective enough for subsequent *in vivo* target detection, principally because the environments during selection and application are not identical. To address this potential issue, an *in vivo* aptamer selection approach was created using intrahepatic colorectal tumor-bearing mice (Figure 3). During their procedure, the tumor-bearing mice were injected with a random library of RNA sequences. After harvesting the tumors, RNA sequences were extracted and amplified with PCR. The resulting RNA pool was again re-introduced into mouse tumors. After this repeated procedure, the resulting RNA molecules demonstrated increasing affinity toward the colorectal tumor protein extract compared to normal tissue cells. The generated aptamers were confirmed to have exceptional *in vivo* selectivity toward the targeted cancerous mouse tissue.

Besides common molecules such as small organics, peptides and proteins, whole intact cells or viruses have been increasingly used as targets for the selection of aptamers, since the prior precise knowledge of target molecules is not required.[6, 24–26] The resulting aptamers can be used for cancer cell detection, although the knowledge of exact targeted molecules on the surface of the cells may not be totally clear. Cell-SELEX has become one of the most widely used methods for screening cells (Fang *et.al.*[6] have given a nice review on this topic). A number of aptamers targeting different cancer cells have since been developed. Most recently, Li *et.al.*[27] reported a slide-based SELEX strategy for *in situ* selection of tissue samples. They demonstrated the effectiveness of their approach by generating an aptamer with high affinity toward infiltrating ductal carcinoma tissues. Similarly, another aptamer system was developed by Wan *et.al.*[28] to capture and enrich EGFR-expressing glioblastoma cells. The cancer is known for its ability to infiltrate human brain regions, and the inability to detect glyoblastoma renders it surgically incurable. The fluorophore-labeled aptamer probe was immobilized on the glass slides and then applied to successfully capture targeted cancer cells with high efficiency and selectivity. Overall, new developments and improvements in the aptamer selection process has made the generation of aptamers a more straightforward and routine practice, leading to greater impact of aptamers and their broader applications in biochemical and medicinal fields.

Aptamer-based Affinity Purification

Chromatography

With extremely high affinity of aptamers to their specific targets, the use of aptamers as a stationary phase in affinity chromatography is totally expected. Other advantages using aptamers in the chromatography include easy modification and immobilization, good stability, and high reproducibility.[29, 30] A variety of aptamer-based chromatographies have been reported for the separation and purification of small molecules, isoforms and proteins by formats of open tubular capillaries, packed bed columns, and monolithic columns.[31, 32] As an example, Huy *et.al.*[33] immobilized anti-17β-estradiol (E2) aptamer onto solid phase beads. These selective affinity beads were then used for the enrichment of E2 from spiked waters sample. The sequential HPLC analysis demonstrated high recovery and reproducibility.

In addition to the separation of simple targets, Tan group reported the selective capture of cancer cells from a flowing suspension on a simple square capillary by immobilizing aptamers in the capillary.[34] More than 90% capture efficiency can be obtained through this device. The method was extended to aptamer-based differential mobility cytometry for highly efficient enrichment of rare cells.[35]

Capillary Electrophoresis

CE has attractive features in affinity studies due to low sample and reagent consumption, short analysis time, high separation performances, ease of automation and the possibility of probing molecular interactions under physiological conditions. Most CE assays are based on laser-induced fluorescence (LIF) detection because of the sensitivity and selectivity of the detection. While a large number of analytes in CE need to be derivatized for LIF detection, aptamers naturally provide a molecular basis for LIF, as long as the binding of analytes can lead to signal changes, a mechanism commonly called structure switching.[36] The strategy was successfully applied for the analyses of inorganic ions such as Pb^{2+} and Hg^{2+}.[37–39] In a separate study, Girardot *et.al.*[40] applied CE to study the interaction between lysozyme and aptamer mediated by both monovalent and divalent cations. They found that the binding between an aptamer and its target was highly dependent on the conformation of the aptamer molecule. The experimental setup allowed them to calculate apparent binding constants for

divalent cations. Zhang *et.al.*[41] introduced another CE technique based on a tunable aptamer for the separation and detection of platelet derived growth factor (PDGF) isomers and their receptors in diluted serum samples. The aptamer can form a stable complex with the B chain but not with the A chain of PDGF, resulting in the difference in electrophoretic mobilities of PDGF isomers. A fine-selected aptamer can even differentiate extremely subtle structural changes, demonstrated by the separation of the enantiomers of an anionic target (adenosine monophosphate, AMP) by an aptamer-based micellar electrokinetic chromatography (MEKC).[42]

Microfluidics

For the past two years, increasing efforts have been made to couple aptamers with microfluidics. Microfluidics integrates one or several laboratory procedures, such as sample preparation, reaction, separation, detection, etc. on a single chip, ranging from millimeters to a few square centimeters in size. The introduction of aptamers on a microchip has been expected to bring several advantages to the field, such as reduced reagent and sample consumption, simplicity, automated processing, faster separation, high throughput and portability. The Tan group[43] immobilized aptamers in a microfluidic channel to capture rare cells to achieve a rapid assay without any pretreatment (Figure 4). Their device has demonstrated both outstanding enrichment purity (97%) and over 80% capture efficiency. They were able to further extend the utility of their microfluidic device to simultaneously sort, enrich, and then detect multiple types of cancer cells in a complex sample.[44] As another example of using aptamer-microfluidic devices for bioanalysis, Reif *et.al.*[45] have designed a microfluidic device for the simultaneous capture and induction of apoptotic cells in Jurkat cells, providing a tool for the study of the mechanisms and temporal dynamics of apoptosis. The Soper group[46] have also utilized an aptamer-immoblized microfluidic device to selectively capture low abundant cancer cells. In their study, aptamers recognizing prostate-specific membrane antigen (PMSA) were selected and immobilized onto the surface of a microchip and fabricated into a high-throughput micro-sampling unit. Such combination of sensitive technology and selective aptamer binding resulted in the 90% recovery rate of rare circulating prostate tumor cells from peripheral blood matrix.

As another interesting development in aptamer-based microfluidics, Soh and co-workers[47] introduced the Microfluidic Electrochemical Aptamer-based Sensor (MECAS) chip by integrating target-specific DNA aptamers that can generate an electrochemical signal through conformational changes in response to the analyte binding. The system was applied to achieve continuous, real-time monitoring of cocaine in blood serum at the physiologically relevant concentration and with physiologically relevant time resolution. In another study, Huang *et.al*.[48] have combined flow cytometry with aptamer-functionalized magnetic microparticles for the detection of adenosine in serum.

Overall, the introduction of aptamers into separation sciences has greatly enriched the field. On the other hand, with wide applications of chromatography, CE and microfluidics, aptamer has the best chance to present itself as an alternative or even a better solution for many separation issues, thus establishing an important role in sample preparations for many practices in academia and industries.

Biosensors

A typical biosensor is comprised of two basic elements: a biological recognition element (enzyme, antibody, receptor, etc.) and a transducer (electrochemical, optical, thermal, etc.). Unlike other analytical methods akin to spectrophotometry or mass spectrometry, which typically need several steps in one analysis, biosensors are simple-to-operate analytical devices. Biosensors based on aptamers as biorecognition elements have been coined as

"aptasensors". The application of aptamers in the field of biosensors takes advantage of the high affinity and tuneable properties of aptamers, while their sensitivity is greatly influenced by the transducer. There are a number of promising transducers currently being used for aptasensor applications, with several recent outstanding reviews available.[5, 49–52] Here, we present an overview of representative approaches for the past two years according to different detection methods.

Electrochemical Detection

Electrochemical detection in aptasensors has attracted increasing attention due to the advantages of high sensitivity, fast response, robustness, low cost and the potential for minituarization (see Figure 5A for an example).[53–68] DNA aptamers are more commonly used for the biosensor development compared to RNA[69] which is less stable and easier to be degraded by ribonucleases. However, efforts have been made by several groups to overcome this drawback through chemical modifications or the use of enantiomeric aptamers with L-nucleotides.[70, 71]

Examples of electrochemical DNA aptamer-based biosensor include the detection of interferon (IFN)-γ,[72] aminoglycosidic antibiotics,[73] and thrombin[74] which was achieved by immobilizing the thrombin-binding aptamer (TBA) onto multi-walled carbon nanotubes as both the molecular recognition element and the carrier of the electrochemical capture probe. An alternative approach to detect thrombin by Zhang *et.al.*[75] was based on a gold electrode modified sequentially with polyamidoamine (PAMAM) dendrimer and with thrombin-binding aptamer through layer-by-layer assembly procedures. Furthermore, thrombin detection was also achieved using a solid-contact potentiometric aptasensor prepared by modifying the single-walled carbon nanotube surface with thrombin aptamer.[76]

An aptamer-gold nanoparticle strip biosensor was reported for the rapid, specific, sensitive, and low-cost detection of Ramos cells spiked into human blood.[77] A similar study by Xu *et.al.*[78] introduced aptamer-based dry reagent strip biosensor probes through chemical immobilization on golden nanoparticles. The approach allowed for the detection of thrombin from human plasma with comparable selectivity and sensitivity to antibody-based methods. In a separate report, a combination of aptamer-based electrochemical nanoparticles and backfiling strategy was utilized for simultaneous detection of small molecules and proteins.[79] The target binding triggered the release of aptamers off the DNA helix, which resulted in the DNA backfiling hybridization on the electrochemically encoded surface. The particular value of the approach is the ability to differentiate unique electrochemical signatures of the individual nanoparticles after binding to different targets at various concentrations.

For most electrochemical detection, the aptamers need be immobilized onto the electrodes. However, there have been several homogeneous methods based on the use of ferrocenyl aptamers[80, 81] for simple and cost-effective detections. Tan *et.al.*[81] compared the heterogeneous switch-on and homogeneous switch-off approaches of aptamer-based electrochemical sensors for thrombin detection.

