Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Nanoparticle-based thermo-dynamic aptasensor for small molecules detection

Feriel Melaine, Clothilde Coilhac, Yoann Roupioz and Arnaud Buhot

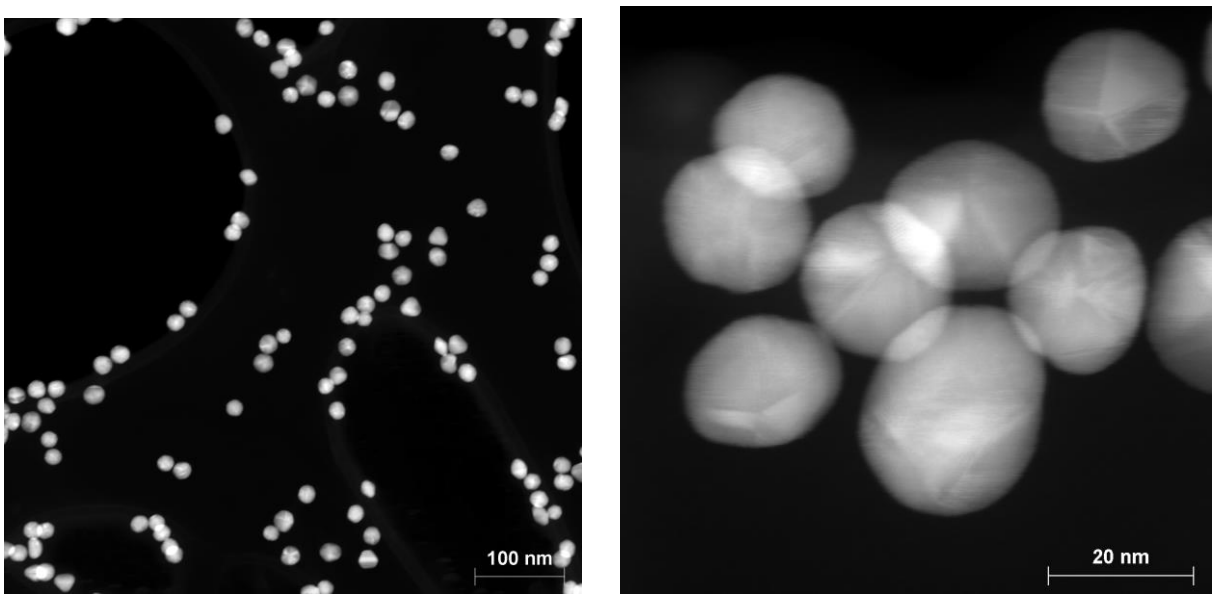
Univ. Grenoble Alpes, INAC, SPrAM, F-38000 Grenoble, France.

CEA, INAC, SPrAM, F-38000 Grenoble, France.

CNRS, SPrAM, F-38000 Grenoble, France.

arnaud.buhot@cea.fr

a)



b)

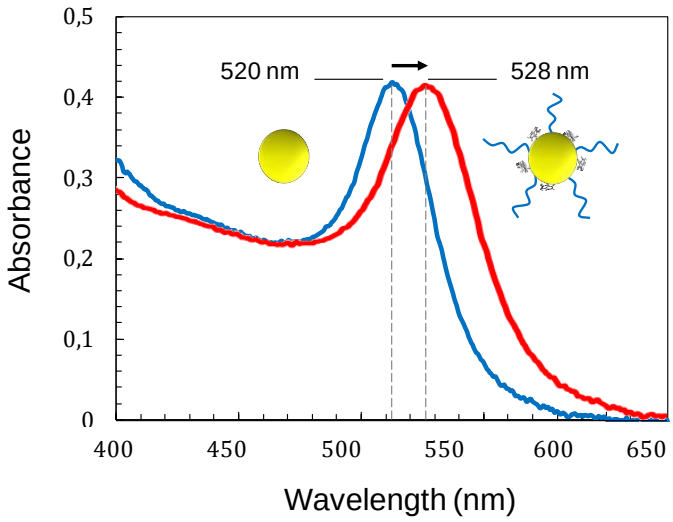


Figure S0. (**a**) TEM images of non-coated AuNPs deposited on a carbone-covered copper grid. (**b**) Absorption spectra of 20 nm non-coated AuNPs (blue line) and AuSplitAPT functionalized-AuNPs (red line). A wavelength shift of 8 nm was observed for the samples AuSplitAPT functionalized-AuNPs compared to the controls (non-coated AuNPs) which is indicative of conjugation to the surface through the thiol moieties.

