

## Aptamer Modules as Sensors and Detectors

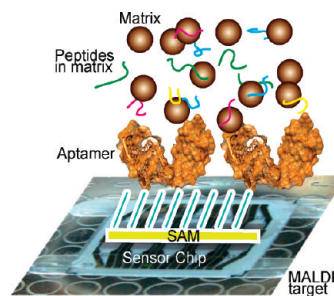
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### CONSPECTUS

Aptamers comprise a range of molecular recognition scaffolds that can be engineered to bind to a legion of different proteins and other targets with excellent specificity and affinity. Because these non-natural oligonucleotides are accessible entirely synthetically, aptamers can be equipped with all sorts of reporter groups and can be coupled to many different carriers, surfaces, nanoparticles, or other biomolecules. They can be used in a highly modular fashion and often recognize their targets by a mechanism in which the aptamer undergoes considerable structural rearrangement, which can be exploited for transducing a binding event into a signal. As a consequence, aptamers have been adapted to a huge variety of “read-out configurations” and are increasingly used as capture agents in many different bioanalytical methods. But despite considerable success with these applications, many remaining challenges must still be overcome for the more widespread incorporation of aptasensors in clinical and environmental biosensing and diagnostics to take place. Some particularly noteworthy progress on this front is currently being made with aptasensor configurations that can be used for the multiplexed sensing of many analytes in parallel.



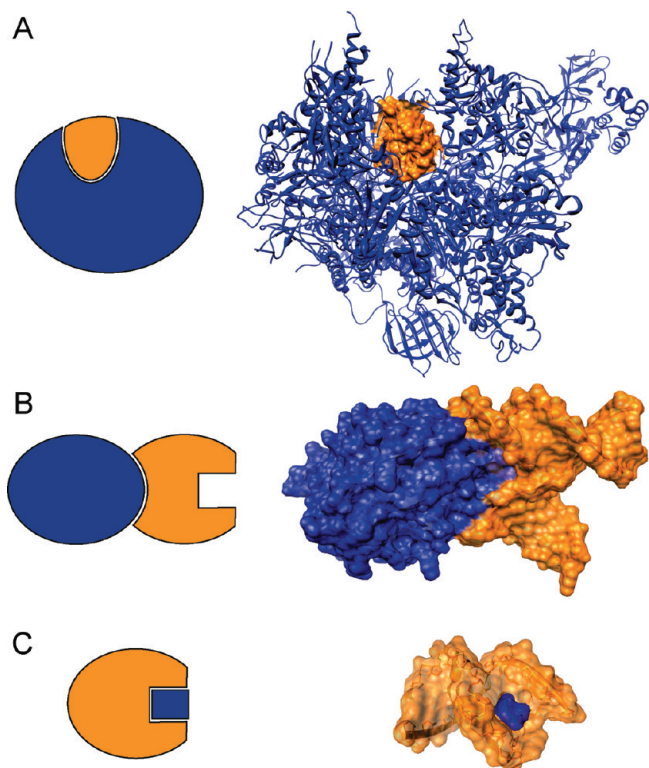
In this Account, we describe some of the concepts involved in transducing the binding of a ligand into a signal through various physico-chemical interactions. Research in this area usually involves the combination of the molecular biology of proteins and nucleic acids with biotechnology, synthetic chemistry, physical chemistry, and surface physics. We begin with a brief introduction of the properties and characteristics that qualify aptamers as capture agents for many different analytes and their suitability as highly versatile biosensor components. We then address approaches that apply to surface acoustic wave configurations, drawing largely from our own contributions to aptasensor development, before moving on to describe previous and recent progress in multiplexed aptasensors.

Obtaining proteome-wide profiles in cells, organs, organisms, or full populations requires the ability to accurately measure many different analytes in small sample volumes over a broad dynamic range. Multiplexed sensing is an invaluable tool in this endeavor. We discuss what we consider the biggest obstacles to the broader clinical use of aptasensor-based diagnostics and our perspective on how they can be surmounted. Finally, we explore the tremendous potential of aptamer-based sensors that can specifically discriminate between diseased and healthy cells. Progress in these areas will greatly expand the range of aptasensor applications, leading to enhanced diagnosis of diseases in clinical practice and, ultimately, improved patient care.

### Introduction

Single-stranded (ss) nucleic acids can assume a wide variety of folding topologies that account for their enormous structural and functional diversity reflecting their participation in a myriad of biological activities. The intricate architectural scaffolds adopted by RNA, ssDNA, or chemically modified nucleic acids provide specific binding sites for molecular recognition by an array of ligands. This is not only true for complexes of naturally evolved nucleic acids and their bound target molecules, but also for a class of

non-natural nucleic acid molecules called aptamers. Aptamers are synthetic oligonucleotides, isolated in vitro from libraries of  $10^{15}$ – $10^{16}$  different sequences by in vitro selection (or SELEX).<sup>1</sup> They can bind proteins and other molecules with high affinity and specificity, including toxic targets. Similar to interactions between antibodies and antigens, their binding characteristics rest upon the conformational plasticity and three-dimensional folding of the nucleic acid sequence that results in a 3D-structure that interacts with the structure of the “aptatope”, the equivalent



**FIGURE 1.** Aptamers can function as either ligands or as receptors. (A) Crystal structure (PDB-ID 2B63) of the 35 nt RNA aptamer FC<sup>2</sup> (orange) bound by yeast RNA polymerase II (blue).<sup>3</sup> (B) Binding mode in the approximately equally sized complex of von Willebrand factor domain A1 (blue) and the DNA aptamer ARC1 172 (orange)<sup>4</sup> (PDB-ID 3HXQ). (C) Mode of recognition between the 33 nucleotide long arginine-binding RNA aptamer (orange)<sup>5</sup> and L-arginine (blue) (PDB-ID 1KOC).<sup>6</sup>

of an “epitope”. Many aptamers recognize their binding partners with nanomolar affinities and below, and they often impair the biological function of their target molecules by antagonistic mechanisms.

