Review

Modelling and development of electrical aptasensors, a short review

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Abstract: Selected by *in vitro* techniques (SELEX, cell-SELEX), aptamers are strands of DNA or RNA molecules able to bind a wide range of targets, from small molecules to live cells, and even tissues, with high affinity and specificity. Due to their efficient targeting ability, aptamers are extensively used in different fields of applications. For example, they ensure high performance as cancer-related markers or in recognizing cancer cells. Actually, they represent a promising way for early diagnosis (biosensors) and to deliver imaging agents and drugs, in both cancer imaging and therapy (therapeutic aptamers). Aptamer-based biosensors (aptasensors) have attracted particular attention over the last decades, so as the possibility of using aptamers in disease therapy in substitution of monoclonal antibodies. The paper briefly reviews the most recent literature on this topic, both concerning the advances in biomedical applications and in the development of electrical aptasensors. The investigation concerning the bioelectronics features of aptamers, to be implemented in the development of electrical nanobiosensors, is also reviewed. To this aim, some recent results of a theoretical/computational framework for modelling the electrical properties of biomolecules (Proteotronics) are reported.

Keywords: aptamer ; aptasensor ; electrical properties; networks; proteotronics

1. Introduction

Nucleic acids are of great importance due to their function in encoding, transmitting, and expressing genetic information [1]. Specific sequences of nucleic acids, referred as aptamers, possess unique binding characteristics to their targets. Usually, they are selected in an *in-vitro* process, termed *Systematic Evolution of Ligands by Exponential Enrichment* (SELEX) and, once selected, they can be synthesized with high reproducibility and purity, in large quantity. Most importantly, it should be possible to generate aptamers for virtually each protein target [2], overcoming the limitations of the needs for cell lines or animals [1].

Aptamers represent an interesting class positioned between small molecules and biologicals. To date, numerous high-affinity aptamers have been selected for a broad range of target molecules, including metal ions, peptides, drugs, proteins, and even whole cells or viruses [3-4, 1]. Furthermore, by understanding the aptamer critical sequence, it becomes much easier to produce aptamers modified for a specific target, making them much more appealing for pharmaceutical applications [1]. Finally, aptamers are very stable and can recover their active conformation after denaturation at high temperature [1].

Due to their high affinity, specificity and stability, along with the benefits originating from the chemical synthesis, aptamers have attracted attention in various applications, including their use in

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nanostructured material. Enormous progress has been made during the past two decades, so aptamers have become true alternative compounds in chemical biology and biomedicine [5].

A number of recent excellent reviews have been published [6-8] that emphasize the bioanalytical applications of aptamers, even if, it has to be pointed out that acceptance and use of aptamers in industry and by (bio)pharmaceutical companies still remains rare [5].

Aptamers are particularly attractive to formulate new targeted therapies in several diseases, like cancer, and offer a satisfactory alternative to monoclonal-antibody-based treatments. Currently, there is one aptamer available in the clinic, specific for the treatment of age-related macular degeneration [9], and 10 under clinical trials. Furthermore, the employment of biosensors using aptamers as recognition units is increasingly promising in clinical practices. A very active research issue consists in drug nanocarriers for *in vivo* applications: their use as targeted cancer therapy can reduce side effects of most chemotherapeutic drugs [9-10]. Recently, do Carmo et al. [11-12] studied a drug delivery system using aptamer against breast tumours in mice. In particular, they studied an aptamer with high affinity for the tumour marker Mucin 1 (MUC1). This aptamer, when included in nanocarriers (here polymeric particles of about 0.1 μm) efficaciously recognized cancer cells, allowing a low cytotoxicity. Furthermore [12] the aptamer nanocarriers when labelled with the radioactive isotope ^{99m}Tc have shown an effective action in detecting the tumour. The reduced side effects and the low production cost of aptamers with respect the analogue antibodies make this research of high interest for medical applications.

Schmøkel et al. [13] addressed criteria for designing an aptamer for albumin receptor interaction. They conceived an albumin-based delivery system for an aptamer that targets blood borne coagulation factor IXa, for site-specific covalent conjugation, at a site distant from the region responsible for human serum albumin's interaction. Then, they performed the investigation on that aptamer functionality and human cellular recycling neonatal Fc receptor (FcRn) engagement, using recombinant human albumin of either a wild type or an engineered human FcRn high binding variant. They conclude that the long circulatory half-life, predominately facilitated by engagement with the cellular recycling neonatal Fc receptor, and ligand transport properties of albumin, promote it as an attractive candidate to improve the pharmacokinetic profile of aptamers [13].

The aim of this short review is to provide a summary of the recent achievements in the selection, development criteria and application of aptamers, focusing on their electrochemical properties. A particular attention will be paid in describing the Proteotronics, a theoretical/computational model able to mimic and foresee the biological aptamer-protein interaction on the electronic surface of biosensors.

2. Aptamer Selection

The SELEX approach (Figure 1) is commonly used to *in vitro* select aptamers of interest in research by an iterative process of selection and amplification [14, 15 and references therein]. The process starts with a chemically synthesized random oligonucleotides library, which contains from 10^{13} to 10^{16} motifs of different sequences. For RNA aptamers, the DNA library is converted into an RNA library, before the SELEX process.

