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Communication

Transmission Line Model Analysis of Transcription Factors Binding to Oligoduplexes - Differentiation of the Effect of Single Nucleotide Modifications

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Advanced impedance spectroscopy analysis based on the transmission line model (TLM) is explored as a novel QCM acoustic biosensing platform for the detection of single point mutations effect on the binding of transcription factor (TFs) to immobilized DNA oligoduplexes and the characterization of the protein-DNA mechanical properties.

The transcription factors (TFs) are proteins that probe the nucleotide sequence of a gene promoter region, bind specifically to its recognition element (RE) and control the gene transcription. Mutations in the RE can thus result in genetic-related diseases, such as cancer [1] and immunological disorders [2], or in the incapacity of a cell to respond to certain stimuli, such as an environmental change [3,4]. The study of the TFs binding to DNA is highly demanded for instance to characterize the binding kinetics, to identify the RE nucleic acid sequences and to assess the effects of mutations on these. Reliable analytical methods and tools are thus needed to test the TFs binding to DNA. To date the interaction of TFs and DNA has been studied by electrophoretic mobility shift assay [5], fluorescence spectroscopy [6], circular dichroism [7], atomic force microscopy (AFM) [8], surface plasmon resonance (SPR) [9], and quartz crystal microbalance (QCM) [9,10]. Among these, biosensors based on the QCM and SPR technologies have become very popular mostly due to the suitability for detection in real time without the need for any labeling procedure. In particular, QCM can be used both to study binding kinetics to quantify association and dissociation constants[11–13] but also to characterize and detect alterations of the mechanical properties of the immobilized biomolecules [10,14].

We have established a novel QCM bioanalytical method based on the transmission line model (TLM) to analyze the binding of TFs to immobilized DNA oligoduplexes[10]. This method rely on the real-time monitoring of impedance spectra, which changes are related with the variations of the dynamic shear modulus and thickness of the biological films [10]. To the best of our knowledge, the present work is the first use of this methodology to study the effect of DNA point mutations on the recognition mechanism of transcription factors. The biological model used is based on the interaction between the *Saccharomyces cerevisae* transcription factor Haa1 with immobilized DNA strands[5,15–17]. Gene and genomic transcription regulation in the yeast model provides an excellent platform to understand the TFs binding to DNA. Indeed, the YEASTRACT database (http://www.yeastract.com) provides to the public up-to-date information on 206.299 documented regulatory associations between TFs and target genes, including 326 DNA binding sites for 113 TFs[18]. Among these DNA binding sites is the Haa1-responsive element (HRE) (5'-(G/C)(A/C)GG(G/C)G-3') which is a part of the promoter region, for instance, of the Haa1-regulated *TPO3* gene that is recognized *in vitro* and *in vivo* by Haa1[5,15–17]. Haa1 has an important role in yeast adaptation and tolerance to stress induced by acetic and propionic acids [15,17,19], with potential implications in the biotechnology and food-processing industries. Because the heterologous expression of the full-length Haa1 in *Escherichia coli* is difficult and leads to very low yield of purified protein, the interaction assays with immobilized DNA oligoduplexes were carried out with a peptide that comprises solely the DNA-binding domain of Haa1 ($Haa1_{DBD}$; mapped to the 123 N-terminal residues). The interaction of this peptide with the HRE motif was proved to mimic the interaction observed with the full-length Haa1 TF [5]. The $Haa1_{DBD}$ used for protein-DNA interaction assays was over-expressed in *Escherichia coli* and purified by affinity chromatography, as described before [5].

Four different 38 bp DNA oligoduplexes were used. The HRE_{wt} contains the Haa1 wild-type RE, and the mutants contain a single point mutation on the RE in its 5th nucleotide (a guanine) starting from the 5'-end (as underlined in the full oligonucleotide sequence, see notes†). In the mutant HRE_{m1} this guanine was replaced by a cytosine, while in the mutant HRE_{m2} it was replaced by an adenine. As control we designed HRE_{neg}, which consists in a random DNA sequence.