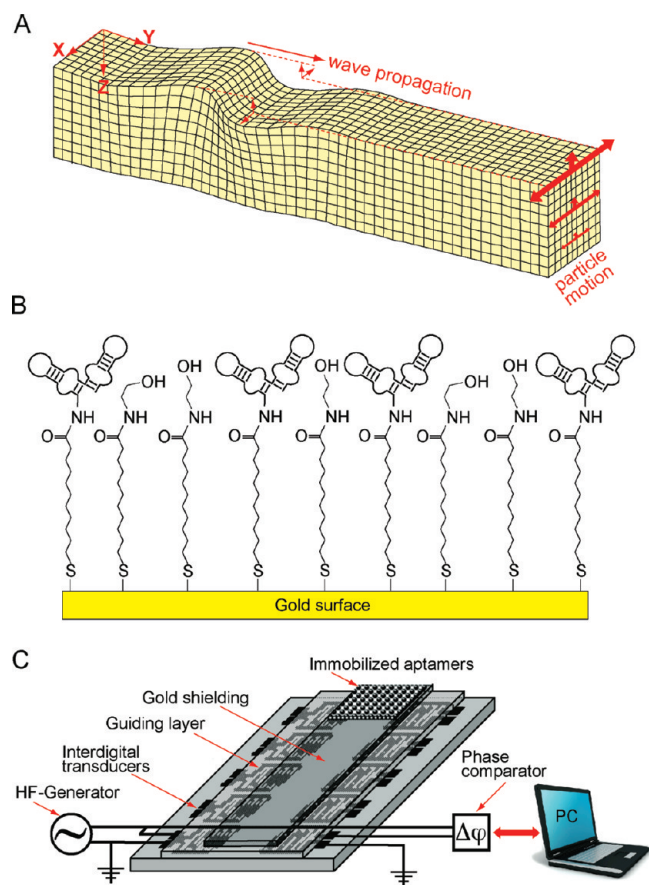
Structural studies have revealed the well-organized structural framework adopted by folded aptamers and the details of the molecular interactions that constitute specific ligand binding sites. Most aptamer sequences are 35–100 nucleotides (nt) in length. In a folded state, this length confers a size that qualifies aptamers to act either as ligands for larger receptors or as receptors for small molecules (Figure 1). Aptameric ligand binding sites are highly modular and can be combined with other functional nucleic acids or non-nucleic acid chemical entities or can be linked to a huge variety of carriers and surfaces. Because they are generated entirely *in vitro*, it is possible to tailor their physicochemical properties to the requirements of their application. Furthermore, it is straightforward to equip aptamers with additional chemical functions that outfit them for a variety of applications in biomedicine, nanotechnology, and cell biology.

These qualities suit aptamers as highly versatile capture agents for the development of a variety of biosensors and sensor arrays and render them advantageous compared with biosensors that apply natural receptors like antibodies or enzymes. Many researchers have made impressive progress in this area and as a consequence the field of aptamer biosensors is currently growing at an enormous pace. In this Account, we discuss some selected recent advances in generating biosensors, multiplexing arrangements, and bioassay methods that rely on aptamer modules and also highlight our own contributions to this expeditiously advancing topic.

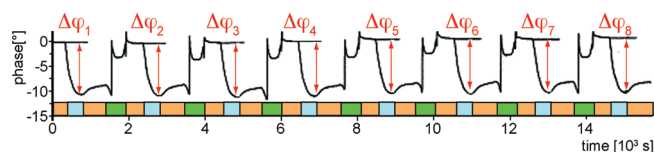
## Surface Acoustic Wave Aptasensors and Others

Since the first report that employed immobilized aptamers in a biosensor to detect a free, unlabeled non-nucleic acid target molecule,<sup>7</sup> many further examples of aptamer-based sensors have been described.<sup>8</sup> While the vast majority of these systems are based on fluorescently labeled aptamers with optical detection, there are many devices and assays, in which ligand-binding by aptamers is transduced into a signal by virtue of other physical principles such as surface acoustic waves,<sup>9</sup> electrochemistry,<sup>10–12</sup> nanoparticles,<sup>13–18</sup> potentiometry,<sup>19</sup> and others.<sup>8,20</sup> One of the earlier examples described a biosensor that applied the anti-IgE DNA aptamer covalently attached to a gold-coated quartz crystal microbalance (QCM).<sup>21</sup> This physical transducer resulted in a detection limit of 3.3 ng cm<sup>−2</sup> of immunoglobulin E.

