Modeling the microscopic electrical properties of thrombin binding aptamer (TBA) for label-free biosensors

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Abstract
Aptamers are chemically produced oligonucleotides, able to bind a variety of targets such as drugs, proteins and pathogens with high sensitivity and selectivity. Therefore, aptamers are largely employed for producing label-free biosensors (aptasensors), with significant applications in diagnostics and drug delivery. In particular, the anti-thrombin aptamers are biomolecules of high interest for clinical use, because of their ability to recognize and bind the thrombin enzyme. Among them, the DNA 15-mer aptamer (TBA), has been widely explored around the possibility of using it in aptasensors. This paper proposes a microscopic model of the electrical properties of TBA and of the aptamer–thrombin complex, combining information from both structure and function, following the issues addressed in an emerging branch of electronics known as proteotronics. The theoretical results are compared and validated with measurements reported in the literature. Finally, the model suggests resistance measurements as a novel tool for testing aptamer-target affinity.

Keywords: aptamers, biomolecule electrical properties, modeling, electrochemical impedance spectroscopy

(Some figures may appear in colour only in the online journal)

1. Introduction
Aptamers are target-specific single-stranded DNA, RNA or peptide sequences mainly generated on demand by an in vitro selection and amplification method called systematic evolution of ligands by exponential enrichment (SELEX), which involves repetitive cycles of binding, recovery and amplification steps [1]. These techniques ensure the production of oligonucleotides specific for targeted disease therapies [2] and medical diagnosis [3]. Recently, computational approaches have been proposed for in silico selection of aptamers with a higher target-binding ability, to be used for clinical diagnosis and medicine [4]. Aptamers can be used under a broad range of experimental conditions as a result of their thermal and chemical stability, furthermore, they can be readily synthesized in large quantities at relatively low cost [1]. This constitutes a great advantage compared to antibody [5] production, which is laborious and requires animal sacrifice. Therefore, academic and commercial interest in the field is growing, thus promising a substantial progress over the coming years [1].

Particular attention is devoted to the DNA 15-mer aptamer which binds the exosite II of thrombin, TBA, whose efficacy in inhibiting platelet thrombus formation has been known for a long time [6]. Traditional therapies against blood coagulation are based on the use of heparin and direct inhibitors like hirudin and bivalirudin [7]. The side effects of these drugs are well known, the most relevant is bleeding [8, 9]. TBA is under clinical trial and its activity and side effects can be controlled by matched antidotes [10].
Really the therapeutic aptamers tested to date have shown good safety margins between the pharmacologically effective dose and the toxicologically established no-adverse-effect levels [11]. Moreover, due to the facility of production and amplification, TBA, as well as many other aptamers, has been recently used in the design of several biosensors, also known as aptasensors [12–14]. Aptasensors are interesting tools offering convenient methodologies for detecting and measuring the levels of specific proteins in biological and environmental samples whose detection, identification and quantification can be very complex, expensive and time consuming [1].

Among aptasensors, a significant role is played by label-free biosensors, designed with high detection sensitivity and selectivity [15]. In particular, electrochemical transduction of biosensors using aptamers as bioreceptors include methods like electrochemical impedance spectroscopy (EIS), differential pulse voltammetry, alternating current voltammetry, square wave voltammetry, potentiometry or amperometry [15, 16].

Xu et al [17] proposed an EIS method for aptamer modified array electrodes as a promising label-free detection for immunoglobulins E (IgE). They found lower background noise, decreased non-specific adsorption, and larger differences in the impedance signals due to the small size and simple structure of the aptamers in comparison to the antibody [17]. Cai et al [18] proposed an aptamer-based biosensing assay for label-free protein detection and quantification by measuring the change in electrochemical impedance upon protein–aptamer complex formation, monitoring the interfacial electron transfer resistance with electrochemical EIS. TBA showed high binding affinity and specificity to its target protein and the impedance detection assay was able to achieve a reliable and sensitive quantification of thrombin [18].

Although much work has been made in last 20 years a deep understanding of the mechanisms of TBA–thrombin recognition and capture is far to be reached.