The selection consists of five steps: 1) binding, i.e. incubation of the library with the target; 2) partition, i.e. isolation of target-bound sequences from unbound ones; 3) elution from a complex *via* chromatography; 4) amplification, generation of a new pool of nucleic acids by polymerase chain reaction (PCR), for DNA libraries, or real time-PCR (for RNA libraries); and 5) conditioning, in which *in vitro* transcription and purification of relevant ssDNA are included [15].

The optimization and development of the SELEX methodology has made the range of targets wider, including small molecules, proteins, viruses, bacteria, live cells, and even tissues [15].

In recent and authoritative reviews [1, 7], many papers dealing with the traditional SELEX process are referenced, with particular attention to those describing strategies arisen for shortening the selection period, while maintaining the aptamer affinity to targets.

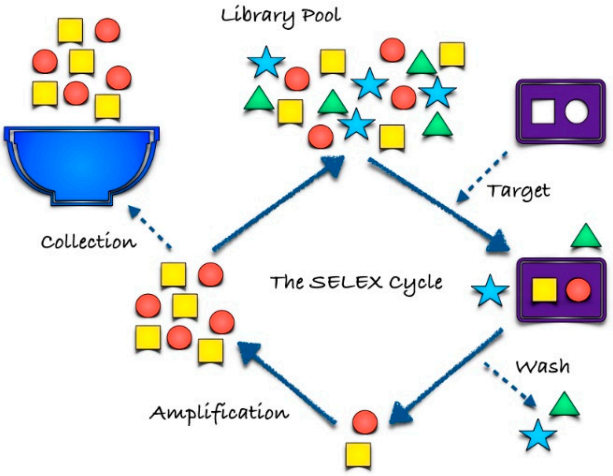

Figure 1. Schematic view of the SELEX procedure.

However, SELEX has many drawbacks, mostly linked to the inherent complexity of the process and to the limits in the application toward cancer detection. This is due, for example, to the absence of aptamers able to bind target cancer cells, or, more specifically, target proteins on the cell surface [16].

To overcome these limitations, cell-SELEX was developed and became a foundational tool widely used for aptamer selection; using this technology, Shangguan and Li [17] identified a DNA aptamer (sgc8c) targeting a T cell line (CCRF-CEM) for acute lymphoblastic leukemia diagnosis. That aptamer demonstrated ability in specifically recognizing leukemia cells in human bone marrow, aspirates in real clinical specimens [17].

The cell-SELEX ability to recognize molecular signatures also on the surface of diseased cells in complex targets, such as red blood cell membranes and whole cells, was demonstrated [18].

SELEX is typically performed using a highly purified target molecule, cell-SELEX uses whole living cells as targets instead. It ensures the selection of aptamers capable of binding the bioactive forms of target proteins on the cell surface, so making the developed aptamers highly suitable for biological applications. Moreover, there is no need to know the quantity or types of proteins on the cell surface, which brings great convenience and simplifies the selection process [1,18].

In 2013, specific aptamers were selected by using whole cancer cells as target, showing the possibility to obtain aptamers able to distinguish one type of cancer cell from another. They were able to identify particular molecular signatures, addressing methods for generating aptamers highly specialized for cancer [7]. Since then, many aptamers have been generated by cell-SELEX [17] targeting from cancer cells, virus-infected cells to bacteria. These aptamers have shown their utility in cell capture, detection and imaging, even in vivo cancer imaging [7].

The inhibition of glucagon receptor (GCGR) is a promising therapy against diabetes mellitus. In fact, when GCGR captures glucagon, a cascade set of events starts, eventually producing hepatic glucose, so arising the levels of glycemia. By using the cell-SELEX procedure, Wang et al. [18] selected a DNA aptamer, GR-3, after 16 rounds of evolved enrichment. The target of this aptamer was preliminarily verified as a membrane protein on the cell surface. It has shown that it can specifically bind those cells, with dissociative constant (K_d) value in the nanomolar range. Specifically, aptamer-mediated pull-down and *GCGR-knockdown* assay confirms that GCGR is the target molecule of GR-3. Furthermore, binding analysis reveals that GR-3 can recognize other cells, with different affinity, in accordance with the protein expression level of GCGR in these cells. A truncated sequence GR-3d maintains recognition and binding to CHO-GCGR, similar to that of full-length GR-3. Imaging of hepatic tissue suggests that GR-3 can bind the cell membrane of hepatic tissues. The Authors conclude that aptamer GR-3 against GCGR is a promising probe molecule for the treatment of

diabetes mellitus, with the advantages of small size, high binding affinity, good stability, lack of immunogenicity, easy synthesis and modification [19].

Civit et al. [20] apply three different screening techniques to characterise the binding properties of selected aptamer candidates. The aim is to optimize the selection of DNA aptamers, rarely enriched in the libraries. From the investigations, four DNA aptamers are identified that exhibit broad-spectrum interaction patterns to different cancer cell lines, derived from solid tumours [20].

Despite performing positive-negative selection cycles of aptamers, a limit of cell-SELEX is that often the selected aptamers do not display specificity for the target cells [21], in other terms they do not discriminate, or poorly discriminate between the target and the control cells [22].