We took an elastodynamic approach by coupling aptamers to the surface of an acoustic Love-wave biosensor in an arrayed format.<sup>22</sup> Love waves are horizontally polarized shear waves, named after Augustus E. H. Love, a British mathematician who first described these waves in a mathematical model. Love waves are confined to the surface of a substrate by a waveguide with an intrinsic shear acoustic speed lower than that of the substrate.<sup>23</sup> Love-wave sensors measure the propagation of acoustic waves through the solid medium of the sensor (Figure 2A) and their disturbance by changes in the adjacent environment that contains the analyte of interest.<sup>24</sup> We prepared a self-assembling monolayer (SAM) on a shielding gold layer using 11-mercaptopundecanoic acid. After activation of the carboxylic acid residues with *N*-hydroxysuccinimide, anti-thrombin DNA and anti-Rev RNA aptamers<sup>25,26</sup> were coupled to the sensor surface (Figure 2B). We constructed a chip consisting of an array of five sensor elements. A high-frequency generator supplied



**FIGURE 2.** Aptamer-based Love-wave biosensor. (A) Love wave propagating in the Y direction through a solid medium with amplitudes in X/Z directions. Particle motion is maximal at the surface and becomes smaller in the interior. (B) Aptamers sensing HIV-1 Rev or  $\alpha$ -thrombin coupled to the Love-wave sensor surface. (C) Arrangement of five sensor elements for detecting an analyte with a Love-wave sensor. HF, high frequency;  $\Delta\phi$ , relative phase change of the sensor signal.



**FIGURE 3.** Binding of  $\alpha$ -thrombin to its DNA aptamer and regeneration of the Love-wave biosensor. Green denotes removal of bound  $\alpha$ -thrombin and sensor regeneration by pH increase. Orange indicates buffer washes. Blue indicates thrombin injection.

voltage to the interdigital transducers, exciting surface acoustic waves (SAW) according to the frequency. Mass loading to the sensor surface caused a phase shift between input and output signal, determined by the phase comparator (Figure 2C).

In Figure 3, the relative phase change  $\Delta\phi$  of a sensor element coupled with the  $\alpha$ -thrombin aptamer is shown for multiple steps of binding and regeneration of the sensor.

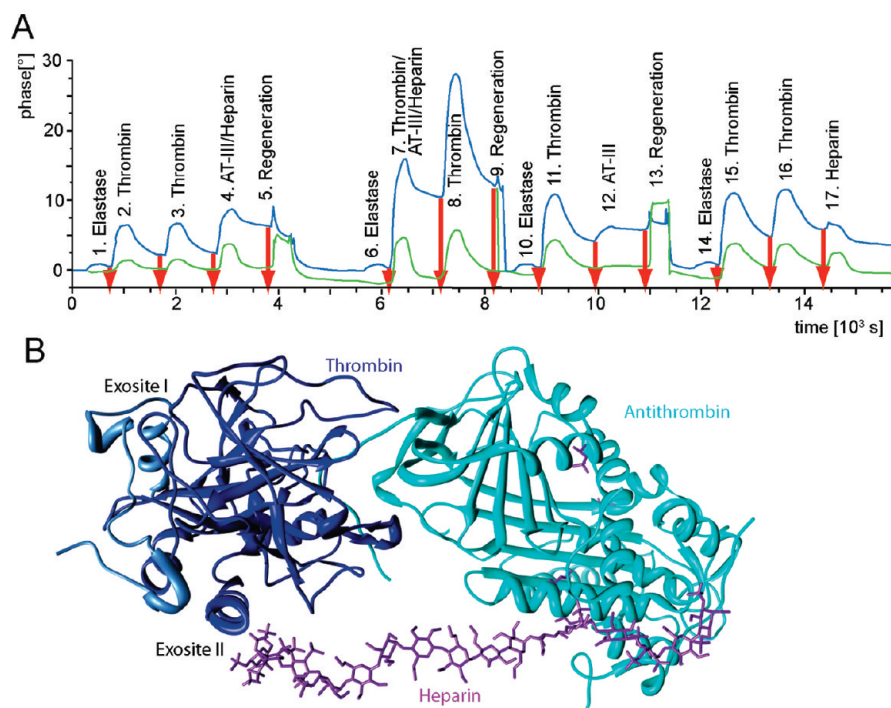
Sensor sensitivity is maintained during repeated cycles of binding and regeneration. This first-generation aptamer-based SAW sensor had a detection limit of  $\sim 75 \text{ pg cm}^{-2}$  for both analytes thrombin and HIV-1 Rev peptide, which was about 2 orders of magnitude better than the SAW-based devices known before.<sup>21</sup>

We then took the prototype Love-wave aptasensor a step forward by applying it to detect individual point mutations in cancer-related DNA gene fragments,<sup>27</sup> and protein–protein interactions to monitor the formation and disassembly of protein complexes involved in blood coagulation.<sup>28</sup> Blood coagulation provides an ideal model for this purpose because of its clinical relevance and the fact that many different protein–protein interactions are involved and because the related processes occur extracellularly under flow. We sought to detect the binding of thrombin to the aptamer immobilized on the sensor surface and the subsequent binding of antithrombin (AT) to thrombin's active site. This was feasible because the aptamer binds exosite II, leaving the active site unoccupied and free for AT binding. The formation of a ternary complex between the mucopolysaccharide heparin, AT, and  $\alpha$ -thrombin was monitored by further signal attenuation. However, we observed differences in the signal intensities that depended on the sequence of addition of either thrombin, or the AT/heparin complex (Figure 4A), suggesting that the binding of the DNA aptamer to exosite II competes with heparin for thrombin binding and thus destabilizes the ternary heparin/AT/thrombin complex (Figure 4B).

