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Supporting Material

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SUPPLEMENTARY INFORMATION

“Quantitative detection of small molecule/DNA complexes employing a force-based and label-free DNA-microarray”

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**DNA surface density.** Quantitative analysis of the fluorescence images relies on the assumption that the amount of fluorescently labeled species per unit area is proportional to the obtained fluorescence intensity per unit area. To investigate this relationship, we titrated identically prepared oligomer 1 spots on a glass slide with different amounts of oligomer 2 ranging from 0 to 0.6 pMol. After 2 hours of incubation the slide was washed thoroughly in 1x PBS and read out via fluorescence. The fluorescence intensities were summed over all pixels of each well divided by the fluorescence spot area, which was on average 1.8 mm of diameter. The fluorescence intensity was proportional to the amount of added ligand for amounts of DNA oligomer 2 less than 0.4 pMol. At higher amounts the fluorescence intensity saturated and deviated significantly from a line fit.

The observed saturation can be explained by electrostatic repulsion between the dsDNA. Short dsDNA is a rod like, cylindrical molecule, which is most densely packed in a parallel arrangement. Close packing of short dsDNA on a surface is thus equivalent to the problem of close packing of hard disks. The total hard disk radius is the sum of the dsDNA radius and the length of electrostatic repulsion. The former is known to be 0.95 nm [1] and the latter is best described by the Debye length that is approximately 0.62 nm at 147 mM Na⁺ [2]. The total disk radius is therefore 1.57 nm. Randomly packing discs results in a packing efficiency of 82% [2]. The packing efficiency and the total disk radius yield the theoretical maximum in short dsDNA surface density $\rho_{\text{Debye}}$ of 0.11 molecules per nm² in good agreement with literature values [3].

To compare whether the onset of fluorescence intensity saturation coincides with $\rho_{\text{Debye}}$ the densities of 1·2 complexes per unit area were determined from the fluorescence spot size and the assumption that all oligomers 2 hybridized to free oligomers 1 immobilized to the surface. Further, the expected maximal fluorescence intensity was determined by extrapolating the line fit for low densities to $\rho_{\text{Debye}}$ yielding $I_{\text{Debye}}$. In Figure S1 the fluorescence intensity ratio $I/I_{\text{Debye}}$ was plotted against the corresponding calculated surface density ranging from 0 to 0.13 molecules per nm². The observed saturation of fluorescence intensity at around 0.09 molecules per nm² is in good agreement with $\rho_{\text{Debye}}$. Remaining free ssDNA strands that also occupy a small fraction of the surface area may explain the slightly lower experimentally determined value.

It is entirely possible that a non-linearity between amount of oligomer 2 per unit area and fluorescence signal at high surface densities contributes to the observed saturation effect. Hence, the molecular setups in the present study were prepared at $\rho_0$ of 0.06 molecular setups per nm². This is a surface density for which we have shown the fluorescence per unit area to
be proportional to the fluorescently labeled species per unit area. Nonetheless, the surface density is rather high: the Flory radius, which is deduced from the radius of gyration, is a good measure of the volume a polymer encompasses [1] [4]. From the actual lengths and the persistence lengths of dsDNA [5], ssDNA [6] and PEG [7] we calculate a Flory radius of 9.38 nm for the 1·2·3 molecular setups used in our experiments. Assuming again a close packing of disks yields an upper limit of 0.003 molecular setups per nm² for the regime wherein the constructs do not interact with each other. The densities used in our experiments are an order of magnitude higher than that. This is a fact that should be kept in mind if the binding of larger and less robust ligands like proteins to dsDNA is going to be investigated. In this case, the surface densities of the molecular setups may have to be decreased further in order to avoid steric hindrance and unwanted interactions.

REFERENCES

**Figure S1.** Different amounts of oligomer 2 were incubated with identically prepared oligomer 1 spots. The fluorescence intensities per unit area are plotted against the calculated surface densities. The dashed line indicates the highest possible density of oligomer 2 per unit area based on the electrostatic repulsion argument. The CUFA experiments are performed at densities of oligomer 2 per unit area, wherein the fluorescence intensity per unit area is proportional to the presence of fluorescently labeled oligomer 2 per unit area (highlighted in grey).