Supporting Information

Control of Probe Density at DNA Biosensor Surfaces using Poly-L-Lysine with Appended Reactive Groups

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SUPPORTING FIGURES

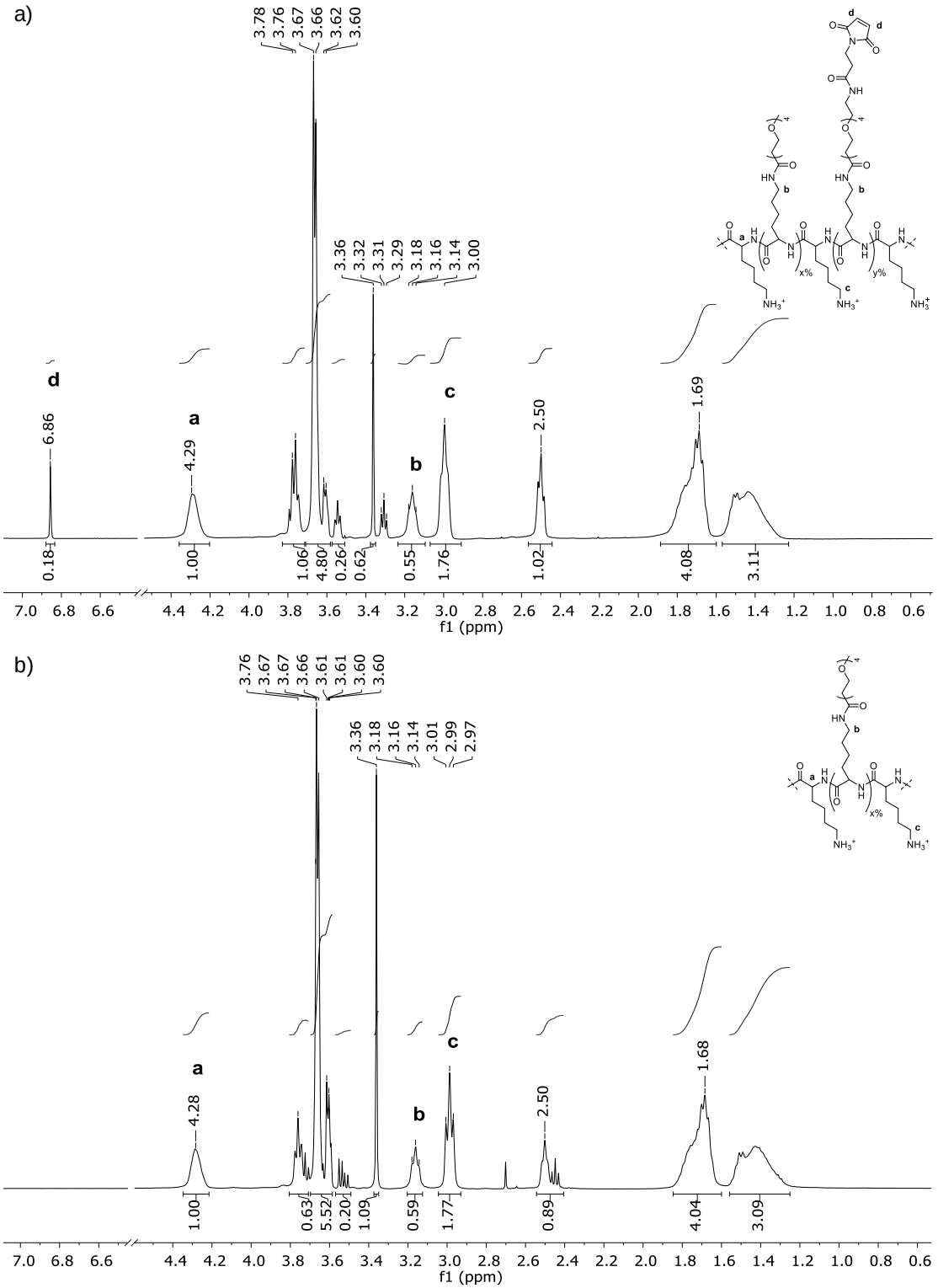

Figure S1. Example of ^{1}H-NMR spectrum of a) PLL-OEG(15.9)-Mal(7.9) and b) PLL-OEG(24.9) after purification.