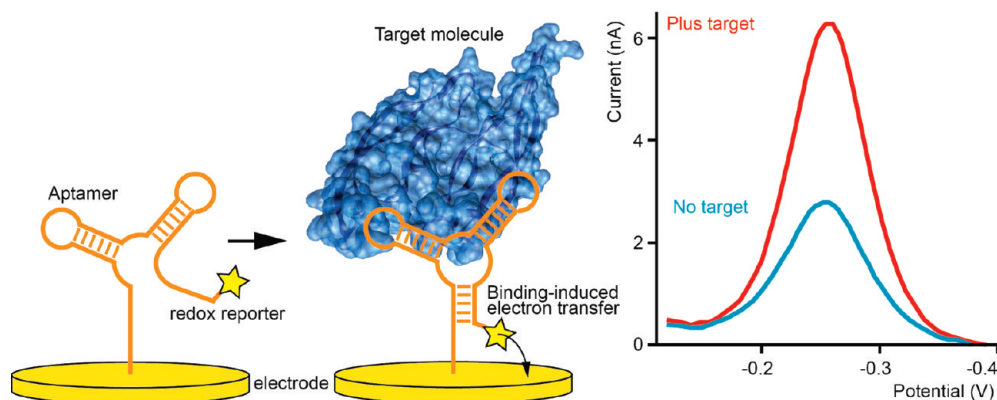
Others have also pursued QCM-based analyte quantification by aptamers. For example, Mascini and colleagues have compared an engineered aptasensor QCM that detects the HIV-1 Tat protein with an analogous surface plasmon resonance (SPR) sensor, and found similar performance of both detection principles.<sup>29</sup> Additional aptamer-regulated physical transducers include micromechanical detection with cantilevers,<sup>30</sup> and many examples based on potentiometric or electrochemical detection,<sup>10–12</sup> or combinations of self-assembled multilayers, nanoparticles, and electrochemistry.<sup>31</sup>

An important challenge in biosensor-development is the ability to detect targets in biological fluids.<sup>8,10</sup> Approaches combining aptasensors with electrochemical detection have proven quite promising. For example, the Plaxco group has developed so-called E-AB sensors for targets such as cocaine,<sup>32</sup> thrombin,<sup>33</sup> or platelet-derived growth factor (PDGF) in biological fluids.<sup>34</sup> In the shown example (Figure 5), the detection signal results from the





**FIGURE 4.** Monitoring complex formation between thrombin, AT, and heparin by the Love-wave sensor. (A) Sequential binding of proteins to the aptamer. Displayed is the difference between sensor (blue) and reference signal (green). From the equilibrium phase shifts (red arrows), concentration and amount of each bound analyte were calculated. (B) Structure of the ternary complex between thrombin, heparin, and AT (PDB-ID 1TB6).



**FIGURE 5.** Electrochemical aptamer-based (E-AB) sensor that detects PDGF at picomolar concentrations directly in blood serum.<sup>34</sup>

redox reporter methylene blue (MB) positioned at the aptamer's 3'-end. Upon target binding, the electrode-attached aptamer undergoes conformational changes that place the MB label more closely to the electrode surface, resulting in more efficient electron transfer.<sup>34</sup>

In collaboration with the Erdem group, we have recently combined aptamer-based electrochemical sensing with magnetic particles for detection of protein analytes.<sup>35</sup> Magnetic particles provide a convenient, automatable means for accumulating and separating specific analytes from samples, which can then be combined with various detection

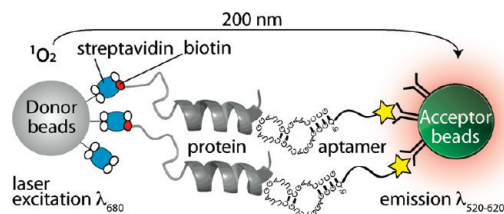
mechanisms. Biotinylated DNA aptamers for lysozyme and  $\alpha$ -thrombin were immobilized on streptavidin-coated magnetic particles and incubated with solutions containing their respective ligands. Quantification was achieved by measuring the oxidation signals of guanine residues in the presence and absence of the analytes. This device comprises cheap starting materials and has potential for increasing sensitivity by accumulating analytes on the magnetic particles.

The coupling of molecular recognition by an aptamer to signal generation enables the production of versatile reporters that can be applied as molecular probes for various

purposes, including high-throughput screening (HTS) for small organic compounds that displace the aptamer from its bound target protein. We have adapted various aptasensor systems for HTS,<sup>36–39</sup> as reviewed in depth previously.<sup>40,41</sup> A new aptasensor that we have recently reported operates on the basis of luminescent  $^1\text{O}_2$  channeling. This read-out configuration was shown to work with both RNA and DNA aptamers that bind the protein cytohesin and was also demonstrated to be HTS-compatible (Figure 6).<sup>42</sup>

## Multiplexed Aptasensors

While some of the sensor systems described above were shown to work in arrayed formats,<sup>22</sup> progress was made in adapting aptamers to sensor arrays capable of detecting different analytes in parallel. In an early example, Ellington