In this respect, Ray and White [22] propose an additional selection pressure, by using RNAse, to remove surface-binding aptamers and select cell-internalizing ones. The experiment consists in evaluating the binding properties of a RNA sequence, identified by means of independent cell-SELEX procedures, against two different pancreatic cancer cell lines. That sequence was indicated by a strong selection pressure among the large pool of other available sequences, present in the aptamer library. The selected aptamer was not specific for the pancreatic cancer cell lines, even if a similar sequence motif was present in previously published internalizing aptamers [21, and references therein]. The identified sequence is able to form a structural motif that binds to a surface protein and exhibits strong affinity, but removing it during cell-SELEX, the probability of identifying aptamers against cell type-specific targets on the cell surface could be increased [21].

Chen et al. [23] reviewed the literature focused on aptamer application in the colonrectal (CRC) cancer. They give a paramount of nanostructures and nano-aptamer-drug delivery system functionalized with aptamers, highly efficient in CRC therapeutic applications. They summarize the potential application of aptamers as biomarkers in CRC diagnosis and therapy, together with a deep explanation of characteristics of aptamer-targeted nanocarriers in CRC [23].

3. Aptamers in electrical nanobiosensors

The identification of a disease-specific marker at an early stage significantly affects the costs and time associated to the drug development and the clinical success rate. Discovering class of diseases in which certain proteins become structurally abnormal, and thereby disrupt the function of cells, tissues and organs of the body, is particularly important in reducing the disease effects, above all when the disease is early detected [24]. To this aim, nanotechnology combined with biotechnology have been excellent in engineering devices extremely sensitive in this area [24-25].

Biosensors (Figure 2) are devices able to detect the presence of a specific analyte, by using a sensing biological element. Detection is the result of the interaction between the sensing element and the target molecule, which produces a change of a measurable physical signal, such as impedance, conductance, light, heat, etc.

The key element of a biosensor is, therefore, the sensing element, which is required to have high affinity with the target. Protein receptors and antibodies have been extensively used to this aim, over the years, and recently, also aptamers, due to the relative ease in production. Specifically, aptasensors, so as immunosensors, are applied to the detection of protein biomarkers, which may be indicative of tumour activity, for example, monitoring binding-specific changes in functionalized electrodes. Those devices appear to be particularly promising with respect to rapid, reagentless detection under realistically complex, realtime conditions [26]. In particular, biosensors using electrical transducers show to be rapid and convenient and are effective solutions in many applications, mainly for point-of-care disease detection [24].

The electrochemical aptasensors are classified into three broad classes, depending on the assay format and the method of detection: amperometric, potentiometric and impedimetric [25, and references therein].

These devices are receiving considerable attention, because of their high sensitivity and selectivity, compatibility with novel fabrication technologies, as, for example, miniaturization, low cost, disposability, minimal simple-to-operate, robust, power requirements, and independence of sample turbidity [26].

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The first electrochemical aptasensor was an amperometric sandwich device, for the detection of thrombin, by using two different aptamers, immobilized onto gold electrodes [27]. Then, new achievements have appeared in construction technologies (electrode-immobilized, redox-tagged, etc.) [28].

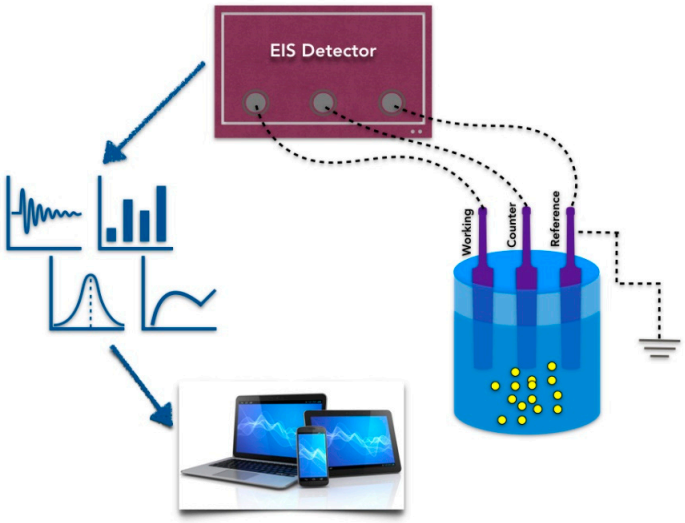

Figure 2. Schematic view of a biosensor.

As regards the detection technique, electrochemical impedance spectroscopy (EIS) is a sensitive and non-destructive technique, widely used to characterise the electrical properties of electrode-aptamer interfaces. The changes in dielectric properties, charge distribution and conductivity are represented by the impedance spectrum (Nyquist plot), whose semicircular part corresponds to an electron transfer limiting process. The diameter of the semicircle describes the interface properties of the electrode, i.e. the electron-transfer resistance.

Zhang el al. [29] investigated an electrochemical biosensor, comprising a tetracycline aptamer immobilised on nano-porous silicon electrode, for the rapid detection of tetracyclines. Electrochemical impedance spectroscopy is used to analyse the behaviour of the sensor. The specific binding of tetracycline to the aptamer biosensor led to a decrease of impedance, and the dose response was quantified with a linear behaviour. Zhang el al. [29] pointed out the importance of concentration on the signal response, enhancing/lowering the resistance value, as proving that this biosensor has the potential to be developed into a fast, simple and sensitive detection technique.