PLL-OEG(x)-Mal(y)

^{1}H NMR (400 MHz D_2O, pH 6.5) δ [ppm] = 1.26–1.55 ((lysine γ-CH_2), 1.63-1.83 (lysine β,δ-CH_2), 2.50 (ethylene glycol CH_2 from both OEG and Mal coupled, -CH_2-C(=O)-NH), 3.00 (free lysine **c**, H_2N-CH_2), 3.16 (ethylene glycol CH_2 of coupled lysine from both OEG and Mal **b**, C(=O)-NH-CH_2-), 3.36 (OEG methoxy, -O-CH_3), 3.65 (oligo ethylene glycol from both OEG and Mal, CH_2-O-), 4.29 (lysine backbone **a**, NH-CH-C(O)-), 6.86 (maleimide from coupled Mal **d**, -C(=O)-CH-CH-C(=O)-).

Quantification of the grafted percentages of compounds OEG and Mal were performed using the integral ratios of the characteristic signals in the ^{1}H NMR spectra (see Figure S1a). All the integrals were normalized using the peak **a** at 4.29 ppm (lysine backbone, NH-CH-C(O)-). The integrals of interest are at 3.00 ppm (free lysine **c**, H_2N-CH_2), 3.16 ppm (functionalized lysine of both Mal and OEG side groups **b**, for both OEG and Mal), and 6.86 ppm (maleimide of Mal **d**, -C(=O)-CH-CH-C(=O)-).

Since all the integrals correspond to two protons and the sum of **b** and **c** equals the total amount of Lys (functionalized and free) at the PLL backbone, eq. 3 and 4 were used to calculate the molfraction of both OEG (x%) and Mal (y%) for each modified PLL polymer:

$$\text{Mal}\% = [\boldsymbol{d}/(\boldsymbol{b}+\boldsymbol{c})] * 100 \qquad \text{Eq.3}$$

$$\text{OEG}\% = [\boldsymbol{b}/(\boldsymbol{b}+\boldsymbol{c})] * 100 - \text{Mal}\% \qquad \text{Eq.4}$$

PLL-OEG(x)

^{1}H NMR (400 MHz D_2O, pH 6.5) δ [ppm] = 1.26–1.55 ((lysine γ-CH_2), 1.63-1.83 (lysine β,δ-CH_2), 2.50 (ethylene glycol CH_2 from both OEG and Mal coupled, -CH_2-C(=O)-NH), 2.99 (free lysine **c**, H_2N-CH_2), 3.16 (ethylene glycol CH_2 of coupled lysine from both OEG and Mal **b**, C(=O)-NH-CH_2-), 3.36 (OEG methoxy, -O-CH_3), 3.65 (oligo ethylene glycol from both OEG and Mal, CH_2-O-), 4.29 (lysine backbone **a**, NH-CH-C(O)-).

For PLL-OEG-Mal, integrals **a**, **b**, and **c** of Figure S1b were used in order to calculate the OEG content of the PLL-OEG, and Eq.4 becomes (Mal% = 0):

$$\text{OEG\%} = [\boldsymbol{b}/(\boldsymbol{b}+\boldsymbol{c})] * 100 \qquad \text{Eq.5}$$

The calculated percentages of OEG and Mal are shown in Table S1.

Table S1 The PLL-OEG-Mal polymers used in this work. The Mal grafting density was varied systematically during the synthesis. Quantification of the grafted percentages of OEG and Mal were done by ^{1}H NMR.

Target composition	Experimental composition	
	% OEG	% Mal
PLL-OEG(25)-Mal(1)	20.3	0.9
PLL-OEG(30)-Mal(2)	30.3	1.8
PLL-OEG(30)-Mal(3)	28.1	3.1
PLL-OEG(30)-Mal(4)	23.0	4.1
PLL-OEG(30)-Mal(6)	29.1	5.5
PLL-OEG(25)-Mal(8)	15.9	7.9
PLL-OEG(25)-Mal(10)	19.4	9.1
PLL-OEG(25)	24.9	-