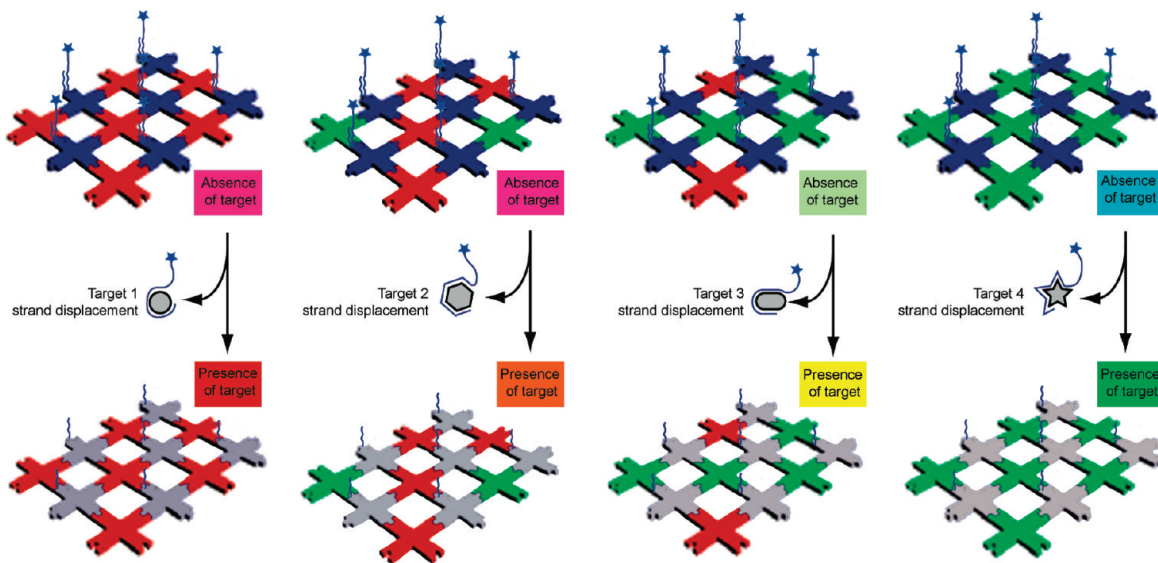


**FIGURE 6.** Streptavidin-coated donor beads bind biotinylated protein; acceptor beads are functionalized with anti-fluorescein isothiocyanate-IgG and interact with the fluorescein-label (star) on the aptamer.<sup>42</sup> Complex formation of protein and aptamer brings donor and acceptor beads in proximity. After laser excitation, singlet oxygen ( $^1\text{O}_2$ ) diffuses to the acceptor beads, which can be detected by light emission.

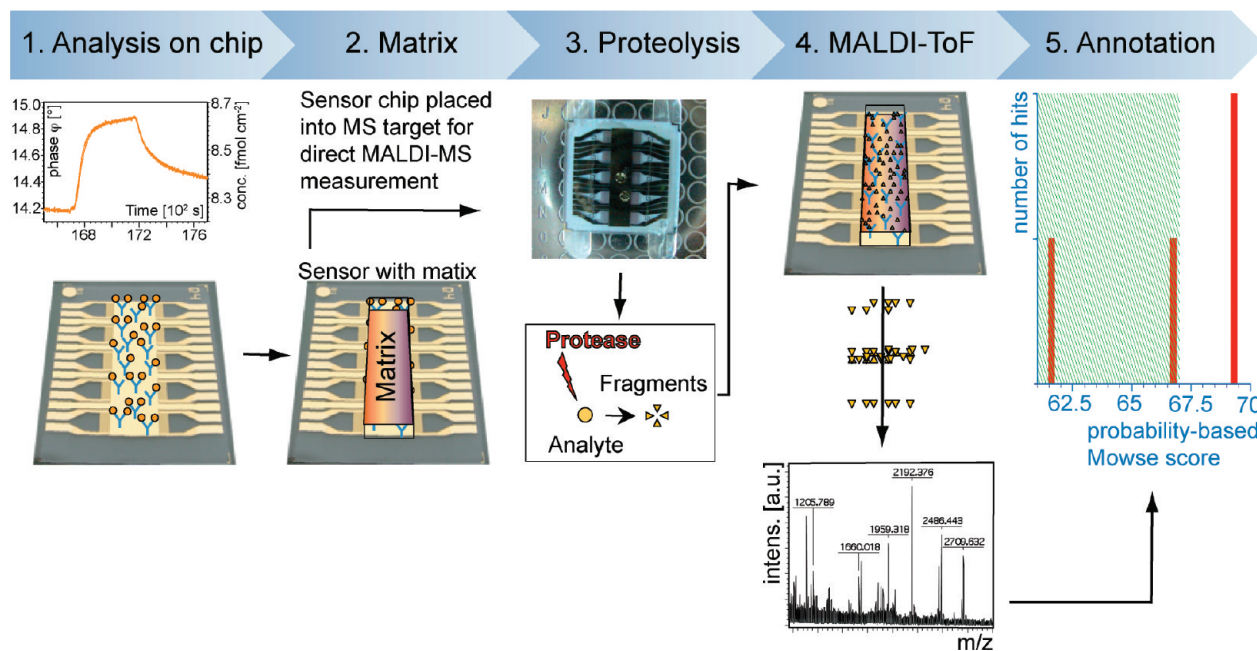
and colleagues conjugated aptamers for lysozyme and the biothreat agent ricin to a sensor array that was based on streptavidine agarose beads placed in micromachined wells on a chip and showed that the cognate analytes could be reliably detected.<sup>43</sup> Other multiplex aptamer assays have been described, including assays that apply 5-bromodeoxyuridine-modified aptamers that allow their photo-cross-linking to the bound targets.<sup>44</sup>

A clever approach by Yan and co-workers combined arrays of self-assembled DNA tiles with hybridization of aptamer probes.<sup>45</sup> Different types of tiles formed 2D nanolattices comprised of a detection tile and different encoding tiles (Figure 7). Each encoding tile was labeled with either a blue or green dye. The detection tile contained oligonucleotides that dangled out of the plane of the formed lattice and could hybridize to an aptamer probe that was labeled with the blue dye. Thus, combinations of encoding tiles with the detection tile into different nanolattices resulted in a broad array of colors according to the RGB color model.