Signal generation in EIS sensor is linked to a change in electron transfer efficiency upon a binding-induced change in flexibility/conformation of the aptamer probe [28]. Pang et al. [28] detailed the effects of complementary length and position of the aptamer-DNA duplex probe on the performance of a model displacement-based electrochemical aptasensor for ATP. They found that greater background suppression and signal gain are observed with longer complementary length of the aptamer-DNA duplex probe. However, sensor equilibration time slows monotonically with increasing complementary length. Furthermore, when too many target binding sites in aptamer sequence being occupied by the complementary DNA, the aptamer-target binding does not occur and no signal gain is observed. They also demonstrated that signal gain of that sensor is strongly dependent on the complementary position of the aptamer-DNA duplex probe, with complementary position located at the electrode-attached or redox-tagged end of the duplex probe [28].

Gosai et al. [30] described how electrical stimulus can modulate the association between the thrombin and its aptamer. They investigated the binding/unbinding of the human thrombin and its 15-mer single stranded DNA aptamer, with the sequence GGTTGGTGTGGTTGG, under the application of electrostatic potential/electric field. Both experimental continuum analysis and atomistic molecular dynamics simulation were employed. Under different experimental conditions,

they observed a decrease in the free energy of binding between the thrombin and aptamer, in presence of positive electric fields. This fact is foreseen also by computations, which showed that the application of positive electric field successfully unbinds the thrombin from the aptamer, confirming the influence of electrostatic potential on the thrombin/aptamer complex, accordingly with the literature [31-32].

Khosravi et al. [33] designed and constructed a carbon nanotube micro-arrays with aptamer, as the molecular recognition element, for detection of Interleukin-6 (IL-6). This protein is primarily produced at sites of acute and chronic inflammation, and associated to a wide variety of disease, including susceptibility to diabetes mellitus and systemic juvenile rheumatoid arthritis. Single wall carbon nanotubes micro-arrays biosensors were functionalized by using different protocol: photo-lithography, metal deposition, and etching techniques. Experiments were conducted in different conditions: non-specific bovine serum albumin, nano-porous silicon samples, and anti-IgG functionalized devices. Real time response of the sensor conductance was monitored with increasing concentration of IL-6, exposure to the sensing surface in buffer solution, and clinically relevant spiked blood samples. Independently on the particular condition, similar signatures in the real time conductance versus time were obtained. Furthermore, unambiguous molecular interaction between IL-6 and nanotube micro-arrays surface was observed, at 1pg/mL concentration. This concentration falls below the diagnostic gray zone for cancer, so that it can be considered an indicator of early stage cancer. Thus, nanotube micro-arrays could potentially be developed for creating multiplexed assays, involving cancer biomarker proteins and possibly circulating tumor cells, all in a single device.

Huang et al. [34] reviewed research trends in disease-related detection with electrochemical biosensors. The focus is placed on the immobilization mechanism of electrochemical biosensors, measurement and relative materials that are used as analytical tools to this aim. They presented the literature related to methodologies in constructing devices for various diseases detection, the commercialization and their applications to clinical analysis. The important role of biosensors in clinical tests is emphasized, especially as regards the impact of electrochemical biosensors in disease-related analysis. Various measurement techniques, used to monitor the electrode, as well as the nanomaterials, magnetic beads that are used for fabrication of electrochemical biosensors were discussed. Although electrochemical biosensors have been proved to offer advantages such as simplicity, low cost, good sensitivity and selectivity, Huang et al. [34] pointed out that they are wider used in clinical laboratory instead of in research laboratory. They answered to this question that the choice of recognition element as well as optimized immobilization strategies need future research, especially in molecular diagnostics and drug delivery agents based on aptamer-nanomaterial fields [34].

Yu and Lai [35] reported the design and fabrication of a “signal-on” electrochemical aptamer-based sensor for detection of ampicillin. Ampicillin (AMP) is a penicillin-like antibiotic, effective in treating certain bacterial infections, such as pneumonia, bronchitis, and ear, lung, skin, and urinary tract infections. Quantification of AMP in biological fluids such as serum, urine, and saliva can help to determine the optimal therapeutic concentration, as well as the most effective method of administration [35, and references therein]. The signalling system is based on target binding-induced changes in the conformation and flexibility of the methylene blue-modified aptamer probe. The engineered sensor shows fast response, since all the components are surface-immobilized, and the possibility to be reused for at least three times. It demonstrates good specificity and is capable of differentiating between ampicillin and structurally similar antibiotics, such as amoxicillin. More importantly, it is selective enough to be employed directly in complex samples, including serum, saliva, and milk. As regards the optimal experimental conditions, although both alternating current voltammetry (ACV) and square wave voltammetry are suitable sensor characterization techniques, that paper shows that ACV is better suited for target analysis [35].

From the above, it is clear that challenges and perspectives of aptamer-integrated biosensors and their biomedical applications are complex and intriguing.

