Supporting Information

Effect of probe-probe distance on the stability of DNA hybrids on surfaces

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A. Procedure for calculation of probe coverage.

We determined probe coverage values for sensors functionalized through coimmobilization of A15*T5-P15 and A15* using SPR and XPS measurements. XPS measurements of the phosphorous and sulfur peak area ratio provided the fractional composition, A15*T5-P15 and A15* (\( \phi_{A15T5P15} \) and \( \phi_{A15} \)), through Equation S1. The numbers 2.8 and 1.0 are the P/S peak area ratios observed from sensors functionalized with just A15*T5-P15 and A15*, respectively. SPR measurements provided the total nucleotide surface density (\( \rho_{\text{Nuc.}, \text{SPR}} \)) of the DNA layer. This value was used with the fractional composition values and lengths of A15*T5-P15 and A15* strands to obtain the probe coverage (Eq. S2).

\[
\left( \frac{P}{S} \right)_{\text{XPS, sample}} = 2.8 \cdot \phi_{A15T5P15} + 1.0 \cdot \phi_{A15} \quad \text{Eq. S1}
\]

\[
\text{Probe coverage} = \frac{\rho_{\text{Nuc.}, \text{SPR}} \cdot \phi_{A15T5P15}}{35 \phi_{A15T5P15} + 15 \phi_{A15}} \quad \text{Eq. S2}
\]
B. Immobilization data from additional samples.

**Figure S1.** SPR measurement of the immobilization of A15*T5-P15 (black line), A15* (red line), and mixtures of A15*T5-P15 and A15* (blue lines). The total DNA concentration of each sample was 4 µmol·L⁻¹. Sensors were exposed to DNA solutions for 4 h, followed by a rinse in DNA-free buffer solution.
C. AFM images of probe immobilization.

**Figure S2.** Representative AFM images obtained from template-stripped gold surfaces (Platypus Technologies) functionalized with (a) A15*, (b) A15*T5-P15, and (c) a 50:50 mixture of A15* and A15*T5-P15. Samples were prepared following procedures identical to those used for SPR sensors. AC-mode AFM images (Agilent 5420) were obtained from random areas of each sample using an 1.0 N·m⁻¹ cantilever (MikroMasch). The images have been flattened to account for slope but are otherwise unprocessed.
D. Melt curves for P15:P15′ hybrids obtained from solution measurements.

**Figure S3.** Melt curves for P15:P15′ hybrids obtained from solution UV absorbance measurements. (a) Plots of hyperchromicity observed at 260 nm for solutions containing P15:P15′ hybrids in 0.10 mol·L⁻¹ and 0.25 mol·L⁻¹ NaCl-TE buffer solution. (b) Processed melt curves showing the fraction of DNA hybridized vs. temperature. Melt temperatures (Tₘ) were taken as the midpoint of each curve, 49.0 and 55.5 °C, respectively.
E. Immobilization and hybridization behavior of a low coverage A15*T5-P15 sensor prepared without A15*.

Figure S4. Immobilization and hybridization behavior of a low coverage A15*T5-P15 sensor prepared without A15*. (a) SPR measurement of the immobilization of A15*T5-P15. The sensor was exposed to a solution containing 4 µmol·L⁻¹ A15*T5-P15 in 1 mol·L⁻¹ NaCl-TE for 4 h, followed by a rinse in DNA-free buffer solution, which results in a probe density of 3.9 × 10¹² cm⁻². (b) Temperature profile of hybridization activity with P15’ targets in 0.10 mol·L⁻¹ NaCl-TE. The scatter plot is the normalized average of experimental data from multiple replicate sensors. The solid line is the result of applying the theoretical model described by Eq. 4 and 5. Values used were Δ𝐻° = -460 kJ·mol⁻¹; Δ𝑆° = -1320 J·mol⁻¹·K⁻¹; 𝑤 = 9.9 × 10⁻¹⁷ J·m²·mol⁻¹, identical to the values used to fit the data in Figure 2.