In the presence of their respective binding partners, the blue labeled aptamers are detached from their hybridization site, resulting in a color change that can be detected in multiplexed format. The blue labeled probes used in a pilot array were the complementary sequences of two viral DNA sequences (probes 1, 2), and the DNA aptamer sequences that bind to  $\alpha$ -thrombin (probe 3) and ATP (probe 4). The presence of the respective displacing target molecule was read-out by fluorescence microscopy.



**FIGURE 7.** Design and detection mechanism of self-assembled combinatorially encoded DNA grids for multiplexed detection of targets by different aptamers. Encoding tiles are in green and red.<sup>45</sup> Detection tile is blue when a blue-labeled aptamer is bound to dangled oligonucleotides (blue). The respective cognate targets (gray) bind the aptamer and displace it with its blue label (star) from the detection tile. The removal of the color blue results in a color change according to the RGB color model.



**FIGURE 8.** Love-wave aptasensor coupled to MS analysis for protein identification.<sup>46</sup> Binding kinetics, analyte quantification, and MS sample preparation is performed on-chip. Analytes are selectively enriched on the sensor; nonbinding proteins are removed (1). The chip surface is covered by a matrix that embeds analytes, absorbs the wavelength of the MALDI laser, and promotes analyte ionization (2). Proteolysis occurs on-chip after transferring it into the mass spectrometer (3). Peptide fragments are analyzed by MALDI-ToF (4) and fragments are annotated (5).

We have recently moved our Love-wave aptasensor system toward multiplexing by coupling it with MALDI-ToF mass-spectrometric detection.<sup>46</sup> This assay allowed monitoring of binding events by the sensor, label-free identification of analyte proteins by MS, and quantification of their amounts from unpurified samples. In a pilot setting aimed at testing the system for clinical applications, we analyzed proteins that originated from patients treated by standard clinical anti-HIV-1 drugs that targeted HIV-1 reverse transcriptase (RT) and the envelope protein gp120, respectively.

We first isolated a set of new RNA and 2'-NH<sub>2</sub>-pyrimidine–RNA aptamers against the third variable loop of gp120 and the RT mutant RT948-2 isolated from a patient by employing an automated robotic in vitro selection workstation. Biotinylated aptamers<sup>47</sup> were coupled to the sensor surface, and values for association- and dissociation-rate constants and the  $K_D$  were quantified directly from the unprocessed signals and found to match the same values measured independently by nitrocellulose filter binding. Reliable identification of the RT948-2 mutant even in mixtures occurred directly on-chip after protease treatment of aptamer-bound proteins, identification of the resulting peptide fragments by MALDI-ToF-MS, and their annotation (Figure 8). This proof-of-principle study suggested that the approach allows for a much broader determination and comparison of mutant viral proteins from different patients in parallel, which would be extremely useful for clinical

research and diagnostics. However, although most aptamer-based assay formats hold great promise for potential clinical applications and certainly have been developed with applications in clinical diagnostics in mind, none of them is currently used in clinics. We discuss below what we think might be possible reasons for that.

## Aptamers for Clinical Diagnostics: Challenges and Perspectives

Indeed, despite the progress that has been made in developing a wide variety of aptasensors, despite the apparent superiority of aptamers over other detector molecules, and even though aptamers are supposedly so easy to obtain,<sup>48</sup> the lack of aptasensors that actually qualify for clinical use is astonishing on one hand but also disappointing on the other. So what are the biggest challenges that need to be overcome on the way to a broader clinical use of aptasensor-based diagnostics and how can this potentially be achieved?

When we analyze this issue, it becomes immediately apparent that there are only a rather limited variation of aptamer–target pairs that have so far been used by researchers who seek to obtain proof-of-principle for their respective new aptasensor diagnostic assays. For example, by far the most frequently applied aptamer to which most assay developers turn is the anti-thrombin aptamer HD1,



**TABLE 1.** Overview of Thrombin-Sensing Assay Systems and Reported Detection Limits<sup>a</sup>

| assay name/<br>format | detection method              | detection<br>limit (pM) | ref |
|-----------------------|-------------------------------|-------------------------|-----|
| nanoparticles         | mass spectrometry             | 10                      | 54  |
| biobarcode            | electrochemical signal        | 6.2                     | 55  |
| Au-electrode          | electrochemical signal        | 0.055                   | 56  |
| quartz sensor         | SAW                           | 400                     | 57  |
| graphite electrode    | voltametry                    | 55500                   | 35  |
| aptaPCR               | qPCR                          | 0.45                    | 58  |
| molecular beacon      | enhanced<br>chemiluminescence | 0.05                    | 59  |
| OECA                  | fluorescence                  | 0.47                    | 60  |
| mag. nanoparticles    | fluorescence                  | 60                      | 61  |
| SERRS                 | Raman spectroscopy            | 100–1000                | 62  |