4. Modelling the Electrical properties of aptamers

The modelling of charge transport in biomolecule is a very intriguing topic which has received, in recent years, continuous attention [36]. The classical theory of charge transport in ordered matter needs to be improved for systems like biomolecules, which, although showing a high degree of organization, have not a crystalline structure. In particular, in systems like these, intrinsically reduced in size, a mean field approach is not significant, and new concepts have been developed to take into account the role of the specific 3D structure. Specifically, the description in terms of Block waves (extended states, ES), which substantially do not account for the space organization of the material is partially or totally substituted by introducing short range waves (localized states, LS). The transition between two LS happens with energy cost by means of tunnelling or hopping mechanisms. In such a way, at low bias and temperature, the dominant transport mechanism is performed by ES, while, increasing bias and/or temperature, the tunnelling/hopping mechanism becomes dominant. Following a backward path, in the early '90s, this kind of local description was overcome by a new proposal which introduced the concept of electron transfer by multiple pathways, a quite effective although simplified view of electronic transport, which, as a consequence, gave value to quite macroscopic aspects like protein motifs and internal interaction [37].

4.1 Complex networks for bioelectronics

In line with these studies, Proteotronics proposed a complex network approach to describe, with different levels of refinement, i.e. atoms, atomic groups, amino acids, bases, etc. [38], the single macromolecule as a set of interacting building blocks which communicate at a distance (the interaction radius, R_C). Information percolates inside the network and produces the macroscopic response as an emergent property which reflects the original biomolecule structure. This is a manifestation of *the structure and function* paradigm, therefore, when, as in many sensing proteins, the activation produces a change of structure (conformational change), also the function changes, and, in turn, this produces detectable changes in the measurable physical quantities (for example, impedance).

These mechanisms are at the basis of biosensors which detect the presence of a specific target by measuring the response of receptor samples which act as the sensing part of the device (see Figure 1).

With the aim to interpret the physical responses produced by biosensors, several years ago a novel kind of investigation in molecular bioelectronics started, receiving the name of Proteotronics [38].

Proteotronics is able to simulate the electrical response of a biomolecule by using an electrical network. The biomolecule can be a protein so as a fragment of DNA/RNA. To start the procedure, the 3D biomolecule structures are needed and, at present, resolving them is still a hard task. Some free databases are available which collect data mainly on proteins [39]. Very few is known about aptamers, which are a relatively young field of investigation but also due to their extreme flexibility in the target-free state. This is one of the reasons of the flourishing of *in silico* methods to predict the structure of proteins and aptamers. To the current state of art, they have to be meant as useful complements to traditional X-ray and NMR methods, since, at present, their predictive capability is not complete, anyway the strong activity in this field gives reason to hope in more and more efficient results [40-42].

Proteotronics uses these data as input to generate a biomolecule-analogue graph. The schematic of the method is the same for both proteins and aptamers: the analysis is performed at the level of single amino acid or nucleobase, the elementary brick, which is identified with the C_α or C_1 carbon atom. Although different choices are possible to identify the centroids of the brick [43], at this level of refinement, differences appear small. Then, collecting the coordinates of each brick and assigning a cut-off distance, R_C, a graph $G(N,L)$, with node set N and link set L is set up: all the Euclidean distances between the couples of elementary bricks are calculated, creating a distance matrix. From the distance matrix, the graph description of the network is represented through its adjacency matrix A of size $N \times N$, with elements $a_{i,j\ (i\neq j)}$ = 1/0 if a link exists or not, and $a_{i,i}$ =0, i.e. no self-loops exist.

321 *A* is a symmetric matrix, the network is undirected. A link between two nodes exists only if their
322 distance is smaller than R_C.
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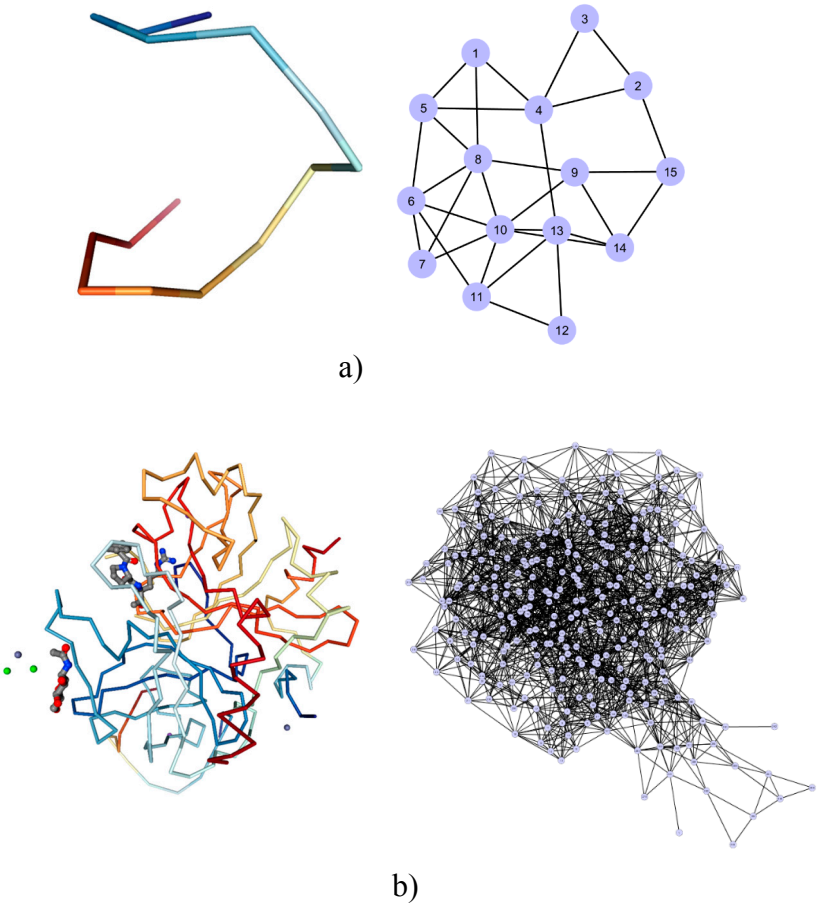