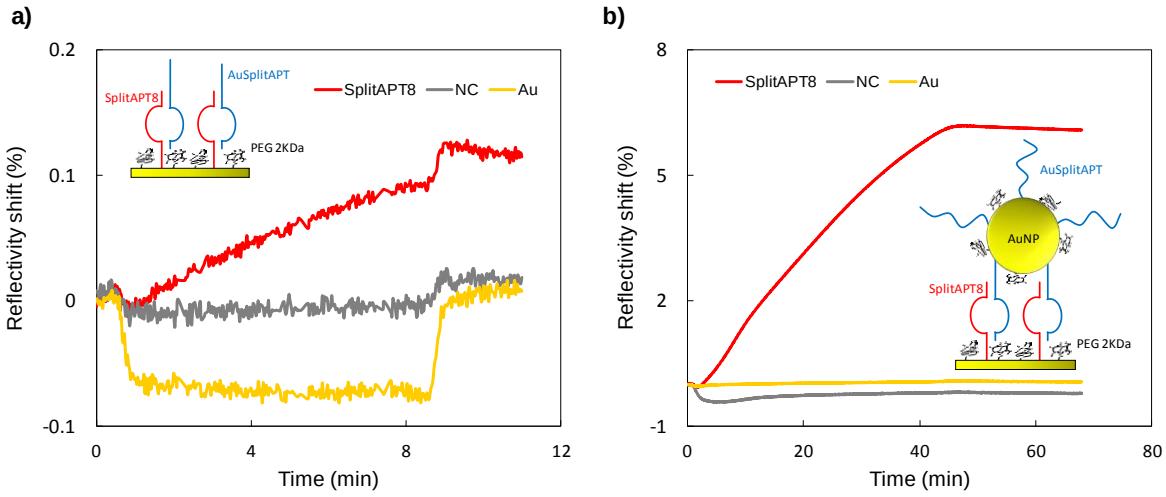


Figure S1. SPR signal amplification due to AuNPs. The red line (SplitAPT8) represents the SPRi signal measured throughout the hybridization of free AuSplitAPT with SplitAPT8 probes (a) and AuSplitAPT functionalized-AuNPs with the same probes (b). The gray line (NC) represents the SPRi signal obtained for the Negative Control spots of the microarray (See Table 1 for DNA sequences). The yellow line (Au) represents the SPRi signal measured on the bare gold spots where no DNA functionalization was performed (presence of PEG molecules as blocking agents). The injection of 250 nM of AuSplitAPT oligonucleotides leads to +0.1% shift in reflectivity measured by SPR imaging (a) while the injection of 200 pM of AuNPs grafted by the same oligonucleotides (AuSplitAPT) leads to a sixty-fold increase of the SPR signal. Inset images correspond to the schematic representation of the detection strategy.