Chemiluminescence Detection

Chemiluminescence detection has been widely applied to numerous fields, since it does not need external light source like fluorescence to produce light signal and offers simplicity, low cost and high sensitivity. It is not surprising, therefore, that chemiluminescence-based aptasensors have recently become a popular choice (see Figure 5B for an example).[82–86] Taking advantage of this detection approach, a chemiluminescence system was constructed based on the catalytic activity of unmodified gold nanoparticles (AuNPs) on the luminal-

H_2O_2 reaction and the interaction of unmodified AuNPs with the aptamer.[87] The binding of the aptamer with its target can induce the AuNP aggregation and further enhance the luminal-H_2O_2 chemiluminescence reaction with close to 26 fM thrombin detection limit being achieved. The measurement had almost 4 orders of magnitude better sensitivity than any current gold nanoparticle-based colorimetric methods for this system. Similarly, detection of other proteins, like α-fetoprotein (AFP), has been reported with a sensitivity limit of 5 pg/mL, which was much lower than the classical enzyme-linked immunosorbent assay (ELISA). In a report by Ahn *et.al.*,[88] an aptamer nanoarray chip-based chemiluminesce immunosorbent assay was developed for sensitive detection of severe acute respiratory syndome (SARS) coronavirus nucleocapsid protein. Such a sensitive detection approach could be highly valuable for the virus detection, particularly in the light of SARS disease outbreaks a few years ago.

Chemiluminescence is also used for the detection of small molecules like Pb^{2+},[89] adenosine and cocaine.[90–92] In a particularly interesting report, *Li et.al.*[93] used isoluminol isothiocyanate (ILITC) as a chemiluminescent tag on an aptamer, which was then used in a CE-based manner to detect the interacting targets. Such an approach has demonstrated the capability to calculate the dissociation constant (K_d) and the number of binding sites.

Fluorescence Detection

Fluorescence detection is one of the most sensitive methods to detect molecular interactions. Since aptamers can be readily modified with fluorescence tags, different approaches have been focused on how to generate fluorescence-labeled aptamers and how to detect fluorescence signal changes in response to aptamer binding to its target (see Figure 5C for an example).[37, 94–106]

A rapid homogeneous assay based on aptamer-conjugated near-infrared fluorescent nanoparticles was developed by Dent *et.al.*[107] for the direct detection of cancer cells in whole blood without prior separation steps. Another aptamer-fluorescent biosensor for intact cell detection was created by the Jaykus group who purified a DNA aptamer with high affinity to pathogenic *Campylobacter jejuni* cells through the cell-SELEX approach.[108] A similar approach was also reposted by Ohk *et.al.*[109] They have developed an Alexa Fluor-conjugated aptamer with high specificity toward pathogenic *Listeria*. These developments offer a novel strategy for pathogen detection in food and environmental samples.

A number of fluorescence aptasensors are label-free and can achieve detection limit at the subpicomolar level. For example, one design was based on a target-induced strand displacement mechanism for cocaine detection.[110] In another report, a homogenous label-free quantitation was achieved through measuring fluorescence decrease of crystal violet via the specific interaction between TBA and thrombin.[111] A highly sensitive aptasensor was also developed by Chang *et.al.*[103] via the utilization of fluorescence resonance energy transfer (FRET) as the mode of detection on a graphene surface immobilized with trombin-selective aptamer. In the resting state, fluorescence is quenched by the dye-linked aptamer, followed by fluorescence recovery after the introduction of thrombin into the system and complex formation.

Although there have been a fair number of publications reporting advances in small molecule biosensor development, very few have actually established their utilization in a biologically-relevant application. One such application was demonstrated by Ozalp, *et.al.*,[112] who have used a novel aptamer-based nanosensor for fluorescence-based measurement of ATP oscillations *in vivo*. Because changes in ATP levels throughout cell metabolism can have various regulating activities on enzymes and overall cellular pathways, obtaining a more detailed understanding of real-time endogenous levels of ATP would be

very beneficial. By using yeast as the model system, the group has not only demonstrated the changes of ATP levels during glycolysis, but also examined other molecules that appeared to be involved in these changes, including mitochondrial F_0F_1 ATPase, plasma membrane ATPase and 2-deoxyglucose. Simultaneous and selective detection of multiple small molecules based on multicolor fluorescent gold nanoprobes takes advantage of the high specificity of aptamers and the unique fluorescence quenching property of AuNPs.[113]

Quantum Dots-based Detection

Besides the common organic fluorescent dyes, quantum dots (QDs) have also been developed as labels for the sensitive detection.[114] Compared to organic fluorescent dyes,[115] QDs are relatively photostable and the wavelength of the emitted light can be controlled by changing the size and composition of the materials. Furthermore, QDs have a very broad excitation range with sharp emission, making it possible to excite different QDs with a single wavelength, while still resulting in a variety of emission wavelengths.

RNA aptamer-functionalized QDs were synthesized for Western blot-like analysis of proteins with histidine tag by combining the revolutionary fluorescence of QDs and the specificity of aptamers.[116] Aptamers were also used in a magnetic bead and red quantum dot-based sandwich assay for *Campylobacter* detection in food.[117] Dong *et.al.*[118] created a platform that combined aptamer-based molecular sensor beacons and QD-based fluorescence resonance energy transfer (FRET) system. Aptamer-functionalized quantum dots interact with graphene oxide, thus resulting in fluorescence quenching due to FRET. After the addition of the aptamer-specific target, the binding to the aptamers causes an increase in QD-graphene oxide distance, inhibiting FRET and enabling fluorescence detection. Such an ability to sensitively detect even minor changes in binding could be particularly useful for many applications. Besides sensitivity, another major advantage of the approach is high specificity. Tennico *et.al.*[119] reported the development of an on-chip aptamer-based fluorescence assay for protein detection and quantification based on sandwich ELISA principles. Here, aptamer-functionalized magnetic beads were utilized to capture the target analyte, while a second aptamer, functionalized with QDs, was employed for on-chip detection.

Colorimetric Detection

As fluorescence-based sensors need the emission of the fluorophores with excitation, it is necessary to have analytical equipment such as fluorometer or fluorescence microscope for detection, which is not always convenient for biosensors. In contrast, it would be more convenient if the detection step can be carried out without any special equipment. Colorimetric detection, though not as sensitive, is a convenient alternative technique for analyte detection with the naked eye (see Figure 5D for an example).[77, 120–122] This approach takes advantage of the high extinction coefficient of metallic nanoparticles, like gold, thus enabling color visualization without a specific instrument. The color of dispersed AuNPs smaller than 100 nm in solution will change from red to blue after aggregation due to the shift of surface plasmon resonance to a higher wavelength. Because of distinctive adsorptive properties of the nucleic acid toward functionalized AuNPs, the binding of the target or conformational changes in the DNA result in assembly of the gold nanoparticles to produce colorimetric signal.

Another interesting colorimetric detection approach recently introduced relies on the DNA base-pair recognition on agent-caging hydrogels.[123] Target-aptamer binding events control the gel-sol transition, leading to the release of previous caged enzyme to take part in its catalytic role in colorimetric reactions. Plaxco group designed a unique colorimetric assay biosensor that conjugated polyelectrolyte and gold nanoparticles.[116] The optical biosensing

is based on different affinities between the cationic polyelectrolyte and various forms of aptamers (bound or unbound). A mixture of conjugated polyelectrolyte and unbound aptamers leads to the aggregation of gold nanoparticles (and, consequently, a readily detectable change in their color), while the mixture of this polymer with target-bound aptamers does not similarly produce a color change. Theoretically, such an approach can be utilized for effective and easy detection of any molecular targets, including nucleic acid (DNA) sequences, proteins, small molecules, and inorganic ions, as demonstrated in the study.

Mass Sensitive Detection

Mass sensitive detection has the advantage of recording mass changes on the sensor surface, requiring no additional reagents for the labeling. More importantly, these detection methods can be used for the real-time detection. However, a number of drawbacks are associated with the mass change-based sensing, and therefore it is normally more applicable for the detection of large analytes like proteins or cells, but not for small molecules.

In SPR, a selective surface is formed by immobilizing an aptamer onto the surface of a sensor-chip.[124, 125] The analyte is then injected at a constant flow rate while the instrument measures changes in the resonance angle that occur at the sensor-chip surface. The angle varies when the aptamer binds to the analyte. It has been found that the signal is proportional to the amount of the bound molecules. As an example, thrombin-binding aptamer was immobilized on gold plasmon resonance (SPR) surface for thrombin detection within the range 0.1–150 nM in human plasma sample.[126] Another SPR-based bioassay strategy was established by Fan group through engineered aptamer modified gold nanoparticles for the detection of adenosine.[122] The specifically designed aptamer consists of two pieces of random-coil like ssDNA, which can induce nanoparticle aggregation in the presence of the target molecule and cause the surface plasmon resonance-based signal change.

Lautner *et.al.* reported the selective detection of the apple stem pitting virus (ASPV) coat proteins (PSA-H, MT32) using aptamer-modified sensor chips with SPR imaging.[127] Tuleuova *et.al.*[101] developed an aptamer beacon for detection of interferon-gamma (IFN-γ) by immobilizing aptamers on avidin-coated surfaces and characterization through SPR detection. In a separate study, Wang *et.al.*[128] demonstrated a modified magnetic nanoparticles (MNPs)-based amplification method. This technique enhanced the signal from SPR bioassays, enabling sensitive adenosine detection on gold substrates. The achieved detection range of 10–10,000 nM was considerably superior to the detection result obtained by a typical SPR sensor, and can be easily extended to detect other biomolecules of interest by introducing corresponding aptamers.

A number of other mass sensitive detection methods, such as surface acoustic wave (SAW) and quartz crystal microbalance (QCM), have also been reported as label-free sensing formats for the aptamer-based bioanalysis.[129–134]

Bioimaging

Given all the advantages of aptamers and their development, in theory, they can be manipulated to detect any molecule of interest with high selectivity and affinity. Additionally, compared to antibodies or other proteins, aptamers are relatively stable in harsh biological environments. These superior characteristics can be utilized for imaging applications.