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325 **Figure 3.** (**a**) The 3D structure of the TBA in the native state (PDB entry 148D_1, [39]) on the left and
326 the corresponding graph at R_C=10.1 Å, on the right; (**b**) the 3D structure of the TBA-thrombin complex
327 (PDB entry 4DII, [39]) on the left, and the corresponding graph at R_C=10.1 Å, on the right.

328 Tuning R_C, a more or less connected graph is produced, although the nature of the graph, i.e. its
329 topological properties, is preserved [44]. In Figure 3, the sketch of the aptamer TBA alone and
330 conjugated with the thrombin is reported and compared with the corresponding graph.

331 *4.2 Resistance to evaluate binding affinity*

332 The topological graph becomes an interaction network by assuming that the links are the
333 physical routes by which the bricks communicate. Specifically, in the common applications to
334 electrical biosensors, two different kinds of electrical measurements are performed: a. at fixed bias
335 intensity with varying frequency, i.e., a standard electrochemical impedance spectroscopy (EIS)
336 measurement, which is represented by impedance spectra [45,46]; b. at fixed frequency and varying
337 bias intensity, which is represented by current (resistance)- voltage plots [47]. In both cases, these
338 measurements, specifically the variation of impedance or current are used to detect, with high
339 specificity and selectivity, the presence of a specific target. The intensity of variation is also related to
340 the target concentration. In general, the threshold of detection is quite low and sometimes very low
341 [46].
342 In the framework of the network approach, this variation is mainly attributed to the change of
343 structure subsequent to the binding of the biomolecule with the target. In some cases, like for the
344 optical receptors, the conformational change is the only structural change in the network, since the
345 target is the photon [48]. In other cases, the target is a material object and so the network changes

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substantially, since, after the sensing action, it represents not a single biomolecule but the whole complex [49,44].

In the present review we describe the results obtained by mimicking the action of an elementary RC (resistance and capacitance in parallel) impedance, Z_{ij}, which is in agreement with most of the EIS experimental outcomes. In these experiments, the capture of the target is detected by an impedance variation, which is macroscopically described by using an analogue electrical circuit, known as Randles cell, whose elements reproduce the observed measure. To obtain information about the microscopic electrical features, we proceed by analysing the experimental results and focusing the investigation on the impedance terms which effectively change during the measure [38,45]. This allows us to postulate the following expression for the elementary impedance:

$$Z_{ij} = f_{ij} \frac{\rho_{ij}}{1+i\omega\varepsilon_0\varepsilon_{ij}\rho_{ij}} \quad (1)$$

with f_{ij} a form factor, ω the frequency of the applied bias, ε_0 the dielectric constant in vacuum, ε_{ij} and ρ_{ij} are the resistivity and dielectric constants of the couple of *ij* nodes.

Concerning the values of resistivity and dielectric constant, they have been calculated as in [49] and describe the mean resistivity/dielectric constant of the couple of bricks which can be made of amino acids only, nucleobases only, or an amino acid and a nucleobase (in the binding region). Once, formally, the network is connected to the bias, the set of corresponding linear equations can be resolved and the total impedance is calculated.

The procedure is applied to the case of TBA aptamer, whose 3D structure is known in the native state (the aptamer is target-free) and also complexed with the thrombin. In particular, the complex state has been resolved in the presence of two different cations, Na^+ and K^+, both of them enforcing the stability of the structure of the aptamers, but with different results concerning the affinity, i.e. the stability of the complex is enhanced by potassium cations [50].

Solving the impedance network, the resistance spectrum can be calculated for each structure. Figure 4 shows the ratio of the resistance of the complex to the native aptamer, r_{comp}/r_{nat} , at increasing values of Rc. The region in which the response is in agreement with experiments (r_{comp}/r_{nat} >1) is well defined, between 8-12 Å, and inside it, the response of the complex resolved in the presence of sodium is definitely the smallest.

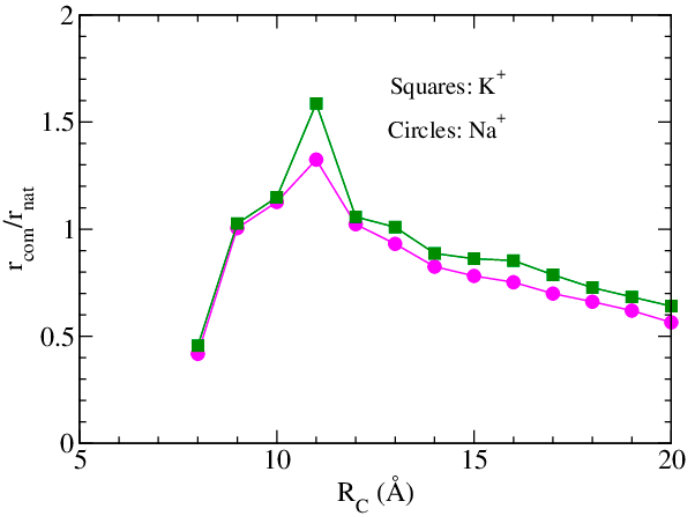

Figure 4. Resistance variation spectra for TBA in the presence of sodium and potassium. The TBA-protein complex has a resistance larger than that of the TBA alone in a range of Rc values close to 11Å. In the whole range of Rc values the resistance ratio of the complex obtained in the presence of sodium is larger than those obtained in the presence of potassium.

