Supplementary Information:

Linear chain formation of split-aptamer dimers on surfaces triggered by adenosine

Chenze Lu[1,2], Christine Saint-Pierre[1], Didier Gasparutto[1], Yoann Roupioz[1], Eric Peyrin[2] and Arnaud Buhot[1].

Tables of the self-folding temperatures of monomers and the melting temperatures of dimers.

Monomers	S5Z	S5*Zc	S6Z	S6*Zc	S8Z	S8*Zc
Tm (in °C)	47.5	52.4	43.8	52.4	47.5	52.4

Table S1: Self-folding temperatures of the more stable secondary structure of the monomers. (Conditions: Salt concentration: 150 mM Na^+, 5 mM Mg^{2+}, strand concentration: 1 μM).

Dimers	S5	S6	S8
Tm (in °C)	74.7	72.0	72.0

Table S2: Self-folding temperatures of the more stable secondary structure of the monomers. (Conditions: Salt concentration: 150 mM Na^+, 5 mM Mg^{2+}, strand concentration: 1 μM).

Image of a prism following grafting solution deposition

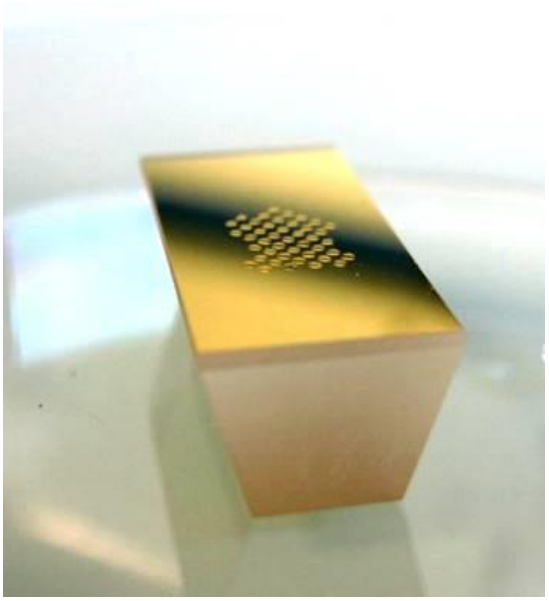

Fig. S1: Image of a Gold coated glass prism for SPRi measurement following grafting solution deposition. Dots represent the grafting spots on the microarray.

Image of the microarray during a SPRi experiment

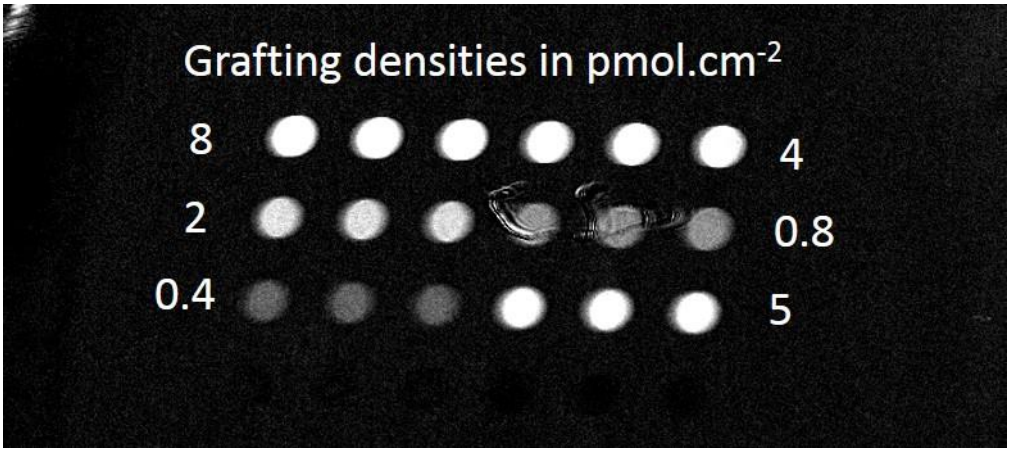

Fig. S2: Surface Plasmon Resonance image after S5 dimer injection.