This paper proposes a microscopic model of the electrical properties of the aptamer–thrombin complex, combining information from both structure and function of the biomolecule. Hereafter we use the term biomolecule for indicating both the aptamer and the aptamer–protein complex.

In particular we analyze the results of the experiment performed by Cai et al [18] that investigated the in vitro response of TBA samples to different thrombin concentrations.

Those experimental data are here described within the framework of proteotronics, i.e. a structure-function representation of the aptamer, able to connect its electrical response at thrombin growing concentration, considering both its structure modification and its internal energy variation [19, 20]. In particular, we argue that the single aptamer electrical response, as well as that of the single enzyme, are well reproduced by an impedance network analog. The differences between these two networks are in the static electrical properties which are specific for the chosen biomolecule. Furthermore, the aptamer–enzyme complex is itself represented by an impedance network and the global electrical response is due to the cooperative action of the component networks.

The present approach strongly improves previous models [21–26]. It aims to verify whether a procedure born for proteins fits well also for different kinds of biomolecules, and whether a single impedance network is a valid model of the expected global electrical response of a biological compound. This novel model takes advantage of recent data concerning some electrical properties of aptamers, i.e. polarizability [27] and resistivity [28]. Furthermore, the proposed electrical description gives a novel powerful tool for testing the aptamer–protein affinity. The results clearly show that a resistance measurement is able to recognize whether the protein–aptamer complex has been performed in the presence of K+ or Na+ ions. Since the complex formed in the presence of K+ is more stable [29], resistance measurements are proposed as a novel tool for resolving different affinities.

As far as we know, there is not another electrical microscopic modeling formulated for aptamers.

2. Materials

The thrombin binding DNA aptamer, 5′-GGT TGG TGT GGT TGG-3′ is a SELEX product able to bind the blood coagulation enzyme thrombin. Its tertiary structure has been resolved with crystallographic [29, 30] and computational [4] techniques.

In performing our investigations we considered:

a. TBA in the native state (without the enzyme, in the wild configuration), hereafter called TBA\textsubscript{nat};

b. TBA complexed (with the enzyme, in the binding configuration), hereafter called TBA\textsubscript{com};

c. TBA in the active state (without the enzyme, in the binding configuration), hereafter called TBA\textsubscript{act}.

The 3D structure we used for describing TBA\textsubscript{nat} is the PDB entry 148D [30, 31] which is an NMR product with 12 different outputs. For TBA\textsubscript{com} we used the entries 4DII and 4DIIH [29, 31], x-ray structures of the aptamer–protein complex resolved in the presence of potassium and sodium ions, respectively. Different effects exerted by Na+ and K+ on the inhibitory activity of TBA are also analyzed within our model. The TBA\textsubscript{act} structures were obtained by cutting off the protein from those of TBA\textsubscript{com}.

The tertiary structures were used as the input of the model shown in section 3.

3. Methods

3.1. Experiment

The experiments performed by Cai et al [18], used Au electrodes functionalized with the aptamer 5′-thiol-TTT TTT GGT TGG TGT GGT TGG-3′ whose core is TBA, and incubated with different concentrations of thrombin. After
incubation electrochemical measurements were performed and the impedance spectra recorded. The plane representation of this spectrum is known as Nyquist plot. The semicircle diameter of the plot gives the electron transfer resistance \( R_{ct} \). The formation of the TBA–thrombin complex monotonically enlarges the value of \( R_{ct} \).

Our aim is to reproduce these data by using the model proposed in the following.

3.2. Theory

The electrical response is here described by using a model that conjugates structure and function of the single biomolecule. This choice aims to mimic a fundamental paradigm in biology, i.e. tertiary structure induces function and vice versa. Accordingly, the model resolution is at the level of the single amino acid/nucleobase. Previous experience gained with sensing proteins (olfactory receptors, opsins, enzymes) has shown that this level of resolution is sufficient for reproducing the electrical properties measured in macroscopic devices [19, 20].