One of the most significant applications that have been extensively exploited would be cancer cell and tumor detection (an example of using an aptamer for cancer tissue imaging is

demonstrated in Figure 6). The ability to sensitively distinguish cancerous tissues at an early stage of development would dramatically improve disease prognosis and change treatment regiment. Because cancer biomarkers and tumor cells exist at particularly low concentrations within the biological system during the early stages, the selectivity and high binding affinity of the aptamers toward their targets could be an optimal detection tool. Indeed, the aptamer developers have answered the call, with more imaging-style aptamers being developed for cancer cell detection than for any other biological tissue. There have already been a number of reports about the aptamers being developed for such cancer-specific oncogenic proteins as EGFR[28] in glioblastoma, HER2 and MUC1 in breast carcinomas,[135] CD30[136–138] and CD4[139] in lymphoma cells. In addition, a novel targeting aptamer is under development for the detection of cancer stem cells[140] – an area currently underserved in the cancer imaging field. In this study, the authors were able to create an aptamer with high affinity toward EpCAM protein, a novel cancer stem cell biomarker. After SELEX-based selection and truncation to the minimum required 19 bases, the new RNA aptamer offered the ability to selectively visualize EpCAM-expressing human breast, colorectal and gastric cancer cells, as was demonstrated through flow cytometry and confocal microscopy.

Another particularly interesting study was performed by Nair *et.al.*[141] They have generated aptamer-conjugated magnetic nanoparticles for selective detection of Tenascin-C in glioma cells. But the true value of the technology comes from the ability to manipulate these nanoparticles via an external magnetic field. Accordingly, the group has developed a “nanosurgeon” for the selective removal of target cancer cells by aptamers. By applying an external three-dimensional rotational magnetic field in different directions they were able to physically pull the attached cancer cells from the surface like a surgical dissection. The result of this operation was 50–60% removal of the attached carcinoma in just 10 minutes (Figure 7). The approach developed here could be especially useful for efficient tumor removal in the places where these cells are not reachable by conventional surgical tools.

As another example of aptamer effectiveness, Zhang *et.al.*[139] have synthesized an RNA aptamer specific to oncogenic CD4 protein, which has exhibited very similar detection properties as the CD4 antibody. Xu *et.al.*[142] expanded this concept by creating a tetravalent aptamer that is capable of mimicking functions of the B52 antibody. The tetramer is capable of binding to two copies of *Drosophila* B52 protein and two copies of streptavidin, thus developing a functional “aptabody” capable of detecting and staining its target in immunochemical and immunohistochemical assays. Though here the aptamers complex was used to mimic an antibody, one could predict that this idea could be used to create functional mimics of numerous proteins.

Protein modifications have also been utilized, though infrequently, as the targets for aptamer development. Lin *et.al.*[143] has generated a DNA aptamer capable of selective binding toward H4 histone protein acetylated at the lysine 16 residue. Using atomic force microscopy, they were able to effectively image acetylation within synthetic microsomal arrays. The technology has demonstrated to be much more selective that the traditional ChIP-quality antibody, demonstrating that aptamers could also provide an alternative to many less-than-efficient post-translational modification (PTM)-directed antibodies. It would be interesting to see whether it is possible to develop aptamers with good selectivity toward general PTMs independent of the protein sequence.

Perhaps some of the greatest success stories have been the aptamers developed for the imaging of prostate cancer through the prostate membrane-specific antigen (PMSA) cell marker.[144–146] These studies have taken advantage of aptamers’ high target specificity and low toxicity not only to effectively create *in vivo* imaging systems, but to further develop

them into effective recognition molecules for delivery of therapeutic nanoparticles.[147, 148] A number of drugs, including doxorubicin[146, 149, 150] and cisplatin,[151] have been delivered to their intended targets through the use of aptamers with very promising results. In every case, aptamer-based targeting improved pharmacokinetics, biodistribution, tolerability and efficacy when compared to conventional drug administration. New developments in coupling of the aptamers to liposomes, bacteriophages, micelles, dendrimers and other nanoparticles have further enhanced the delivery efficiency by improving aptamer-drug endocytosis.[151–153] Additionally, Ferreira *et.al.*[154] have demonstrated that aptamers alone, when properly modified, can achieve selective cancer toxicity without the use of any biochemical drug. In their study, *O*-glycan-peptide specific DNA aptamers were modified at the 5′ end with the photodynamic therapy agent Chlorin e6. After targeted delivery of the synthesized molecule to epithelial cancer cells, the photodynamic agent was activated with UV light, resulting in >500-fold increased toxicity in the cancer cells. Similar studies were also carried out by others.[155, 156]

The potential use of siRNA to specifically silence target genes involved in a disease has generated much excitement in the scientific community.[157] However, nonspecific distribution of the siRNA throughout the body can often result in toxic side-effects. There have been multiple promising developments in the apamer-siRNA delivery systems described by Dassie, *et.al.*[144] and others.[146, 158, 159] In these studies, aptamer-siRNA chimeras have been created against PMSA-expressing tumors. The ability to target cancer-specific molecules within a living organism can be a very promising tool for future cancer treatment with low chemotoxicity. The development of such biological imaging and delivery molecules aims at not only driving forward early diagnosis of cancer and other diseases, but providing target molecules for therapeutics.

Despite many successful applications of aptamers in cell imaging based on protein markers, broader progress has been made with the development of cell-SELEX in the field.[6] As discussed above, the introduction of cell-SELEX made it possible to generate diseased cell-specific aptamers without the prior need for a specific biomarker or molecular signature information, by taking advantage of disease-related changes in cell morphology and physiology. This procedure is particularly useful when biomarkers are not known or are not present in a specific cancer set, a feature often observed as a result of the heterogeneous nature of cancers. Most recently, such an approach has been utilized to develop selective and sensitive imaging molecules for T-cell Acute Lymphocytic Leukemia,[160, 161] lung cancer,[162, 163] liver cancer,[164] B-cell lymphoma[165] and many more. Kunii, *et.al.*[162] have developed a DNA aptamer capable of recognizing the molecules that exist predominantly on target small cell lung cancer (SCLC). After labeling with a fluorophore (FITC), the aptamer showed great promise in recognizing and imaging of the lung carcinoma. This was, reportedly, the first such DNA sequence to bind to potential surface biomarkers on SCLC cells. In a similar report, Tan group has generated a number of DNA aptamers capable of selective binding to acute myeloid leukemia (AML), but not to control cells.[160] Interestingly, they discovered that some of these aptamers can detect AML cells within a complex mixture of bone marrow aspirates, while other recognized targets associated with monocytic differentiation. Such a variety of aptamer properties could provide a particularly useful toolbox for effective examination of leukemia and its subcategories. In a separate study, fluorescence-labeled quantum dots were conjugated to an aptamer for selective imaging of mouse liver hepatoma cells, resulting in particularly sensitive recognition approach.[164] Another interesting development was reported by the Shangguan group who generated an aptamer capable of recognizing adenocarcinoma cells, a sub-type of non-small cell lung cancer.[163] Their tetramethylrhodamine-based TAMRA-fluorophore-labeled aptamer could easily differentiate adenocarcinoma from other sub-categories of lung

cancers. The ability to discriminate a single type of lung cancer from normal cells or other lung cancers shows great promise for effective prognosis.

One interesting example was the development of a multiplex system-based aptamers capable of imaging different types of cancer cells simultaneously. Chen *et.al.*[166] have combined the selectivity of aptamers (for T-cell leukemia and B-cell lymphoma) and the sensitivity of fluorescence resonance energy transfer (FRET) to create a set of nanoparticles for imaging. Such technology could be useful for monitoring multiple cancer and cancer biomarkers in the early stages of tumor development.

The continuous development of the aptamer field has also produced promising *in vivo* imaging technologies. The Tan group devised a fluorescent Cy5-labeled aptamer as the probe for *in vivo* imaging of B-cell lymphoma xenograph nude mice.[165] After intravenous injection of the conjugate into the mice, they were able to effectively monitor the aptamer dictribution within the organism via fluorescence. This was the demonstration of the first cell-SELEX generated aptamer to be used for *in vivo* tumor fluorescence imaging. Another *in vivo* imaging system was developed by Mi, *et.al.*[23] who demonstrated a novel *in vivo* aptamer selection procedure using intrahepatic bearing mice and generating an aptamer specific to hepatic colon cancer metastasis. They further validated their approach by identifying the biomarker responsible for the aptamer selectivity, which turned out to be p68 RNA helicase, a protein previously shown to be upregulated in colorectal cancer.[23] Double-staining procedure of the tumor tissues has further confirmed the localization of the newly developed apamer with the p68 protein. The demonstrated ability of the aptamers to localize to specific tumor sites in a living mouse holds great promise for better early diagnosis, characterization of disease states, prediction of patient outcomes and formulating specific treatment regiments, thus further advancing to the ultimate goal of personalized medicine.

While there have been increasing efforts on the development of new imaging tools, aptamer holds a unique position with its high specificity and tunable features. We expect to see continuously great efforts for applications and fundamental developments of aptamers in the imaging field in the coming years.

Aptamers as Discovery Tools

While aptamer is very effective at recognition of target molecules with high specificity, it typically needs to couple with another tool such as mass spectrometry to become a discovery tool. So far, there have been only several reports of aptamers being coupled with mass spectrometry-based analysis. The most likely reason being the limited availability of aptamers. To overcome such a predicament, instead of studying aptamer-protein interactions, Mann group[167] has used aptamers as a tag for other RNA molecules to create functional baits that can capture RNA-interacting proteins on the proteomics scale (Figure 8). The quantitative proteomics technique based on stable isotope labeling by amino acids in cell culture (SILAC) was used to distinguish the interacting proteins from background by comparing the isotopic ratios between bait and control, thus providing a powerful tool to study specific RNA–protein interactions. It enables the quantitative study of *in vivo*-assembled RNA–protein complexes by transfecting the corresponding plasmid followed by aptamer-based purification from crude cell extracts. They have demonstrated the utility of the approach to retrieve interaction partners for a number of RNA sequences, including the HuR interation motif, H4 stem loop, “zipcode” sequence, tRNA and an RNA fold in DGCR-8/Pasha mRNA. Besides identifying the major known interacting proteins, a novel set of translational machinery interactors were identified for the DGCR-8/Pasha mRNA sequence. In a similar study, Said *et.al.*[168] used RNA aptamers as a tagging tool to study small non-coding RNAs (sRNA) and their potential protein interaction partners. Many

sRNA often function as gene regulators, and it would be highly significant to obtain a more in-depth understanding of their mode of action. Though in their project large-scale quantitative proteomic analysis was not used, they were still able to identify some interesting sRNA-interacting proteins in *Salmonella*.