Triplicates of spots with the same grafting density are consecutive and present similar reflectivity intensities. The grafting densities are indicated on the image in pmol.cm^{-2}. The spots with 5 pmol.cm^{-2} have been made from a grafting solution of 10 μM of Z strands without the NC strands while the other spots are a mixture of Z and NC strands with a total DNA concentration of 20 μM in appropriate ratio (see Materials and Methods). On this image, the reflectivity signal (white intensity) is decreasing with the grafting density as expected.

Gel electrophoresis experiments

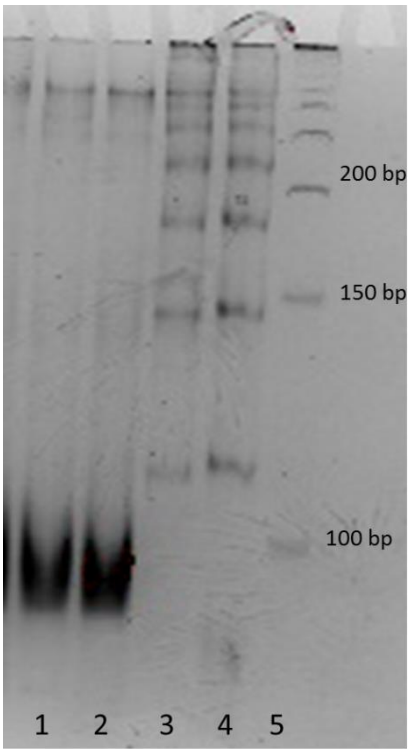

Fig. S3: Electrophoresis results on acrylamide gels 10%. Lines 1 and 2 were the S6 dimers (hybridization of monomers S6Z and S6*Zc) with 1mM of adenosine in the gel, lines 3 and 4

were the fully commentary S6 dimers (hybridization of monomers S6Z and S6cZc). Line 5 was the DNA ladder for indicating the length of the structure.

The lines 1 and 2 presented a broad band corresponding to the length of the S6 dimers with hybridization of the Z and Zc complementary strands. No adenosine bridges were present while the concentration of adenosine in the gel is set at 1mM. On the contrary, the lines 3 and 4 presented several bands corresponding to the binding of multiple dimers through the S6 and S6c hybridization. Those electrophoresis experiments suggested that the 1D chains with adenosine bridges were not formed in solution or were not strong enough to support migration through acrylamide gel even in presence of a large amount of adenosine (1 mM).

Experimental details:

TBE buffer, GelRed as fluorescent dye. 10 pmol of sample in 15 μL of loading buffer.

