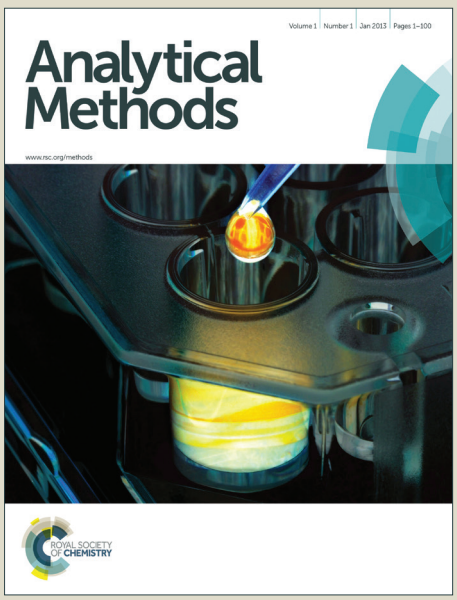
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Analytical Methods ARTICLE

Cite this: DOI: 10.1039/x0xx00000x

Received 00th
Accepted 00th

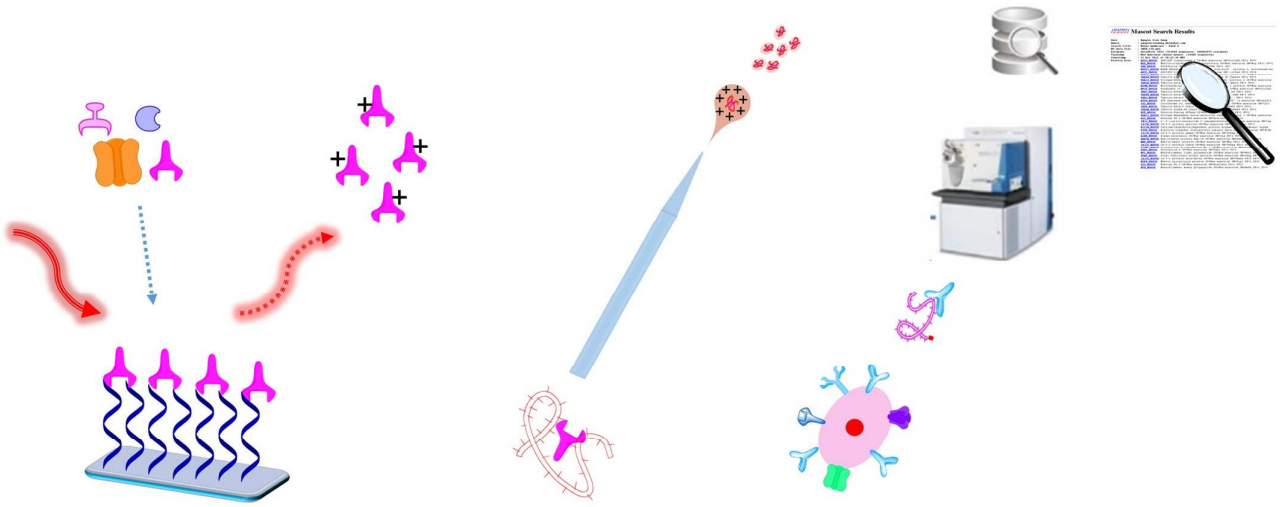
DOI: 10.1039/x0xx00000x

www.rsc.org/

Oligonucleotide aptamers: emerging affinity probes for bioanalytical mass spectrometry and biomarker discovery

Basri Gülbakan[a]

Selective isolation of biological important molecules and their functional characterization is one of the primary goals of bioanalytical chemistry. Several different affinity tools such as antibodies, affimers, nanobodies, DARPins have been explored to achieve these goals. In recent years, oligonucleotide based affinity tools called aptamers have become progressively attractive and the research in this area has seen an exponential increase. Aptamer probes have been explored in many different areas of bioanalytical chemistry such as electrical and optical biosensor development, targeted drug delivery, logic gates, DNA nanotechnology, and point of care diagnostics. However aptamers are still largely overlooked in mass spectrometry (MS) and biomarker discovery. After the completion of human genome project, the focus has shifted towards functional genomics and to understand the living systems by deciphering the functions of proteins and metabolites. Therefore identification and functional characterization of these molecules is of outmost importance. While identification of isolated biomolecules and analysis of simple biological mixtures using MS has become relatively simple, the power of MS gradually decreases as the complexity of the biological mixtures increases. Therefore development of selective and targeted approaches is at the forefront of mass spectrometry. Aptamers have great potential in affinity mass spectrometry to improve selectivity, specificity and throughput. Herein, bioanalytical mass spectrometry and biomarker discovery applications of aptamers will be reviewed.



Journal Name

RSCPublishing

ARTICLE

1 Introduction

In the past two decades, the potential of using oligonucleotides as molecular probes and recognition elements has greatly expanded as a result of the discovery of a new class of affinity molecules called "aptamers".[1, 2] The word aptamer is derived from the Latin words "*aptus*" meaning to fit and *"mer"* meaning the repeating unit. Aptamers are single-stranded oligonucleotides (DNA or RNA) which have the ability to bind to other molecules with high affinity and specificity. They are evolved from random oligonucleotide pools with an iterative process called Systematic Evolution of Ligands by EXponential enrichment (SELEX). Oligonucleotide aptamers adopt a unique, target-dependent three-dimensional structure for recognition. Secondary interactions such as van der Waals forces, electrostatic pairing, hydrogen bonding and π-π stacking collectively infer their affinity, selectivity and specificity. Since after the inception of SELEX, oligonucleotide aptamers have been generated for variety of targets, ranging from small molecules; such as metal ions, organic dyes and amino acids, antibiotics, and peptides, to large biomolecules including proteins, bacteria, virus infected and cancer cells.[3] Based on their unique target recognition capabilities, their selective binding and affinity, aptamers are functionally similar to antibodies. On the other hand they are structurally different from antibodies in that they don't have a predefined and conserved structural motif for recognition[4]. Aptamers do present several significant advantages over antibodies. In the first place, time and material needed for the generation of aptamers by the SELEX process is comparatively low. This attribute makes aptamer production less labor-intense and economically more favorable. Second, as opposed to antibodies; aptamers are chemically synthesized and there is no need for animals. This largely eliminates batch-to-batch variations which is regarded as a big disadvantage of antibodies. Third, chemical synthesis of aptamers renders the biochemical manipulation possible. They can be uniquely tailored with a wide range of chemistries without compromising their affinity and function. Therefore, aptamers can easily be conjugated with other molecules and can be immobilized onto various surfaces. Nuclease resistant bases can be incorporated into their structure by using commercially available phosphoramidites. Locked nucleic acids and 2'-O-methyl nucleotide analogues are such examples to enhance nuclease resistance when adopted for in vivo studies.[5] Other attributes, such as long shelf life and controllable or cyclical denaturation and renaturation, expanded the flexibility of aptamers in various experimental designs. Owing to all these listed advantages, aptamers became very useful in applications in variety of disciplines, including biotechnology, medicine, pharmacology, cell biology, microbiology and chemistry. [6-16] In the past 10 years, aptamers have become progressively more attractive and the number of papers published in the field have seen an exponential increase. In parallel, research in aptamer-based mass spectrometry and biomarker discovery is also gaining great momentum. Herein, bioanalytical mass spectrometry and biomarker discovery applications of aptamers will be reviewed.

2 Bioanalytical mass spectrometry and aptamers

Development of the "soft" desorption-ionization techniques namely, Matrix-Assisted Laser Desorption/ionization (MALDI) and Electrospray Ionization (ESI) was a major breakthhrough in bioanalytical chemistry as they made the study of biological macromolecules in the gas phase possible. [17, 18] Prior to these developments, mass spectrometric analysis of biological molecules such as proteins, glycans, lipids, and DNA were very difficult and in most cases impossible. These innovative mass spectrometry (MS) techniques have revolutionized proteomics[19, 20] and metabolomics fields and have opened up new research directions.[21, 22]

Tremendeous improvements in instrumentation and bioanalytical methods is seen in MS in the past 20 years. It would be very fair to say that, no other bioanalytical technique experienced such rapid and diverse developments as MS did. Today, MS is an indispensable platform for biomolecule analysis and became an integral part of life science research. It is also heavily used in clinical laboratory diagnosis.[23-25] While MS made great strides, certain technical and experimental difficulties still exist. Current efforts focus on more

systematic and targeted approaches to use MS more effectively and ameliorate its sensitivity, selectivity and throughput. Regardless of the technique used, bioanalytical chemistry- in the simplest terms- goes after 2 major questions:

"What" molecules exist in the biological sample? And *"how much"* of these biomolecules exist in the biological sample?

Biological complexity of blood, urine, cerebrospinal fluid, and tissue lysates is the major road blocker for mass spectrometry. These samples contain enormous amount of different biomolecules each with a different quantity which is also defined as the dynamic range. Even though MS systems greatly evolved, they are still not at the required sensitivity level and not reached the dynamic range to directly deal with biological complexity. Therefore the question of *"what"* and *"how much"* cannot be adequately answered by MS yet. One way of circumventing this complexity problem is to break apart the original sample into pieces by fractionation and apply multidimensional separation prior to MS. This strategy forms the basis of modern MS-based proteomics and MS-based metabolomics studies. The second approach is selective molecular isolation by using specific capturing probes. As noted above, aptamers have significant advantages as affinity probes. They can therefore be successfully implemented in the context of complexity reduction.

Applications of aptamers in bioanalytical MS can be condensed into two main categories. In the first one, aptamers are surface immobilized onto a solid support and MS is used as a read-out probe. In the second one, MS is directly used to characterize the aptamer-ligand interactions.

3 Surface immobilized aptamer platforms for mass spectrometry

3.1 Aptamer-conjugated planar surfaces for mass spectrometry

Immobilization of DNA probes on various surfaces is quite well established. Essentially DNA chips were the transforming technology for genomics and transcriptomics. Today, thousands of gene products can be screened on DNA array surfaces with very high throughput. As aptamers are DNA and RNA oligonucleotides, this idea was applied to aptamers as well. The first successful application of aptamers in MS in this category was reported by McGown group in 2004. [26] In that study, thrombin-binding DNA aptamer was covalently bound to a fused-silica glass surface. Upon incubation and washing steps, nonspecific proteins, such as albumin, were largely removed from the aptamer modified spots and thrombin and prothrombin was captured from plasma. The same group applied the same strategy for specific capture of serum immunoglobulin IgE which is used as a biomarker for allergy and they were able to capture IgE at picomolar levels. [27] In another very interesting work, an "aptamer like" sequence of insulin-linked polymorphic region (ILPR) was immobilized in a similar fashion onto glass slides and used for the affinity extraction of insulin from the nuclear extracts of human pancreatic cells.[28] The authors suggested that insulin itself might play a role in regulation of its own gene through association with G-quadruplexes formed in the ILPR region. Cho et al. reported a microbead based affinity chromatography method coupled to MALDI-MS.[29] In that study, photo-cleavable linker modified HCV RNA polymerase aptamer was immobilized onto magnetic beads. Target protein HCV RNA polymerase was then captured from human serum with the aptamer modified beads. After capturing, enriched protein was eluted from the bead upon exposure to near-UV light. Finally captured protein was digested with trypsin and peptide profile was analyzed using MALDI-TOF**. (Figure 1)**

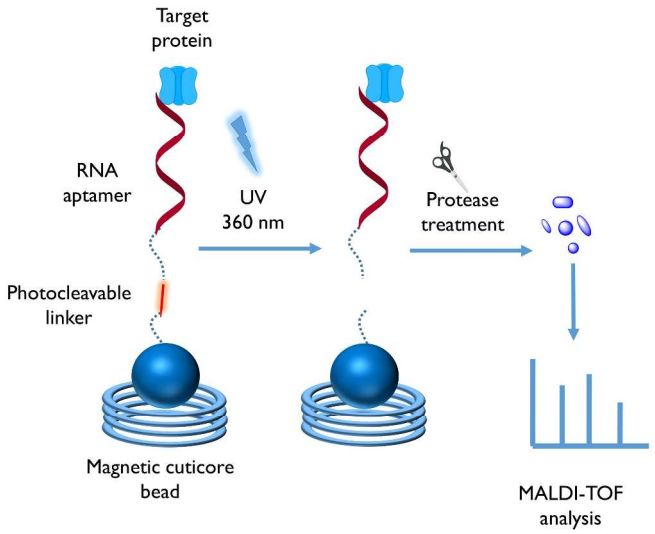


Figure 1. Aptamer based affinity microbead MALDI-MS [29]

In a surface enhanced laser desorption ionization (SELDI-MS) study, amino linked thioaptamer XBY-S2 was precoupled to each spot on a PS20 ProteinChip array (Ciphergen) containing epoxy functional group. Lipopolysaccharide (LPS)-stimulated mouse 70Z/3 pre-B cell nuclear extracts were then incubated with these spots. After washing, these spots were digested with trypsin and analyzed by SELDI-MS. Using this ''on-chip'' capture and digestion approach, five hnRNPs were identified.[30] Laurell group used their solid phase extraction method called as Integrated Selective Enrichment Target (ISET)-MALDI-MS for aptamer based enrichment. The aptamer/ISET-MALDI-MS platform displayed a

detection limit of 10 fmol for thrombin in five different human serum samples. [31]