10 MHz QCMs from International Crystal Manufacturing Company were cleaned, activated with a mixture of 10% biotin-PEG disulfide (LCC Engineering & Trading) and 90% 11-hydroxy-1-undecanethiol (Dojindo) and saturated with streptavidin (Roche). The oligoduplexes were ordered with a biotin tag in the forward primer 5'-end (Sigma-Aldrich, see sequence details on notes) to mediate their immobilization in the streptavidin-coated surface of the QCM electrodes (Fig. 1).

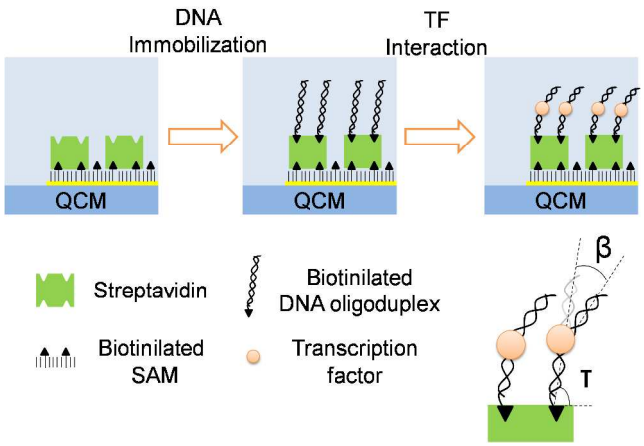

Fig. 1 Schematic representation of the QCM methodology for real-time detection and assessment of transcription factors interaction mechanics. The biosensor consists in DNA oligoduplexes probes previously immobilized on sensor surface via SAM gold modification and biotin-strepavidin affinity coupling. These probes are oriented with a tilting angle (τ) away from the surface. The interaction of the studied transcription factor, Haa1, with DNA oligoduplexes containing the specific recognition element is known to promote a bending angle (β) in the DNA structure. We have designed other DNA oligoduplexes with single point mutations to evaluate their influence in the interaction mechanics.

The assays were run at a constant flow rate of 100 µl.min^{-1} using Tris buffer pH 8.0 (10 mM Tris, 100 mM KCl, 0.005 % Tween20) as running buffer and the binding of Haa1$_{DBD}$ at 50 nM to each DNA oligoduplex was monitored. The temperature was controlled with a PID controlled system with a resolution of 0.1ºC. No temperature changes, which may induce significant changes of the viscosity or the density of the liquid media or biomolecules, were observed during the course of the experiments. A schematic diagram of this experimental procedure is represented in Fig. 1. The complex reflection coefficient (S_{11}) was acquired with an Agilent 4395A impedance analyzer and used as an input for the application of a mathematical algorithm based on TLM [10]. This algorithm enables the calculation of the dynamic shear modulus (G^*_{film}) and film thicknesses (h_{film}) of the immobilized biological layers at the sensor surface [10]. The accuracy of the calculated data is certainly dependent on the accuracy and resolution of the algorithms used that depends on the resolution of the measured data. Thus, the measurement settings (power, span, IFBW, number of points) were selected based on an optimum balance between admittance spectra resolution and reading time and also minimizing uncertainty of the original data in terms of signal to noise ratio. Furthermore, to avoid systematic errors the data presented is the result of three independent replications, including the respective independent S11 and algorithm solutions.

The density of the immobilized biomolecules layers is a required input parameter to the model [10]. We used the weighted density of the films at the QCM sensor surface (Table 1), estimated from the densities of water (d=1000 g.dm^{-3}), of the proteins (d=1350 g.dm^{-3}), and of the DNA (d=1700 g.dm^{-3}) [9,20], from structural considerations and stoichiometry determination of the number of immobilized molecules at equilibrium[10],as detailed in the electronic supporting information.

The TLM calculated mechanical properties of the QCM biosensors with immobilized HRE$_{wt}$, HRE$_{m1}$, HRE$_{m2}$ or HRE$_{neg}$ are summarized in Table 1. The magnitude of the dynamical shear modulus ($|G^*_{film}|$) as well as the h_{film} are similar for all these DNA oligoduplexes, which was expected given the identical contour length of the immobilized DNA oligoduplexes (12.92 nm). Therefore, these results demonstrate the good method reproducibility and robustness. We determined that at equilibrium, the 38 bp DNA oligoduplexes adsorb in average with a h_{film} of 10.8±0.8 nm and tilting angle of 57.2º±6.0º, presenting a $|G^*_{film}|$ of 91.4±4.9 kPa.