Regeneration of the functional microarray for successive detections of adenosine

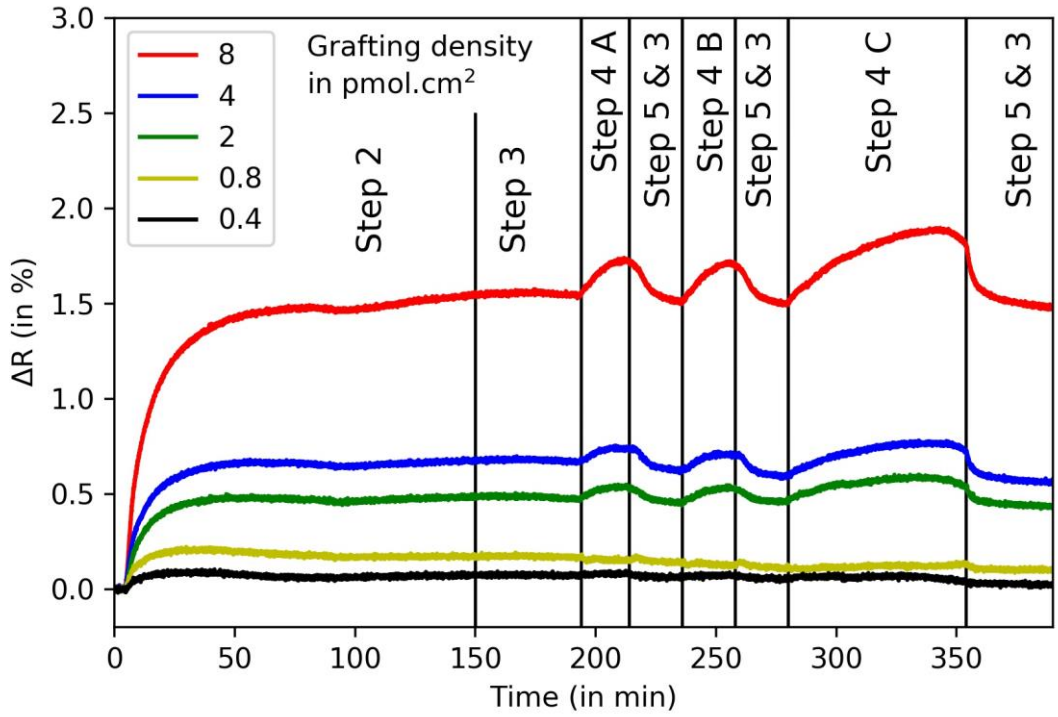

Fig. S4: Real-time SPRi signal following successive injections demonstrating the regeneration of the functional microarray for successive adenosine detection by S5 dimer injections.

Step2: starting from an initial microarray with Z probes at various grafting densities, the injection of S5*Zc allowed for the preparation of a functional microarray with S5* probes. **Step 3:** with a buffer injection, the stabilization of the functional microarray was reached. **Step 4 A:** The detection of adenosine was obtained by an injection of S5 dimer (concentration = 1 μM) and adenosine (concentration = 100 μM). **Step 5 & 3:** Injection of the buffer without adenosine

and S5 dimers allowed for a regeneration of the functional microarray. **Step 4 B:** A second adenosine detection was possible consecutively by an injection of S5 dimers (concentration = 1 μM) and adenosine (concentration = 100 μM) without regenerating with NaOH solution the microarray back to its initial state. **Step 5 & 3:** Injection of the buffer without adenosine and S5 dimers allowed for a regeneration of the functional microarray. **Step 4 C:** A third adenosine detection was obtained by an injection of S5 dimers (concentration = 1 μM) and adenosine (concentration = 1 mM). **Step 5 & 3:** Injection of the buffer without adenosine and S5 dimers allowed for a regeneration of the functional microarray.

