Electronic Supplementary Material (ESI) for Analyst.

Electronic Supporting Information

A – Experimental Setup

The QCM crystal sensors were mounted on a 25 µL acrylic cylindrical flow cell which was costume designed and optimized by computational fluid dynamics tools. A syringe pump system (Cetoni GmbH, Germany) and a selector valve (Valco Instruments, USA) were connected to it and used to control the flow onto the acoustic QCM sensor cell. During the experiments, the flow cell was placed inside a homemade environmental chamber with a peltier element connected to a 5C7-19S PID temperature controller (OVEN industries, USA) set to 25.0ºC. QCMs were connected to an impedance analyzer (Agilent 4395A) for complex reflection coefficient (S_{11}) monitoring and impedance spectroscopy analysis. The impedance analyzer was interfaced with a computer through a GPIB-USB acquisition board from National Instruments.

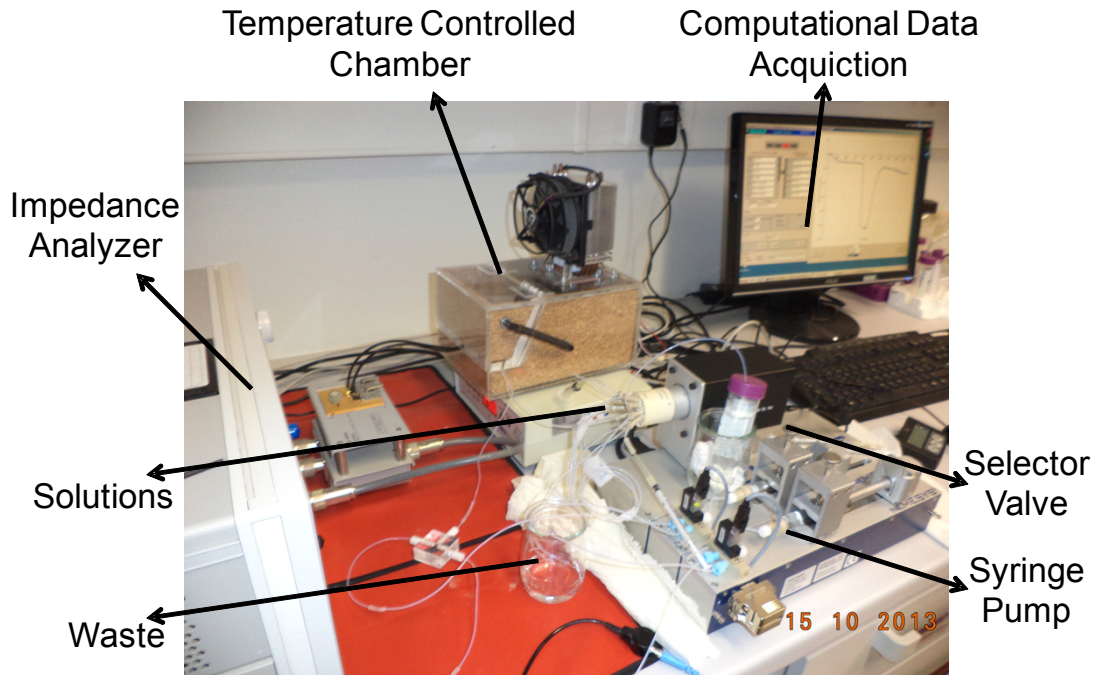

Fig. S1 – Experimental setup apparatus used for the monitoring of biomolecular interactions.

B – Estimation of the biological layer density

The biological layer density is an important input value in the mathematical algorithm for the mechanical parameters extraction. It can be obtained using alternative techniques or published values in the literature. We have decided to estimate an effective density because our biological layers are binary (DNA + buffer) or ternary (DNA + protein + buffer) mixtures and it is assumed that its density value changes along the adsorption transient.

The quartz crystal microbalance (QCM), respond linearly with the increase of the mass deposited (m) at the sensor surface as described by the Sauerbrey equation:

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\rho_{quartz}\mu_{quartz}}}\Delta m \qquad \text{(S1)}$$

where f_0 is the fundamental resonance frequency, ρ_{quartz} (2651 g.dm^{-3}) is the quartz density and μ_{quartz} (2.947x10^{11} g.cm^{-1}.s^{-2}) is the quartz shear modulus.