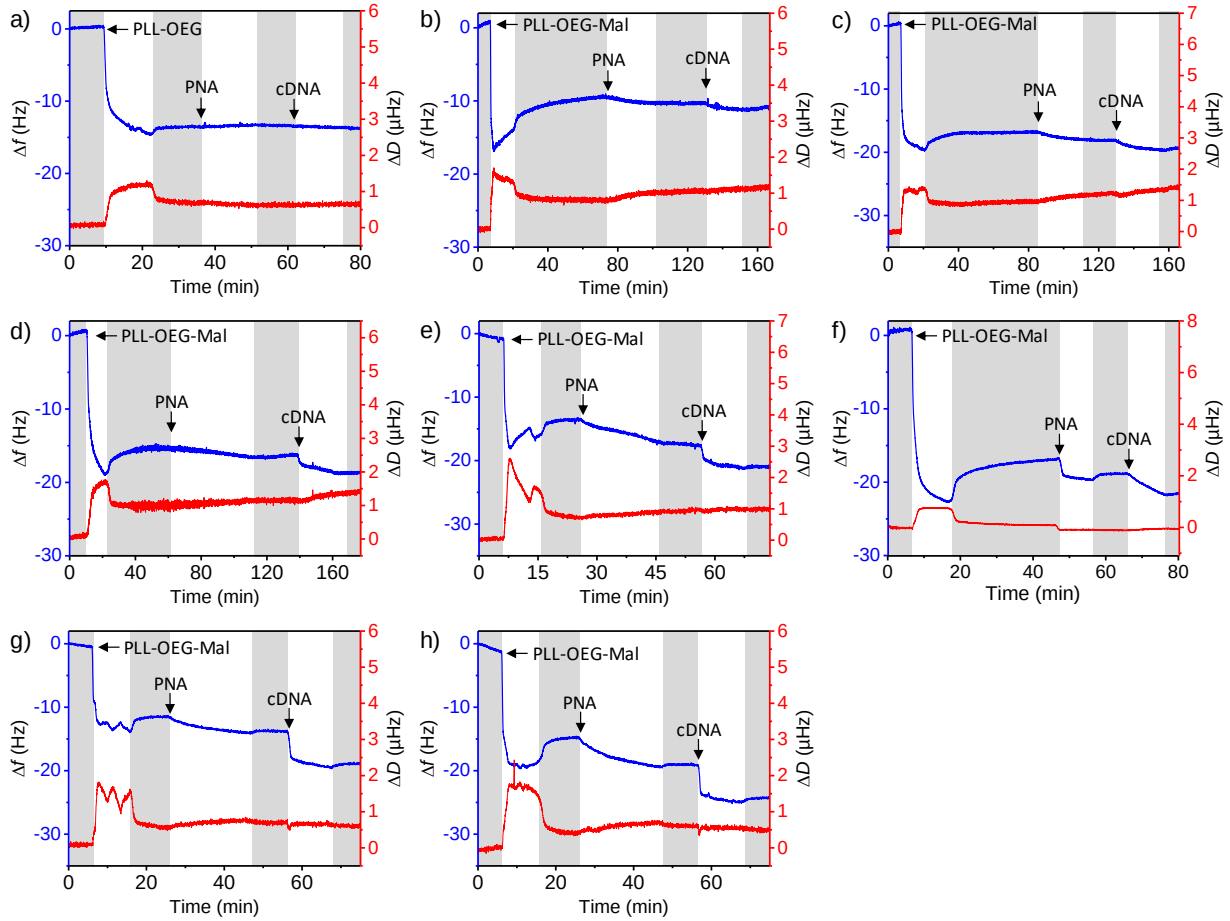

Figure S2. QCM measurements employed for the study of the relationships between the functionalization and the adsorbed masses of modified PLL polymers, thiol-PNA and cDNA over gold substrate. The PLL used were: a) PLL-OEG(24.9), b) PLL-OEG(20.3)-Mal(0.9), c) PLL-OEG(30.3)-Mal(1.8), d) PLL-OEG(28.1)-Mal(3.1), e) PLL-OEG(23.0)-Mal(4.1), f) PLL-OEG(29.1)-Mal(5.5), g) PLL-OEG(16.6)-Mal(7.9) and h) PLL-OEG(19.4)-Mal(9.1). The concentrations used in these experiments were 0.3 mg/mL for all the PLL solutions, 1 μM for both PNA-thiol and cDNA solutions, in PBS (pH 7.2). A PBS washing step (gray bar) was flushed before and after every adsorption step. Only the 5th overtone is displayed for both Δf and ΔD. The frequency shifts for each step were calculated subtracting the plateau average value of the frequency prior to the injection of the molecule (modified PLL, PNA probe, cDNA) to the average plateau value after the following PBS washing step.

