Electronic Supplementary Material (ESI) for Analyst.


# Supporting material

## Kinetic characterization of retinoic X receptor binding to specific and unspecific DNA oligoduplexes with quartz crystal microbalance

Rogerio M. M. Rodrigues, Jorge de-Carvalho, Guilherme N.M. Ferreira

## Electrical model characterization of QCM

The quartz crystal microbalance can be represented as equivalent circuit or Butterworth-Van Dyke model. The serial resonance frequency, $f_s = 1/(2\pi\sqrt{LC})$, corresponds to the maximum of spectrum conductance ($G_{max}$) and at the half height of the maximum conductance correspond two frequencies, $f_{Bmax}$ and $f_{Bmin}$ (fig S1).

When the QCM is load with rigid mass (ΔR=0) then $\Delta f_s = \Delta f_{Bmin}$. However, a load with viscous properties (ΔR>0) induces differences in both frequency variations. In this case, $\Delta f_{Bmin} \approx R/(4\pi L) + \Delta f_s$, a frequency which is less influenced by resistance variation.

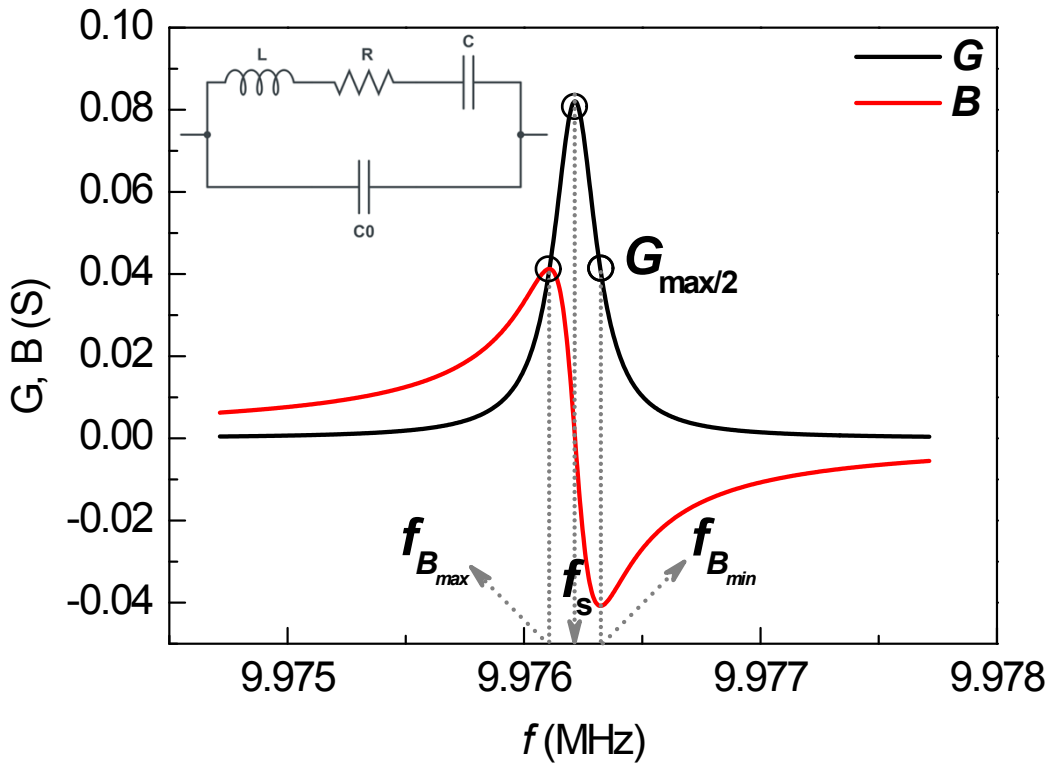


**Figure S1**. Real (*G*) and imaginary (*B*) parts of admittance (Y) spectrum for a 10 MHz quartz crystal near fundamental resonance ($f_s$) at air medium. The $f_s$ correspond to the higher frequency at $G_{max/2}$. The inset represents the BVD equivalent circuit of a unload crystal.

## RXRαDBD purification

The DBD domain of RXRα was purified in two-steps chromatographic processes (affinity and cationic-exchange) obtained a high purity protein fragment, >98% (figure S2), with a yield of 20 mg/L of initial growth medium.