This ideal response is however affected by the loss of energy of the propagating acoustic wave due to interfering interfacial factors [13]. When detecting analytes in liquid environments, the viscoelasticity of the DNA biomolecules contributes to the measured resonance frequency variations. So, we have applied the Sauerbrey equation for the streptavidin layer, which is known to rigid, thin and compact. The determination of streptavidin adsorbed mass allows the calculation of number of protein molecules. Thus, it becomes possible to determine indirectly by the reaction stoichiometry of 1:2 (strepavidin:biotin) the number of immobilized DNA oligoduplexes.

$$n^{o}_{DNA} = 2\,N_A\frac{m_{strept}}{M_{strept}} \qquad \text{(S2)}$$

where, N_A is the Avogadro constant (6.022x10^{23} molécules.mol^{-1}) and M_{strep} is the streptavidin molecular weight (52.8 kDa).

The area occupied by DNA oligoduplexes (A_{DNA}) can then be obtained using the published diameter of the double helix (2 nm). The remaining electrode area is occupied by the buffer (A_{buffer}). Thus, the effective density of the DNA oligoduplexes layer can be estimated by:

$$d_{eff,DNA\ layer} = \frac{d_{DNA}A_{DNA} + d_{buffer}A_{buffer}}{A_{electrode}} \qquad \text{(S3)}$$

where d_{DNA} is the published buoyant density of DNA (1700 g.dm^{-3}) and d_{buffer} is assumed similar to water density, 1000 g.dm^{-3}.

An analogous estimation can be performed using the layer volume instead of the surface area. Nevertheless, since the layer thickness at this point is still undetermined, and because it would be equal for both components of the binary mixture, it is redundant.

The density estimation for the ternary mixture is more difficult. Here, we assumed that $Haa1_{DBD}$ adsorption on DNA layers behaves accordingly the Sauerbrey equation for its mass determination. Molecular modeling based on the primary aminoacid sequence was performed for the estimation of the $Haa1_{DBD}$ diameter. The transcription factor adsorbed mass and theoretical diameter allow the estimation the volume of water in the layer being replaced by protein. Hence, it becomes possible to estimate the effective density of the ternary mixture by:

$$d_{eff,DNA+TF\ layer} = \frac{d_{DNA}V_{DNA} + d_{Haa1_{DBD}}V_{Haa1_{DBD}} + d_{buffer}V_{buffer}}{V_{total}} \qquad \text{(S4)}$$

where the $d_{Haa1DBD}$ (1350 g.dm^{-3}) is the published density of buoyant density of protein. The volumes of DNA (V_{DNA}) and buffer (V_{buffer}) are calculated using the film thickness determined by applying the algorithm based on TLM of the preceding reference data point.

These effective densities are calculated for the equilibrium state. Therefore, their values during the adsorption transient are normalized by the variation of the resonance frequency.

$$deff,n = deff,eq\frac{\Delta f_n}{\Delta f_{eq}} \quad \text{(S5)}$$

where the index n represents a time data point during the adsorption transient and eq represents the data in equilibrium.

C – DNA oligoduplexes tilting and bending angle determination

A mathematical algorithm based on the transmission line model was applied for impedance spectroscopy data analysis to determine film thicknesses. The theoretical contour length based on the DNA molecular model is higher than the film thicknesses obtained for the 38bp DNA sequences (HRE_{wt}, $HRE_{m1,}$ HRE_{m2} and HRE_{neg}). This indicates that DNA adsorbs with a tilting angle (τ) between the center of the DNA strand and the surface (Fig. S2). Considering the contour length (2a) and the film thickness (h_i) we can apply the trigonometric equation for τ estimation:

$$\sin(\tau) = \frac{h_i}{2a} \quad \text{(S6)}$$

The interaction of the transcription factor with the DNA oligoduplexes provoked a decrease of the film thickness (h_f), which is in accordance with general accepted biological model for TF binding to DNA describing a bending of the DNA structure in the recognition motif. This bending angle (β) can also be estimated, since this motif is located at the center of the HRE sequence (a - half of the contour length). More, it is known the initial and final thicknesses. So, this allows the calculation of the opposite side of the triangle (b) formed with β (Fig. S2):

$$b = h_f - h_i/2 \quad \text{(S7)}$$

Similarly, the angle of such triangle (φ) can be determined using the trigonometric equation:

$$\sin(\varphi) = \frac{b}{a} \quad \text{(S8)}$$

The projection of a normal vector on the angles τ and β allow the identification of a common angle (ζ). Since there are right angles, the bending angle can be estimated through the equations:

$$\tau + \zeta = 90° \quad \text{(S9)}$$

$$\beta + \varphi + \zeta = 90° \quad \text{(S10)}$$

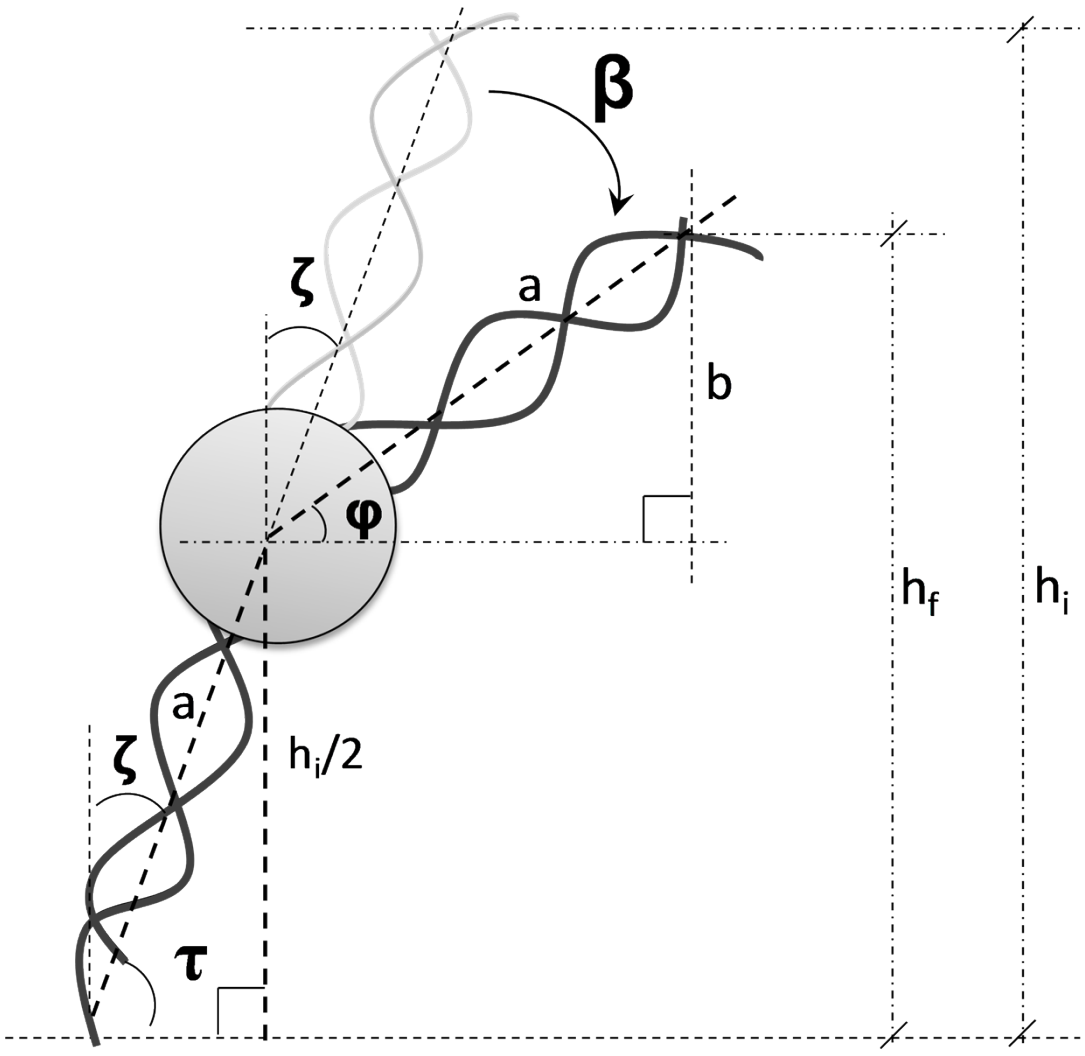

Fig. S2 - Scheme used to estimate the DNA bending angle after $Haa1_{DBD}$ protein binding. h_i and h_f are the thickness of the film before and after TF binding, respectively, and 2a is total contour length of the DNA. τ is the DNA tilting angle and β is the DNA bending angle.

D –Processing and presentation of experimental data

The mechanical parameters of the different complexes Haa1-HRE were calculated through the TLM algorithm, with a temporal range of approximately 800 seconds. For each Haa1-HRE complex were performed three independent experiments. However, the data do not always coincide in triplicate to the same measurement times. Thus, in order to obtain an average response and its deviation, a 6^{th} order polynomial fitting was performed to the experimental data by the least squares method. The average curves were obtained and the respective errors calculated from the standard deviation. Figure S3 shows an example of the polynomial fittings made to $\Delta|G^*_{film}|$ for $Haa1_{DBD}$-HRE_{wt} complex. For all variations of G^*_{film} and bending presented in the main paper the same procedure was performed.