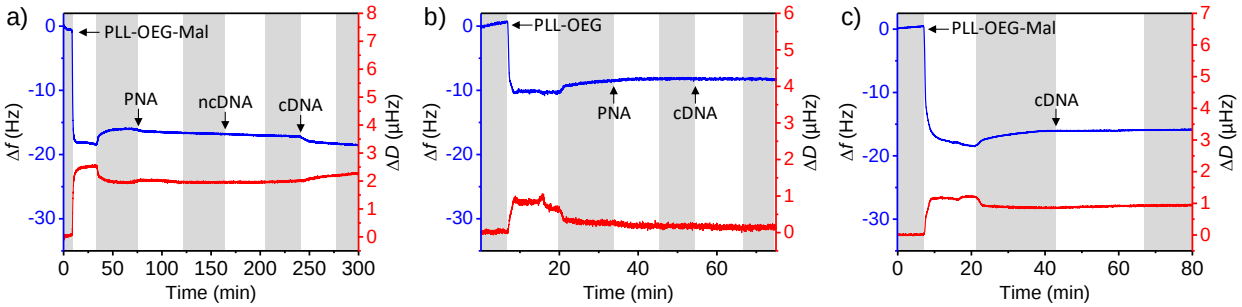

Figure S3. QCM time traces for the evaluation of the selectivity of the biorecognition layer consisting of PLL on a gold substrate. a) Selectivity experiment using PLL-OEG(30.3)-Mal(1.8), and flushing ncDNA and cDNA, after anchoring the PNA probes. Control experiments to test the absence of non-specific interactions using b) PLL-OEG with low content of OEG groups (19.9%) including the PNA step and c) PLL-OEG(28.1)-Mal(3.1) without PNA step. In all the multistep adsorption experiments, the concentrations were 0.3 mg/mL for all modified PLL solutions, 1 μM for PNA-thiol, ncDNA and cDNA solutions. A PBS washing step at pH 7.2 (gray bar) was flushed before and after every adsorption step. The 5^{th} overtone was used for both Δf and ΔD.

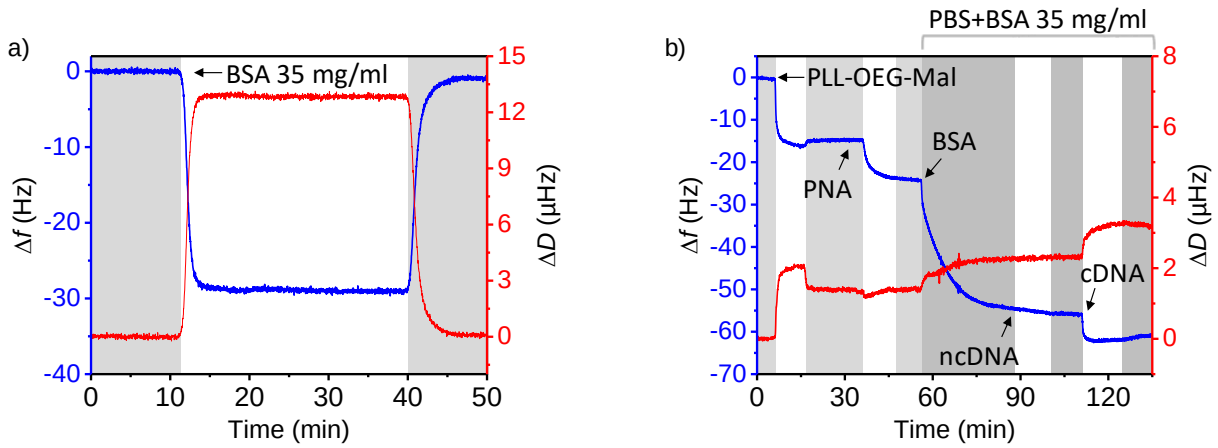

Figure S4. a) Antifouling experiments at QCM flushing a highly concentrated BSA solution (35 mg/mL in PBS 7.2) over a substrate coated with PLL-OEG(29.1)-Mal(5.5). The gold substrate was functionalized outside the QCM chamber by immersion of the substrate, after oxygen plasma activation, in the solution (0.3 mg/mL) of modified PLL. b) Control experiments to test the selectivity of the binding of DNA at a PNA-modified chip in the presence of BSA. PLL-OEG(19.4)-Mal(9.1) (0.3 mg/mL) and PNA (1 μM PNA-thiol) solutions were flushed over the activated gold substrate. BSA solution (35 mg/ml, in PBS pH 7.2) was injected to equilibrate the surface; then ncDNA and cDNA (1 μM; in the presence of the same concentration of BSA as the preceding buffer) were flushed consecutively. In both experiments, light gray bars and dark grey bars stand for PBS and PBS + BSA 35 mg/ml washing steps. Only the 5th overtone is displayed for both Δf (blue) and ΔD (red).

