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Electronic Supplementary Information

Label-Free Detection of DNA Single-Base Mismatches using a Simple Reflectance-Based Optical Technique

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- **Details about data analysis**
- **Effect of spotting concentration on probe surface density**
- **Effect of polyA spacers on affinity estimates**

Details about data analysis

The data as obtained from the experimental procedure consist of a time-lapse video (final effective frame rate: 0.2 Hz) of the surface along all the measurement period. The data reduction procedure presented here converts the raw intensity values of the pixels in a surface density signal relative to binding of the target on a specific probe.

Detection of reflected intensity from sensitive areas. The amount of immobilized probes was so low that their spots were hardly distinguishable from the non-spotted surface before hybridization took place. Therefore, the parts of the surface image that supply information about the adhesion process were identified by subtracting the first frame from the last one. Figure S1 shows a subset of such spots, for various probe sequences and spotting concentrations. To correct for intensity variations not corresponding to a specific adhesion process, coronas immediately around every spot were also monitored (see Fig. 1 in the main text). For every frame, the pixel values inside every spot and corresponding corona were averaged to obtain the mean value.

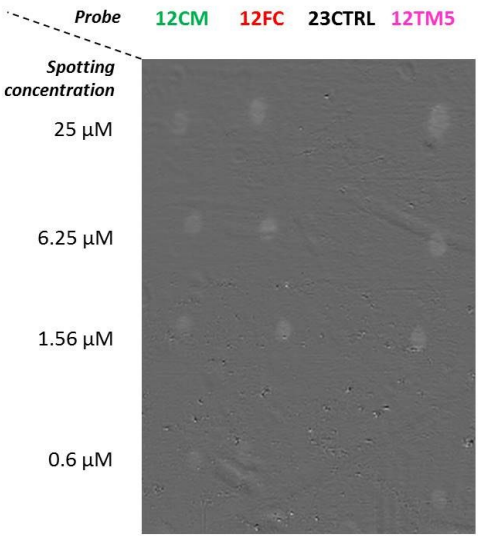

Figure S1 A series of spots for various probe sequences and spotting concentrations, upon full hybridization of the same target sequence 12T. The last frame before first target addition was subtracted, so that the image effectively shows only the intensity increase corresponding to 12T binding.

Intensity to surface density conversion. The system was equilibrated for some hours before the first injection, and a baseline signal was acquired, to check and correct for possible drifts. At each injection, stray light and turbulence caused an intensity jump lasting a few seconds, which was removed before the analysis. The reflected intensity was then converted into the surface density of the mass at the interface according to [Giavazzi *et al.*, *Proc. Natl. Acad. Sci.* U.S.A., **110**, 9350 (2013)]:

$$\sigma = \sigma_0 \sqrt{u/u_0 - 1} \qquad (1)$$

where $\sigma_0 \approx 4{,}9 ng/mm^2$ is a constant representing the density of mass which yields doubling of reflected intensity as compared to the bare surface; it depends on the surface material, ~~and~~ buffer refractive index and setup optics. u is the intensity level of a spot and u_0 is the intensity of the bare surface. In our experimental condition, u_0 is not directly measured, but it can be obtained from the intensity of copolymer-coated coronas as $u_0 = 0.8\ u_{cor}$ [Giavazzi *et al.*, *Biosens. Bioelectron.*, 58, 395 (2014)]. This figure corresponds to a copolymer film about 6 nm thick, with 85% of volume occupied by water. [Yalçin *et al.*, *Anal. Chem.* 81, 625 (2009), Pirri *et al.*, *Anal. Chem.* 76, 1352 (2004)]

Average of equivalent traces. To ensure adequate reduction of noise, in each experiment at least 4 equivalent spots were produced and measured on the prism, with the same probe sequence and spotting concentration. Traces of the replicas were averaged and the resulting curves were then fitted to determine the thermodynamic parameters specific for the hybridization process, as discussed in the main text. Figure S2 displays the single spot traces and their average value for the 12FC-12T binding in Fig. 2a, showing good internal consistency.