Besides analyzing RNA-protein interactions, aptamers have also been utilized for the discovery of novel disease-relevant proteins. A number of laboratories have utilized the combination of aptamer-based technology with proteomics for multiplex discovery of novel biomarkers. A group from SomaLogic has introduced a sensitive aptamer-based proteomics array technology for multiplex target selection. The approach was applied for a clinical study of chronic kidney disease, resulting in the identification of 2 known and 58 new potential biomarkers.[169] In another study from the same group, the technology was used for non-small cell lung cancer biomarker discovery among a population of long-time smokers.[170] In addition to identifying 44 candidate biomarkers, the group was able to develop a 12-protein panel that discriminates cancer-induced samples from controls with 84% specificity. The study allowed the group to identify novel serum proteins that have not been previously identified as lung cancer biomarkers; these biomarkers were verified within a large testing sample. In a related study, Ahn et al.[171] reported an aptamer microarray-based affinity capture and mass spectrometry identification of biomarkers in serum samples. Microarrays were spotted with aptamers specific to TATA Box Protein (TBP) by sol-gel approach. Serum samples containing TBP protein were used for target capture. The captured protein was then digested *in situ* and the peptides were analyzed by electrospray ionization mass specrometry (ESI-MS). The binding sites and consecutive binding constants of alkali metal ions to thrombin-binding aptamer DNA were studied by fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS). Such combination of aptamers and proteomics technologies is clearly capable of providing significant input in the field of early disease diagnosis.

Beside the capture and identification of proteins, aptamer-MS based analysis has recently been applied for detection of small molecules and single proteins. As an example, Nguyen *et al.*[172] devised a system by integrating a microchip capable of specific capture, enrichment, and isocratic elution of biomolecular analytes for mass spectrometry detection. Aptamers were immobilized on microbeads packed inside a microchamber, where a model analyte, adenosine monophosphate (AMP), was used for specific capture and enrichment via the affinity binding to aptamers, followed by offline MALDI-MS label-free detection. Similarly, Tan group[173] used aptamer-conjugated graphene oxide as both an affinity extraction media and a MALDI matrix, which enabled them to enrich and analyze by mass spectrometry cocaine and adenosine from plasma. In another study, Zhao *et.al.*[174] coupled the sandwich binding of two affinity aptamers (for increased specificity) and inductively coupled plasma mass spectrometry (ICP-MS) for highly sensitive analyte detection. This technology resulted in thrombin detection limit of as low as 0.5 fmol with a three orders of magnitude dynamic range.

Despite a few reports, at the current stage, mass spectrometry is not being frequently coupled with aptamer-based capturing techniques. The main reason is that an aptamer is usually selected against a known target, and therefore is often used only for the detection of this target. We believe that selection of aptamers against a class of multiple targets (such as certain types of compounds or protein modifications) or intact cells would warrant a nice combination with mass spectrometric analyses to facilitate comprehensive identification of actual targets and interacting biomolecules.

Perspectives

One important issue which limits the application of aptamers is relatively inadequate availability compared to antibodies. Additionally, most method developments are still focused on several well-known targets like thrombin. Nonetheless, recent studies have demonstrated the beginning stages of the aptamer development focusing on the examination of important therapeutic targets (e.g. oncogenic proteins). Continuation along this path may result in multiple novel therapeutic leads and drug targets. Additionally, we believe it is extremely appealing to increase efforts on the development of aptamers targeting certain modification groups, including post-translational modifications of proteins, such as phosphorylation or glycosylation. Along with single molecule detection, these expansions would potentially provide a more comprehensive scope of analysis.[175]

Further developments of cell-SELEX have offered very promising results in recent years, but it will be of high importance to have the knowledge of the actual recognition mechanism for specific targets of certain cancer cells. Unlike other aptamer applications, the recognition of cells is based on multi-target interactions. Uncovering these precise modes of interactions would provide a more detailed view of disease development and present researchers with novel biomarkers.

High affinity and selectivity are the most important properties of an aptamer, which is also the reason they are called “chemical antibodies”. As long as a valid screening method for aptamer generation is available, its utilization for complex sample targeting should be readily achievable. Due to the relatively simple chemistry, aptamer-based applications will become more tunable and accessible compared to antibodies. The ability to be easily modified or attached to a support also makes aptamers an invaluable analytical tool. As the aptamer field progresses, we expect the acceleration in areas of biomarker discovery, early disease detection and diagnosis.

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Literature Cited

1. Tuerk C, Gold L. Science. 1990; 249:505–510. [PubMed: 2200121]
2. Ellington AD, Szostak JW. Nature. 1990; 346:818–822. [PubMed: 1697402]
3. Robertson DL, Joyce GF. Nature. 1990; 344:467–468. [PubMed: 1690861]
4. Keefe AD, Pai S, Ellington A. Nat Rev Drug Discovery. 2010; 9:537–550.
5. Cho EJ, Lee JW, Ellington AD. Annu Rev Anal Chem. 2009; 2:241–264.
6. Fang XH, Tan WH. Acc Chem Res. 2010; 43:48–57. [PubMed: 19751057]
7. Bouchard PR, Hutabarat RM, Thompson KM. Annu Rev Pharmacol Toxicol. 2010; 50:237–257. [PubMed: 20055704]
8. Chen T, Shukoor MI, Chen Y, Yuan QA, Zhu Z, Zhao ZL, Gulbakan B, Tan WH. Nanoscale. 2011; 3:546–556. [PubMed: 21109879]
9. Yang XB, Li X, Prow TW, Reece LM, Bassett SE, Luxon BA, Herzog NK, Aronson J, Shope RE, Leary JF, Gorenstein DG. Nucleic Acids Res. 2003; 31:8.
10. Tok J, Lai J, Leung T, Li SFY. Electrophoresis. 2010; 31:2055–2062. [PubMed: 20564698]
11. Misono TS, Kumar PKR. Anal Biochem. 2005; 342:312–317. [PubMed: 15913532]
12. Miyachi Y, Shimizu N, Ogino C, Kondo A. Nucleic Acids Res. 2010; 38
13. Miyachi Y, Shimizu N, Ogino C, Kondo A. J Biosci Bioeng. 2009; 108:S64–S64.

14. Ogino C, Miyachi Y, Hayase T, Nosaka K, Kondo A. Nucl Acids Res. 2009; 38:e21. [PubMed: 19955232]
15. Berezovski M, Drabovich A, Krylova SM, Musheev M, Okhonin V, Petrov A, Krylov SN. J Am Chem Soc. 2005; 127:3165–3171. [PubMed: 15740156]
16. Berezovski M, Musheev M, Drabovich A, Krylov SN. J Am Chem Soc. 2006; 128:1410–1411. [PubMed: 16448086]
17. Berezovski MV, Musheev MU, Drabovich AP, Jitkova JV, Krylov SN. Nat Protoc. 2006; 1:1359–1369. [PubMed: 17406423]
18. Javaherian S, Musheev MU, Kanoatov M, Berezovski MV, Krylov SN. Nucleic Acids Res. 2009; 37
19. Kanoatov M, Javaherian S, Krylov SN. Anal Chim Acta. 2010; 681:92–97. [PubMed: 21035608]
20. Lou XH, Qian JR, Xiao Y, Viel L, Gerdon AE, Lagally ET, Atzberger P, Tarasow TM, Heeger AJ, Soh HT. Proc Natl Acad Sci U S A. 2009; 106:2989–2994. [PubMed: 19202068]
21. Qian JR, Lou XH, Zhang YT, Xiao Y, Soh HT. Anal Chem. 2009; 81:5490–5495. [PubMed: 19480397]
22. Cho M, Xiao Y, Nie J, Stewart R, Csordas AT, Oh SS, Thomson JA, Soh HT. Proc Natl Acad Sci U S A. 2010; 107:15373–15378. [PubMed: 20705898]
23. Mi J, Liu YM, Rabbani ZN, Yang ZG, Urban JH, Sullenger BA, Clary BM. Nat Chem Biol. 6:22–24. [PubMed: 19946274]
24. Zheng JA, Li YB, Li JX, Wang J, Su YQ. Key Eng Mater. 2010; 439–440:1456–1462.
25. Tang ZW, Parekh P, Turner P, Moyer RW, Tan WH. Clin Chem. 2009; 55:813–822. [PubMed: 19246617]
26. Chen F, Hu YL, Li DQ, Chen HD, Zhang XL. PLoS One. 2009; 4
27. Li SH, Xu H, Ding HM, Huang YP, Cao XX, Yang G, Li J, Xie ZG, Meng YH, Li XB, Zhao Q, Shen BF, Shao NS. Journal of Pathology. 2009; 218:327–336. [PubMed: 19291713]
28. Wan Y, Kim YT, Li N, Cho SK, Bachoo R, Ellington AD, Iqbal SM. Cancer Res. 70:9371–9380. [PubMed: 21062984]
29. Ravelet C, Peyrin E. Integrated Analytical Systems. 2009; 10:271–286.
30. Peyrin E. J Sep Sci. 2009; 32:1531–1536. [PubMed: 19370736]
31. Zhao Q, Li XF, Le XC. Anal Chem. 2008; 80:3915–3920. [PubMed: 18363332]
32. Zhao Q, Li XF, Shao YH, Le XC. Anal Chem. 2008; 80:7586–7593. [PubMed: 18759461]
33. Huy GD, Jin N, Yin BC, Ye BC. Bioprocess Biosyst Eng. 34:189–195. [PubMed: 20734205]
34. Martin JA, Phillips JA, Parekh P, Sefah K, Tan W. Mol BioSyst. 7:1720–1727. [PubMed: 21424012]
35. Liu Y, Bae SW, Wang K, Hong JI, Zhu Z, Tan WH, Pappas D. Anal Chim Acta. 673:95–100. [PubMed: 20630183]
36. Zhu ZY, Ravelet C, Perrier S, Guieu V, Roy B, Perigaud C, Peyrin E. Anal Chem. 2010; 82:4613–4620. [PubMed: 20446673]
37. Liu CW, Huang CC, Chang HT. Anal Chem. 2009; 81:2383–2387. [PubMed: 19219985]
38. Lee KH, Huang MF, Liu CW, Chang HT. Electrophoresis. 2010; 31:1101–1107. [PubMed: 20151395]
39. Liu XJ, Qi C, Bing T, Cheng XH, Shangguan DH. Anal Chem. 2009; 81:3699–3704. [PubMed: 19323492]
40. Girardot M, Gareil P, Varenne A. Electrophoresis. 2010; 31:546–555. [PubMed: 20119964]
41. Zhang HQ, Li XF, Le XC. Anal Chem. 2009; 81:7795–7800. [PubMed: 19691297]
42. Ruta J, Perrier S, Ravelet C, Roy B, Perigaud C, Peyrin E. Anal Chem. 2009; 81:1169–1176. [PubMed: 19128144]
43. Phillips JA, Xu Y, Xia Z, Fan ZH, Tan WH. Anal Chem. 2009; 81:1033–1039. [PubMed: 19115856]
44. Xu Y, Phillips JA, Yan JL, Li QG, Fan ZH, Tan WH. Anal Chem. 2009; 81:7436–7442. [PubMed: 19715365]