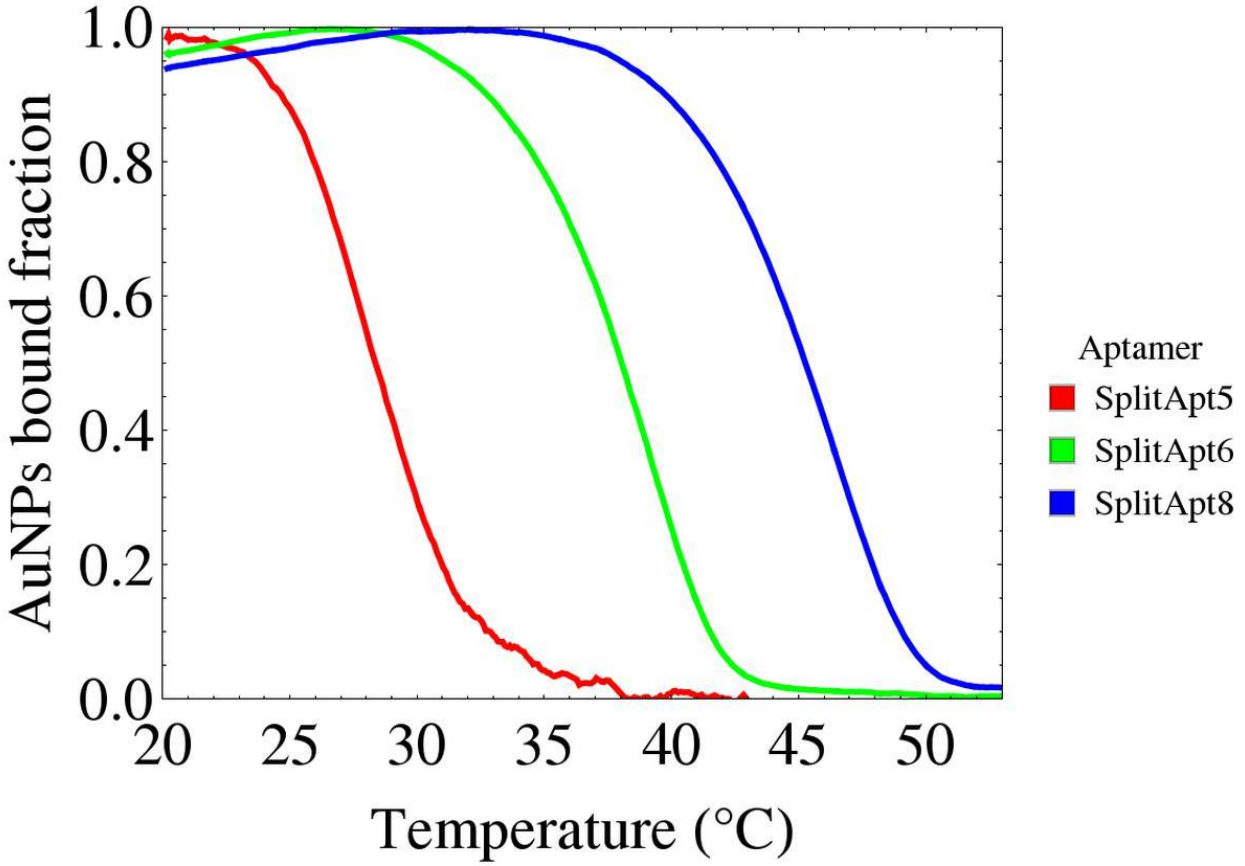


Figure S2. Melting temperatures of the complex with AuNPs on the various functionalized surfaces (**SplitApt5**, red; **SplitApt6**, green; **SplitApt8**, blue). The increased lengths of the hybridized stem lead to an increase of the stability of the complexes formed and thus the observed melting temperatures.