Biomolecule structure is reproduced by using a graph. By taking the C\(_1\) and C\(_\alpha\) carbon atoms as the centroids of each nucleobase/amino acid, their position is mapped into the position of a node of the graph. Then, two nodes are connected to form a link only if their distance is smaller than an assigned interaction radius, \( R_C \).

Figure 1 (a) represents the graph of the TBA\(_{nat}\) (PDB entry 148D_1, \( R_C = 10.1 \) Å). Figure 1 (b) shows the graph of the TBA\(_{com}\) (PDB entry 4DII, \( R_C = 10.1 \) Å).

Each link mimics a specific electrical interaction, as observed in experiments [19, 20]. Specifically, the impedance measurements performed by Cai et al [18], are interpreted in terms of a macroscopic Randles cell made of 4 passive elements, i.e., the electron-transfer resistance, \( R_{et} \), the solution resistance, the double-layer capacitance, \( C_{dl} \), and the Warburg impedance, which is the linear part of the lowest frequency range and represents the diffusion-limited electron-transfer process [18].

In the actual experimental design the Warburg impedance is not foreseen, thus the thrombin injection changes the values of only two of these elements, say \( R_{et} \) and \( C_{dl} \), connected in parallel. Therefore, only \( R_{et} \) and \( C_{dl} \) were taken into account for modeling the electrical properties of the biomolecules. In other words, each link is replaced by an elemental impedance, describing the charge transfer and/or the charge polarization across the aptamer or the aptamer–protein complex. Since this is the relevant information for analyte detection, this is also the only electrical interaction we take into account. Therefore, each link is replaced by an elemental impedance, describing the charge transfer and/or the charge polarization across the aptamer or the aptamer–protein complex.

The elemental impedance is that of an \( R_C \) parallel circuit:

\[
Z_{a,b} = \frac{l_{a,b}}{A_{a,b}} = \frac{\rho_{a,b}}{1 + \frac{i\rho_{a,b} \varepsilon a,b \beta 0}{\omega}}.
\]

where \( A_{a,b} = \pi (R_C^2 - l_{a,b}^2/4) \) is the cross-sectional area between spheres of radius \( R_C \) centered on the \( a \)th and \( b \)th node, respectively; \( l_{a,b} \) is the distance between these centers, \( i = \sqrt{-1} \) is the imaginary unit, \( \varepsilon_0 \) is the vacuum permittivity, \( \omega \) is the circular frequency of the applied voltage, \( \rho_{a,b} \) is the resistivity of the \( a \)th, \( b \)th node couple. The relative dielectric constant of the couple of \( a \)th and \( b \)th node, \( \varepsilon_{a,b} \), is expressed in terms of the intrinsic polarizability of each isolated amino acid/nucleobase, \( \alpha_{elec} \).
In particular,

\[
\varepsilon_{a,b} = 1 + \frac{4\pi}{6} (\alpha_{\text{elec},a} + \alpha_{\text{elec},b}).
\]

The resistivity of the \(l\)th nucleobase is \(\rho_l = \rho \delta_l\), where \(\rho\) is the mean value calculated upon the AGCTU set [28]. The resistivity of the link between the \(a\)th and the \(b\)th nucleobase is defined as

\[
\rho^N_{a,b} = \frac{\rho (\delta_a + \delta_b)}{2}.
\]

Analogous data are not given for amino acids, therefore we assume that their resistivities are proportional to \(\bar{\rho}\), \(\rho^A = \gamma \bar{\rho}\), with \(\gamma\) the same for all the amino acids. The possible value of \(\gamma\) will be briefly discussed in the following. The link resistance of a couple of amino acids is, again, \(\rho^A\). For both relative dielectric constants and resistivities, we assume that previous formulas also hold in the aptamer–thrombin contact regions in which amino acids and nucleobases are connected between them. In particular, for the couple of the \(a\)th nucleobase and \(b\)th amino acid, the link resistance is:

\[
\rho^N_{a,b} = \frac{\rho (\delta_a + \gamma)}{2}.
\]