Table1. **Surface immobilized aptamer platforms for mass spectrometry**

Aptamer	Application	Platform used	Reference
Thrombin, IgE, aptamers, "aptamer like" insulin-linked polymorphic region (ILPR)	Selective isolation of target proteins from complex mixtures and MALDI-MS analysis	Planar fused silica surfaces	26, 27, 28
RNA aptamer for HCV RNA polymerase	HCV RNA polymerase capture from serum with the aptamer and MALDI-MS analysis	microbead based affinity chromatography	29
XBY-S2 thioaptamer	(LPS)-stimulated mouse 70Z/3 pre-B cell nuclear extracts and SELDI-MS identification of human ribonucleoproteins hRNPs	PS20 ProteinChip array	30
Thrombin aptamer	Selective isolation of thrombin from complex mixtures and MALDI-MS analysis on ISET chips	Integrated Selective Enrichment Target (ISET)	31
Insulin aptamer, lysozyme aptamer	Laser assisted proteolysis and detection of proteotypic peptides by MALDI-MS	Nanoporous gold chip	32, 33
yTBP aptamer	Fluorescence and MALDI-MS detection of selectively captured proteins	Sol-gel based aptamer microarray	34
Thrombin, gp120 aptamers	Surface acoustic wave and MALDI-MS detection of selectively captured proteins	SAW-MS chips	35, 36
Vasopressin aptamer	Selective capture of vasopressin	silica coated magnetic nanoparticles	39
ATP aptamer	Surface assisted laser desorption ionization of ATP from cell lysate	Aptamer conjugated gold nanoparticles	50
Cocaine, adenosine aptamer	Selective isolation of cocaine and adenosine from human plasma and direct laser desorption ionization	Graphene oxide	51
ATP aptamer	Selective isolation of adenosine from CEM cancer cells and direct laser desorption ionization	Au@MnO nanoflowers	52
Thrombin aptamer	Selective isolation of thrombin from human plasma	Gold nanorods	53
Thrombin aptamer	Specific enrichment and rapid analysis of thrombin in biological samples using MALDI-TOF-MS	Magnetic graphene/gold nanoparticles nanocomposites	54

Zhang group fabricated MALDI targets with nanoporous gold and tethered them with insulin binding aptamers. Their reported sensitivity and dynamic range of detection was superior to previous reports.[32] Same group has extended this approach for selective isolation and MALDI-MS based detection of lysozyme. Instead of detecting the pseudo molecular ion peak of the proteins, they applied laser assisted proteolysis to the captured proteins and used liberated proteotypic peptides for quantification. [33]

3.2 Dual platforms in Aptamer-based MS

In addition to MS-only aptamer approaches, dual formats in which MS detection is coupled with another analytical method, were also reported. J.Y.Ahn et al. developed a sol-gel based platform in which cy3-labelled TATA box binding protein (yTBP) aptamers were entrapped into sol-gel micro-particles. These were then arrayed onto 96-well plate. (Figure 2) After protein incubation, the resulting aptamer microarray was scanned and analyzed using a 96-well fluorescence scanner. These array spots were then treated with trypsin to perform proteolytic cleavage.

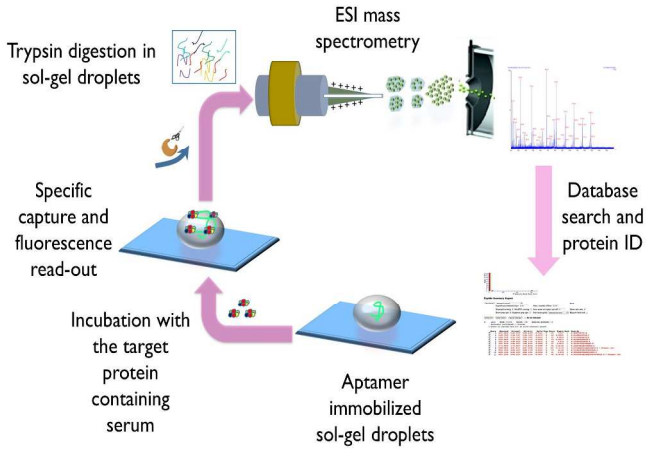


Figure 2. Schematic diagram of the "aptamer microarray mediated capture and identification" approach.[34]

After completion of the digestion, peptides were retrieved and analyzed in an off-line manner with LC-MS/MS.[34] The authors reported that they were able to capture and detect yTBP in serum even if the percentage of yTBP was at 0.001%. Treitz et. al. reported a surface acoustic wave biosensor coupled with mass spectrometry (SAW-MS) for the analysis of a protein complex consisting of human blood clotting cascade factor thrombin with an aptamer immobilized SAW chip. [35] A similar SAW-MS-aptamer method was also reported for HIV-1 envelope protein gp120. The novel part of these studies is that both specificity and reliable quantitative analysis of binding can be obtained in a single experiment. [36]

3.3 Aptamer-conjugated nanomaterials for mass spectrometry

Apart from these planar surface based aptamer methods, nanoparticles were also utilized in aptamer based-MS. Nanoparticles have compelling advantages over planar surfaces. First, the immobilization chemistry of different receptors (antibody, lectin, aptamer) onto nanoscale surfaces is quite well established. Second, nanoparticles have larger surface area-to-volume ratios. This increases probe density and in many cases impart multivalency and avidity. Finally, as nanoparticles have three dimensional shapes, probe immobilized nanoparticles function similar to free solution phase probes. As a result of all these advantages, a new field called nano-proteomics has emerged [37, 38] Tan group was the first to apply nanoparticles for aptamer enhanced MS. In their study, silica coated magnetic nanoparticles modified with vasopressin aptamers were used for selective extraction of vasopressin prior to atmospheric pressure MALDI analysis.[39] Nanomaterials are not as common as conventional MALDI matrices, but they are also used as matrices for laser desorption ionization. This attribute of nanomaterials actually date back to pioneering and Nobel Prize winning work of Tanaka where cobalt nanoparticles dispersed into glycerol was used as the matrix.[40] However the use of nanomaterials was largely overlooked until Siuzdak's seminal work on laser desorption ionization on nanoporous silicon (DIOS).[41] After this work, various forms of silicon surfaces and different nanoparticles were tested as matrices for LDI.[42-47] Nanomaterials have several advantages in LDI. First of all they introduce very little or no background ions. Therefore a very clean mass spectra can be obtained in the low mass region (<1000 Da). This makes them amenable for small molecule (metabolomics, fluxomics) applications. Second their sensitivity surpass the conventional methods.[48, 49] These properties of nanomaterials were combined with aptamers to unify selective capture and ionization on single platforms. Huang et al. used ATP aptamer modified gold nanoparticles for surface assisted laser desorption/ionization and reported sub-micromolar sensitivities.[50] Gulbakan et. al. reported a dual approach in which aptamer modified graphene oxide was used affinity extraction and at the same time probe for ionization. [51] They used ATP and cocaine aptamer tethered graphene oxide for selective capturing and attained significantly improved S/N ratios. In a conceptually similar but more developed version, they used dual aptamer-conjugated multifunctional nanoflowers as a platform for targeting, capture, and detection in laser desorption ionization MS. In that study, manganese oxide petals of gold manganese oxide (Au@MnO) hybrid

nanoflowers were decorated with leukemia cancer cell targeting sgc8 aptamers and gold core was decorated ATP aptamers. They showed that these particles serve as i) an efficient ionization probe and ii) high level of selectivity in cells can be obtained by means of two different aptamers. [52] The same group used aptamer conjugated gold nanorods for selective enrichment of thrombin from human plasma and were able to detect as low as 1 ng of protein. [53] In a similar study, Xiong et.al used aptamer-conjugated magnetic graphene/gold nanoparticles nanocomposites (MagG@Au) for specific enrichment and rapid analysis of thrombin in biological samples using MALDI-TOF-MS. [54]

All these examples show that aptamer-conjugated surfaces significantly improve MS detection from both sensitivity and selectivity respects.

4 Mass spectrometry for direct characterization of aptamer-ligand interactions

Mass spectrometry is usually regarded as the most versatile tool in bioanalytical chemistry. McLafferty has often referred to the ''S'' advantages of mass spectrometry for solving problems: specificity, sensitivity and speed. Apart from giving structural information, MS can also be faithfully used to study non-covalent interactions adding another "S" to this list which is stoichiometry. The most important advances in non-covalent mass spectrometry were realized with ESI-MS as it allows gentle transfer of solution phase species to the gas phase directly. These studies are currently known as "native ESI-MS" referring to preservation of the native structures of biomolecules in ESI mass spectrometry. As previously noted, MS methods were also used for the characterization of aptamer-ligand interactions. Cassiday et.al was the first to apply MS for identification of the stoichiometry of aptamer-ligand binding. They used ESI-MS and showed that 31-nucleotide RNA aptamer specifically binds to human transcription factor NF-κB p50 homodimer. Gross group demonstrated that the G-quadruplex formation of the 15-mer thrombin-binding aptamer can be probed by MS. This study is of particular importance as it is one of the first reports that a distinct solution phase feature (G-quadruplex) can be successfully probed in the gas phase.[55]

Brodbelt group studied the tobramycin, adenosine triphosphate (ATP) and flavin mononucleotide (FMN) binding aptamers with electrospray ionization. They claimed that, although aptamer-ligand complexes were detected, the relative binding affinities determined by MS did not fully correlate with results obtained from solution experiments.[56] However, ESI-MS could be successfully used to calculate binding constants provided that sample preparation and instrumental conditions are fine-tuned and appropriate mathematical models are used to treat the data. [57] Guo et.al has showed that binding affinity of L-argininamide aptamer could be calculated by Electrospray Ionization Fourier Transform Mass Spectrometry (ESI-FTMS).[47] This was in sharp contrast to the work of Brodbelt. The discrepancy lies in the fact that the mass spectrometers and ionization conditions were completely different. Brodbelt group used a quadrupole ion trap mass spectrometer with relatively high interface temperatures. Key to the success in native ESI is the use of mild instrumental conditions. Low interface temperatures, low collision voltages, and optimized ion guide pressures are mandatory to preserve biomolecule complexes. It is quite likely that instrumental conditions in quadrupole ion trap were not suited to preserve aptamer-ligand interactions. Gross group has quantified $Sr^{2+}$and K^+ binding affinities of thrombin binding aptamer by native-MS titration method.[58] This work was extended to calculate Na^+, K^+, Rb^+, and Cs^+ affinities of the same aptamer. [59] The results showed that binding constant of potassium was 5-8 times greater than those of other alkali metal ions, and the potassium binding site was different from other metal binding sites. In a quite recent example, Ruigrok et.al used native MS as a probe for SELEX. [60] After seven rounds, they selected streptavidin-binding oligonucleotides, they then used native MS to both rank the affinities and stoichiometry of the 5 different evolved aptamers. The results revealed that streptavidin was found to bind a maximum of two aptamer units simultaneously, regardless of the aptamer used. Hydrogen-deuterium exchange MS (HDX-MS) has become a very successfully method to study protein–ligand interactions in recent years.[61, 62] Gross group modified the traditional H/D exchange protocol to study the aptamer-protein interactions. They utilized a strong anion exchange column through rapid removal of the oligonucleotides from the solution prior to MS analysis. [63] In quite recent work, HDX-MS was employed to study the effect of RNA aptamers on the structural flexibility of the serpin plasminogen activator inhibitor-1 (PAI-1). [64]

All these ESI studies support the notion that solution phase chemistry is well reflected in the gas phase and MS could be used for structural study of oligonucleotides from very different angles.