45. Reif RD, Martinez MM, Wang K, Pappas D. Anal Bioanal Chem. 2009; 395:787–795. [PubMed: 19685232]
46. Dharmasiri U, Balamurugan S, Adams AA, Okagbare PI, Obubuafo A, Soper SA. Electrophoresis. 2009; 30:3289–3300. [PubMed: 19722212]
47. Swensen JS, Xiao Y, Ferguson BS, Lubin AA, Lai RY, Heeger AJ, Plaxco KW, Soh HT. J Am Chem Soc. 2009; 131:4262–4266. [PubMed: 19271708]
48. Huang PJJ, Liu JW. Anal Chem. 2010; 82:4020–4026. [PubMed: 20405823]
49. Zhou J, Battig MR, Wang Y. Anal Bioanal Chem. 2010; 398:2471–2480. [PubMed: 20644915]
50. Li BL, Dong SJ, Wang EK. Chem Asian J. 2010; 5:1262–1272. [PubMed: 20408164]
51. Li D, Song SP, Fan CH. Acc Chem Res. 2010; 43:631–641. [PubMed: 20222738]
52. Sefah K, Phillips JA, Xiong X, Meng L, Van Simaeys D, Chen H, Martin J, Tan W. Analyst. 2009; 134:1765–1775. [PubMed: 19684896]
53. Sassolas A, Blum LJ, Leca-Bouvier BD. Electroanalysis. 2009; 21:1237–1250.
54. Hianik T, Wang J. Electroanalysis. 2009; 21:1223–1235.
55. Ikebukuro K, Kiyohara C, Sode K. Anal Lett. 2004; 37:2901–2909.
56. Zelada-Guillen GA, Bhosale SV, Riu J, Rius FX. Anal Chem. 82:9254–9260. [PubMed: 20961052]
57. Min K, Song KM, Cho M, Chun YS, Shim YB, Ku JK, Ban C. Chem Commun (Cambridge, U K). 46:5566–5568.
58. Tran DT, Vermeeren V, Grieten L, Wenmackers S, Wagner P, Pollet J, Janssen KP, Michiels L, Lammertyn J. Biosens Bioelectron. 26:2987–2993. [PubMed: 21185167]
59. Hua M, Tao M, Wang P, Zhang Y, Wu Z, Chang Y, Yang Y. Anal Sci. 26:1265–1270. [PubMed: 21157095]
60. Shao Z, Li Y, Yang Q, Wang J, Li G. Anal Bioanal Chem. 398:2963–2967. [PubMed: 20865403]
61. Zhang JK, Zhang BB, Wu Y, Jia SR, Fan T, Zhang ZY, Zhang CZ. Analyst. 135:2706–2710. [PubMed: 20714519]
62. Ding CF, Ge Y, Zhang SS. Chem--Eur J. 16:10707–10714.
63. Li LD, Zhao HT, Chen ZB, Mu XJ, Guo L. Anal Bioanal Chem. 398:563–570. [PubMed: 20607523]
64. Kuang H, Chen W, Xu DH, Xu LG, Zhu YY, Liu LQ, Chu HQ, Peng CF, Xu CL, Zhu SF. Biosens Bioelectron. 26:710–716. [PubMed: 20643539]
65. Wang JA, Zhang P, Li JY, Chen LQ, Huang CZ, Li YF. Analyst. 135:2826–2831. [PubMed: 20830327]
66. Jeong S, Han SR, Lee YJ, Kim JH, Lee SW. Oligonucleotides. 20:155–161. [PubMed: 20565241]
67. Oguro A, Ohtsu T, Nakamura Y. Anal Biochem. 2009; 388:102–107. [PubMed: 19250914]
68. Wang JL, Munir A, Li ZH, Zhou HS. Talanta. 81:63–67. [PubMed: 20188888]
69. Ferapontova EE, Olsen EM, Gothelf KV. J Am Chem Soc. 2008; 130:4256–4258. [PubMed: 18324816]
70. Vaught JD, Dewey T, Eaton BE. J Am Chem Soc. 2004; 126:11231–11237. [PubMed: 15355104]
71. Mascini M, Papamichael K, Mevola I, Pravda M, Guilbault GG. Anal Lett. 2007; 40:403–430.
72. Liu Y, Tuleouva N, Ramanculov E, Revzin A. Anal Chem. 2010; 82:8131–8136. [PubMed: 20815336]
73. Rowe AA, Miller EA, Plaxco KW. Anal Chem. 2010; 82:7090–7095. [PubMed: 20687587]
74. Liu XR, Li Y, Zheng JB, Zhang JC, Sheng QL. Talanta. 2010; 81:1619–1624. [PubMed: 20441948]
75. Zhang ZX, Yang W, Wang J, Yang C, Yang F, Yang XR. Talanta. 2009; 78:1240–1245. [PubMed: 19362182]
76. Duzgun A, Maroto A, Mairal T, O'Sullivan C, Rius FX. Analyst. 2010; 135:1037–1041. [PubMed: 20419254]
77. Liu GD, Mao X, Phillips JA, Xu H, Tan WH, Zeng LW. Anal Chem. 2009; 81:10013–10018. [PubMed: 19904989]

78. Xu H, Mao X, Zeng QX, Wang SF, Kawde AN, Liu GD. Anal Chem. 2009; 81:669–675. [PubMed: 19072289]
79. Qian XQ, Xiang Y, Zhang HX, Chen Y, Chai YQ, Yuan R. Chem Eur J. 16:14261–14265.
80. Fukasawa M, Yoshida W, Yamazaki H, Sode K, Ikebukuro K. Electroanalysis. 2009; 21:1297–1302.
81. Tan ESQ, Wivanius R, Toh CS. Electroanalysis. 2009; 21:749–754.
82. Park H, Paeng IR. Anal Chim Acta. 685:65–73. [PubMed: 21168553]
83. Zhu X, Zhang Y, Yang W, Liu Q, Lin Z, Qiu B, Chen G. Anal Chim Acta. 684:121–125. [PubMed: 21167993]
84. Qi YY, Li BX. Chem Eur J. 2011; 17:1642–1648.
85. Li Y, Qi HL, Gao QA, Yang J, Zhang CX. Biosens Bioelectron. 26:754–759. [PubMed: 20650626]
86. Ouyang X, Yu R, Jin J, Li J, Yang R, Tan W, Yuan J. Anal Chem. 83:782–789. [PubMed: 21207953]
87. Qi YY, Li BX. Chem--Eur J. 2010; 17:1642–1648.
88. Ahn DG, Jeon IJ, Kim JD, Song MS, Han SR, Lee SW, Jung H, Oh JW. Analyst. 2009; 134:1896–1901. [PubMed: 19684916]
89. Li T, Wang E, Dong S. Anal Chem. 2010; 82:1515–1520. [PubMed: 20095579]
90. Yan XL, Cao ZJ, Lau CW, Lu JZ. Analyst. 2010; 135:2400–2407. [PubMed: 20652180]
91. Zhang SS, Yan YM, Bi S. Anal Chem. 2009; 81:8695–8701. [PubMed: 19788280]
92. Yan XL, Cao ZJ, Kai M, Lu JZ. Talanta. 2009; 79:383–387. [PubMed: 19559894]
93. Li HY, Deng QP, Zhang DW, Zhou YL, Zhang XX. Electrophoresis. 2010; 31:2452–2460. [PubMed: 20564271]
94. Book B, Chen J, Irudayaraj J. Biotechnol Bioeng.
95. Wang KY, Zeng YL, Yang XY, Li WB, Lan XP. Eur J Clin Microbiol Infect Dis. 30:273–278. [PubMed: 20936492]
96. Mallikaratchy PR, Ruggiero A, Gardner JR, Kuryavyi V, Maguire WF, Heaney ML, McDevitt MR, Patel DJ, Scheinberg DA. Nucleic Acids Res.
97. Ma C, Huang H, Zhao C. Anal Sci. 26:1261–1264. [PubMed: 21157094]
98. Wang LB, Chen W, Ma WW, Liu LQ, Ma W, Zhao YA, Zhu YY, Xu LG, Kuang H, Xu CL. Chem Commun (Cambridge, U K). 2010; 47:1574–1576.
99. Xu JP, Song ZG, Fang YA, Mei J, Jia L, Qin AJ, Sun JZ, Ji JA, Tang BZ. Analyst. 135:3002–3007. [PubMed: 20877906]
100. Huang J, Zhu Z, Bamrungsap S, Zhu G, You M, He X, Wang K, Tan W. Anal Chem. 82:10158–10163. [PubMed: 21080638]
101. Tuleuova N, Jones CN, Yan J, Ramanculov E, Yokobayashi Y, Revzin A. Anal Chem. 2010; 82:1851–1857. [PubMed: 20121141]
102. Zhang LB, Wei H, Li J, Li T, Li D, Li YH, Wang EK. Biosens Bioelectron. 25:1897–1901. [PubMed: 20106653]
103. Chang HX, Tang LH, Wang Y, Jiang JH, Li JH. Anal Chem. 2010; 82:2341–2346. [PubMed: 20180560]
104. Chen SJ, Huang CC, Chang HT. Talanta. 81:493–498. [PubMed: 20188952]
105. Tuleuova N, Ramankulov E, Revzin A. Cytokine. 52:97–97.
106. Tuleuova N, Revzin A. Cell Mol Bioeng. 3:337–344. [PubMed: 21170394]
107. Deng T, Li JS, Zhang LL, Jiang JH, Chen JN, Shen GL, Yu RQ. Biosens Bioelectron. 2010; 25:1587–1591. [PubMed: 20022484]
108. Dwivedi HP, Smiley RD, Jaykus LA. Appl Microbiol Biotechnol. 87:2323–2334. [PubMed: 20582587]
109. Ohk SH, Koo OK, Sen T, Yamamoto CM, Bhunia AK. Journal of Applied Microbiology. 109:808–817. [PubMed: 20337767]
110. He JL, Wu ZS, Zhou H, Wang HQ, Jiang JH, Shen GL, Yu RQ. Anal Chem. 2010; 82:1358–1364. [PubMed: 20078091]