Detection range from 10 μM up to 1 mM for adenosine

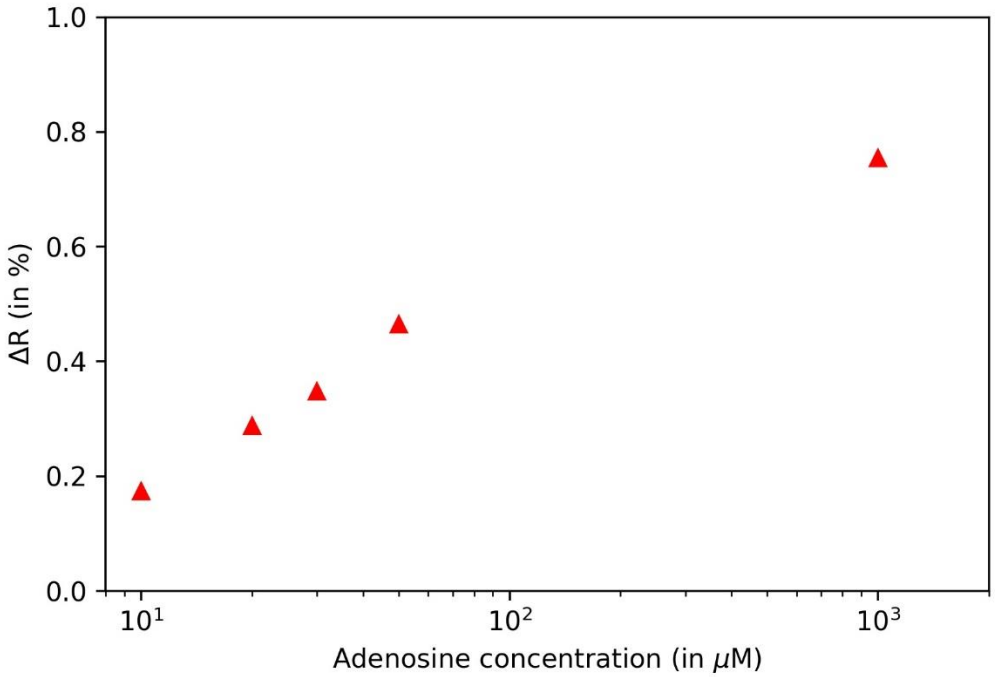

Fig. S5: Detection range from 10 μM to 1 mM for adenosine with S5 dimer injections.

Signal off: Signal SPR obtained through injection of dimers without adenosine

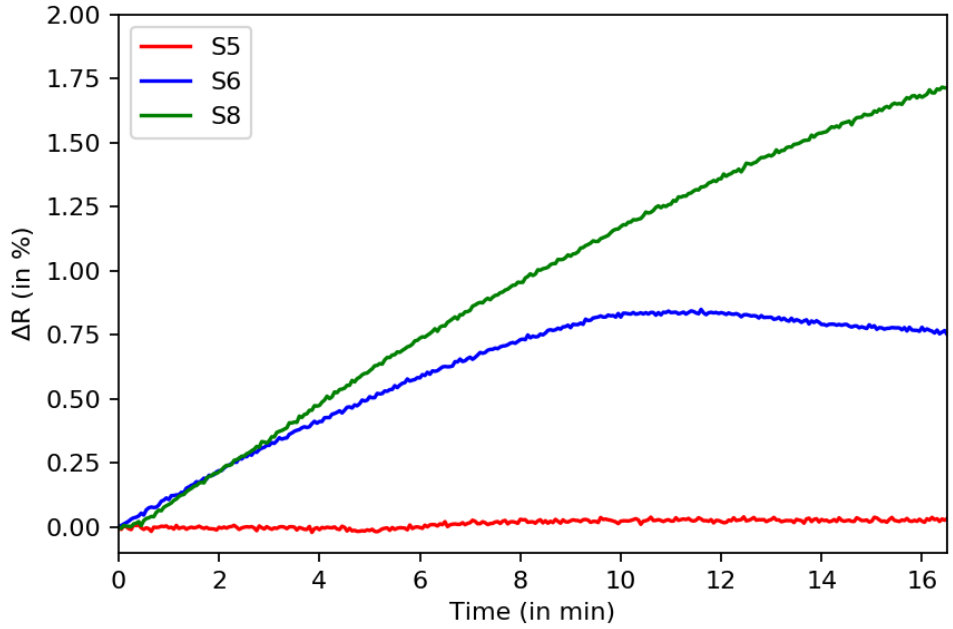

Fig S6: SPR signal observed upon injection of S5, S6 and S8 dimers (concentration 1 µM) without adenosine for the spots at 8 pmol.cm^{-2} grafting density.

The injections of S6 and S8 dimers at 1 µM lead to a significant SPR signal even without adenosine. The number of hybridizing bases were large enough to lead to bridges without adenosine. This effect impaired low detection limits for those S6 and S8 dimer sequences since the increased of signal by a small amount of adenosine only slightly increased the background signal. On the contrary, the SPR signal for the injection of S5 dimers without adenosine was close to the noise level. Thus, no bridges were present and a small amount of adenosine trigger the formation of bridges leading to an observable SPR signal. In conclusion, sequence engineering of the split-aptamer allowed us to obtain a Signal OFF - Signal ON behavior of the biosensor.