The other soft ionization technique MALDI also allows the sensitive detection of large, non-volatile, and labile molecules by mass spectrometry. On the other hand, MALDI-MS is not as commonly used for probing non-covalent complexes as native ESI-MS. The

primary reason for this is that non-covalent interactions are disrupted during either in sample preparation or in the ionization process. However, it was shown that under appropriate conditions, MALDI-MS can be successfully used and allow for the detection of non-covalent complexes.[65] Chen and Gulbakan reported that the aptamer-protein interactions could also be studied by high resolution MALDI MS provided that sample preparation conditions were fine-tuned and -aza-2 thiothymine was used as the MALDI matrix.[66]

5 SELEX against live targets

As noted before, aptamers can be selected to variety of targets. One of these important targets are prokaryotic and eukaryotic cells. Most of the aptamer based biomarker discovery efforts are conducted with aptamers selected against live eukaryotic cells. So this section devoted to a brief description of the live cell selection. After the development of SELEX, several different groups have selected aptamers for single targets such as ATP, IgE, thrombin and PDGF. The idea of using live cells for selection dates back to the work of Morris et.al where red blood cell membranes were used as a complex target for SELEX.[67] This was then followed by the selection of Blank et.al that used rat brain tumor micro-vessels.[68] The first aptamer selection for a live organism was against African trypanosomes, *Trypanosoma brucei* a parasite causes African sleeping sickness.[69] This parasite is known to express a cell surface shield, known as variable surface glycoprotein (VSG). The same group also reported the generation of aptamers for different VSG variants.[70] Ulrich et al. generated aptamers using another live parasite, American trypanosomes, *Trypanosoma cruzi*.[71] Similarly, bacteria has also been the subject of aptamer selection. The early work on this was carried out for *Bacillus anthracis* spores.[72] The first aptamer selection against live bacteria was for *Mycobacterium tuberculosis*.[73]

Bacterial SELEX has attracted huge interest in recent years especially in the context of detecting food pathogens. For example high quality aptamers were selected *Salmonella enteritidis*[74], *Campylobacter jejuni*[75]. Comprehensive discussion of bacterial SELEX is beyond the scope of this review and can be accessed elsewhere.[76, 77]

First study of eukaryotic cell-SELEX whose target was unknown priori was carried out by Wang et.al. They selected aptamers with capability to distinguish differentiated PC12 cells from normal PC12 cells by using a subtractive SELEX strategy.[78] Tan group has systematized the selection against live cancer cells by also implementing the subtractive strategy. They termed this process as cell-SELEX.[79]

Envision was that cell-SELEX could be a useful tool for finding ligands to specific biological markers that distinguish different cancer cells. Technical aspects of the selection procedure has been explained in greater details elsewhere.[80]

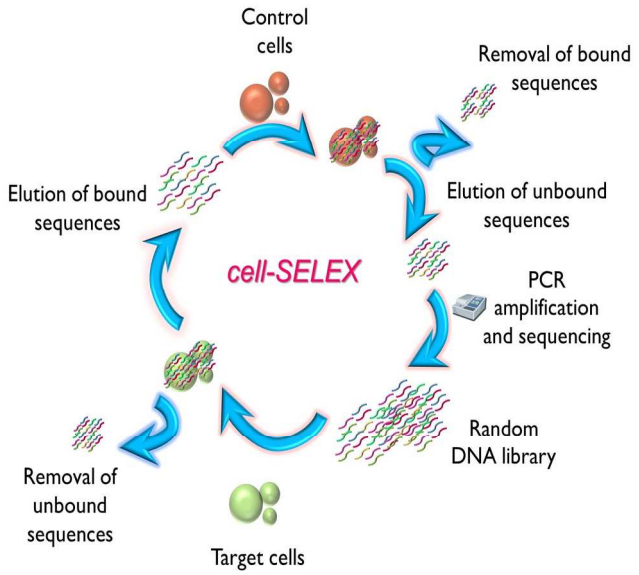


Figure 3. Schematics of cell-SELEX

Briefly, the strategy uses a positive cancer cell line (target cell line) for the selection. However, cancer cell lines have a lot of commonalities with other cancer cell lines and normal cell lines on their cell surface make-up. Therefore a counter selection was employed in which a negative cell line (control cell line) was used in this subtractive step. The success of cell-SELEX and specificity depends highly on the subtractive step. This steps ensures that evolved probes are specific to only target cell lines. This strategy is a particularly promising scheme for various diseases including cancer in research and therapy. To date, live cells of many different cancers such as T-Cell, B-Cell leukemia, lung cancer, liver cancer, ovarian cancer, prostate cancer, colon cancer and glioblastoma multiform have been selected by this process. As a result, over 400 different aptamers have been generated for many of the cancer lines.[81, 82] Considering the lack of specific probes for live cell recognition, cell-SELEX derived aptamers have emerged as a very useful tools.

6 Cell-SELEX derived aptamers for biomarker discovery

In the past twenty years, biomarker discovery and in particular protein biomarker discovery has become a new research focus after the completion of Human Genome Project. Proteomics research aims to interpret the function of genes in biological systems by understanding the role and function of proteins. In parallel to this impetus, MS instrumentation and proteomics methods advanced at an unprecedented level. Thousands of proteins in very complex biological specimen such as plasma, cerebrospinal fluid, saliva, cell and tissue lysates can be identified and even be quantified in quite short periods.[83] Many of the previously unknown mysteries of biological systems have now been resolved thanks to the advances in proteomics and mass spectrometry. However, despite the huge investment in proteomics research, the progress in bringing protein biomarkers into clinical practice is still not very successful. [84] Literature is flooded with studies reporting long list of "biomarker candidates". As a result, MS-based discovery proteomics turned out to be more like a "my protein list is longer than yours" research. [85] While all these reported lists of proteins might undeniably be useful, the question of which proteins are the most important and which of them are the real biomarkers yet remains to be answered. MS is the most advanced tool to conduct biomarker discovery and will likely remain as such in the coming years. On the other hand, FDA approved clinically useful biomarker discovered by MS is still scarce. One of the primary reasons for this problem is that the biological mixtures are way more complex than current analytical MS technologies can cope with. Biological mixtures span a dynamic range of 9 orders (in some cases even >9) of magnitude, while MS tools can still only reach to 4-5 orders of magnitude. Moreover, most of the "putative biomarkers" are hidden in a sea of other biomolecules at a concentration level (ng/ml) that current MS instruments cannot faithfully detect. [86] If they do, this comes as a result of great effort and with many hundred hours of machine time which is very labor intense and expensive. Unfortunately, no PCR analogue is available for proteins to enable amplification. Moreover, the protein lists discovered by MS don't always represent "what is really in" the biological mixtures. This problem has recently been addressed by Human Proteome Organization (HUPO) with a pilot test study to understand the impact of the human and instrumentation in protoemics.[87] The results indicated that even with a relatively small proteome, samples could not be successfully analyzed albeit the best instrumentation was used. While discovery proteomics is still an active research field, focus is shifting more towards targeted proteomics. Prior bimolecular knowledge is used to derive and validate protein biomarkers rather than looking at the problem from a global and untargeted perspective.[88, 89] Apart from serving as molecular recognition tools, aptamers are also very promising in the context of biomarker discovery for a number of reasons. First, prior knowledge of the target is unnecessary. The need to know the molecular composition of the cell surface does not play an important role in cell-SELEX. Second, the cell membrane surface has numerous different proteins. In cell-SELEX, each of these molecules is a potential target. At the end of a successful selection, several aptamers can be generated for many different cellular targets. This feature is very important, as some of these molecules may play roles in the development of the cell or the disease they cause. Producing a similar panel of monoclonal antibodies in such a short time without purified antigens is very difficult. Third, membrane proteins are the least represented subclass of the proteome.[82, 90, 91] Membrane proteins are intrinsically very difficult to isolate and to identify. They are buried in a highly dense pack of lipid bilayers and membrane proteins are highly hydrophobic. This poses another level of difficulty in MS based identification platforms as hydrophobic compounds are quite In contrast to the technical difficulties to analyze them, membrane proteins are very important for disease diagnosis and therapeutics and they have been extensively targeted for drug design, and it accounts for about 70% of all known drug targets (e.g., HER2 and G-protein coupled receptors). The underpinning hypothesis in aptamer based biomarker discovery is that the fished-out proteins might have important functions in disease diagnosis and therapeutics. Because, in cell-SELEX the selection is done blindly i.e. without any bias towards a particular protein target.

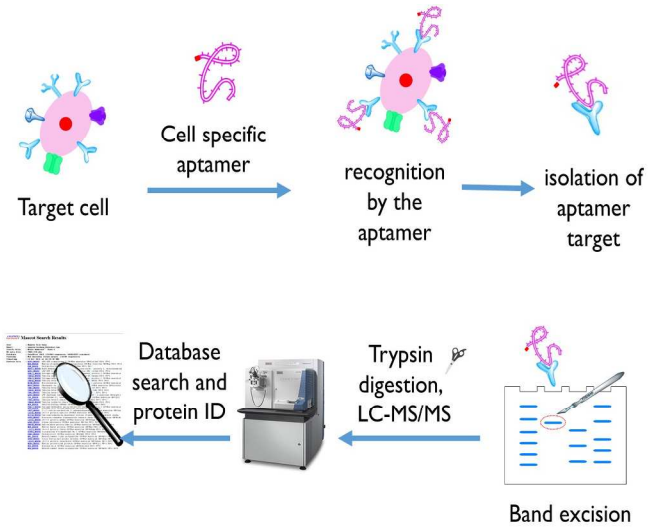


Figure 4. Principle of aptamer-based biomarker discovery methods

Because of all these advantages, aptamers are becoming more and more involved in membrane protein elucidation. (Figure 4) The very first report of using aptamers for cancer biomarkers is the identification of the aptamer selected against YPEN-1 endothelial cells. [68] In this SELEX study, 25 different aptamers were analyzed and one aptamer was found to selectively bind micro vessels of rat brain glioblastoma but not the vasculature of the normal rat brain including peritumoral areas. The molecular target protein of aptamer named as III.1 was isolated from endothelial cells by ligand-mediated magnetic DNA affinity purification. This protein was then identified by MS as rat homologue of mouse pigpen, a not widely known endothelial protein the expression. Another nice example was the discovery of tenascin-C aptamers using glioblastoma cell line, U251.[92] In that study, DNA aptamer named as GBI-10 was found to interact with tenascin C which is an extracellular protein found in the tumor matrix. However, this was more of a proof of concept type of study as this aptamer had been selected tenascin C in an earlier report.[93] In the most striking example, protein tyrosine kinase PTK7 was identified as the binding receptor on the cell membrane for aptamer sgc8 which was selected against CCRF-CEM leukemia cells. [94] The authors also used the PTK7 plasmid to express PTK7 in a negative cell line in which sgc8 aptamer had no affinity. They found that after this expression sgc8 was able to recognize negative cells as well suggesting another level of proof. Protein tyrosine kinase-7 (PTK7), also known as colon carcinoma kinase-4 (CCK4), is a relatively new and little studied member of the RTK superfamily. Two years after PTK7 was reported as a novel biomarker candidate for T-ALL cells using aptamers, it was identified as a novel regulator of non-canonical WNT or planar cell polarity (PCP) signaling. [95, 96] this report was particularly important in that it has generated totally new insights about cancer. Following this report, this marker has now been tested against several other cancer cell lines as well to use it as a generic biomarker. [97-101] Krylov group at York University reported a quite similar strategy which they term as AptaBiD (aptamer-facilitated biomarker discovery).[102] Their aptamer selection method for immature and mature dendritic cells presented a dual approach. In the first place, it was a better optimized version of the previous reports for mining the biomarkers. Aptamer selection and biomarker discovery were simultaneously performed. AptaBiD approach also reversed the order of the cell-SELEX and negative selection was followed by positive selection. It also employed a long masking ssDNA (synthetic scrambled unlabeled 80-mer deoxyoligonucleotide) to suppress nonspecific binding of aptamers to cells and cell debris. As a result of aptamer based fishing out, known biomarkers of mDCs (CD40, CD80) as well as previously unknown biomarkers of iDCs (CXorf17 protein, galectin-3, glycoprotein NMB, and lipoprotein lipase) and mDCs (copine-2) were identified. AptaBID method was recently applied for identification of a new biomarker on primary cultured mouse tumor endothelial cells (mTECs).[103] The authors identified troponin-T via (MALDI-TOF) MS the molecular target of aptamer AraHH00. Its presence was also confirmed by measuring mRNA, protein levels, western blot, immunostaining, a gel shift assay of AraHH001 with troponin T. Dua et.al selected RNA aptamers for pancreatic ductal adenocarcinoma (PDAC) cells. After their selection they applied (i) aptamer-based target pull-down and (ii) genome-wide microarray-based identification of differentially expressed mRNAs in aptamer-positive and -negative cells. Alkaline phosphatase placental-like 2 (ALPPL-2), an oncofetal protein was identified as the target by mass spectrometry. [104] Cerchia et.al selected a RNA-based aptamer, named GL21.T. After a phospho-receptor tyrosine kinase (RTK) array analysis and filter binding analysis with the soluble extracellular domain of human Axl, Dtk (Tyro3) and Mer as targets, Axl was found to be the target protein.[105] However the general applicability of this method is questionable as it was more like a trial and error approach. All of these examples rely on cell lysis, aptamer-based pull down using magnetic beads, gel electrophoresis and MS. Cell lysis liberates the membrane proteins to a non-native conformation. Moreover, very little is known on how aptamers recognize their targets on the cell surface, therefore how and under which conditions aptamer-target interactions can be

maintained is still an open question. Therefore, these successful cases do not warrant that all aptamer targets can be identified in this way. To address this particular problem, crosslinking is applied as an alternative approach. In this strategy, permanent contacts are formed between the aptamer and the target before the cell lysis is applied. This method could be advantageous as more stringent washing conditions could be used to eliminate the non-specific binding. This strategy was first applied to B-cell leukemia recognizing aptamer TDO5.[106] In this approach, the aptamer probe was chemically modified with a photoactive 5-iodo-deoxyuridine (5-dUI) nucleotide for covalent binding of the aptamer with cells. Subsequent enrichment of the target protein by magnetic extraction using a biotin-streptavidin interaction was followed by identification of collected protein by MS and database search. Finally, the identity of the target protein was confirmed using an existing antibody. The selected aptamer. Immunoglobin heavy mu chain (IgM) was identified as the target of the TDO5. The major drawback of this approach was the need for precise positioning of the photoreactive bases into aptamer sequence without compromising the binding. This approach enabled the formation of a covalent bond between aptamer and its target but it was very labor intense and needed very rigorous optimization. Therefore its general applicability appears to be limited. Famulok group reported another photo-crosslinking strategy utilizing photocrosslinkable phenyl azide moiety. (Figure 5) This approach eliminated the need for tedious optimization steps of the aforementioned method. Photoreactive cross-linking moiety was attached to the 5-end of the aptamer.[107] They validated their chemistry by applying it to three different aptamers whose secondary structures are completely different. In all three cases, the XL strategy was reported to give very high crosslinking efficiencies. This report is quite attractive as it is less labor intense and easy to perform. The major drawback is that it has not been applied to an aptamer whose target was previously unknown. Formaldehyde based crosslinking approach has been suggested as another way of aptamer-protein crosslinking on live cells.[108] Formaldehyde induced reversible crosslinking has been widely used in the method called as chromatin immunoprecipitation (ChIP) for many decades. This method temporarily freezes transient DNA-protein contacts in living cells determining whether a certain protein-DNA interaction is present at a given location, condition, and time point. ChIP assays are particularly useful for the identification of transcription factors and their target genes.