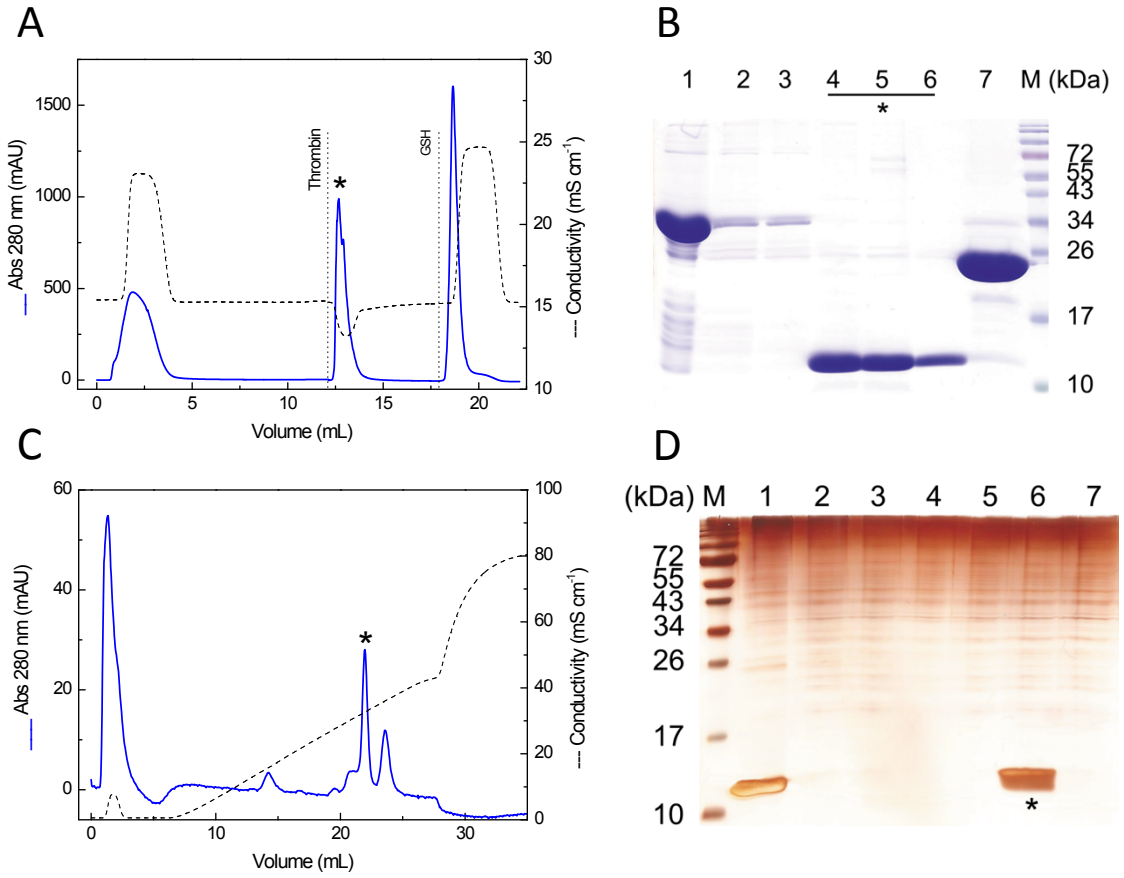


**Figure S2**. RXRDBD two-steps purification. (A) GST affinity chromatography with on-column thrombin cleavage. The GST-RXRDBD protein was immobilized on matrix, cleaved with 11.5 U of thrombin and GST moiety was eluted with 15 mM of GSH. (B) SDS-PAGE analysis of affinity chromatography. Lane 1: soluble fraction; lanes 2 and 3: flow-through; lanes 4, 5 and 6: RXRDBD cleaved; lane 7: GST. Gel stained with Coomassie Brillant Blue method. (C) Cationic exchange chromatography (1 ml column-Resource S). Linear (0-500 mM NaCl) and non-linear (500-1000 mM) elution gradients. (D) SDS-PAGE analysis of cation exchange chromatography. Lane 1: RXRDBD after cleavage column; lanes 2 and 3: excluded fractions (peak 1); lanes 4 and 5: washout; lane 6: fraction eluted with 400 mM NaCl containing RXRDBD (peak 2 *); lane 7: fraction corresponding to peak 3. Stained gel by the method of silver staining.

**Two analytes model analysis**

The kinetics parameters for the protein-DNA interactions at 100 mM NaCl were calculated based in a model of two analytes

$$A_1 + B \underset{k_{d1}}{\overset{k_{a1}}{\rightleftarrows}} A_1B \qquad A_2 + B \underset{k_{d2}}{\overset{k_{a2}}{\rightleftarrows}} A_2B$$

The association rates were calculated by fitting two analytes model to the association transients:

$$\Delta f_{A1} = \frac{\Delta f_{max} k_{a1} C_1}{Kf - Ks} \times \left( \frac{k_{d2}(Kf - Ks)}{KfKs} + \frac{k_{d2} - Kf}{Kf} e^{-Kf\,t} - \frac{k_{d2} - Ks}{Ks}\, e^{-Ks\,t} \right)$$

$$\Delta f_{A2} = \frac{\Delta f_{max} k_{a2} C_1}{(Kf - Ks)MW} \times \left( \frac{k_{d1}(Kf - Ks)}{KfKs} + \frac{k_{d1} - Kf}{Kf} e^{-Kf\,t} - \frac{k_{d1} - Ks}{Ks}\, e^{-Ks\,t} \right)$$

$$Kf = 0.5\ \left( K_A + K_B + \left( (K_A - K_B)^2 + 4k_{a1}k_{a2}C_1C_2 \right)^{0.5} \right)$$

$$Ks = 0.5\ \left( K_A + K_B - \left( (K_A - K_B)^2 + 4k_{a1}k_{a2}C_1C_2 \right)^{0.5} \right)$$

$$K_A = k_{a1}C_1 + k_{d1}$$

$$K_B = k_{a2}C_2 + k_{d2}$$

$k_{a1}$ and $k_{a2}$ are the apparent association rate constants, and $k_{d1}$ and $k_{d2}$ are the dissociation rate constants for the monomers and dimers, respectively.

$C_1$ and $C_2$ are the concentrations of monomer and dimer, and are calculated based on the equilibrium constant:

$$2\ \text{RXR}\alpha\text{DBD} \rightarrow \text{RXR}\alpha\text{DBD}_2$$

$$K_D = \frac{[RXR\alpha DBD]^2}{[RXR\alpha DBD - RXR\alpha DBD]}$$