111. Jin Y, Bai JY, Li HY. Analyst. 2010; 135:1731–1735. [PubMed: 20467654]
112. Ozalp VC, Pedersen TR, Nielsen LJ, Olsen LF. J Biol Chem. 285:37579–37588. [PubMed: 20880841]
113. Zhang J, Wang LH, Zhang H, Boey F, Song SP, Fan CH. Small. 2010; 6:201–204. [PubMed: 19957283]
114. Wagner MK, Li F, Li JJ, Li XF, Le XC. Anal Bioanal Chem. 2010; 397:3213–3224. [PubMed: 20532875]
115. Algar WR, Tavares AJ, Krull UJ. Anal Chim Acta. 2010; 673:1–25. [PubMed: 20630173]
116. Shin S, Kim IH, Kang W, Yang JK, Hah SS. Bioorg Med Chem Lett. 2010; 20:3322–3325. [PubMed: 20457520]
117. Bruno JG, Phillips T, Carrillo MP, Crowell R. J Fluoresc. 2009; 19:427–435. [PubMed: 19052851]
118. Dong HF, Gao WC, Yan F, Ji HX, Ju HX. Anal Chem. 2010; 82:5511–5517. [PubMed: 20524633]
119. Tennico YH, Hutanu D, Koesdjojo MT, Bartel CM, Remcho VT. Anal Chem. 2010; 82:5591–5597. [PubMed: 20545301]
120. Kim YS, Kim JH, Kim IA, Lee SJ, Jurng J, Gu MB. Biosens Bioelectron. 26:1644–1649. [PubMed: 20829027]
121. Lu WT, Arumugam R, Senapati D, Singh AK, Arbneshi T, Khan SA, Yu HT, Ray PC. ACS Nano. 4:1739–1749. [PubMed: 20155973]
122. Li F, Zhang J, Cao XN, Wang LH, Li D, Song SP, Ye BC, Fan CH. Analyst. 2009; 134:1355–1360. [PubMed: 19562201]
123. Zhu Z, Wu CC, Liu HP, Zou Y, Zhang XL, Kang HZ, Yang CJ, Tan WH. Angew Chem, Int Ed. 2010; 49:1052–1056.
124. Scarano S, Mascini M, Turner APF, Minunni M. Biosens Bioelectron. 2010; 25:957–966. [PubMed: 19765967]
125. Shen GY, Chen Y, Zhang YM, Cui JA. Prog Chem. 2010; 22:1648–1655.
126. Polonschii C, David S, Tombelli S, Mascini M, Gheorghiu M. Talanta. 2010; 80:2157–2164. [PubMed: 20152466]
127. Lautner G, Balogh Z, Bardoczy V, Meszaros T, Gyurcsanyi RE. Analyst. 2010; 135:918–926. [PubMed: 20419239]
128. Wang JL, Munir A, Zhu ZZ, Zhou HS. Anal Chem. 2010; 82:6782–6789. [PubMed: 20704367]
129. Yao CY, Qi YZ, Zhao YH, Xiang Y, Chen QH, Fu WL. Biosens Bioelectron. 2009; 24:2499–2503. [PubMed: 19188059]
130. Gronewold TMA, Baumgartner A, Hierer J, Sierra S, Blind M, Schafer F, Blumer J, Tillmann T, Kiwitz A, Kaiser R, Zabe-Kuhn M, Quandt E, Famulok M. J Proteome Res. 2009; 8:3568–3577. [PubMed: 19469583]
131. Chen QA, Tang W, Wang DZ, Wu XJ, Li N, Liu F. Biosens Bioelectron. 2010; 26:575–579. [PubMed: 20692147]
132. Yao CY, Zhu TY, Qi YZ, Zhao YH, Xia H, Fu WL. Sensors. 2010; 10:5859–5871.
133. Lee D, Yoo M, Seo H, Tak Y, Kim WG, Yong K, Rhee SW, Jeon S. Sens Actuators B Chem. 2009; 135:444–448.
134. Pan YL, Guo ML, Nie Z, Huang Y, Pan CF, Zeng K, Zhang Y, Yao SZ. Biosens Bioelectron. 2010; 25:1609–1614. [PubMed: 20031387]
135. Gupta S, Thirstrup D, Jarvis TC, Schneider DJ, Wilcox SK, Carter J, Zhang C, Gelinas A, Weiss A, Janjic N, Baird GS. Appl Immunohistochem Mol Morphol.
136. Zeng Z, Zhang P, Zhao N, Sheehan AM, Tung CH, Chang CC, Zu Y. Mod Pathol. 23:1553–1558. [PubMed: 20693984]
137. Zhang P, Zhao NX, Zeng ZH, Feng YD, Tung CH, Chang CC, Zu YL. Lab Invest. 2009; 89:1423–1432. [PubMed: 19823169]
138. Zhao N, Bagaria HG, Wong MS, Zu Y. J Nanobiotechnology. 9:2. [PubMed: 21281497]

139. Zhang P, Zhao NX, Zeng ZH, Chang CC, Zu YL. American Journal of Clinical Pathology. 134:586–593. [PubMed: 20855639]
140. Shigdar S, Lin J, Yu Y, Pastuovic M, Wei M, Duan W. Cancer Sci.
141. Nair BG, Nagaoka Y, Morimoto H, Yoshida Y, Maekawa T, Kumar DS. Nanotechnology. 21:455102. [PubMed: 20947949]
142. Xu DY, Shi H. Nucleic Acids Res. 2009; 37
143. Lin LY, Fu Q, Williams BAR, Azzaz AM, Shogren-Knaak MA, Chaput JC, Lindsay S. Biophys J. 2009; 97:1804–1807. [PubMed: 19751687]
144. Dassie JP, Liu XY, Thomas GS, Whitaker RM, Thiel KW, Stockdale KR, Meyerholz DK, McCaffrey AP, McNamara JO, Giangrande PH. Nat Biotechnol. 2009; 27:839–U895. [PubMed: 19701187]
145. Min K, Jo H, Song K, Cho M, Chun YS, Jon S, Kim WJ, Ban C. Biomaterials. 32:2124–2132. [PubMed: 21147500]
146. Kim E, Jung Y, Choi H, Yang J, Suh JS, Huh YM, Kim K, Haam S. Biomaterials. 31:4592–4599. [PubMed: 20206379]
147. Cerchia L, de Franciscis V. Trends Biotechnol. 28:517–525. [PubMed: 20719399]
148. Yan AC, Levy M. RNA Biol. 2009; 6:316–320. [PubMed: 19458497]
149. Savla R, Taratula O, Garbuzenko O, Minko T. J Control Release.
150. Kim D, Jeong YY, Jon S. ACS Nano. 4:3689–3696. [PubMed: 20550178]
151. Dhar S, Kolishetti N, Lippard SJ, Farokhzad OC. Proc Natl Acad Sci U S A. 108:1850–1855. [PubMed: 21233423]
152. Wu YR, Sefah K, Liu HP, Wang RW, Tan WH. Proc Natl Acad Sci U S A. 107:5–10. [PubMed: 20080797]
153. Cao ZH, Tong R, Mishra A, Xu WC, Wong GCL, Cheng JJ, Lu Y. Angew Chem, Int Ed. 2009; 48:6494–6498.
154. Ferreira CS, Cheung MC, Missailidis S, Bisland S, Gariepy J. Nucleic Acids Res. 2009; 37:866–876. [PubMed: 19103663]
155. Stephanopoulos N, Tong GJ, Hsiao SC, Francis MB. ACS Nano. 4:6014–6020. [PubMed: 20863095]
156. Shieh YA, Yang SJ, Wei MF, Shieh MJ. ACS Nano. 4:1433–1442. [PubMed: 20166743]
157. Guo P, Coban O, Snead NM, Trebley J, Hoeprich S, Guo S, Shu Y. Adv Drug Deliv Rev. 62:650–666. [PubMed: 20230868]
158. Neff CP, Zhou J, Remling L, Kuruvilla J, Zhang J, Li H, Smith DD, Swiderski P, Rossi JJ, Akkina R. Sci Transl Med. 3:66ra66.
159. Zhou JH, Swiderski P, Li HT, Zhang J, Neff CP, Akkina R, Rossi JJ. Nucleic Acids Res. 2009; 37:3094–3109. [PubMed: 19304999]
160. Sefah K, Tang ZW, Shangguan DH, Chen H, Lopez-Colon D, Li Y, Parekh P, Martin J, Meng L, Phillips JA, Kim YM, Tan WH. Leukemia. 2009; 23:235–244. [PubMed: 19151784]
161. Medley CD, Bamrungsap S, Tan W, Smith JE. Anal Chem. 83:727–734. [PubMed: 21218774]
162. Kunii T, Ogura S, Mie M, Kobatake E. Analyst. 136:1310–1312. [PubMed: 21321690]
163. Zhao ZL, Xu L, Shi XL, Tan WH, Fang XH, Shangguan DH. Analyst. 2009; 134:1808–1814. [PubMed: 19684903]
164. Zhang J, Jia X, Lv XJ, Deng YL, Xie HY. Talanta. 81:505–509. [PubMed: 20188954]
165. Shi H, Tang ZW, Kim Y, Nie HL, Huang YF, He XX, Deng K, Wang KM, Tan WH. Chemistry-an Asian Journal. 5:2209–2213.
166. Chen XL, Estevez MC, Zhu Z, Huang YF, Chen Y, Wang L, Tan WH. Anal Chem. 2009; 81:7009–7014. [PubMed: 19572554]
167. Butter F, Scheibe M, Morl M, Mann M. Proc Natl Acad Sci U S A. 2009; 106:10626–10631. [PubMed: 19541640]
168. Said N, Rieder R, Hurwitz R, Deckert J, Urlaub H, Vogel J. Nucleic Acids Res. 2009; 37
169. Gold L, Ayers D, Bertino J, Bock C, Bock A, Brody EN, Carter J, Dalby AB, Eaton BE, Fitzwater T, Flather D, Forbes A, Foreman T, Fowler C, Gawande B, Goss M, Gunn M, Gupta S,