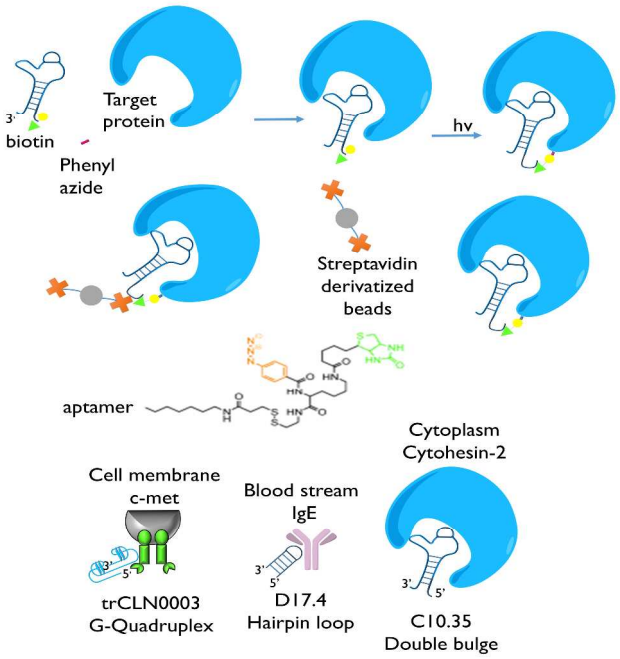


Figure 5. Principle of aptamer-protein crosslinking with phenylazide moiety [107]

In that example, the very well-known chemistry borrowed from ChIP method was applied to ovarian cancer cell line TOV-21G and the selected aptamer against it. After binding of TOV6 to its cognate target on the cell surface membrane, the TOV6/target interaction was fixed by formaldehyde. The protein–aptamer hybrid was then extracted from the cell lysate and recovered. The protein was identified as stress-induced phosphoprotein 1 (STIP1) by MS. Identity of the target was also confirmed through siRNA silencing and antibody binding. The method is attractive as formaldehyde crosslinking could easily be reversed simply by heating. However, formaldehyde is a very small and non-specific cross linker and any transient protein-DNA contact within the cell and also aptamer/non-specific protein contacts might also be cross-linked. This appears to be the major drawback of the method. Apart from cancer cell targeting aptamers, several aptamers that target viral proteins have also been developed. [109] In the context of biomarker discovery, a fluorescence method called alpha-screen assay was reported to identify the target of aptamer probes that can recognize cells infected with vaccinia virus (VV).[110] The results revealed that hemagglutinin was highly expressed on the surface of the cells and was the marker recognized by the aptamer. To provide further evidence that HA was the target of aptamer PP3, an experiment was performed using BSR

T7 cell. These cells were infected with rabbit pox virus and transfected with plasmids containing either tagged VV SPI-3 or VV HA under the control of T7 promoter. No signal was observed for SPI-3 overexpressing RPV-infected BSR T7 cells, thus eliminating SPI-3 and other proteins from the entry fusion complex that interacts directly with the SPI-3-HA multimer. Only the tagged HA sample showed a signal with aptamer PP3 further suggesting HA as the target of aptamer PP3.

7 Aptamer arrays for biomarker discovery

Apart from these MS-assisted proteomics efforts, another useful modality for aptamer based biomarker discovery is array platforms. Microarrays can be defined as a functional element (DNA, RNA, antigen, antibody, aptamer, and small molecule) being attached to a solid substrate in an ordered manner at high probe density. Hundreds to thousands of these products can be immobilized on a very small area with a specialized robotic arraying tool. The immobilized probe is generated by labeling it with a fluorescence dye, radioisotope, or a chemo luminescence agent. Products (i.e., the array) serve as interaction targets for a labeled probe. Even though the application of the array platforms for genomics and transcriptomics is quite successful, they are

Table 2. Biomarker discovery based on cell-SELEX

Aptamer	Cell line	Method	Identified target	Reference
aptamer III.1 (DNA)	YPEN-1, endothelial cells	Cell lysis+aptamer based pull down+ gel electrophoresis+ nanoLC-MS/MS	Pigpen	66
GBI-10	U251, glioblastoma cell line	Cell lysis+aptamer based pull down+ gel electrophoresis+ nanoLC-MS/MS	Tenascin C	90
Sgc8	CEM, lymphoblastic leukemia cells	Cell lysis+aptamer based pull down+ gel electrophoresis+nanoLC-MS/MS	PTK7	92
Several aptamers	Dendritic cells	Cell lysis+aptamer based pull down+ nanoLC-MS/MS	Several	100
AraHH001	mTECs, mouse tumor endothelial cells	Cell lysis+aptamer based pull down+ gel electrophoresis + MALDI-TOF	troponin T	101
SQ-2	pancreatic ductal adenocarcinoma (PDAC) cells	Cell lysis+aptamer based pull down+ gel electrophoresis+ nanoLC-MS/MS	ALPPL-2	102
GL21.T	U87MG, human primary glioblastoma cells	RTK array analysis and filter binding analysis	Axl, Dtk	103
TD05	Ramos, Burkitt's lymphoma	UV crosslinking, aptamer based pull down+ gel electrophoresis+ nanoLC-MS/MS	IgM heavy chain	104
Several	H1838 on-small-cell lung carcinoma (NSCLC)	UV crosslinking, aptamer based pull down+ gel electrophoresis+ nanoLC-MS/MS	c-Met, sec7	105
TOV6	TOV-21G ovarian cancer cells	Formaldehyde crosslinking, aptamer based pull down+ gel electrophoresis+ nanoLC-MS/MS	STIP1	106
PP3	Vaccinia virus (VV) infected HeLa cells	Alpha screen assay	hemagglutinin	108

slowly emerging for proteins and are not widely used in proteomics yet. [111, 112] One of the leading figures in proteomics and founding member of HUPO, Samir Hanash has stated in an excellent review that "various microarray formats- in which protein-capture agents, recombinant or natural proteins, cell or tissue lysates are arrayed and then interrogated with patient samples-are slowly complementing MS as a high-throughput tool for biomarker discovery and validation. So, MS in combination with liquid chromatography will remain as the main proteomic workhorse until microarrays and capture agents can comprehensively interrogate complex proteomes".[113]

There are couple of interconnected factors which limit the widespread use of array platforms for biomarker discovery. The first one is the lack of available specific probes. To address this problem, HUPO has initiated several different projects for the development of antibodies for proteomic targets. [114, 115] However, in order for the antibodies to be useful in array based platforms, they should be immobilized onto a solid surface without affecting the functionality and specificity. Antibodies are relatively large proteins. Development of reproducible and orientation-specific immobilization protocols are still not fully optimized and this is a very active research area.[116] Details of this topic is beyond the scope of this review and it is perfectly reviewed elsewhere.[117] Another problem is multiplexing capabilities of antibody based platforms is still not up to the needs of the post-genomic era. It is extremely desirable to measure multiple biomarker candidates quantitatively in one single platform.

Aptamers are quite advantageous over antibodies and holds great promise in array applications for several reasons. First, very well optimized protocols for making DNA arrays could be implemented to fabricate aptamer arrays with small modifications. Secondly, as also briefly explained before, a wide range of different chemistry options are available for aptamers. Therefore many different substrates and detection tools (fluorescence, colorimetric detection, magnetic relaxation, and radioisotope based detection) could be utilized. There have been different attempts to realize this goal. In one of the earliest designs, photoaptamers were used. Photoaptamers were produced with a modified version of SELEX process. Bases bearing photo-crosslinkable moieties were used during the selection. Photoaptamers were discovered for proteins with a wide range of characteristics, including acidic, basic, large, small, glycosylated, chemically modified, and hydrophobic. The photo-SELEX process has been successfully automated as a high-throughput process. Wide range of proteins have yielded active photoaptamers that exhibit nanomolar or better affinities. In the earliest designs, photoaptamers were synthesized with an amine on the 5′ terminus to provide a covalent anchor to an array surface. [118] After incubation and washing, the array was irradiated at 308 nm with a XeCl excimer laser to photo cross-link the photoaptamer to its cognate protein. Exposing the protein/aptamer complex to UV light induced covalent bond formation between the photoaptamer and cognate protein.

After that step, fluorescence was quantified from covalently bound protein on each photoaptamer feature. In that study, each cognate protein concentration was varied from 0.01 to 10 nmol/L, whereas seven other proteins were varied over the same concentration range. The total concentration in each microarray experiment was 11.1 nmol/L protein, containing various concentrations of endostatin, bFGF, thrombin, angiogenin, tumor growth factor-β1, interleukin-4, p-selectin, and serum amyloid p component. In a follow up study, data was presented for a 17-plex photoaptamer array exhibiting limits of detection below 10 fM for several analytes including interleukin-16, vascular endothelial growth factor, and endostatin and they were able to measure proteins in 10% serum samples. [119] One of the distinct advantages of the photoaptamer arrays is that, after binding and crosslinking, the only protein molecules present on the array are those that are covalently crosslinked to their cognate aptamer. Hence a global labelling step that targets protein-specific chemical moieties could be employed. In these initial reports, photoaptamer microarrays were a defined as a paradigm shifting methodology in the field of proteomics on several grounds. First the ability to select highly specific binding reagents by directed methods provides a powerful tool for protein quantitation that is not easily attainable by other tools. Second, the ease of manufacture and photoaptamer stability allows a wide range of applications that is unlimited by many of the constraints traditionally associated with biological reagents. The acquisition of photoaptamers is limited only by the availability of individual proteins. While all these claims are true, there has been a hiatus in this field until a new generation of SELEX technology is introduced in 2010. The leading aptamer company Somalogic created a new class of aptamer, which they

termed as the Slow Off-rate Modified Aptamer (SOMAmer). This strategy enabled efficient selection of high-affinity aptamers for almost any protein target and the development of novel highly-multiplexed assays for high-performance proteomics.[120]

There are a couple of very innovative modifications to the original SELEX in the SOMAmer selection. The protein alphabet has 20 different letters and therefore statistically way more different words (proteins) can be derived from this alphabet. As opposed to this, DNA alphabet consists only of 4 letters (A, G, C, and T/U) and the chemical words generated with these letters from SELEX is relatively limited. For some important clinical targets, SELEX has therefore failed to yield high affinity aptamers. This has been a major road-blocker for diagnostic and clinical applications of aptamers.

In an attempt to address this problem, nucleotide triphosphate analogs chemically modified at the 5-position (R) of uridine (dUTP): 5-benzylaminocarbonyl-dU (BndU); 5 naphthylmethylaminocarbonyl-dU (NapdU): 5-tryptaminocarbonyl-dU (TrpdU); and 5-isobutylaminocarbonyl-dU (iBudU) bases were used in the SELEX experiments. [121]

The underlying idea behind this modification was to increase the chemical space and to improve the strength of the secondary interactions against the aptamer targets which accordingly would produce better binders.[121]

To test their hypothesis, the authors used thirteen "difficult" human proteins that had repeatedly failed SELEX with unmodified DNA and used GA733-1 protein as a control, which had yielded high-affinity aptamers with unmodified DNA-SELEX. Their results showed that only SELEX with modified nucleotides yielded high-affinity aptamers to these difficult proteins. The second innovative aspect of the work was the selection of aptamer based on their off-rates. They showed that off-rate kinetics of SOMAmers against the targets they were selected, were around 1 h as opposed to their binding to histones which was around 1 minute. This vast difference in dissociation rates between cognate and non-cognate interactions contributes to specificity very significantly. After all these optimizations, a multistep proteomic assay was developed. Assay principles is shown in Figure 6**.**

Briefly, the sample is incubated with a mixture of SOMAmers each containing a biotin, a photocleavable group, and a fluorescent tag followed by capture of all SOMAmer-protein complexes on streptavidin beads (*this step is called as Catch-1*).