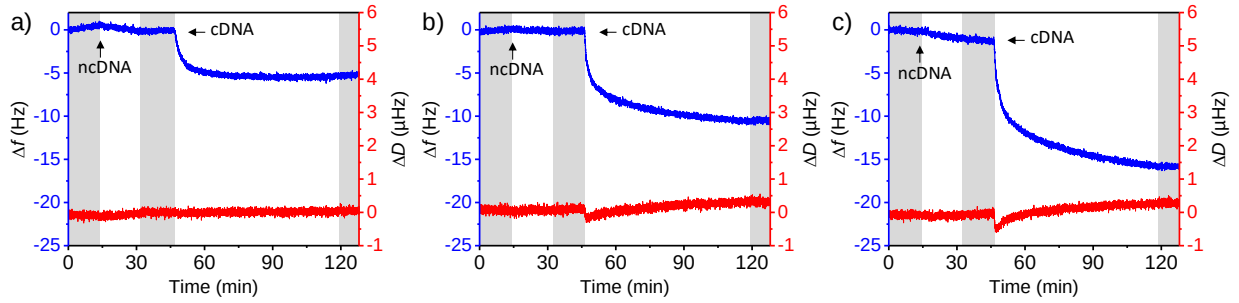

Figure S5. QCM measurements for ncDNA and cDNA binding step over SiO_2 substrates using a) PLL-OEG(30.3)-Mal(1.8), b) PLL-OEG(29.1)-Mal(5.5) and c) PLL-OEG(19.4)-Mal(9.1). After oxygen plasma treatment, the SiO_2 chips were functionalized by dipping them in the corresponding PLL solution and, after a washing step with Milli-Q water, in PNA-thiol solution, respectively for 30 min and 1 h. The graphs show the selectivity of the systems by injection of 1 μM ncDNA (44 nt) in PBS solution and, after rinsing with PBS, 1μM cDNA (43 nt). DNA quantification was done using the Sauerbrey equation. Concentration of modified-PLLs and PNA in PBS (pH 7.2) were 0.3 mg/mL and 1 μM, respectively. Only the 5th overtone is displayed for both Δf and ΔD.

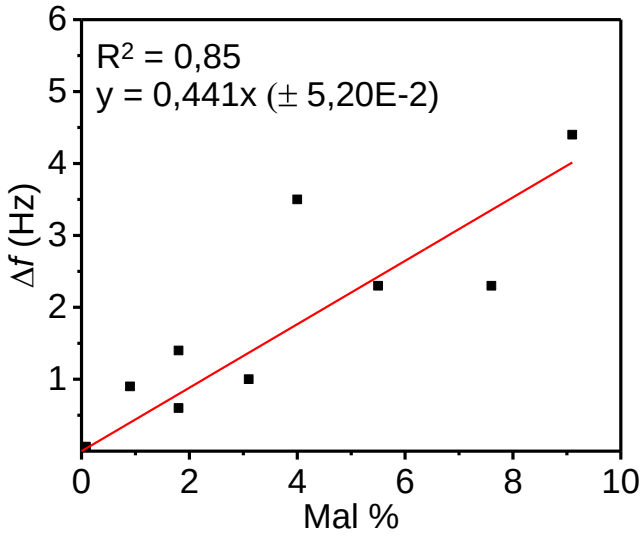

Figure S6. Frequency change of the PNA-thiol deposition step obtained by QCM versus the fraction of Mal grafted to the PLL polymer, quantified by ^{1}H NMR. All the experiments were performed using 0.3 mg/mL of modified PLL, 1 µM PNA thiol solution (activated by TCEP) and 1 µM cDNA solution in PBS at pH 7.2. PLL-OEG-Mal polymers with different degrees of Mal (0.0-10.9%) were used.

