Supporting Material

Biosensor Regeneration

We explored the strong affinity of streptavidin to biotin to construct the presented biosensor. As explained in the manuscript (see scheme 1 and associated discussion), this constitutes the molecular base for the biosensor surface functionalization. After the immobilization of streptavidin this strong binding is used to further immobilize the desired DNA oligoduplex probe and generate the aimed biosensors. The strong biotin-streptavidin coupling was used to ensure that the biomolecular probe construction is not disrupted along the experiments. The biosensors are then used to monitor the TFs interaction mechanism with DNA. The sensor regeneration is possible through the bounded TF removal by simply increasing the buffer ionic strength (Fig. S2). The motional series resonance frequency signal returned to its initial value after performing the regeneration with binding buffer + NaCl 500 mM. Fig. S2 suggests that all the TF has been removed from immobilized DNA oligoduplexes.



Fig. S1 – Motional series resonance frequency variations upon TF-DNA interaction and regeneration of the sensor with 500 mM of NaCl.

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} 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:

$$\sin(\tau) = \frac{h_i}{2a} \quad (S1)$$

The interaction of the transcription factor with the HRE_{wt} 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_{wt} 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):

$$b = h_f - h_i/2 \qquad (S2)$$

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

$$\sin(\varphi) = \frac{b}{a}$$
 (S3)

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

$$\tau + \zeta = 90^{\circ} (S4)$$
$$\beta + \varphi + \zeta = 90^{\circ} (S5)$$



Fig. S2 - Scheme used to estimate the DNA bending angle after Haa1p_{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.