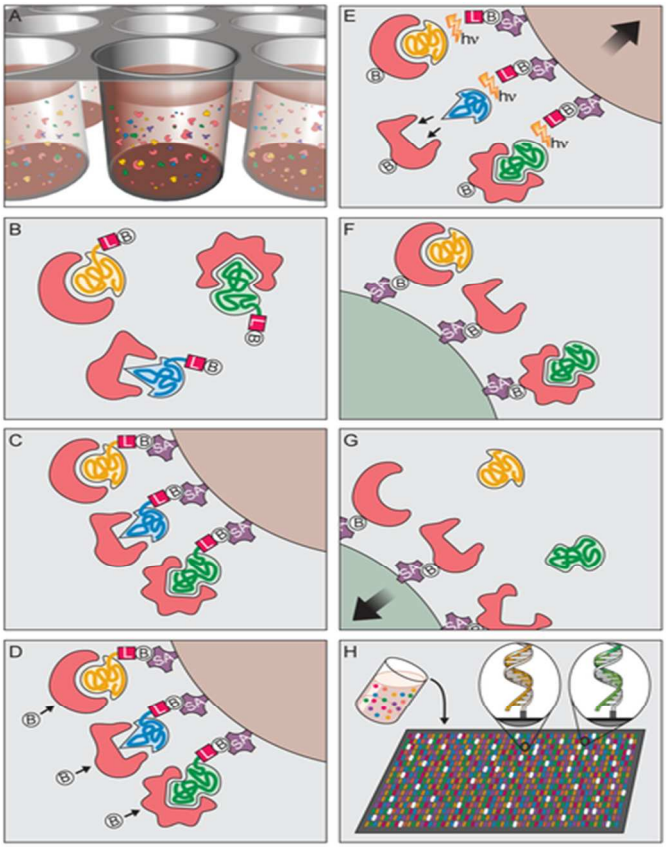


Figure 6. Principle of multiplex SOMAmer affinity assay[120]

After stringent washing of the beads to remove unbound proteins and labeling of bead-associated proteins with biotin under controlled conditions, the complexes are released from the beads back into solution by UV light irradiation and diluted into a high concentration of dextran sulfate, an anionic competitor. The biotin that was originally part of the SOMAmer remains on beads. The anionic competitor coupled with dilution selectively disrupts non-cognate complexes and because only the proteins now contain biotin, the complexes are re-captured on a second set of beads *(the step is called as Catch-2)* from which unbound SOMAmers are removed by a second stringent washing. The SOMAmers that remain attached to beads are eluted under high pH-denaturing conditions and hybridized to sequence-specific complementary probes printed on a standard DNA microarray. So, by combining unnatural bases, kinetic modulations a unique proteomic array was developed. The assay takes advantage of the dual nature of aptamers as molecules capable of both folding into complex three-dimensional structures and hybridization to specific

capture probes. (Figure 5). One of the really promising aspects of the SOMAmer assay was capability to measure 813 proteins with 1 pM median LLOQ, 7-log overall dynamic range (~100 fM–1 μM). This is really an unprecedented performance for aptamer-based assays. After optimizing the assay, it was applied to find biomarkers for chronic kidney disease (CKD) to demonstrate its utility for clinical studies and they identified 60 proteins that varied significantly between early and late stage CKD, which could provide a foundation for developing CKD diagnostics. To mimick ELISA type of assays, a plate version of the SOMAmer assay was also developed.[122]

After this initial report, SOMAmer assay has been applied to several other clinical problems. The findings of the lung cancer study showed that 44 potential lung cancer biomarkers that discriminate stages I-III NSCLC cases from at-risk heavy smoker controls.[123, 124] The results were quite unique in that most of the proteins identified in this study had not been identified previously as serum lung cancer biomarkers. In a very recent study some of these markers have been validated. Apart from lung cancer, other thoracic diseases such as malignant pleural mesothelioma[125], pulmonary tuberculosis[126] were also tested with these assay. Recently, it has also been used for the discovery of age related changes in cerebrospinal fluid and blood.[127, 128]

8 Conclusion and future opportunities

There has been quite remarkable progress in aptamer development in the last two decades. Numerous examples were published where aptamers were demonstrated to perform very well in selective and sensitive bioanalytical platforms. It is very clear from all the accumulating evidence reviewed herein that aptamers will have important implications in bioanalytical mass spectrometry as well. Despite their potential, their use is still not comparable to that of other affinity binding agents such as antibodies. Certain major barriers still exists that prevent aptamers from becoming the affinity agents of choice. In the first place, selection method is often labor intensive and time consuming. Second high affinity aptamers are still lacking for many of the important clinical targets. Aptamers selected with natural bases often lack the desired binding affinity and specificity to target proteins. Expanding the chemistry is one way to circumvent this problem. Non-natural bases have been introduced and used for aptamer selection. Affinities were >100-fold improved over those of aptamers containing only natural bases for vascular endothelial cell growth factor-165 (VEGF-165) and interferon-γ (IFN-γ).[129] A similar and more expanded version called artificial expanded genetic information systems (AEGIS) were used in cell-SELEX to generate triple negative breast cancer cells.[130] These innovative approaches are expected to become more common in the coming years for aptamer selection. Intellectual property rights of aptamers were retained under multiple patents and this was one of the major obstacles in this field. However, the base patents on aptamer selection (SELEX) are expired and the constraints by patent protections associated with their development are now lifted. Therefore the future holds great promise and it is anticipated that more companies invest in the aptamer field and output will exponentially grow. From the bioanalytical mass spectrometry point of view, almost all aptamer applications were conducted with ATP, thrombin, platelet-derived growth factor and immunoglobin E aptamers. Although these targets are sufficient for proof of principle experiments, the focus now has to shift to real applications. In addition, rather than single target isolation, multiplexed aptamer-MS platforms are needed to demonstrate the power and broad utility of the methods. Especially MALDI is quite suitable to realize this goal. It is successfully to analyze multiple proteins in a single sample preparation, in particular, in the direct analysis of biological fluids. ELISA-like aptamer arrays and targeted MS platforms are still areas to be explored. Recently a new method called micro-arrays for mass spectrometry (MAMS) was introduced as a high throughput and ultrasensitive MALDI approach.[131] This kind of high throughput and sensitive platforms can be used in concert with aptamer affinity capture for screening multiple targets. Similar platforms exists with antibodies. Immunoaffinity capture prior to mass spectrometry is for example demonstrated to be a quite robust tool and been widely applied [132, 133]. These immuno-MALDI methods are particularly useful for targeted quantitation of proteotypic peptides. Similar methods could in principle be developed with the availability of high quality peptide-specific aptamers. Characterization of aptamer-ligand complexes is another very important topic. To date research on aptamers been mostly application centered and very little attention was given on how aptamers recognize their targets. Only a handful aptamers exist whose structures are fully

understood. Native-MS and ion mobility spectrometry have proven to be very powerful tools for structural biology. Probing conformational changes associated with ligand binding and would definitely be of great value. These structural methods are expected to shed more light onto the binding mechanisms of aptamers. Biomarker discovery of cancer using aptamer probes is a very exciting area. Given the paucity of disease biomarkers, development of new methods for the discovery of new markers will be very important. Most of the FDA-approved clinically proven cancer drugs target cell surface proteins and inhibit their functions. Moreover membrane proteins are the least represented subclass of the proteome. As aptamers can specifically differentiate cancer cells from normal cells, this could a very unique way to determine molecular characteristics of cells. However no universal method exists that could be used to unambiguously identify aptamer targets by MS. As the cell SELEX ends up with multiple aptamers, a universal method might help finding a panel of surface markers rather than just a single marker. This could also enable pattern recognition where the expression levels of multiple targets could be probed. Although exciting reports exist, it is yet not very clear whether the targets of aptamers are indeed disease biomarkers or just "aptamer binding proteins". Therefore the candidate biomarkers need to be validated with a large cohort of clinical samples. The functional roles of these proteins should also be elucidated by complementary follow-up studies. These proteins directly or indirectly pave the way for biomarker discovery. In conclusion, the potential of aptamers will be more realized in the near future in mass spectrometry and an exponential increase is expected in this field.

Acknowledgements

Basri Gulbakan is grateful to Dr.Konstantin Barylyuk, Dr.Ilker Avan, Dr. Bora Inci and Dr. Cagatay Dengiz and for careful reading of the manuscript and corrections. Financial support from TUBITAK BIDEB is also greatly acknowledged.

Notes and References

[a] Institute of Child Health, Division of Pediatric Basic Sciences, Hacettepe University 06100 Ankara, Turkey, E-mail: basri@hacettepe.edu.tr
Phone: +90 312 305 11 41 Fax: (312) 324 32 84

1. A. D. Ellington and J. W. Szostak, *Nature*, 1990, 346, 818-822.
2. C. Tuerk and L. Gold, *Science*, 1990, 249, 505-510.
3. G. Mayer, *Angewandte Chemie International Edition*, 2009, 48, 2672-2689.
4. R. Mukhopadhyay, *Analytical Chemistry*, 2005, 77, 114 A-118 A.
5. K. S. Schmidt, S. Borkowski, J. Kurreck, A. W. Stephens, R. Bald, M. Hecht, M. Friebe, L. Dinkelborg and V. A. Erdmann, *Nucleic Acids Research*, 2004, 32, 5757-5765.
6. A. D. Keefe, S. Pai and A. Ellington, *Nat Rev Drug Discov*, 2010, 9, 537-550.
7. E. W. M. Ng, D. T. Shima, P. Calias, E. T. Cunningham, D. R. Guyer and A. P. Adamis, *Nat Rev Drug Discov*, 2006, 5, 123-132.
8. P. R. Bouchard, R. M. Hutabarat and K. M. Thompson, *Annual Review of Pharmacology and Toxicology*, 2010, 50, 237-257.
9. T. Šmuc, I.-Y. Ahn and H. Ulrich, *Journal of Pharmaceutical and Biomedical Analysis*, 2013, 81–82, 210-217.
10. V. C. Özalp, K. Bilecen, M. Kavruk and H. Avni Öktem, *Future Microbiology*, 2013, 8, 387-401.
11. D. H. J. Bunka and P. G. Stockley, *Nat Rev Micro*, 2006, 4, 588-596.
12. A. Baumstummler, D. Lehmann, N. Janjic and U. A. Ochsner, *Letters in Applied Microbiology*, 2014, 59, 422-431.
13. J. A. Phillips, D. Lopez-Colon, Z. Zhu, Y. Xu and W. Tan, *Analytica Chimica Acta*, 2008, 621, 101-108.
14. Z. Hou, S. Meyer, N. E. Propson, J. Nie, P. Jiang, R. Stewart and J. A. Thomson, *Cell Res*, 2015, 25, 390-393.
15. M. Famulok and G. Mayer, *Chemistry & Biology*, 21, 1055-1058.
16. J. Zhou and J. J. Rossi, *Oligonucleotides*, 2010, 21, 1-10.
17. J. B. Fenn, *Angewandte Chemie International Edition*, 2003, 42, 3871-3894.
18. M. Karas and F. Hillenkamp, *Analytical Chemistry*, 1988, 60, 2299-2301.
19. A. Bensimon, A. J. R. Heck and R. Aebersold, *Annual Review of Biochemistry*, 2012, 81, 379-405.
20. T. E. Angel, U. K. Aryal, S. M. Hengel, E. S. Baker, R. T. Kelly, E. W. Robinson and R. D. Smith, *Chemical Society Reviews*, 2012, 41, 3912-3928.
21. H. P. Benton, J. Ivanisevic, N. G. Mahieu, M. E. Kurczy, C. H. Johnson, L. Franco, D. Rinehart, E. Valentine, H. Gowda, B. K. Ubhi, R. Tautenhahn, A. Gieschen, M. W. Fields, G. J. Patti and G. Siuzdak, *Analytical Chemistry*, 2015, 87, 884-891.
22. A. Zhang, H. Sun, P. Wang, Y. Han and X. Wang, *Analyst*, 2012, 137, 293-300.
23. B. Gülbakan, K. Barylyuk and R. Zenobi, *Current Opinion in Biotechnology*, 2015, 31, 65-72.
24. M. H. Gelb, C. R. Scott and F. Turecek, *Clinical Chemistry*, 2015, 61, 335-346.
25. E. Scolamiero, C. Cozzolino, L. Albano, A. Ansalone, M. Caterino, G. Corbo, M. G. di Girolamo, C. Di Stefano, A. Durante, G. Franzese, I. Franzese, G. Gallo, P. Giliberti, L. Ingenito, G. Ippolito, B. Malamisura, P. Mazzeo, A. Norma, D. Ombrone, G. Parenti, S. Pellecchia, R. Pecce, I. Pierucci, R. Romanelli, A. Rossi, M. Siano, T. Stoduto, G. R. D. Villani, G. Andria, F. Salvatore, G. Frisso and M.