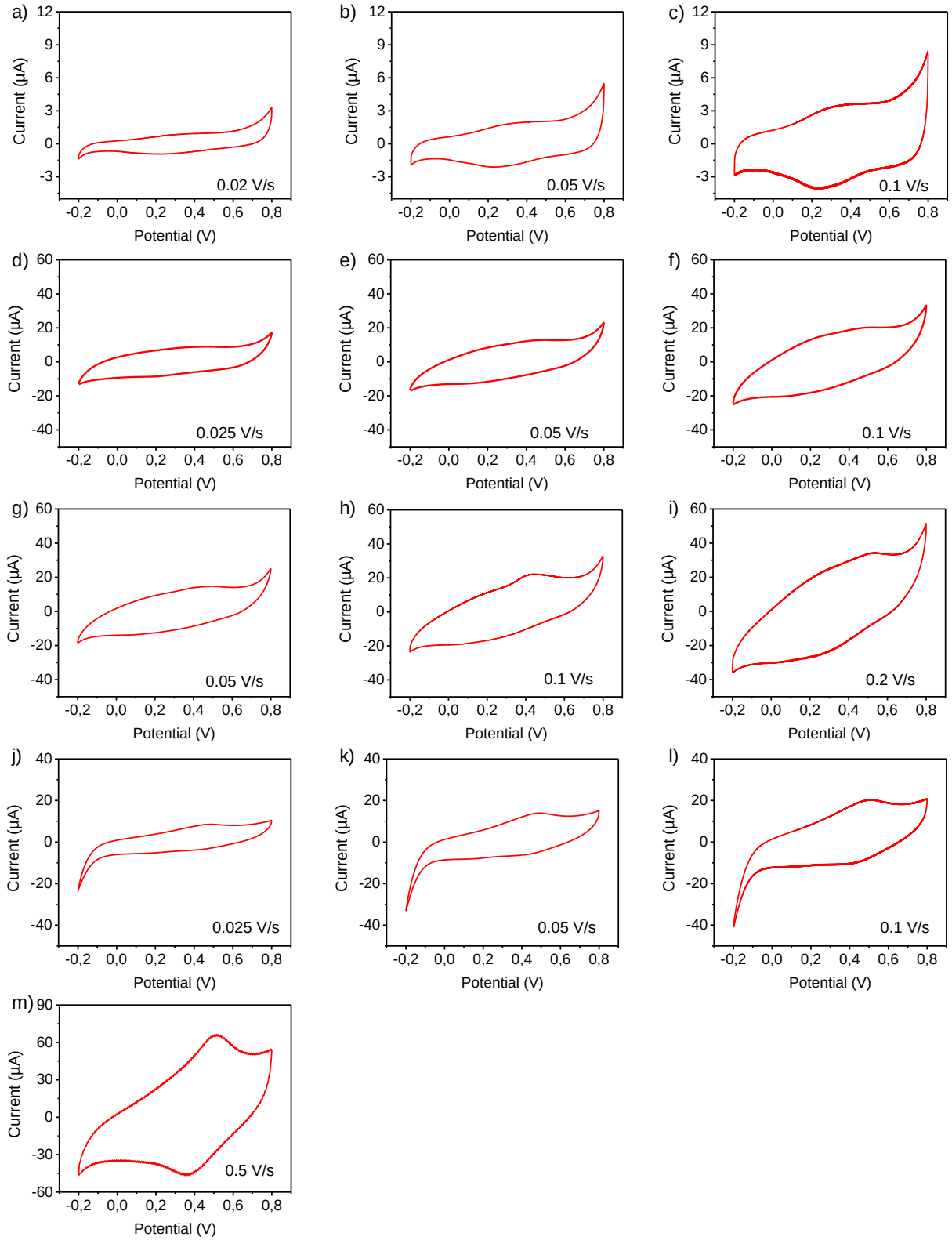

Figure S7. Cyclic voltammograms of (a-c) PLL-OEG and (d-m) PLL-OEG-Mal-functionalized Au substrates after anchoring of PNA and hybridization with Methylene Blue-functionalized