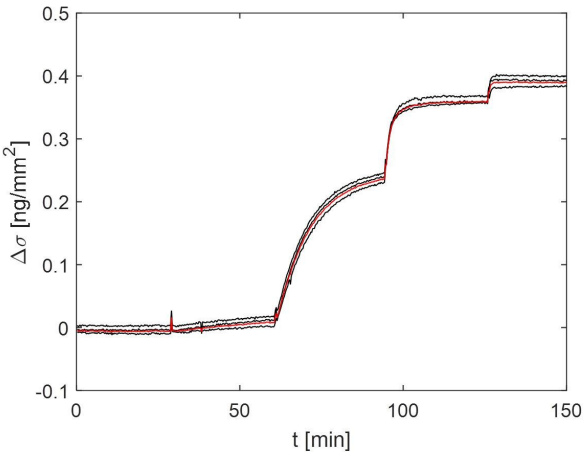

Figure S2. Increase of the surface density of bound target strand 12T upon hybridization to 12FC probe, as assessed from single spots (black lines) and from their average (red line). Same conditions as in Fig. 2a.

Effect of spotting concentration on probe surface density

In principle, RPI enables measuring the probe surface density prior to hybridization on the basis of the initial difference in reflectivity between spot and corona (typically lower than $0.25u_0$). This measurement, performed in previous RPI experiments in which protein receptors were used [Giavazzi 2013], was not possible in this case because the lower molecular weight of the probes and their sparseness leads to a very weak contrast of the spot and thus to extremely noisy estimates. However, for each chosen spotting concentration c_S we measured the value $\Delta\sigma_{sat}$, the maximum surface density of bound targets, which effectively expresses the surface density of active probes. The data are plotted in Fig. S3, expressed in mass surface concentration (left-hand axis) and number surface density (right-hand axis). Fig. S1 shows a saturating behaviour, in which the probe density cannot be increased above about 1 probe per 10 nm^2, corresponding to a mean distance between probes on the order of their length.

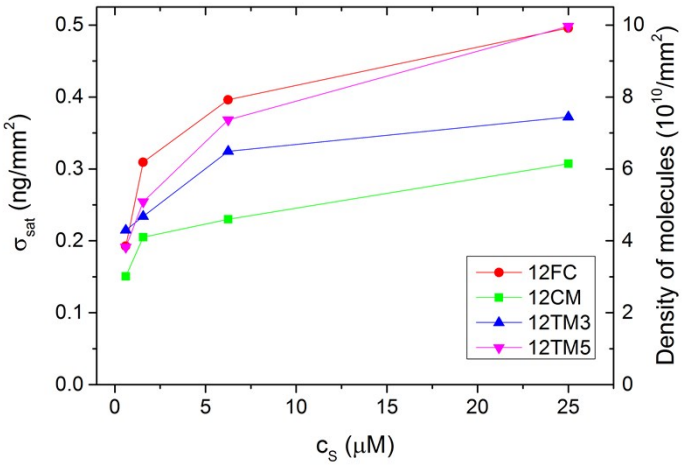

Figure S3 Target surface density (left axis) and the equivalent number density (right axis) as a function of probe spotting concentration.

Effect of polyA spacers on affinity estimate

To test the effect of the probe distance from the surface, we studied sequences with polyA spacers on the tethered end (A_n-12FC: NH_2-5'-$(A)_n$CAGGACTGTCGT-3').
In Figure S4, we show K_{diss} for n = 0, the usual probe, and for n = 6,12. The hybridization occurring at the surface is not favoured by increasing the spacer distance; it is rather slightly disfavoured, possibly because of the increased mass and linear charge, producing an effectively higher probe crowding at the same probe density.

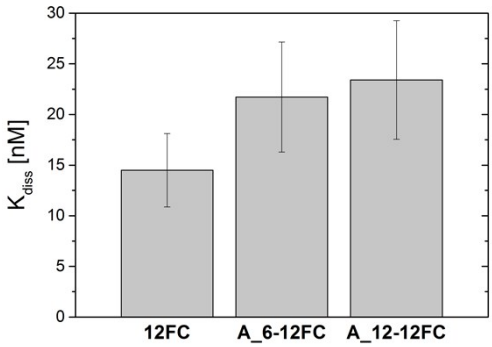

Figure S4 Dissociation constants for different spacer lengths; data obtained at ionic strength I_{Na} = 160 mM, T = 33 °C, c_S= 6.25 µM.