Ruoppolo, *Molecular BioSystems*, 2015, DOI: 10.1039/C4MB00729H.
26. L. W. Dick and L. B. McGown, *Analytical Chemistry*, 2004, 76, 3037-3041.
27. J. R. Cole, L. W. Dick, E. J. Morgan and L. B. McGown, *Analytical Chemistry*, 2007, 79, 273-279.
28. A. C. Connor, K. A. Frederick, E. J. Morgan and L. B. McGown, *Journal of the American Chemical Society*, 2006, 128, 4986-4991.
29. S. Cho, S.-H. Lee, W.-J. Chung, Y.-K. Kim, Y.-S. Lee and B.-G. Kim, *ELECTROPHORESIS*, 2004, 25, 3730-3739.
30. H. Wang, X. Yang, G. C. Bowick, N. K. Herzog, B. A. Luxon, L. O. Lomas and D. G. Gorenstein, *Biochemical and Biophysical Research Communications*, 2006, 347, 586-593.
31. S. J. Lee, B. Adler, S. Ekström, M. Rezeli, Á. Végvári, J.-W. Park, J. Malm and T. Laurell, *Analytical Chemistry*, 2014, 86, 7627-7634.
32. X. Zhang, S. Zhu, C. Deng and X. Zhang, *Chemical Communications*, 2012, 48, 2689-2691.
33. X. Zhang, S. Zhu, Y. Xiong, C. Deng and X. Zhang, *Angewandte Chemie International Edition*, 2013, 52, 6055-6058.
34. J.-Y. Ahn, S. W. Lee, H. S. Kang, M. Jo, D.-k. Lee, T. Laurell and S. Kim, *Journal of Proteome Research*, 2010, 9, 5568-5573.
35. G. Treitz, T. M. A. Gronewold, E. Quandt and M. Zabe-Kühn, *Biosensors and Bioelectronics*, 2008, 23, 1496-1502.
36. T. M. A. Gronewold, A. Baumgartner, J. Hierer, S. Sierra, M. Blind, F. Schäfer, J. Blümer, T. Tillmann, A. Kiwitz, R. Kaiser, M. Zabe-Kühn, E. Quandt and M. Famulok, *Journal of Proteome Research*, 2009, 8, 3568-3577.
37. L. Jia, Y. Lu, J. Shao, X.-J. Liang and Y. Xu, *Trends in Biotechnology*, 2013, 31, 99-107.
38. F. H. Kobeissy, B. Gulbakan, A. Alawieh, P. Karam, Z. Zhang, J. D. Guingab-Cagmat, S. Mondello, W. Tan, J. Anagli and K. Wang, *OMICS: A Journal of Integrative Biology*, 2014, 18, 111-131.
39. K. Turney, T. J. Drake, J. E. Smith, W. Tan and W. W. Harrison, *Rapid Communications in Mass Spectrometry*, 2004, 18, 2367-2374.
40. K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida, T. Yoshida and T. Matsuo, *Rapid Communications in Mass Spectrometry*, 1988, 2, 151-153.
41. J. Wei, J. M. Buriak and G. Siuzdak, *Nature*, 1999, 399, 243-246.
42. B. Gulbakan, D. Park, M. Kang, K. Kececi, C. R. Martin, D. H. Powell and W. Tan, *Analytical Chemistry*, 2010, 82, 7566-7575.
43. Y. Chen and A. Vertes, *Analytical Chemistry*, 2006, 78, 5835-5844.
44. Y. Wang, Z. Zeng, J. Li, L. Chi, X. Guo and N. Lu, *J. Am. Soc. Mass Spectrom.*, 2013, 24, 66-73.
45. X. Wen, S. Dagan and V. H. Wysocki, *Analytical Chemistry*, 2007, 79, 434-444.
46. R. Arakawa and H. Kawasaki, *Analytical Sciences*, 2010, 26, 1229-1240.
47. C.-K. Chiang, W.-T. Chen and H.-T. Chang, *Chemical Society Reviews*, 2011, 40, 1269-1281.
48. S. A. Trauger, E. P. Go, Z. Shen, J. V. Apon, B. J. Compton, E. S. P. Bouvier, M. G. Finn and G. Siuzdak, *Analytical Chemistry*, 2004, 76, 4484-4489.
49. A. Nordström, E. Want, T. Northen, J. Lehtiö and G. Siuzdak, *Analytical Chemistry*, 2008, 80, 421-429.
50. Y.-F. Huang and H.-T. Chang, *Analytical Chemistry*, 2007, 79, 4852-4859.
51. B. Gulbakan, E. Yasun, M. I. Shukoor, Z. Zhu, M. You, X. Tan, H. Sanchez, D. H. Powell, H. Dai and W. Tan, *Journal of the American Chemical Society*, 2010, 132, 17408-17410.
52. I. Ocsoy, B. Gulbakan, M. I. Shukoor, X. Xiong, T. Chen, D. H. Powell and W. Tan, *ACS Nano*, 2013, 7, 417-427.
53. E. Yasun, B. Gulbakan, I. Ocsoy, Q. Yuan, M. I. Shukoor, C. Li and W. Tan, *Analytical Chemistry*, 2012, 84, 6008-6015.
54. Y. Xiong, C. Deng and X. Zhang, *Talanta*, 2014, 129, 282-289.
55. L. A. Cassiday, L. L. Lebruska, L. M. Benson, S. Naylor, W. G. Owen and L. J. Maher Iii, *Analytical Biochemistry*, 2002, 306, 290-297.
56. K. M. Keller, M. M. Breeden, J. Zhang, A. D. Ellington and J. S. Brodbelt, *Journal of Mass Spectrometry*, 2005, 40, 1327-1337.
57. K. Barylyuk, B. Gülbakan, X. Xie and R. Zenobi, *Analytical Chemistry*, 2013, 85, 11902-11912.
58. X. Guo, Z. Liu, S. Liu, C. M. Bentzley and M. F. Bruist, *Analytical Chemistry*, 2006, 78, 7259-7266.
59. E. Hong, H.-J. Yoon, B. Kim, Y.-H. Yim, H.-Y. So and S. Shin, *J Am Soc Mass Spectrom*, 2010, 21, 1245-1255.
60. V. J. B. Ruigrok, E. van Duijn, A. Barendregt, K. Dyer, J. A. Tainer, R. Stoltenburg, B. Strehlitz, M. Levisson, H. Smidt and J. van der Oost, *ChemBioChem*, 2012, 13, 829-836.
61. J. R. Engen, *Analytical Chemistry*, 2009, 81, 7870-7875.
62. G. F. Pirrone, R. E. Iacob and J. R. Engen, *Analytical Chemistry*, 2015, 87, 99-118.
63. J. Sperry, J. M. Wilcox and M. Gross, *J Am Soc Mass Spectrom*, 2008, 19, 887-890.
64. M. B. Trelle, D. M. Dupont, J. B. Madsen, P. A. Andreasen and T. J. D. Jørgensen, *ACS Chemical Biology*, 2014, 9, 174-182.
65. F. Chen, B. Gülbakan, S. Weidmann, S. R. Fagerer, A. J. Ibáñez and R. Zenobi, *Mass Spectrometry Reviews*, 2015, DOI: 10.1002/mas.21462, n/a-n/a.
66. F. Chen, B. Gulbakan and R. Zenobi, *Chemical Science*, 2013, 4, 4071-4078.
67. K. N. Morris, K. B. Jensen, C. M. Julin, M. Weil and L. Gold, *Proceedings of the National Academy of Sciences*, 1998, 95, 2902-2907.
68. M. Blank, T. Weinschenk, M. Priemer and H. Schluesener, *Journal of Biological Chemistry*, 2001, 276, 16464-16468.
69. M. Homann and H. U. Göringer, *Nucleic Acids Research*, 1999, 27, 2006-2014.
70. M. Lorger, M. Engstler, M. Homann and H. U. Göringer, *Eukaryotic Cell*, 2003, 2, 84-94.
71. H. Ulrich, M. H. Magdesian, M. J. M. Alves and W. Colli, *Journal of Biological Chemistry*, 2002, 277, 20756-20762.
72. J. G. Bruno and J. L. Kiel, *Biosensors and Bioelectronics*, 1999, 14, 457-464.
73. F. Chen, J. Zhou, F. Luo, A.-B. Mohammed and X.-L. Zhang, *Biochemical and Biophysical Research Communications*, 2007, 357, 743-748.
74. M. Labib, A. S. Zamay, O. S. Kolovskaya, I. T. Reshetneva, G. S. Zamay, R. J. Kibbee, S. A. Sattar, T. N. Zamay and M. V. Berezovski, *Analytical Chemistry*, 2012, 84, 8114-8117.