In other terms, we assume the concept of a linear superposition of the effects, i.e. each couple of nodes has a specific value of polarizability and resistivity which depends only on the selected nodes. The presence of the other nodes in the network or the position of the selected couple does not affect this result. This also means that, in this study, the electric and dielectric effects of water and dissolved electrolytes are explicitly not considered. It is well known that this matter is quite hard to investigate also by using the effective methods of molecular dynamics calculations [32]. In particular, the presence of solvent may play a role in the dielectric properties of the biomolecules and has been described by using both distance-dependent permittivity models and continuous permittivity models [33]. The inclusion of solvent mainly affects the surface regions of the molecules and may be described like a non-uniform distribution of polarizabilities inside it [27, 33, 34]. In particular, the polarizability values are larger close the surfaces (due to the presence of water) and smaller inside the protein. Finally, concerning the binding, the solvent effect is described by an increasing of the solvation energy of the conformations with larger surface areas, i.e. by favoring the conformations with smaller surface areas [33].

For a biomolecule small as the TBA, the concept of surface area is not well defined and the choice of using the isolated polarizabilities for the whole aptamer is not in contrast with the presence of the solvent, because it acts uniformly on the biomolecule. For the sake of simplicity, the same choice has been made for the protein and the aptamer–protein complex. On the other hand, the role of electrolytes in the formation of the aptamer–protein complex is here implicitly considered, by analyzing the electrical properties of complexes obtained in the presence of two different cations. Finally, as far as we know, an explicit role of solvent in the electrical transport properties is not verified.

The network is connected to an external bias by means of ideal contacts put on the first and last nucleobase and electrically solved by using standard techniques. In particular, by using the node Kirchhoff’s law, the problem statement is expressed by a set of linear equations that are numerically solved by a standard computational procedure [20, 35, 36].

Concerning the process of TBA–thrombin conjugation, it is here described by mimicking protein activation due to the specific ligand. The mechanism of protein activation is a long

**Figure 2.** (a) The relative resistances of the aptamer activated in the presence of potassium ions and in the native state. The active state is taken by the PDB entry 4DII [29], while the native states come from the PDB entry 148D [31]. Calculations are performed at growing values of \(R_c\). The structures of the native states which produce the largest differences with respect the activated state (1, 5, 6, 8) are in black. (b) The relative resistances of the aptamer activated in the presence of sodium ions and in the native state. The active state is taken by the PDB entry 4DII [29], while the native states come from the PDB entry 148D [31]. Calculations are performed at growing values of \(R_c\). The structures of the native states which produce the largest differences with respect the activated state (1, 5, 6, 8) are in black.
time debated problem [38–41]. The most recent models go beyond the lock-and-key scheme, by considering protein activation as the result of multiple pathways along and between energy funnels [25, 37–41]. Each energy funnel is built up on a specific stable (ground) state, the native state (native funnel) or the binding state (binding funnel), for example. Analogously, we argue that when an aptamer folds from the molten state to the native state, its free energy, so as its conformational entropy, becomes smaller and smaller. This process is described by the aptamer sliding down an energy funnel (the native funnel), toward its minimum energy state (the native state). The interaction with thrombin may produce the binding or simply a variation of its free energy. In terms of the energy funnel description, the aptamer changes its state from native to binding, moving from the native funnel toward the binding funnel, or it stays in the initial funnel, but goes toward a higher energy state. In the impedance network analog, both these kinds of dynamics go with a change of the value of the interaction radius, \( R_C \). This is also in analogy with protein dynamics, since in going up and down among the states, the protein changes the number of its internal bonds, retaining only those useful for stabilizing its final configuration [40].

Furthermore, when a sample receives its specific target, in general and if the target concentration is not huge, only a fraction of the aptamers binds the target and this is at the origin of the dose-response mechanism.

In particular, the dose-response given by experiments may be reproduced by using a Hill-like relation between the fraction of aptamer–protein complex, \( f \), and the value of \( R_C \) [25]:

\[
 f = \frac{x^a}{b + x^a}, \tag{5}
\]

where \( x = (R_0 − R_C)/R_0 \), \( R_0 \) is the value of \( R_C \) corresponding to \( f = 0 \) i.e. a sample consisting only of TBAs in their native state, and \( a \) and \( b \) are numerical fitting parameters.