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Finally, the resistance measurement is proposed to estimate the binding affinity of the aptamer to its target [49, 44]. The effectiveness of this kind of test has been confirmed in a different analysis performed on a set of 5 different anti-angiopoietin aptamers [51]. Their structures were produced *in silico* with a cascade procedure able to furnish a number of possible configurations both for the aptamer alone and complexed with the protein. The mean features of these configurations were analysed by using the strategy previously outlined. In particular, and in good agreement with experiments, the resistances calculated for these aptamers are in line with the affinity performances observed in experiments. The ratio r_{comp}/r_{apt} of the resistance of the complex to the aptamer, at increasing values of R_C, is reported in Figure 5: the behaviour of the four angiopoietin-2 (Ang-2) specific aptamers is well separated by that of the only angiopoietin-1 (Ang-1) specific aptamer.

This scheme is quite general and can be used for different kinds of interactions (chemical, physical, mechanical etc.,). Till now, the modelling of several ligands, receptors, and the complexes ligand-receptor have been developed [38], and the gained experience in this field allows to understand that, in describing the ligand-receptor docking, beyond the primary role played by the conformational change, a not subordinate part has to be attributed to the change of the interaction itself [46,49,52]. As a matter of fact, on the physical point of view, biomolecules are open systems in continuous interaction with the environment, therefore, also the description of their electrical properties necessarily refers to assigned external conditions. When these conditions change (the solute kind or solute concentration, temperature, and finally the binding to a different molecule) we have to expect that this also affects the information exchange, promoting or depressing the macroscopic response.

At the level of a single biomolecule the resistance spectrum shown in Figure 4 can be interpreted as an activation spectrum, i.e. it reports to what extent the presence of the target changes the aptamer response.

At the level of a sample, the increasing concentration of target molecules changes the intensity of the response (in the case of TBA , the resistance becomes larger and larger [49]). This is an effect of two different phenomena: a. increasing the number of targets, the number of complexed biomolecules increases and, in turn, the response becomes greater; b. increasing the number of targets produces a variation of the environment which, in the network description, is expressed by a change of the value of R_C, for both aptamer and complex.

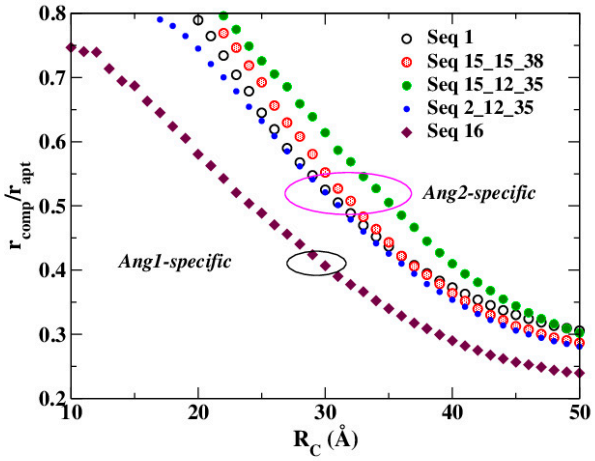

Figure 5. The resistance variation spectra of 5 different anti- angiopoietin aptamers. Circles identify the Ang-2 specific aptamers; diamonds identify the Ang-1 specific aptamer.

Specifically, we postulate that, for an assigned concentration of the target, the fraction of aptamer-protein complex is a function, g, of the interaction radius:

$$g = \frac{1}{1+bx^{-a}} \qquad (2)$$

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with $x = (R_0 - R_C)/R_0$, R_0 is the value of Rc corresponding to the absence of target, and a,b are fitting parameters [49]. The value of Rc changes with the target concentration.

Finally, the expected resistance response of the sample:

$$r_{sample} = [g \times r_{comp} + (1 - g) \times r_{nat}] \quad (3)$$

depends on the target concentration, i.e. on Rc. The spectrum of the total impedance of the aptamer alone and complexed is then calculated and represented like a standard Nyquist plot. In particular, in Figure 6 we report the Nyquist plot calculated by assuming g =0 (corresponding to the absence of thrombin), and g=0.93 which corresponds to a thrombin concentration of 1 μM [49]. The shape of the calculated Nyquist plot and its variation depends on the specificity of the ligand and on its concentration [45-47] .

4.3 Hierarchy and assortativity as a measure of binding affinity

Assortativity defines networks in which highly/lowly connected nodes are also bound to highly/lowly connected nodes. High assortativity limits information circulation in the network. In other terms, high assortativity characterizes a closed system, i.e. a system that is not able to exchange information with the environment, so as low assortativity describes an open system, i.e. a system which efficaciously exchange information with outside [44].