75. H. Dwivedi, R. D. Smiley and L.-A. Jaykus, *Appl Microbiol Biotechnol*, 2010, 87, 2323-2334.
76. C. L. A. Hamula, H. Zhang, F. Li, Z. Wang, X. Chris Le and X.-F. Li, *TrAC Trends in Analytical Chemistry*, 2011, 30, 1587-1597.
77. K. L. Hong and L. J. Sooter, *BioMed Research International*, 2015, 2015, 31.
78. C. Wang, M. Zhang, G. Yang, D. Zhang, H. Ding, H. Wang, M. Fan, B. Shen and N. Shao, *Journal of Biotechnology*, 2003, 102, 15-22.
79. D. Shangguan, Y. Li, Z. Tang, Z. C. Cao, H. W. Chen, P. Mallikaratchy, K. Sefah, C. J. Yang and W. Tan, *Proceedings of the National Academy of Sciences*, 2006, 103, 11838-11843.
80. K. Sefah, D. Shangguan, X. Xiong, M. B. O'Donoghue and W. Tan, *Nat. Protocols*, 2010, 5, 1169-1185.
81. X. Xiong, Y. Lv, T. Chen, X. Zhang, K. Wang and W. Tan, *Annual Review of Analytical Chemistry*, 2014, 7, 405-426.
82. J. Barman, *RSC Advances*, 2015, 5, 11724-11732.
83. N. Rifai, M. A. Gillette and S. A. Carr, *Nat Biotech*, 2006, 24, 971-983.
84. R. D. Smith, *Clinical Chemistry*, 2012, 58, 528-530.
85. V. Marx, *Nat Meth*, 2013, 10, 19-22.
86. P. Horvatovich, N. Govorukhina and R. Bischoff, *Analyst*, 2006, 131, 1193-1196.
87. A. W. Bell, E. W. Deutsch, C. E. Au, R. E. Kearney, R. Beavis, S. Sechi, T. Nilsson and J. J. M. Bergeron, *Nat Meth*, 2009, 6, 423-430.
88. G. Rosenberger, C. C. Koh, T. Guo, H. L. Röst, P. Kouvonen, B. C. Collins, M. Heusel, Y. Liu, E. Caron, A. Vichalkovski, M. Faini, O. T. Schubert, P. Faridi, H. A. Ebhardt, M. Matondo, H. Lam, S. L. Bader, D. S. Campbell, E. W. Deutsch, R. L. Moritz, S. Tate and R. Aebersold, *Scientific Data*, 2014, 1.
89. L. C. Gillet, P. Navarro, S. Tate, H. Röst, N. Selevsek, L. Reiter, R. Bonner and R. Aebersold, *Molecular & Cellular Proteomics*, 2012, 11.
90. Y. Kim, C. Liu and W. Tan, *Biomarkers in Medicine*, 2009, 3, 193-202.
91. H. Ulrich and C. Wrenger, *Cytometry Part A*, 2009, 75A, 727-733.
92. D. A. Daniels, H. Chen, B. J. Hicke, K. M. Swiderek and L. Gold, *Proceedings of the National Academy of Sciences*, 2003, 100, 15416-15421.
93. B. J. Hicke, C. Marion, Y.-F. Chang, T. Gould, C. K. Lynott, D. Parma, P. G. Schmidt and S. Warren, *Journal of Biological Chemistry*, 2001, 276, 48644-48654.
94. D. Shangguan, Z. Cao, L. Meng, P. Mallikaratchy, K. Sefah, H. Wang, Y. Li and W. Tan, *Journal of Proteome Research*, 2008, 7, 2133-2139.
95. G. Jiang, M. Zhang, B. Yue, M. Yang, C. Carter, S. Z. Al-Quran, B. Li and Y. Li, *Leukemia Research*, 2012, 36, 1347-1353.
96. T. Prebet, A.-C. Lhoumeau, C. Arnoulet, A. Aulas, S. Marchetto, S. Audebert, F. Puppo, C. Chabannon, D. Sainty, M.-J. Santoni, M. Sebbagh, V. Summerour, Y. Huon, W.-S. Shin, S.-T. Lee, B. Esterni, N. Vey and J.-P. Borg, *Blood*, 2010, 116, 2315-2323.
97. R. Chen, P. Khatri, P. K. Mazur, M. Polin, Y. Zheng, D. Vaka, C. D. Hoang, J. Shrager, Y. Xu, S. Vicent, A. J. Butte and E. A. Sweet-Cordero, *Cancer Research*, 2014, 74, 2892-2902.
98. Y. Liu, J. Chen, A. Sethi, Q. K. Li, L. Chen, B. Collins, L. C. J. Gillet, B. Wollscheid, H. Zhang and R. Aebersold, *Molecular & Cellular Proteomics*, 2014, DOI: 10.1074/mcp.M114.038273.
99. H.-W. Na, W.-S. Shin, A. Ludwig and S.-T. Lee, *Journal of Biological Chemistry*, 2012, 287, 25001-25009.
100. H. Zhang, A. Wang, S. Qi, S. Cheng, B. Yao and Y. Xu, *International Journal of Molecular Sciences*, 2014, 15, 11665-11677.
101. S. Gärtner, A. Gunesch, T. Knyazeva, P. Wolf, B. Högel, W. Eiermann, A. Ullrich, P. Knyazev and B. Ataseven, *PLoS ONE*, 2014, 9, e84472.
102. M. V. Berezovski, M. Lechmann, M. U. Musheev, T. W. Mak and S. N. Krylov, *Journal of the American Chemical Society*, 2008, 130, 9137-9143.
103. M. N. Ara, M. Hyodo, N. Ohga, K. Akiyama, K. Hida, Y. Hida, N. Shinohara and H. Harashima, *Cancer Medicine*, 2014, 3, 825-834.
104. P. Dua, H. S. Kang, S.-M. Hong, M.-S. Tsao, S. Kim and D.-k. Lee, *Cancer Research*, 2013, 73, 1934-1945.
105. L. Cerchia, C. L. Esposito, S. Camorani, A. Rienzo, L. Stasio, L. Insabato, A. Affuso and V. de Franciscis, *Mol Ther*, 2012, 20, 2291-2303.
106. P. Mallikaratchy, Z. Tang, S. Kwame, L. Meng, D. Shangguan and W. Tan, *Molecular & Cellular Proteomics*, 2007, 6, 2230-2238.
107. J. L. Vinkenborg, G. Mayer and M. Famulok, *Angewandte Chemie International Edition*, 2012, 51, 9176-9180.
108. D. Van Simaeys, D. Turek, C. Champanhac, J. Vaizer, K. Sefah, J. Zhen, R. Sutphen and W. Tan, *Analytical Chemistry*, 2014, 86, 4521-4527.
109. T. Wandtke, J. Woźniak and P. Kopiński, *Viruses*, 2015, 7, 751-780.
110. P. Parekh, Z. Tang, P. C. Turner, R. W. Moyer and W. Tan, *Analytical Chemistry*, 2010, 82, 8642-8649.
111. V. Romanov, S. N. Davidoff, A. R. Miles, D. W. Grainger, B. K. Gale and B. D. Brooks, *Analyst*, 2014, 139, 1303-1326.
112. M. Cretich, F. Damin and M. Chiari, *Analyst*, 2014, 139, 528-542.
113. S. M. Hanash, S. J. Pitteri and V. M. Faca, *Nature*, 2008, 452, 571-579.
114. G. S. Omenn, *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 2014, 1844, 866-873.
115. G. S. Omenn, *Journal of Proteomics*, 2014, 100, 3-7.
116. E. Steen Redeker, D. T. Ta, D. Cortens, B. Billen, W. Guedens and P. Adriaensens, *Bioconjugate Chemistry*, 2013, 24, 1761-1777.
117. A. Makaraviciute and A. Ramanaviciene, *Biosensors and Bioelectronics*, 2013, 50, 460-471.
118. D. Zichi, T. Koga, C. Greef, R. Ostroff and H. Petach, *Clinical Chemistry*, 2002, 48, 1865-1868.
119. C. Bock, M. Coleman, B. Collins, J. Davis, G. Foulds, L. Gold, C. Greef, J. Heil, J. S. Heilig, B. Hicke, M. Nelson Hurst, G. M. Husar, D. Miller, R. Ostroff, H. Petach, D. Schneider, B. Vant-Hull, S. Waugh, A. Weiss, S. K. Wilcox and D. Zichi, *PROTEOMICS*, 2004, 4, 609-618.
120. L. Gold, D. Ayers, J. Bertino, C. Bock, A. Bock, E. N. Brody, J. Carter, A. B. Dalby, B. E. Eaton, T. Fitzwater, D. Flather, A. Forbes, T. Foreman, C. Fowler, B. Gawande, M. Goss, M. Gunn, S. Gupta, D. Halladay, J. Heil, J. Heilig, B. Hicke, G. Husar, N. Janjic, T. Jarvis, S. Jennings, E. Katilius, T. R. Keeney, N. Kim, T. H. Koch, S. Kraemer, L. Kroiss, N. Le, D. Levine, W. Lindsey, B.

Lollo, W. Mayfield, M. Mehan, R. Mehler, S. K. Nelson, M. Nelson, D. Nieuwlandt, M. Nikrad, U. Ochsner, R. M. Ostroff, M. Otis, T. Parker, S. Pietrasiewicz, D. I. Resnicow, J. Rohloff, G. Sanders, S. Sattin, D. Schneider, B. Singer, M. Stanton, A. Sterkel, A. Stewart, S. Stratford, J. D. Vaught, M. Vrkljan, J. J. Walker, M. Watrobka, S. Waugh, A. Weiss, S. K. Wilcox, A. Wolfson, S. K. Wolk, C. Zhang and D. Zichi, *PLoS ONE*, 2010, 5, e15004.

121. J. D. Vaught, C. Bock, J. Carter, T. Fitzwater, M. Otis, D. Schneider, J. Rolando, S. Waugh, S. K. Wilcox and B. E. Eaton, *Journal of the American Chemical Society*, 2010, 132, 4141-4151.
122. S. Kraemer, J. D. Vaught, C. Bock, L. Gold, E. Katilius, T. R. Keeney, N. Kim, N. A. Saccomano, S. K. Wilcox, D. Zichi and G. M. Sanders, *PLoS ONE*, 2011, 6, e26332.
123. R. M. Ostroff, W. L. Bigbee, W. Franklin, L. Gold, M. Mehan, Y. E. Miller, H. I. Pass, W. N. Rom, J. M. Siegfried, A. Stewart, J. J. Walker, J. L. Weissfeld, S. Williams, D. Zichi and E. N. Brody, *PLoS ONE*, 2010, 5, e15003.
124. M. R. Mehan, D. Ayers, D. Thirstrup, W. Xiong, R. M. Ostroff, E. N. Brody, J. J. Walker, L. Gold, T. C. Jarvis, N. Janjic, G. S. Baird and S. K. Wilcox, *PLoS ONE*, 2012, 7, e35157.
125. R. M. Ostroff, M. R. Mehan, A. Stewart, D. Ayers, E. N. Brody, S. A. Williams, S. Levin, B. Black, M. Harbut, M. Carbone, C. Goparaju and H. I. Pass, *PLoS ONE*, 2012, 7, e46091.
126. M. A. De Groote, P. Nahid, L. Jarlsberg, J. L. Johnson, M. Weiner, G. Muzanyi, N. Janjic, D. G. Sterling and U. A. Ochsner, *PLoS ONE*, 2013, 8, e61002.
127. G. S. Baird, S. K. Nelson, T. R. Keeney, A. Stewart, S. Williams, S. Kraemer, E. R. Peskind and T. J. Montine, *The American Journal of Pathology*, 180, 446-456.
128. S. J. Kiddle, M. Sattlecker, P. Proitsi, A. Simmons, E. Westman, C. Bazenet, S. K. Nelson, S. Williams, A. Hodges, C. Johnston, H. Soininen, I. Kłoszewska, P. Mecocci, M. Tsolaki, B. Vellas, S. Newhouse, S. Lovestone and R. J. B. Dobson, *Journal of Alzheimer's Disease*, 2014, 38, 515-531.
129. M. Kimoto, R. Yamashige, K.-i. Matsunaga, S. Yokoyama and I. Hirao, *Nat Biotech*, 2013, 31, 453-457.
130. K. Sefah, Z. Yang, K. M. Bradley, S. Hoshika, E. Jiménez, L. Zhang, G. Zhu, S. Shanker, F. Yu, D. Turek, W. Tan and S. A. Benner, *Proceedings of the National Academy of Sciences*, 2014, 111, 1449-1454.
131. P. L. Urban, K. Jefimovs, A. Amantonico, S. R. Fagerer, T. Schmid, S. Madler, J. Puigmarti-Luis, N. Goedecke and R. Zenobi, *Lab on a Chip*, 2010, 10, 3206-3209.
132. U. A. Kiernan, D. Nedelkov and R. W. Nelson, *Journal of Proteome Research*, 2006, 5, 2928-2934.
133. J. D. Reid, D. T. Holmes, D. R. Mason, B. Shah and C. H. Borchers, *J. Am. Soc. Mass Spectrom.*, 2010, 21, 1680-1686.

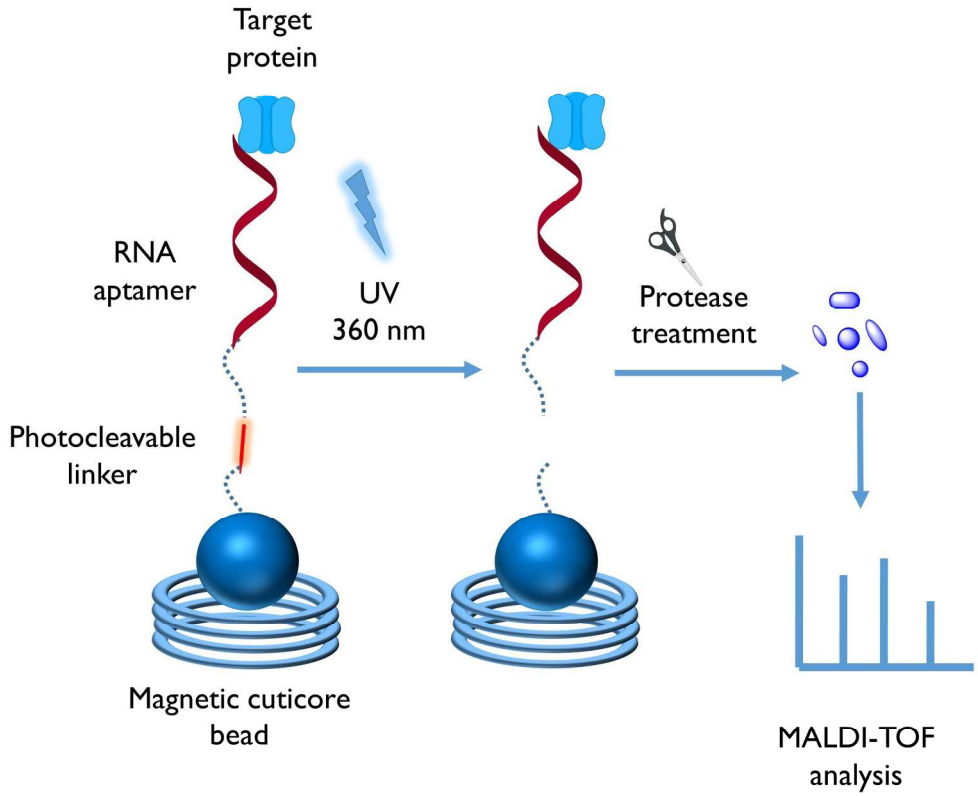


Figure 1
460x387mm (120 x 120 DPI)

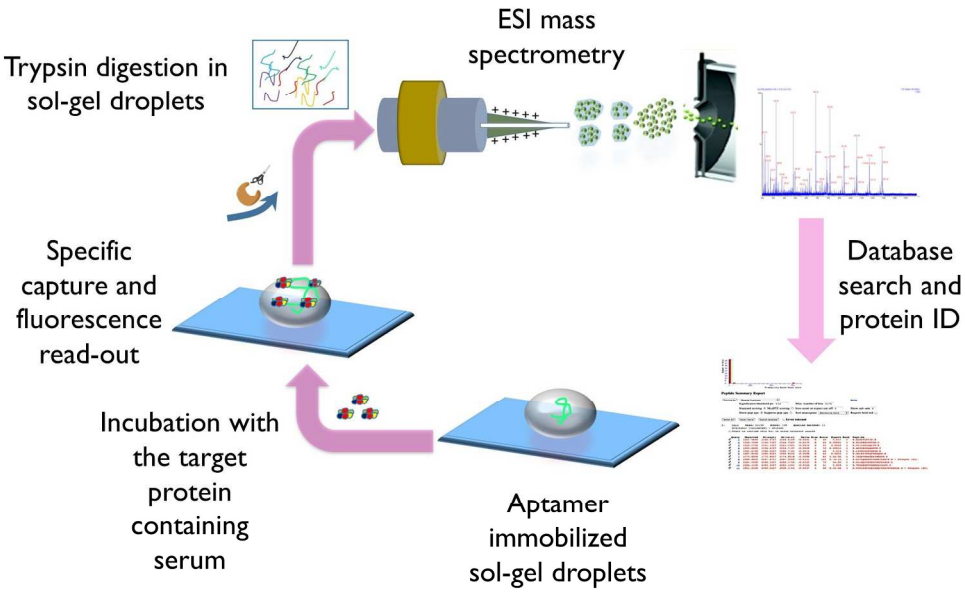


Figure 2
542x332mm (120 x 120 DPI)