<sup>a</sup>Abbreviations: SAW, surface acoustic wave; SERRS, surface-enhanced resonance Raman scattering; OECA, oligonucleotide-based enzyme capture assay.

also known as TBA, and HD22.<sup>25,49</sup> This  $\alpha$ -thrombin-targeting DNA aptamer was selected in 1992 and thoroughly characterized in subsequent studies. Since then, it has been employed in >400 publications.<sup>25,50–53</sup> Over 250 of these discuss its use as a capturing agent in bioanalytical assays, some of which are summarized in Table 1. This number by far exceeds what has been published with other aptamer–target pairs for diagnostic purposes.<sup>12</sup> The second, third, and fourth most frequently applied aptamer–target pairs sense cocaine, nucleotides (mainly ATP), and platelet-derived growth factor. Again, these are all well-characterized and well-behaved aptamer–ligand pairs that can be accessed by anybody as easily as a PCR primer or a fine chemical. But there is nobody to blame for this lack of variation; certainly not those researchers who first and foremost seek to investigate the basic principles of a new assay for which they can revert to well-established aptamer modules rather than getting involved with the anything but marginal efforts of isolating and characterizing new aptamers for all sorts of clinically relevant targets. The more so as, similar to monoclonal antibodies, these efforts will hardly be rewarded by high-impact publications, since the selection of new aptamers is viewed by most colleagues (and reviewers) as a routine process, which it is actually not if done properly, notwithstanding statements suggesting the contrary.

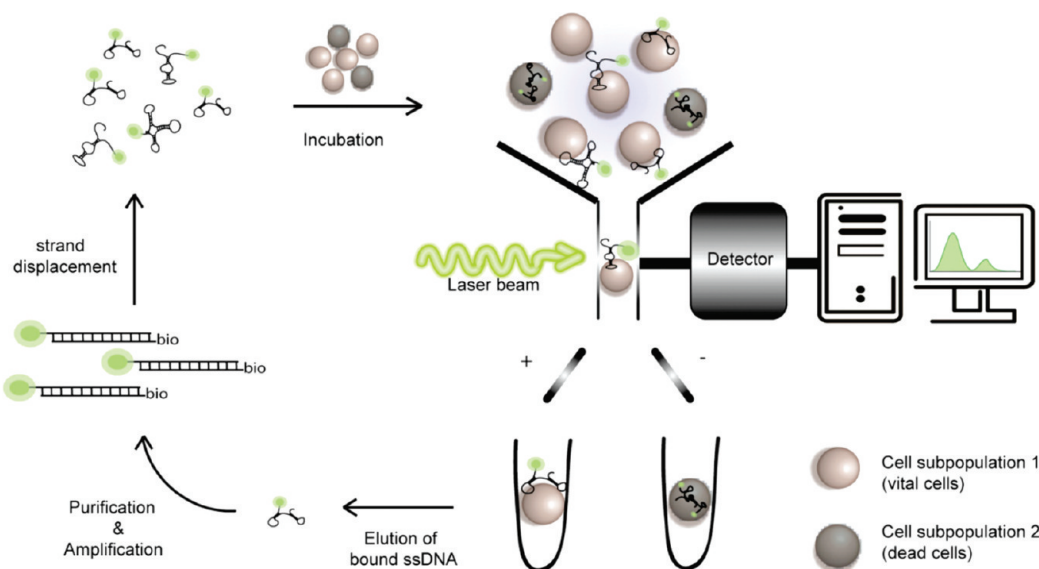
The flipside of using the same aptamer/target pairs over and over again is that it potentially generates strong bias in aptasensor development. In case of the thrombin aptamer, this scenario has been termed recently as the “thrombin problem” in an excellent essay by Geoffrey Baird.<sup>48</sup> Indeed, while not being intricate at first sight, most assays that sense thrombin share the same limitation: none of them has been shown to measure endogenous levels of thrombin in “real” samples, such as whole blood or plasma derived from

patients. No reported assay goes beyond measuring *ex vivo* constituted samples, often with exogenously added thrombin. Scrutinizing this issue is not trivial, but some reasons might appeal. As said before, aptamers recognize defined three-dimensional shapes, in contrast to most epitope-recognizing antibodies. Therefore, aptamer-based sensor systems crucially depend on preanalytical handling procedures that guarantee the preservation of protein structure during the entire diagnostic process, from sampling through work up toward detection. An important step into this direction has been described recently by combining protease inhibitors and bivalent aptamers in a aptasensor format. This combination enabled the quantification of thrombin *in vivo*, exemplified by monitoring thrombin concentration during hip replacement surgery.<sup>60</sup> Aptasensor assays should also be compatible with current equipment of diagnostics units in clinics to facilitate their broader acceptance. An issue here may be the alleged fragility of aptamers, which may be true for RNAs, but most aptamers for clinical use result from nuclease-resistant chemically modified libraries. Finally, because it is likely the paucity of good aptamers for clinically relevant targets that explains the current lack of aptamer-based diagnostic setups used in daily clinical practice, aptasensors are required that measure marker proteins for which alternatives are not available.