Finally, the sample resistance is given by:

\[
 r_{\text{sample}} = N \times \left[ f \times r_{\text{com}} + (1 − f) \times r_{\text{nat}} \right], \tag{6}
\]

where \( N \) is the number of aptamers in the sample, \( r_{\text{nat}} \) and \( r_{\text{com}} \) are the single aptamer and the single aptamer–thrombin complex resistances, respectively.

4. Results

4.1. General results: single biomolecule

As a first step, we calculated the global resistances of \( TBA_{\text{nat}} \) and \( TBA_{\text{act}} \) at increasing values of \( R_C \). Numerical results are reported in figures 2(a) and (b).

We found that all the 12 native structures show a resistance smaller than that of the active state, in particular for \( R_C \) values close to 10 Å. Specifically, \( TBA_{\text{act}} \) in the presence of both \( \text{Na}^+ \) and \( \text{K}^+ \), exhibits a relative resistance variation, \( r_{\text{rrv}} = r_{\text{act}}/r_{\text{nat}} − 1 \) greater than \( 300\% \) when compared with the four native structures 148D_1, 148D_5, 148D_6, 148D_8.

Such a greater resistance of \( TBA_{\text{act}} \) with respect to \( TBA_{\text{nat}} \) indicates it has a more disconnected or dilated structure. Since we are interested in selecting one couple of structures with large resistance resolution, as shown by experiments, we focus on the first sequence of the native states, 148D_1, whose \( r_{\text{rrv}} \) value is intermediate among that of the four aforementioned native structures. We notice that the results are quite independent on the kind of ions used for resolving the \( TBA_{\text{act}} \) structures.

Thrombin significantly changes these outcomes. To this purpose, figures 3(a) and (b) report \( r_{\text{rrv}} = r_{\text{com}}/r_{\text{nat}} − 1 \) of the structures \( TBA_{\text{com}} \) and 148D_1.

The presence of the thrombin strongly reduces the differences observed in previous simulations, i.e., by using \( TBA_{\text{com}} \) instead of \( TBA_{\text{act}} \), \( r_{\text{rrv}} \) falls from about 300% to 50%
for the complex obtained in the presence of K$^+$ and to 25% for the complex obtained in the presence of Na$^+$.

As a matter of fact, thrombin completes the aptamer network, thus enhancing the number of connections and reducing the value of global resistance. This happens in different ways for the two structures, thus revealing that the binding is different.

In particular, the formation of the aptamer–thrombin complex is promoted by the presence of K$^+$ [29]. Therefore, this result suggests resistance measurements as a complementary test of the aptamer–thrombin affinity.

We have also tested the role of the resistivity proportionality factor $\gamma$, introduced in section 3 (see figures 3(a) and (b)). By increasing this value, the resistance of the aptamer–enzyme complex grows, as expected. On the other hand, this variation is not so massive to definitely suggest a value of $\gamma$ larger than 1.

### 4.2. Comparison with experimental dose-response

To compare theory and experiment, it is necessary to rescale the results obtained for the single biomolecule to the sample size. In particular, as described in the Theory subsection, we conjecture that, by increasing the thrombin concentration, the number of aptamers in the complexed state becomes larger and that also the free energy of each aptamer becomes higher.

Accordingly, we select $R_0 = 13.3$ Å as the interaction radius useful to calculate the resistance of the single TBA in the native state. This value is the largest corresponding to the region in which, in agreement with experiments, the resistance of TBA$_{com}$ is larger than that of TBA$_{nat}$ (see figure 3). The sample resistance is given by equation (6) with $f = 0$, up to $N$. When thrombin is added, the free energy of each aptamer changes and the $R_C$ value decreases [25, 37]. Equation (5) produces the fraction of aptamer–thrombin complexes, $f$, corresponding to the new value of $R_C$, to be used in equation (6). Finally, equation (6) allows us to reproduce the resistance variation of the sample as given by experiments (dose-response). The rate $r/r_0$ of the sample electron-transfer resistance, $R_{et}$, measured for different thrombin concentrations with respect the sample $R_C$ without thrombin, the corresponding values of $R_C$ and the fraction, $f$, of aptamer–thrombin complexes are summarized in table 1.