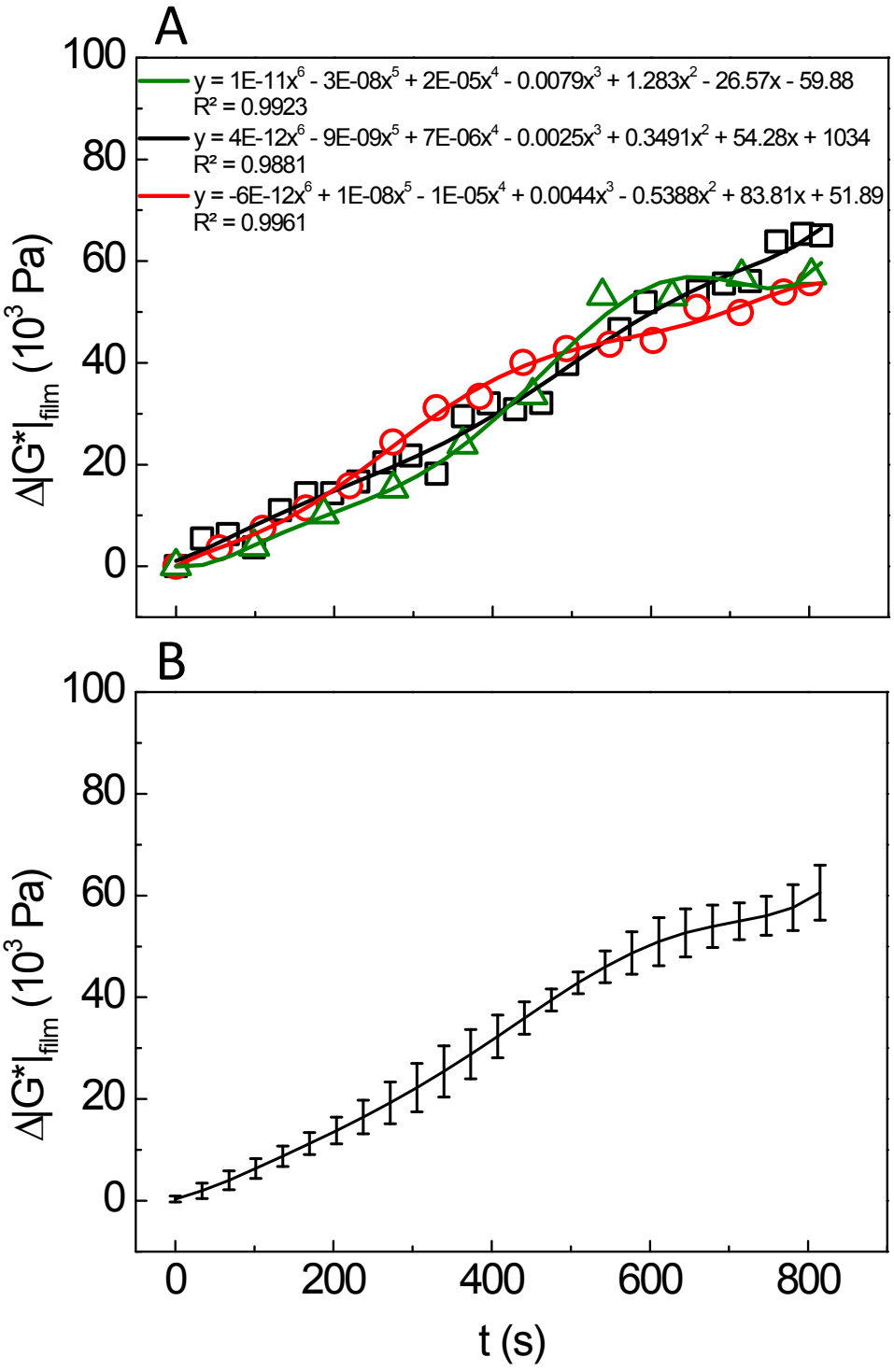

Fig. S3 - A) Calculated values for $\Delta|G^*_{film}|$ to Haa1-HREwt complex formed on the surface of QCM sensor as a function of time. The 6th order polynomial curves (line) were fitted to each of three experiments (symbol) using the least squares method. B) Average curve $\Delta|G^*_{film}|$ obtained from the three polynomial fittings.