Supporting Information

Determination of the probe grafting density. The probe concentration during grafting must be carefully chosen to have a density allowing the highest hybridization rate without steric hindrance between each oligonucleotide. The density of grafted DNA probes is usually electrochemically estimated by the Cottrell's experiment used by Steel *et al*. with ion exchange between $Ru(NH_3)_6$ and DNA counterions in weak electrolytes.[1] However, this method is not convenient for neutral PNA probes. In our case, grafting density was determined according to Porter's work on the electrochemical desorption of n-alkanethiol monolayers.[2] Thiolated probes gave a peak at -1208 mV vs Ag/AgCl when cycling toward reduction potential in 0.5 M KOH solutions.

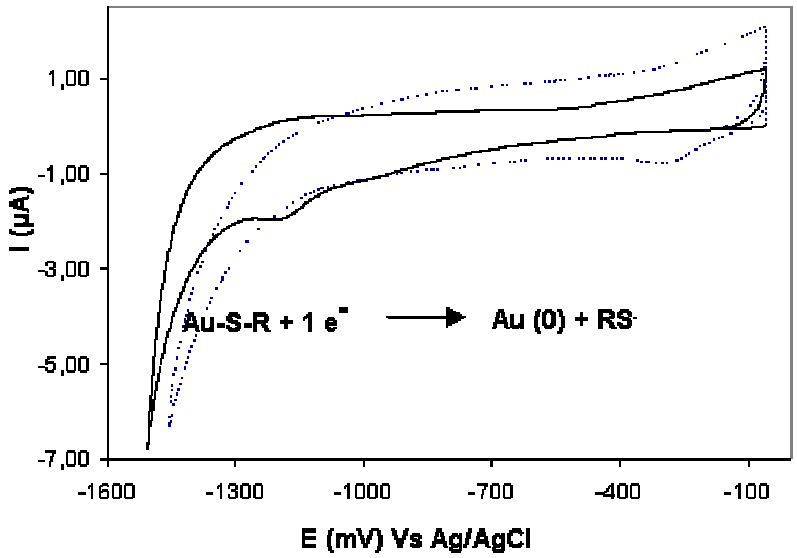

Figure 1. Cyclic voltammograms for bare gold electrode (dashed line) and for the reductive desorption of thiolated PNA monolayer on gold electrode (plain line). The voltammograms were recorded at 100 $mV.s^{-1}$ in 0.5 M KOH. The electrode area is 2 mm^2. Solutions were purged with N_2 for 15min.

The coverage Γ (mol cm^{-2}) can be obtained from cyclic voltammetry (CV) measurements as a linear relation between the observed charge Q (C), corresponding to the integration of the redox wave, and the

number of electroactive unit, α, which is generally one for each bounded DNA.

$$Q = \alpha F N_0 \quad (1)$$

$$\Gamma = N_0 / A \quad (2)$$

With N_0 corresponding to the number of electrolyzed moles of molecules and A corresponding to the electrode surface area (cm^2) taken as 1.2 times the geometric surface area. In these case of electroactive adsorbed species irreversibly reduced, the voltammograms can also be described with the following relations[3]:

$$i_p = \frac{\alpha F^2 A \nu \Gamma}{2.718 RT} \quad (3)$$

and $$\Delta E_{p,1/2} = \frac{62.5}{\alpha} mV(25°C) \quad (4)$$

Where i_p is the peak intensity, F the Faraday constant (C/equiv) and $\Delta E_{p,1/2}$ is the peak width at half-height of the wave. Grafting concentrations were optimized by determining the better ratio signal/noise. This simple direct method of reductive desorption reactions gave values of $\Gamma = 2.0 \pm 0.4\ 10^{-11}$ $mol.cm^{-2}$ for aptamer DNA probes used for the direct detection and $\Gamma = 2.3 \pm 0.3\ 10^{-11}$ $mol.cm^{-2}$ for PNA probes in the indirect detection system.

Influence of the pH on method 1. For acidic and neutral pH, no electrochemical peak is obtained independently of the nature of the grafted oligonucleotide. This means that polymer 1 is not electrostatically bound to the non-specific oligonucleotide and consequently, that something blocks the access of the polymer to the oligonucletide.

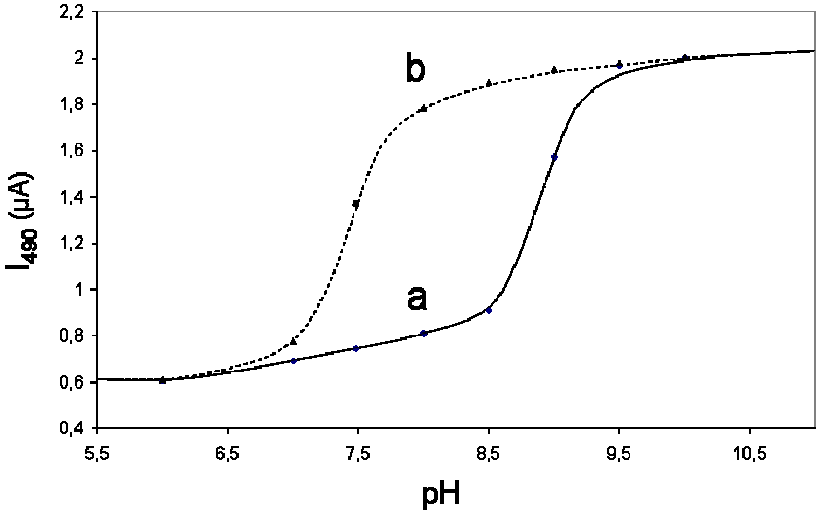

Figure 2. Current Intensity at 490 mV as function of pH for two sequences grafted on gold electrodes after putting in contact with human α-thrombin and then polymer 1. a) Specific human α-thrombin aptamer (X1); b) Non-specific human α-thrombin sequence (X2).

In fact, the isoelectric point of thrombin protein is in the range of pH from 6.3 to 7.55 and the major isoform is 7.3 (according to the product data sheet of Aldrich-Sigma). Low pH provokes protonation of the arginine and lysine moieties of the protein that induce the non-specific binding with the negatively-charged modified electrode. If the pH is inferior to 7.3, the human α-thrombin is positively charged and interacts with the negatively-charged single-stranded DNA (ss-DNA) on the gold surface and that leads to a "blocking of the surface" where the cationic polythiophene (polymer 1) cannot bind to ss-DNA. Therefore, there is no electrochemical signal for both sequences X1 and X2. In contrast, if the working pH is superior to 9.5, the protein is negatively charged, thus repulsed from the surface and easily eliminated by washing. At basic pH, polymer 1 freely interacts with the grafted oligonucleotides and the current intensity recovered its maximum as if there was no human α-thrombin in the solution. In both cases (pH inferior to 7.3 or superior to 9.5), there is no discrimination between sequences X1 and X2.

References

(1) Steel, A. B.; Herne, T. M.; Tarlov, M. J. *Anal. Chem.* **1998**, *70*, 4670-4677.

(2) Widrig, C.A. ; Chung, C. ; Porter M. D. *J. Electroanal. Chem.* **1991**, *310*, 335-339.

(3) Bard, A. J.; Faulkner, L. R. *Electrochemical methods: Fundamentals and Applications*, 2nd Edition, Wiley, New York, **2001**, chapt. 14, 594-595.