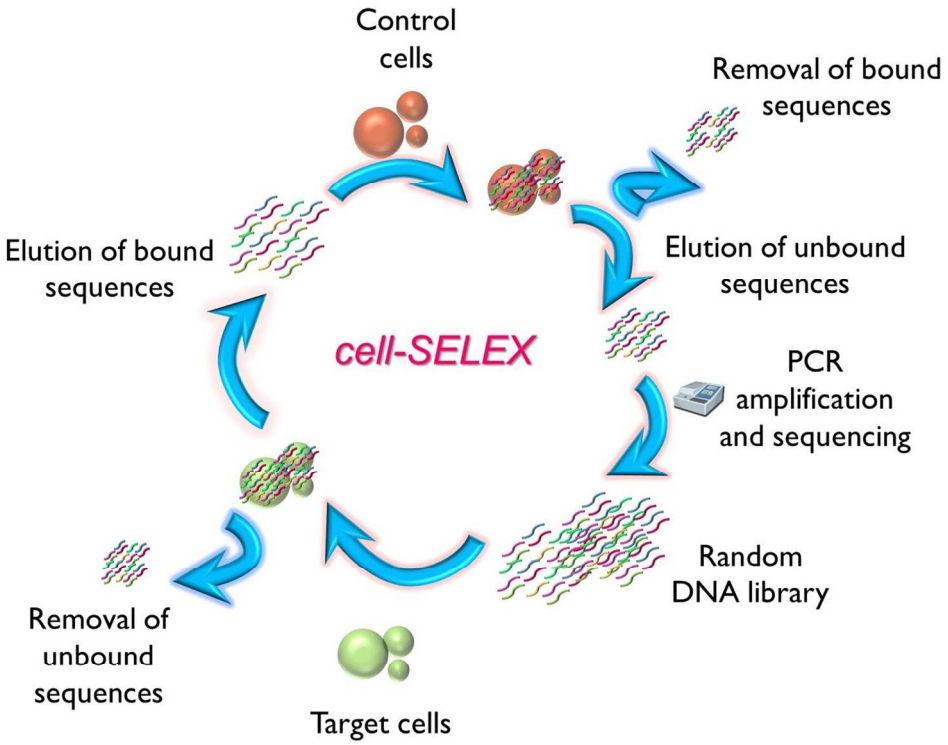


Figure 3
320x246mm (150 x 150 DPI)

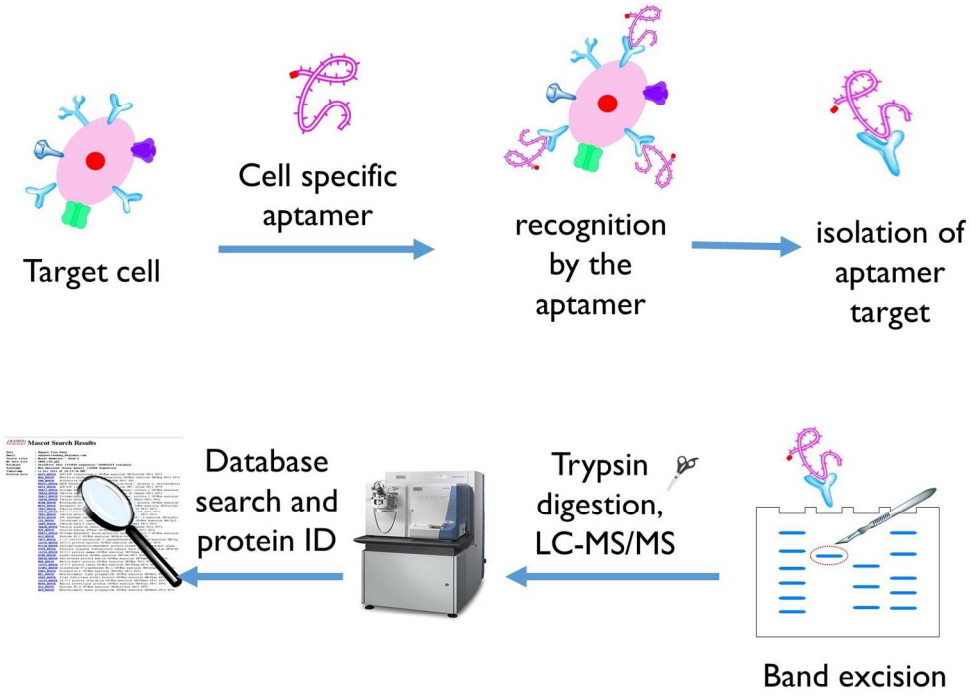


Figure 4
383x273mm (120 x 120 DPI)

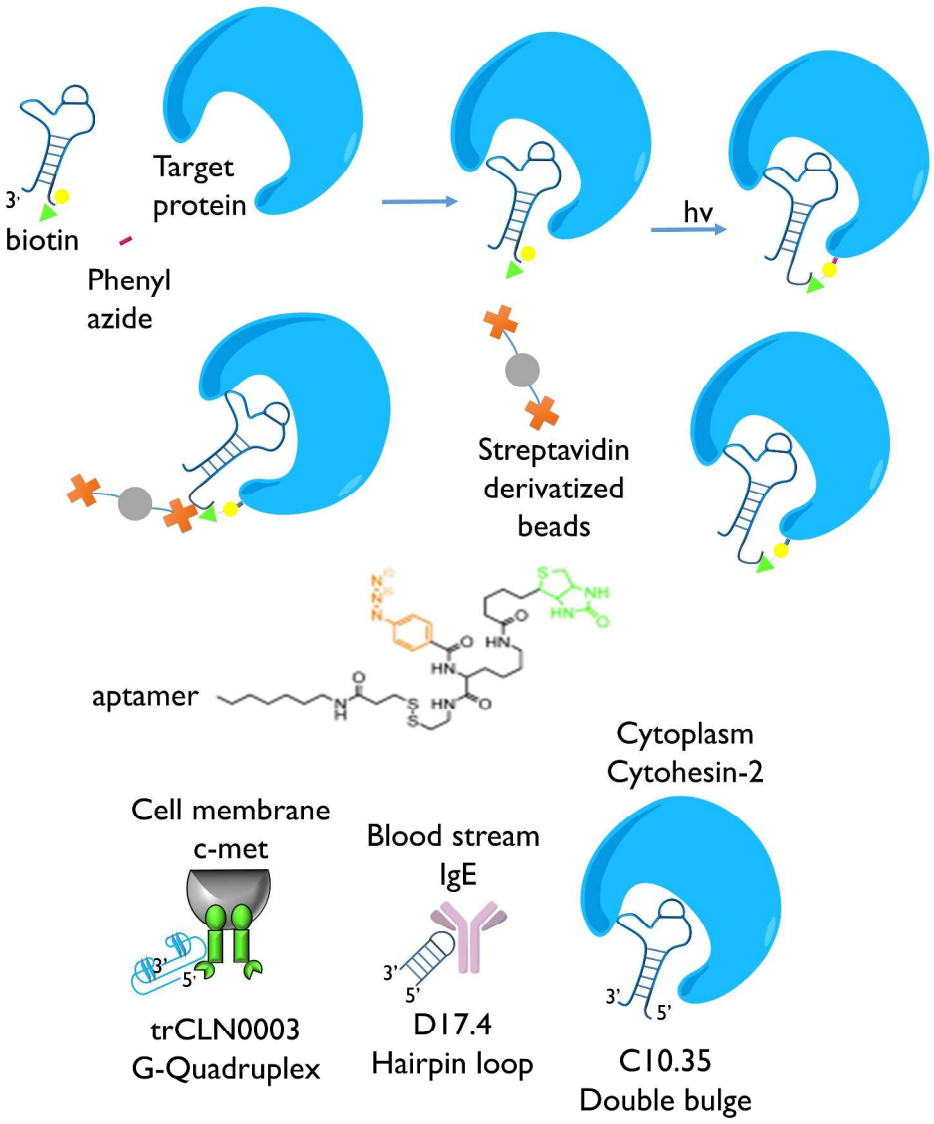


Figure 5
882x1041mm (120 x 120 DPI)

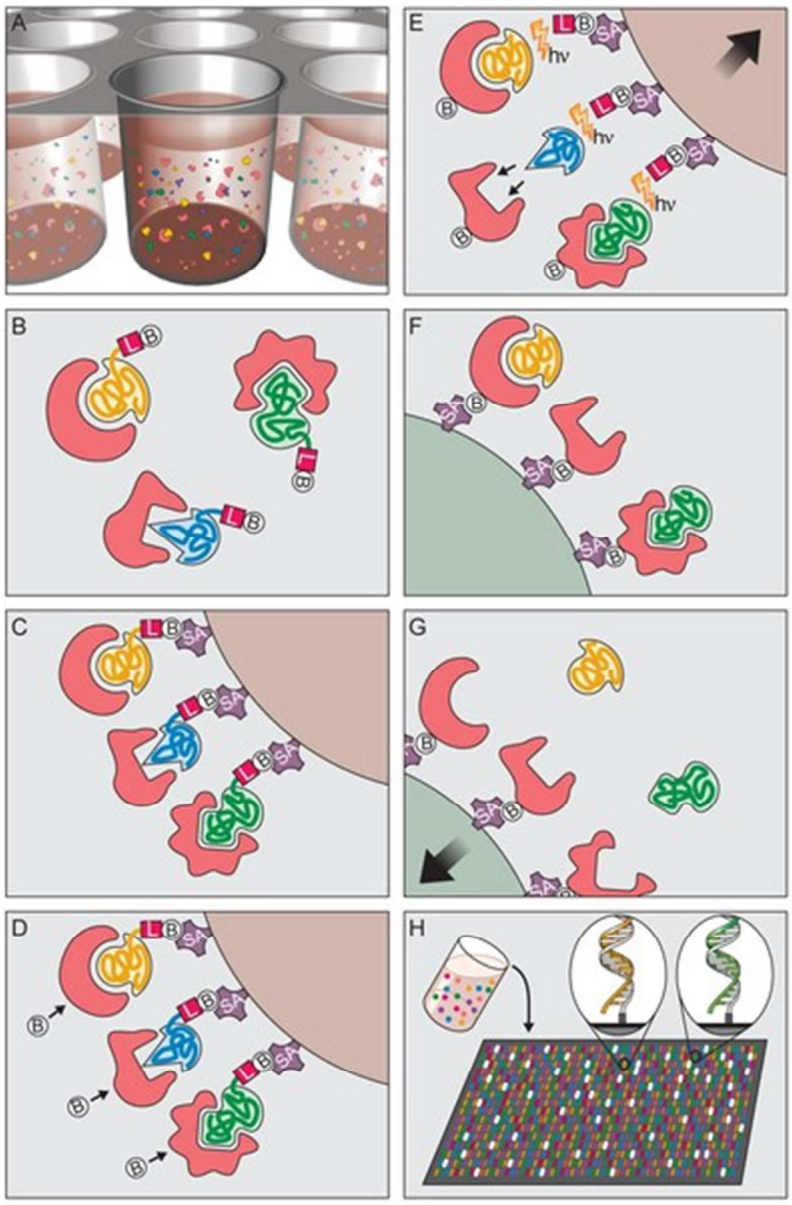
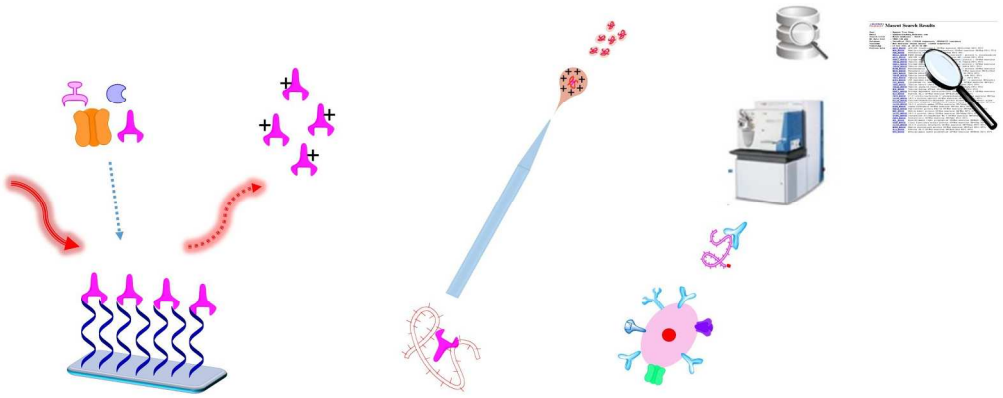


Figure 6
85x129mm (118 x 118 DPI)



Aptamer-based mass spectrometry
528x209mm (120 x 120 DPI)