Halladay D, Heil J, Heilig J, Hicke B, Husar G, Janjic N, Jarvis T, Jennings S, Katilius E, Keeney TR, Kim N, Koch TH, Kraemer S, Kroiss L, Le N, Levine D, Lindsey W, Lollo B, Mayfield W, Mehan M, Mehler R, Nelson SK, Nelson M, Nieuwlandt D, Nikrad M, Ochsner U, Ostroff RM, Otis M, Parker T, Pietrasiewicz S, Resnicow DI, Rohloff J, Sanders G, Sattin S, Schneider D, Singer B, Stanton M, Sterkel A, Stewart A, Stratford S, Vaught JD, Vrkljan M, Walker JJ, Watrobka M, Waugh S, Weiss A, Wilcox SK, Wolfson A, Wolk SK, Zhang C, Zichi D. PLoS One. 5:e15004. [PubMed: 21165148]

170. Ostroff RM, Bigbee WL, Franklin W, Gold L, Mehan M, Miller YE, Pass HI, Rom WN, Siegfried JM, Stewart A, Walker JJ, Weissfeld JL, Williams S, Zichi D, Brody EN. PLoS One. 5:e15003. [PubMed: 21170350]
171. Ahn JY, Lee SW, Kang HS, Jo M, Lee DK, Laurell T, Kim S. J Proteome Res. 2010; 9:5568–5573. [PubMed: 20806970]
172. Nguyen TH, Pei RJ, Qiu CM, Ju JY, Stojanovic M, Lin Q. J Microelectromech Syst. 2009; 18:1198–1207.
173. Gulbakan B, Yasun E, Shukoor MI, Zhu Z, You MX, Tan XH, Sanchez H, Powell DH, Dai HJ, Tan WH. J Am Chem Soc. 2010; 132:17408–17410.
174. Zhao Q, Lu XF, Yuan CG, Li XF, Le XC. Anal Chem. 2009; 81:7484–7489. [PubMed: 19670869]
175. Seo YJ, Chen SL, Nilsen-Hamilton M, Levinea HA. Bull Math Biol. 2010; 72:1623–1665. [PubMed: 20077028]

Biographies

Anton Iliuk received his B.S. (2004) and M.S. (2006) in chemistry (with biochemistry emphasis) from Eastern New Mexico University in Portales, New Mexico under the supervision of Dr. Newton Hilliard. He started the pursuit of his Ph.D. degree in 2006 at the Department of Biochemistry under the supervision of Dr. W. Andy Tao. His current research focus is the development of new technologies for more effective analysis of protein phosphorylation and mass spectrometry-based proteomics.

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Dr. W. Andy Tao, Associate Professor of Biochemistry at Purdue University, earned his PhD (2001) from the Department of Chemistry, Purdue University. He worked for three years as a Damon Runyon Postdoctoral Fellow in the Institute for Systems Biology at Seattle, before starting his research group at the Department of Biochemistry, Purdue University in 2005. Tao's research interest is in proteomics and biological mass spectrometry. His research group has developed various techniques for quantitative proteomics and phosphoproteomics. In particular, the Tao group has introduced soluble nanopolymers as a platform for the study of intracellular molecular signaling and for the identification of intracellular drug targets.

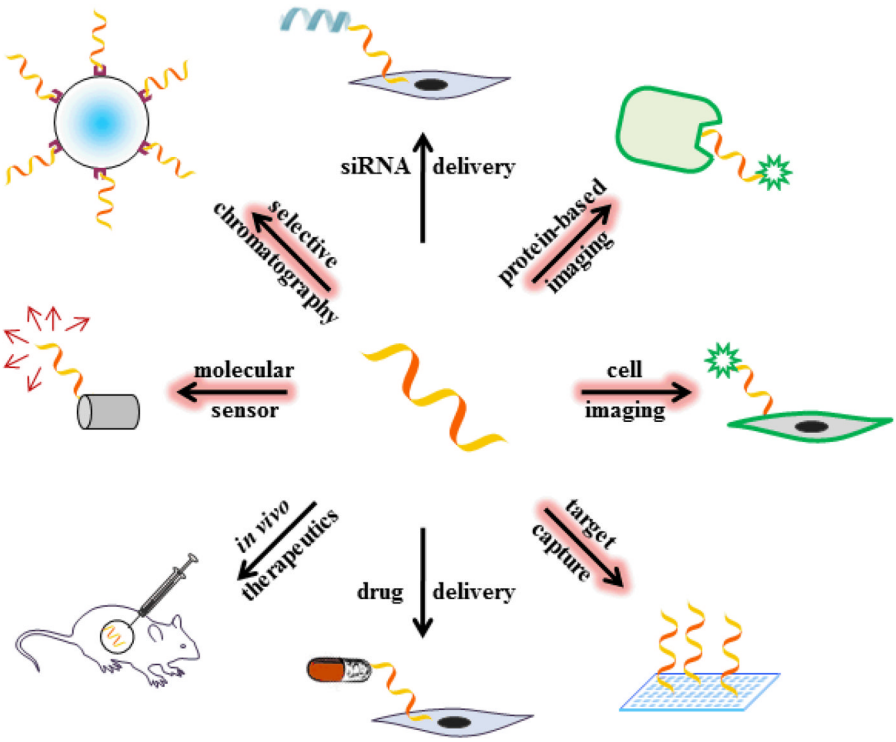

Figure 1.
The widespread use of aptamers for numerous analytical and biological applications. Bioanalytical applications of aptamers are highlighted in red.

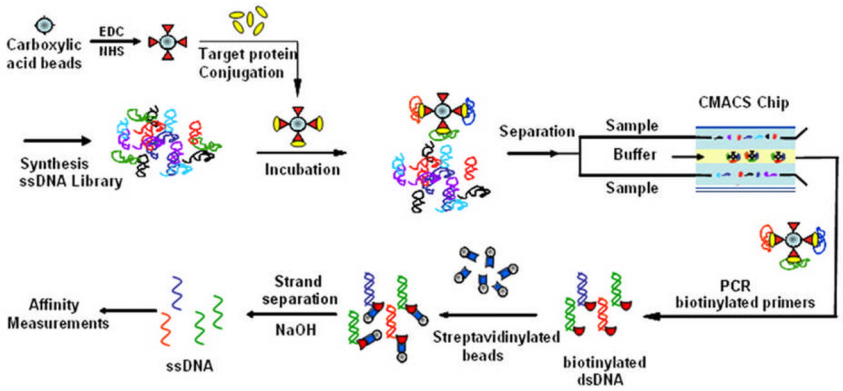

Figure 2.

Flowchart for the M-SELEX process.[20] A target protein is first conjugated to magnetic beads and then incubated with ssDNA library. Aptamers that bind to the target protein are then separated using the CMACS (continuous-flow magnetic activated chip-based separation) device. Finally, the aptamers bound on the target-coated beads are amplified via PCR.

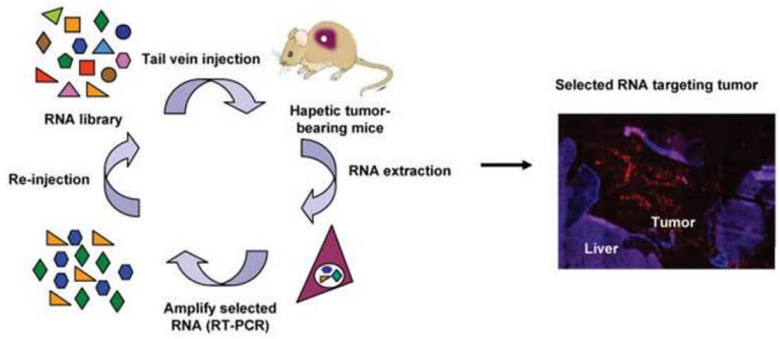

Figure 3.

In vivo selection of aptamers.[23] Tumor-bearing mice are intravenously injected with a random library of RNA sequences. After harvesting the tumors and extracting and amplifying the bound RNA molecules, the re-injection procedure is repeated several more times, resulting in increasing specificity of the bound aptamers. The selected RNA aptamers can finally be used for *in vivo* tumor imaging.

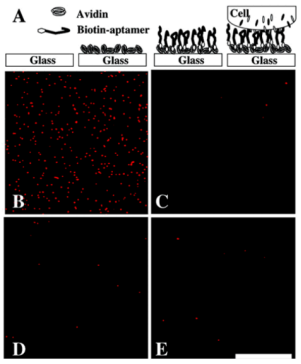

Figure 4.

Aptamer-based capture and enrichment.[43] Immobilized sgc8 aptamer was used to capture its target cells. A. Schematic representation of the aptamer immobilization and target capture. B. Specific capture of the target cells using the sgc8 aptamer. C. Representative capture of the control cells using the sgc8 aptamer. D. Capture of the target cells using immobilized random DNA sequence. E. Capture of the control cells using immobilized random DNA sequence.

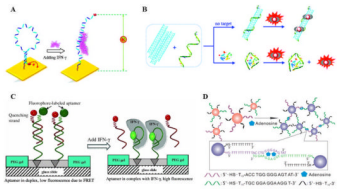

Figure 5.