Table 1- Summary of the extracted mechanical parameters using the mathematical algorithm based on TLM for characterization of the different tested biomolecular films.

Film	Density (g cm^{-3})	G^*_{film} (kPa)	h_{film} (nm)	Tilting (º)	Bending (º)
HRE$_{wt}$	1180 ± 10	87.9 ± 3.8	11.6 ± 0.2	63.5 ± 1.8	NA
HRE$_{m1}$	1160 ± 36	95.9 ± 3.3	10.6 ± 1.4	55.9 ± 10.2	NA
HRE$_{m2}$	1157 ± 31	86.5± 8.6	11.2 ± 0.1	59.9 ± 1.0	NA
HRE$_{neg}$	1150 ± 26	95.3 ± 4.6	9.8 ± 0.9	49.6 ± 6.5	NA
HRE$_{wt}$-Haa1$_{DBD}$	1213 ± 6	148.5 ± 6.6	8.4 ± 0.6	NA	39.0 ± 6.0
HRE$_{m1}$-Haa1$_{DBD}$	1193 ± 25	156.0 ± 13.8	9.5 ± 1.4	NA	18.4 ± 1.6
HRE$_{m2}$-Haa1$_{DBD}$	1197 ± 21	99.1 ± 11.0	10.6 ± 0.1	NA	9.3 ± 0.8
HRE$_{neg}$-Haa1$_{DBD}$	1157 ± 31	89.6 ± 6.0	11.2 ± 1.2	NA	NA

NA – not applicable

The binding of the TFs to the specific DNA RE was proven to significantly change the mechanical properties of the immobilized DNA film which becomes more ordered and compact when complexed with the TF peptide [10]. As previously published [10], we can monitor these alterations of the film mechanical properties when the Haa1$_{DBD}$ peptide binds to the

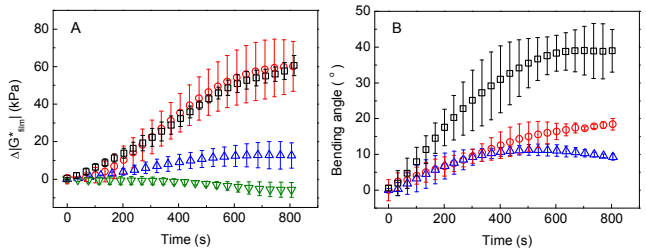

Fig. 2 - Monitoring of the $Haa1_{DBD}$ interactions with HRE_{wt} (□), HRE_{m1} (○), HRE_{m2} (Δ) or HRE_{neg} (▽). The determinations of the dynamical shear modulus (A) and the DNA bending angle (B) variations have shown that binding mechanism of the $Haa1_{DBD}$ with DNA oligoduplexes differing in point mutations can be distinguished using QCM technology. The curves represent the averaging of three independent experiments as explained on the electronic supporting information.

immobilized DNA oligoduplexes. These alterations are expected to be significantly different for the HRE_{wt} and HRE_{neg}, but the single point mutated sequences HRE_{m1} and HRE_{m2} introduced a new detection threshold challenge of mechanics differentiation. Indeed, it was previously shown by SPR measurements that the equilibrium dissociation constant of the Haa1-HRE complex is higher for HRE_{neg} (K_D>10000 nM) than for HRE_{m2} (K_D=6780.0±865 nM), HRE_{m1} (K_D=38.5±4.5 nM) and HRE_{wt} (K_D=2.0±0.2 nM) [5]. Here, the QCM data analysis of the binding of $Haa1_{DBD}$ peptide to the wild type (HRE_{wt}), mutated (HRE_{m1} and HRE_{m2}), and negative control (HRE_{neg}) oligoduplexes with the mathematical algorithm based on the TLM [10] clearly shows that these different kinetics [5] are associated with significant differences in the layers mechanical properties.

In Fig. 2A we represent the change over time of the $|G^*_{film}|$ for the binding of $Haa1_{DBD}$ at 50 nM to the immobilized oligoduplexes. These transients reveal that the binding of $Haa1_{DBD}$ to its specific HRE_{wt} and point-mutation HRE_{m1} sequences (higher kinetic affinity) always results in stiffer films against QCM mechanical oscillation showing higher $|G^*_{film}|$ as compared with binding to HRE_{m2} and HRE_{neg} (lower kinetic affinity). In fact, this is in accordance with previous observations of the $Haa1_{DBD}$ interaction with an immobilized random and nonspecific DNA sequence (HRE_{neg})[10].