Gold and colleagues recently took a very promising move toward this end. They described a high-content aptasensor-based proteomics configuration that allowed the simultaneous measurement and profiling of 813 different proteins found in small amounts of blood samples from cancer patients.<sup>63,64</sup> Aptamers with particularly slow off rates were selected from libraries of DNA sequences containing chemically modified nucleotides (others and we have developed DNA-selection technologies allowing for incorporating modified nucleotides and reliable copying of the respective DNA templates<sup>65</sup>). The feasibility of this multiplexed aptasensor in clinical application was demonstrated by identifying biomarkers for various diseases, including chronic kidney disease and lung cancer. Without doubt, this assay is a significant step forward in multiplex aptamer-based protein detection; a caveat may be the lack of sequence information on any of the 813 aptamers, potentially hampering compatibility with daily clinical routine, reproducibility of the reported assay, or its further development by others.

## Aptamers That Recognize Cell Types

Apart from measuring plasma proteins with aptasensors, where else will aptamer-based sensors likely have high



**FIGURE 9.** Selection scheme by FACS.<sup>68,69</sup> Cell mixtures consisting of vital and dead cells are incubated with a ssDNA library. Dead and vital cells are separated in a cell sorter according to their ability to activate calcein. This procedure selects cell surface-binding aptamers associated with the vital cell phenotype.

impact in the future? Recently several groups have started endeavors to identify aptamers that interact with a malignant cell population<sup>66</sup> or with malignant tissues<sup>67</sup> without knowledge of the recognized target *a priori*. This approach provides access to smart aptamers that can distinguish between pathogenic and healthy conditions in cells and tissues. Besides their potential for diagnostic purposes, these molecules will also be useful as delivery vehicles. Being entirely accessible by organic synthesis, they can be generated bearing site-directed modifications like dyes, affinity tags, or other functionalities. These modifications allow using aptamers equipped with fluorescent tags for flow cytometry as an alternative to the currently more common but more expensive antibodies. The challenge thus is to identify sophisticated and selective aptamers that recognize defined cell subpopulations.

Toward this goal, we have developed a novel selection scheme that uses fluorescent-activated cell sorting (FACS) to separate bound from unbound nucleic acids during the SELEX process (Figure 9).<sup>68,69</sup> This approach efficiently overcomes the so-called “dead cell-problem”. Dead cells or cells with reduced membrane integrity take up nucleic acids in an unspecific manner. This leads to an inferior progress of enrichment when centrifugation is used as separation method, because this cannot discriminate live from dead cells. FACS–SELEX overcomes the problems associated with the centrifugation method and thus allows enrichment of cell-targeting aptamers quite effectively. We have successfully

applied FACS–SELEX and identified Burkitt's lymphoma B-cell specific aptamers. Aptamer C10 selectively recognized B-cell lymphoma with high affinity whereas B-cells from healthy volunteers were not bound. These characteristics make C10 a promising candidate for flow cytometry-based diagnostics but also a potential vehicle for selectively delivering therapeutics to B-cell tumors.

## Conclusions and Outlook

There is now an armada of proof-of-principle studies showing that aptasensors are not far from becoming an indispensable tool for biomedical research and diagnostics. Aptamer modules can be adapted to a huge variety of read-out configurations and are in many respects equal or even superior to antibody-based diagnostic tools currently used in clinical and environmental diagnostics. The challenges that aptasensor developers will have to meet in the future are in identifying well-behaved and selective aptamers for many more clinically and environmentally relevant target molecules than are presently available. The combination of automated and highly sophisticated selection techniques with new chemically modified and SELEX-compatible library designs might turn out to be highly beneficial for improving the qualities of aptasensors and the acceleration of clinically more acceptable aptasensors. We also envisage a high future impact for aptasensors that are specifically adapted to targeting tissues and whole cells. This requires the development of new chemical and selection tools that improve



aptamer selections in whole organisms like mouse tumor models, xenografts, and individual organs or tissues. Likewise, improved selection strategies targeting whole cells and specific cell subpopulations will greatly expand the range of aptasensor applications. Because these lead to aptamers that recognize unknown targets, smart chemical or biophysical approaches that facilitate rational, rapid, and proteome-wide profiling for target identification are also required. Many researchers are now engaged in aptasensor development; we believe that what we have seen so far in this important area of research is only the tip of an enormous aptasensor iceberg.

*We thank all co-workers and collaborators who have contributed to our projects over the past 10 years. Their names appear throughout the references. The research described here was supported by the DFG, the Alexander von Humboldt Foundation, ESF, BMBF, Scottish Research Council, and SULSA.*

## BIOGRAPHICAL INFORMATION

**Michael Famulok** studied chemistry at Marburg University until 1986 and completed his Doctorate in 1989. He did postdoctoral work at MIT under Julius Rebek (1989–1990) and at Massachusetts General Hospital under Jack Szostak (1990–1992). His independent career began at LMU Munich in 1992. Since 1999 he has been Professor of Biochemistry and Chemical Biology at Bonn University. His research interests include aptamer and intramolecular technology, the chemical biology of guanine nucleotide exchange factors (GEFs), receptor tyrosine kinase signaling, and DNA nanoarchitectures.

**Günter Mayer** studied chemistry at the LMU Munich until 1998. After his doctorate at Bonn University, he spent 3 years in industry before starting his independent academic career in Bonn in 2004. In 2009, he accepted a Reader position of Translational Biology at Strathclyde University, Glasgow. Since 2010, he has been Professor of Chemical Biology & Chemical Genetics at Bonn University. His research interests include the chemical biology of aptamers, riboswitches, and the development of oligonucleotide-based diagnostic and therapeutic strategies.

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