Hierarchy ranks node connectivity: high hierarchy means that few nodes (hubs) rule the link distribution in the network. In general, this is identified as a mark of the network weakness [53-54], since removing a small number of hubs the network becomes unstable. On the contrary, a flat structure (low hierarchy) is more stable, but also flexible and adaptable in handling changes (resilience).

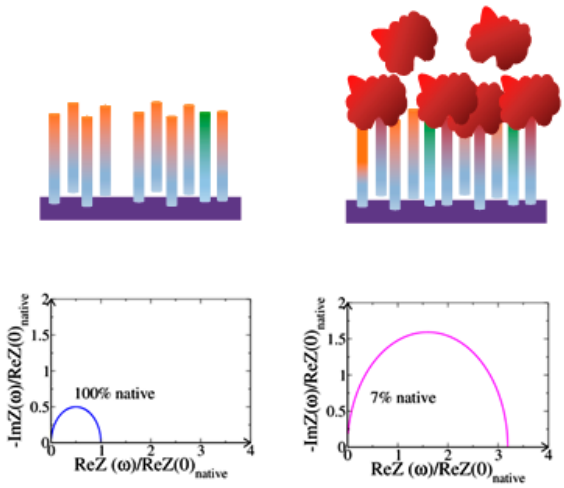

Figure 6 A sketch of an EIS measurement. On the top, an electrode functionalized with aptamers, before and after the injection of the receptor. On the bottom, the corresponding Nyquist plots calculated for a sample of aptamers in the native state and with 93% of TBA-thrombin complex, and 7% of TBA in the native state [49].

By using these two paradigms we can try to find a new key to interpret the binding affinity of aptamers and, specifically, of TBA. We observed that the network analogue of the aptamer changes its assortativity from negative to positive by adding the thrombin. It means that the network describing the aptamer complexed with the protein is less prone to external influences, i.e. it is more stable than the network describing the aptamer alone. Furthermore, when the free aptamer is in the presence of potassium, it has higher hierarchy and assortativity than the same aptamer in the presence of sodium. In other terms, the aptamer in the presence of sodium is more stable than the aptamer in the presence of potassium, i.e. it has a reduced necessity to bind the protein to enforce its stability (low binding affinity). These results are resumed in Figure 7, which gives a sketch of the

TBA hierarchy and assortativity scheme: on the left, negative assortativity, we found only the target-free structures, on the right, positive assortativity, we found only the complex structures [44]. The target-free structures have higher hierarchy with respect the complex, i.e. they are less stable.

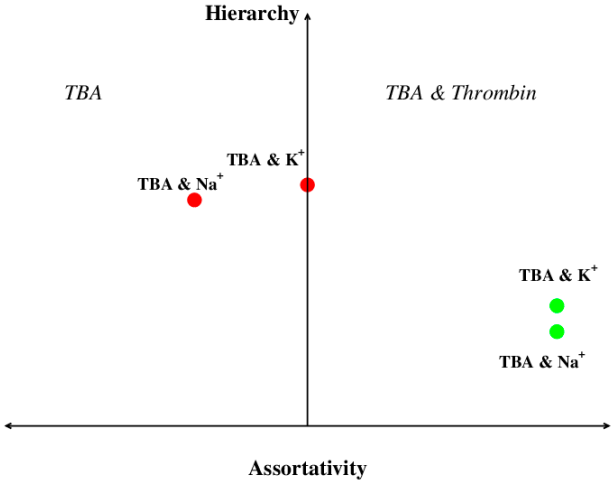

Figure 7 Hierarchy-assortativity plot of the aptamer TBA. On the left the aptamer alone, in the native state or in the configuration it assumes when bound the receptor, in the presence of two different cations. On the right the couple TBA-thrombin, in the presence of the cations.

5. Conclusion

The interest in developing biosensors is an active area of analytical chemistry and physics research, as evidenced by the amount of published papers. Biosensors for detection of various diseases have aroused a great deal of interest, so that they play a crucial role in clinical tests, as largely discussed in the literature.

Aptamers are greatly attractive, for their peculiarity to be employed in both diagnosis and therapy. This is extremely important for increasing the survival rate of cancer patients, for example, as well as the therapeutic efficiency of treatment, which could be well adjusted for individual, in general.

Electrochemical biosensors have been proved to offer advantages such as simplicity and cost-effectivity. Furthermore, they exhibit good sensitivity and selectivity, under optimized conditions. In many cases, a very low concentration of biological samples is sufficient.

Here, we highlighted the recent achievements in the selection, development criteria and application of aptamers, focusing on the impact of electrochemical biosensors, especially for disease-related analysis.

As regards the modelling of the electrical properties of aptamers, the Proteotronics approach is described. It is a theoretical/computational model, able to mimic the electronic responses given by biomolecules solicited in vitro by static and dynamic bias. We presented cases dealing with the analysis of affinity performance of the complex constituted by the aptamer and its specific ligand. Independently on the specific results, the emphasis is placed on the principles of Proteotronics that can be generalized to evaluate binding affinity in other aptamer-ligand complexes.

In conclusion, electrochemical biosensors are widely studied/used in the research laboratory, but some critical aspects, such as toxicity and pharmacokinetics, remain to be deeply investigated, before entering clinical practices. However, aptamer-nanomaterials composites are in pole position for diagnosis and therapy, as well as for imaging and drug delivery.

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