The mechanism of TFs binding to its RE is known to be initiated by an electrostatic attraction to the DNA and TF's sliding through the nucleotide sequence, probing the pattern of hydrogen bond donors and acceptors present laterally in the purine and pyrimidine aromatic nucleobases. The later are easily accessible to the TF in the major groves of the DNA double helix or becomes exposed through a bending of the DNA structure at the minor groves [23]. The initial sliding process was observed during the initial 100 s after starting the adsorption of the $Haa1_{DBD}$ peptide. As shown in Fig. 2 the lag variations of the $|G^*_{film}|$ (Fig. 2A) and bending angle (Fig. 2B) occurred during this phase. After the initial sliding and probing process, it is commonly accepted that the TF mechanism of binding to DNA goes through the establishment of hydrogen bounds and water release from the hydration spheres of both interacting molecules. This is expected to lead to higher film viscosity, which is signaled by the QCM-TLM as an increase of $|G^*_{film}|$ for the HRE_{wt}, HRE_{m1} and HRE_{m2} (Fig. 2A). Another effect of the specific binding of the TF to the DNA REs is the forced bending of the DNA structure, particularly if the RE is located in a minor grove of the DNA structure. As such, the degree of DNA bending can be used to identify the specific binding of the TFs and possibly revealing also the location of the REs in the minor grove. As shown in Fig. 2B, the specific binding of the $Haa1_{DBD}$ to HRE_{wt} resulted in a significant structural bending (>30º), suggesting thus the RE location at a minor grove. Consistent with this hypothesis, Ace1, a close homologue of Haa1, has been found to contact DNA through minor groove interactions [24]. The estimated structural bending is however lower for the immobilized oligoduplexes containing a single nucleotide modification (Fig.2B). It is conceivable that the nucleotide modification for HRE_{m1} and HRE_{m2} alters the pattern of available chemical groups to establish hydrogen bonds with the aminoacids of the $Haa1_{DBD}$ peptide thus interfering with its binding to the oligoduplexes. In the case of the negative control (HRE_{neg}) the $|G^*_{film}|$ decreases, while the film thickness increases and thus it is not possible to estimate a bending angle of the oligoduplex structure. This reveals that there is an established link between the mechanical properties of the $Haa1_{DBD}$ binding to immobilized oligoduplexes and the binding kinetics described elsewhere [5].

Conclusions

The complex impedance analysis of QCM is a reliable and efficient technique to infer about biological phenomena occurring at the sensor surface. When associated with the TLM, the QCM is suitable to characterize the mechanical properties changes associated to the binding of TFs to DNA. Single point mutations on the DNA recognition element can be detected. The model establishes a link between molecular interaction kinetics and mechanics, extending the application of QCM to more than mass variation sensing and qualitative assessment of viscoelasticity changes.

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Notes and references

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† The complementary oligoduplex sequences were 5'-[biotin]-TTC TCT GTG CTT GGC **GAG GGG** TTT ACT GGA GCC CAA TC and 5'-GA TTG GGC TCC AGT AAA **CCC CTC** GCC AAG CAC AGA GAA-3' for HRE_{wt}; 5'-[biotin]-TTC TCT GTG CTT GGC **GAG G<u>C</u>G** TTT ACT GGA GCC CAA TC -3' and 5'- GA TTG GGC TCC AGT AAA **C<u>G</u>C CTC** GCC AAG CAC AGA GAA -3' for HRE_{m1}, 5'-[biotin]-TTC TCT GTG CTT GGC **GAG G<u>A</u>G** TTT ACT GGA GCC CAA TC -3' and 5'- GA TTG GGC TCC AGT AAA **C<u>T</u>C CTC** GCC AAG CAC AGA GAA -3' for HRE_{m2}.

Electronic Supplementary Information (ESI) available: experimental set-up details, estimation of the density of the layers, determination of tilting and bending angles, and experimental data processing See DOI: 10.1039/c000000x/

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