The corresponding Nyquist plots, as calculated within this model, are drawn in figure 4. In particular, we report the Nyquist plot corresponding to a sample in which all the aptamers are in the native state, $R_C = R_0 = 13.3$ Å, and the Nyquist plot of a sample with the 93% of aptamers binding thrombin and $R_C = 11.3$ Å, as given by equation (5).

Finally, the plot of the Hill-like equation (5), is drawn in figure 5. The fitting parameters are $a = 2.99$ and $b = 2.7 \times 10^{-4}$. In the inset of this figure we report the relative

| Concentration (M) | $r/r_0$ | $R_C$ (Å) | $f$ |
|------------------|---------|-----------|-----|
| $1.0 \times 10^{-6}$ | 3.2 | 11.3 | 0.93 |
| $1.0 \times 10^{-8}$ | 2.1 | 11.5 | 0.90 |
| $1.0 \times 10^{-10}$ | 1.8 | 11.7 | 0.87 |
| $1.0 \times 10^{-12}$ | 1.2 | 12.7 | 0.26 |

Note: First column indicates the thrombin concentration in molar, by Cai et al experiment [18]; column 2 is the ratio between the resistance ($r_0$) of a sample not exposed to the thrombin, and the resistance ($r$) of a sample at the thrombin concentrations indicated in column 1 experiment. Column 3 gives the values of the interaction radius ($R_C$) used for calculating the fraction $f$ of aptamer–protein complexes theory, column 4 gives the $f$ values (see equation (5) theory.)
difference \( r_T/r_R - 1 \) of the sample resistance measured at different concentrations of thrombin, \( r_T \), and the resistance of the reference sample (without thrombin), \( r_R \), as function of the \( R_C \) values we used for fitting the percentage of aptamer–thrombin complex.

5. Discussion and conclusions

Aptamers are a new promising group of bioreceptors, because of their outstanding selectivity, sensitivity and stability [15]. Especially aptamer-based clinical label-free devices are widely recognized as the new frontier in diagnosis as well as in therapy. Unfortunately, poor information is at present available concerning the mechanisms of aptamer activation, so going deeper in this field is extremely challenging.

In this paper we detailed and tested a microscopic model useful for the description of the electrical properties of aptamers and aptamer–protein complexes, by using analog impedance networks. The plug-in of aptamer and protein activates an electrical communication between them which results in the modification of the electrical response of the aptamer alone.

In particular, we analyzed the electrical response of three different structures, the aptamer in the native state, \( TBA_{nat} \), and the aptamer in the binding state, without the thrombin, \( TBA_{act} \), and with the thrombin, \( TBA_{com} \). The relative resistance variation, \( r_{rel} = r_{act}/r_{nat} - 1 \) calculated for \( TBA_{nat} \) and \( TBA_{act} \) is large, when compared with experiments but when the protein is added (\( TBA_{act} \) is substituted by \( TBA_{com} \)) it becomes comparable with experiments. This result suggests that the resistance growth is not only a passivation effect due to the addition of a hydrophobic layer of proteins [18], but that the role of the specific structural docking is relevant, since the protein completes the impedance network and reduces the resistance which would have been measured in a sample with the aptamers activated but not bound to the protein.

Furthermore, the model predicts that a resistance measurement is a good test of the protein–aptamer affinity. The model is validated on EIS measurements performed by Cai et al. [18] on TBA samples exposed to growing concentration of thrombin.

Finally, monitoring the change of \( R_R \) obtained from EIS promises a sensitive and reliable protein label-free biosensor. The interpretation of these data by using a simple network impedance is a new success of proteotronics methods. All these findings could accelerate the development of label-free biosensors of practical relevance.

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