cDNA (cDNA-MB), obtained varying the scan rate. PLL-OEG(24.9)-functionalized chips were scanned with a scan rates of a) 0.025, b) 0.05 and c) 0.1 V/s. Three PLL-OEG-Mal were used: PLL-OEG(28.1)-Mal(3.1) modified substrates were scanned at d) 0.025, e) 0.05 and f) 0.1 V/s; PLL-OEG(29.1)-Mal(5.5) ones at g) 0.05, h) 0.1 and i) 0.2 V/s, and PLL-OEG(19.4)-Mal(9.1) at j) 0.025, k) 0.05, l) 0.1 and m) 0.5 V/s. Gold substrates were previously activated by oxygen plasma and immersed for 1h in the corresponding modified PLL and PNA-thiol solution, respectively. Before the CV experiments, the substrates were covered by a 1µM cDNA-MB solution (5'-MB-AG CTG GTG GCG TAG-3') in PBS at pH 7.2 for 1 h. Freshly prepared 0.1 M $NaClO_4$ solution was degassed for 5 min and used as the electrolyte for the all CV experiments. The concentrations of the modified-PLLs and PNA in PBS were 0.3 mg/mL and 1 µM, respectively.

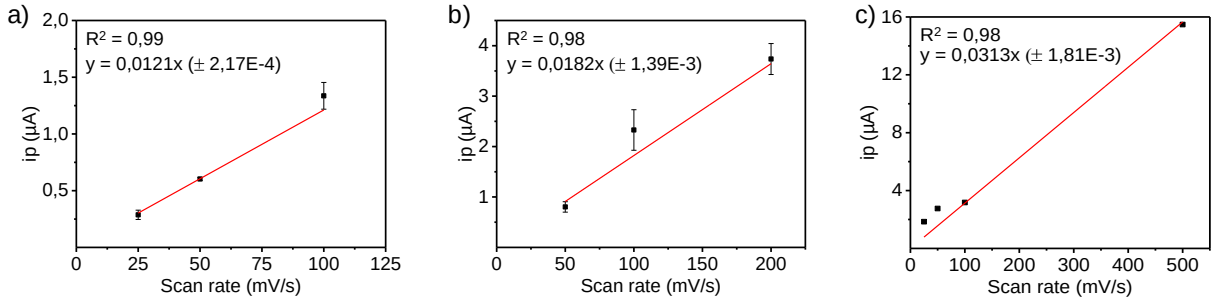

Figure S8. Dependence of the anodic peak current densities on scan rate, derived from the experiments in Figure S7. The modified PLL used were: a) PLL-OEG(28.1)-Mal(3.1), b) PLL-OEG(29.1)-Mal(5.5), c) PLL-OEG(19.4)-Mal(9.1). Prior to the CV experiments, freshly prepared 0.1 M $NaClO_4$ solution was degassed for 5 min and used as the electrolyte. The concentrations of all the modified-PLLs in PBS were 0.3 mg/mL.

Electrochemical analysis

To obtain the points of the anodic currents (Figure S8), the cyclic voltammograms in Figure S7 were treated as follows: the first derivative was applied to the anodic segment of interest to find the peak potential (E_p). A range of ± 0.2 V was defined from the E_p; the baseline was consequently established between these potentials and the peak intensity (i_p) was recorded. All the passages were done by means of the CHI760D software (CH Instruments, Inc. Austin, USA). In order to obtain the surface coverage (Γ), a linear regression was performed for each set of Au substrates, functionalized by the modified PLL with the same Mal%, from 0% to 9.1% (Figure S8). The linear fitting was forced through the origin and the surface coverages were calculated from each slope, exploiting Eq. 2 (see Experimental section). The linear dependence of the i_p on the scan rate, as well as the high value of all the R^2, confirmed that the electrochemical reaction was surface-confined. Afterwards, each Γ was converted in molecules per unit area (cm^2) and plotted versus the Mal% grafted to the modified PLL used in the CV experiments (Figure 4).

KRAS-WT: SPDP-dPEG$_4$-CTA CGC CAC CAG CT ε (260 nm): 127900 $M^{-1}cm^{-1}$

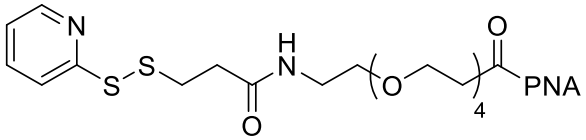

Figure S9. Spacer SPDP-dPEG$_4$. Protecting group SPDP = 3-(2-pyridyldithio)propionyl).