Application of aptamers as biosensors. A. Schematic representation of an eletrochemical biosensor.[72] An aptamer hairpin is self-assembled on gold electrodes with a redox label near the electrode surface. Addition of the target (IFN-γ in this case) causes conformational change in the hairpin and shift of the redox label away from the electrode, resulting in lower electron-transfer efficiency. B. Schematic representation of luminescent biosensor.[86] In the absence of target, the SWNTs are in complex with the present aptamers, resulting in the quenching of the luminescence signal from the chelated Eu3+. After the addition of the target (LYS in this case), which binds with the aptamers, the SWNTs aggregate and can be easily removed, resulting in the strong unquenched luminescence signal from the added Eu^{3+}. C. Aptamer biosensor based on fluorescence quenching.[106] Fluorophore-labeled aptamer and quencher-labeled complementary strand beacons are immobilized in a well. In the pre-detection form, fluorescence signal on the aptamer is quenched due to the FRET effect of the duplex. The addition of the target disrupts the DNA binding and FRET, resulting in strong fluorescence. D. Schematic representation of a colorimetric biosensor.[122] Gold nanoparticles are functionalized with aptamer molecules. Addition of the target results in nanoparticles linking together and aggregating, thus causing the change in color.

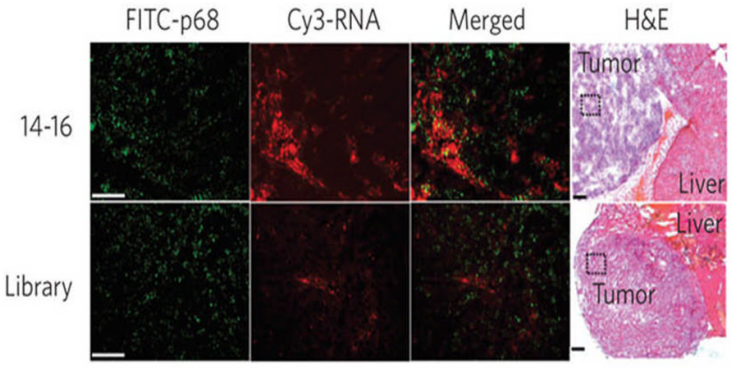

Figure 6.

Aptamer-based cancer tissue imaging.[23] Fluorescence microscopy-based detection of the fluorescence-labeled aptamer (red) co-localizing with its target, p68 (green), expressed in liver tumors (upper panel). Random RNA library was injected as a control (lower panel).

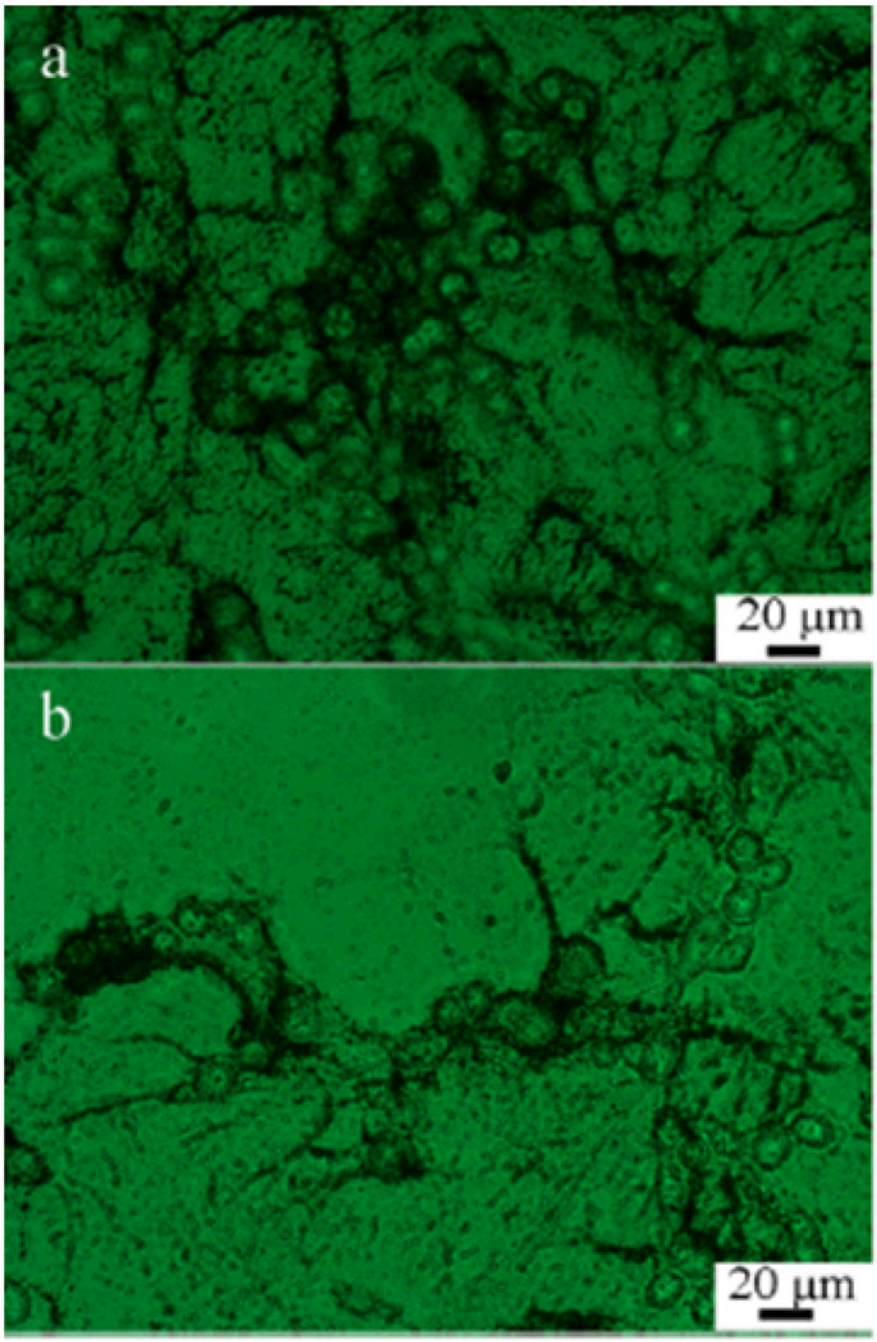

Figure 7.
Aptamer-based cell manupilations.[141] Aptamer-based magnetic nanosurgeons were used to pull the glioma cells out of the selected area by applying rotational magnetic field. A. The starting point of the target cells. B. The selected area after 10 min nanosurgery was conducted to clear the glioma cells.

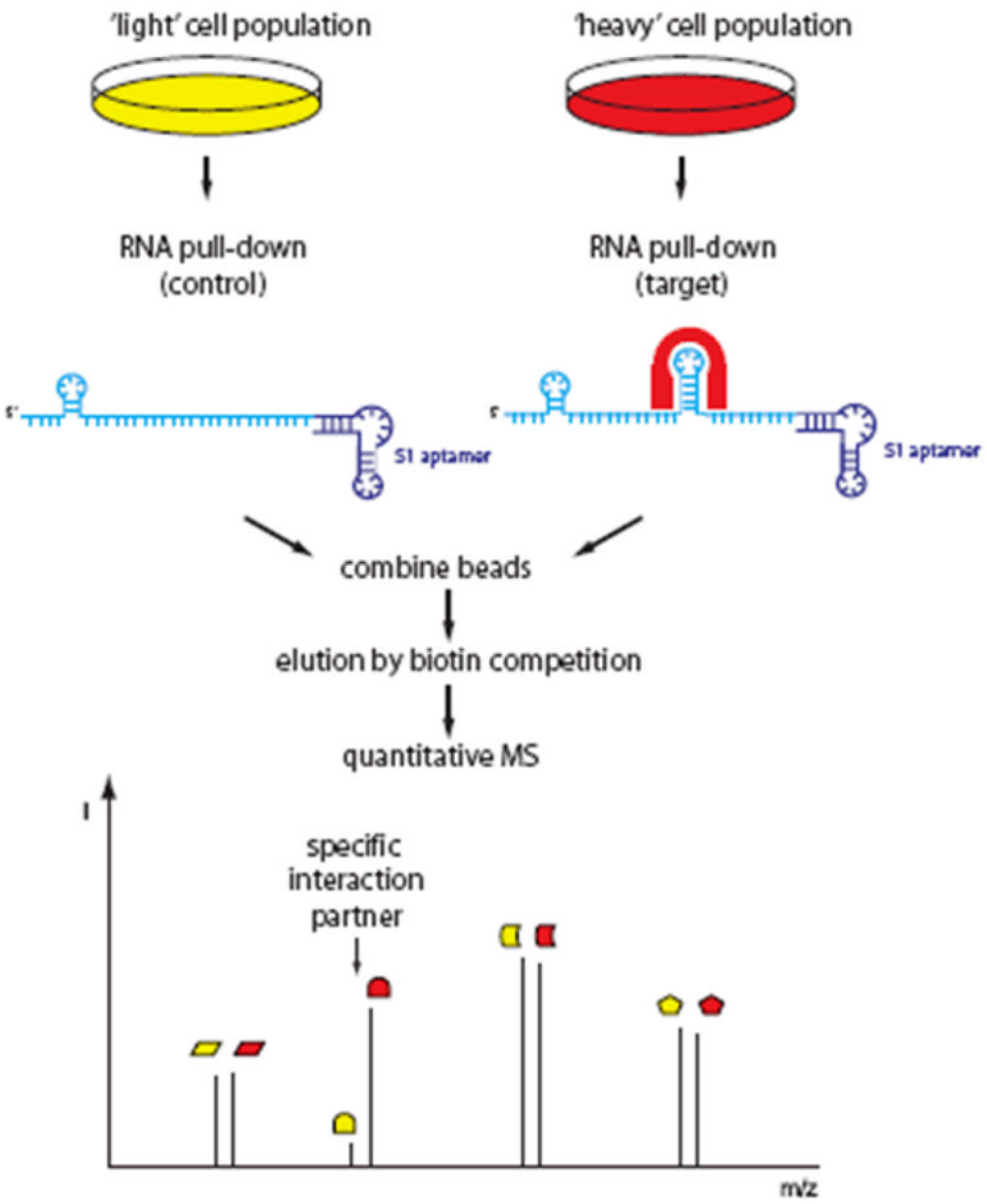

Figure 8.

Aptamers as a discovery tool for RNA-protein interactions.[167] RNA molecules (control and target) were immobilized on the beads by an aptameric tag, which were then incubated with either “light” or “heavy” SILAC lysates and combined. After RNA-protein complex elution, digestion and MS analysis, the specific target RNA interacting partners can be identified and quantitatively distinguished from the control sample.