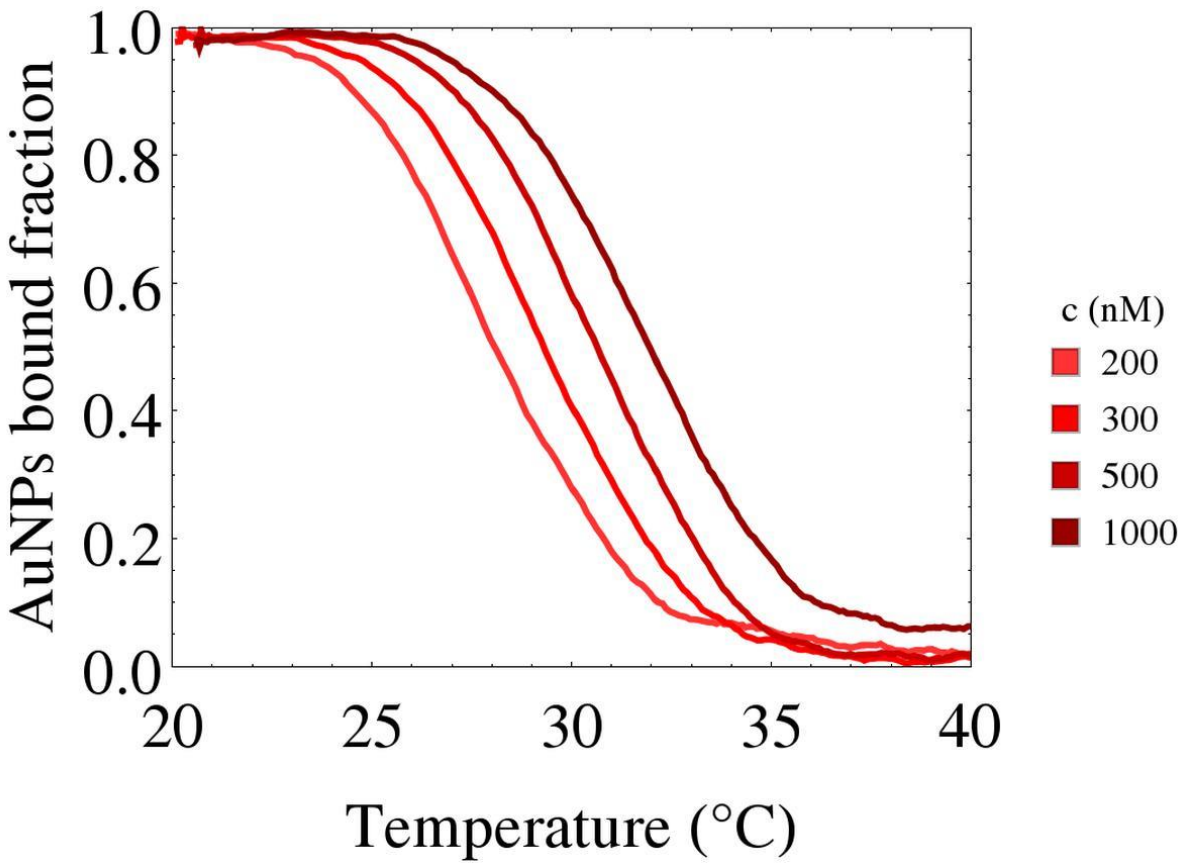


Figure S3. Melting curves for the complex formed by AuNPs functionalized with **AuSplitAPT** and the arrayed **SplitApt4** spots for various concentration of Adenosine (200, 300, 500 and 1,000 nM).

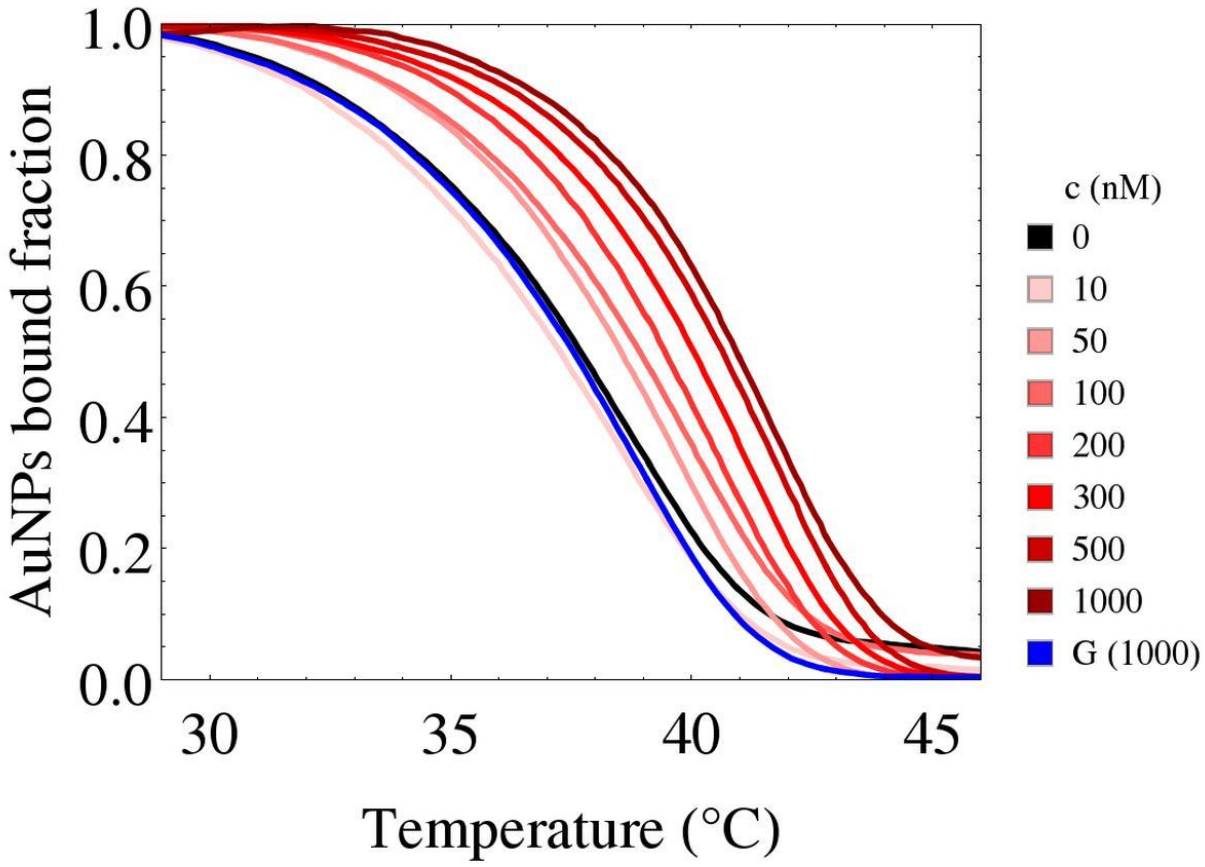


Figure S4. Melting curves for the complex formed by AuNPs functionalized with **AuSplitAPT** and the arrayed **SplitApt6** spots for various concentration of Adenosine (from 10 nM light pink to 1,000 nM dark red) or with 1,000 nM Guanosine (blue) or without Adenosine nor Guanosine (black).

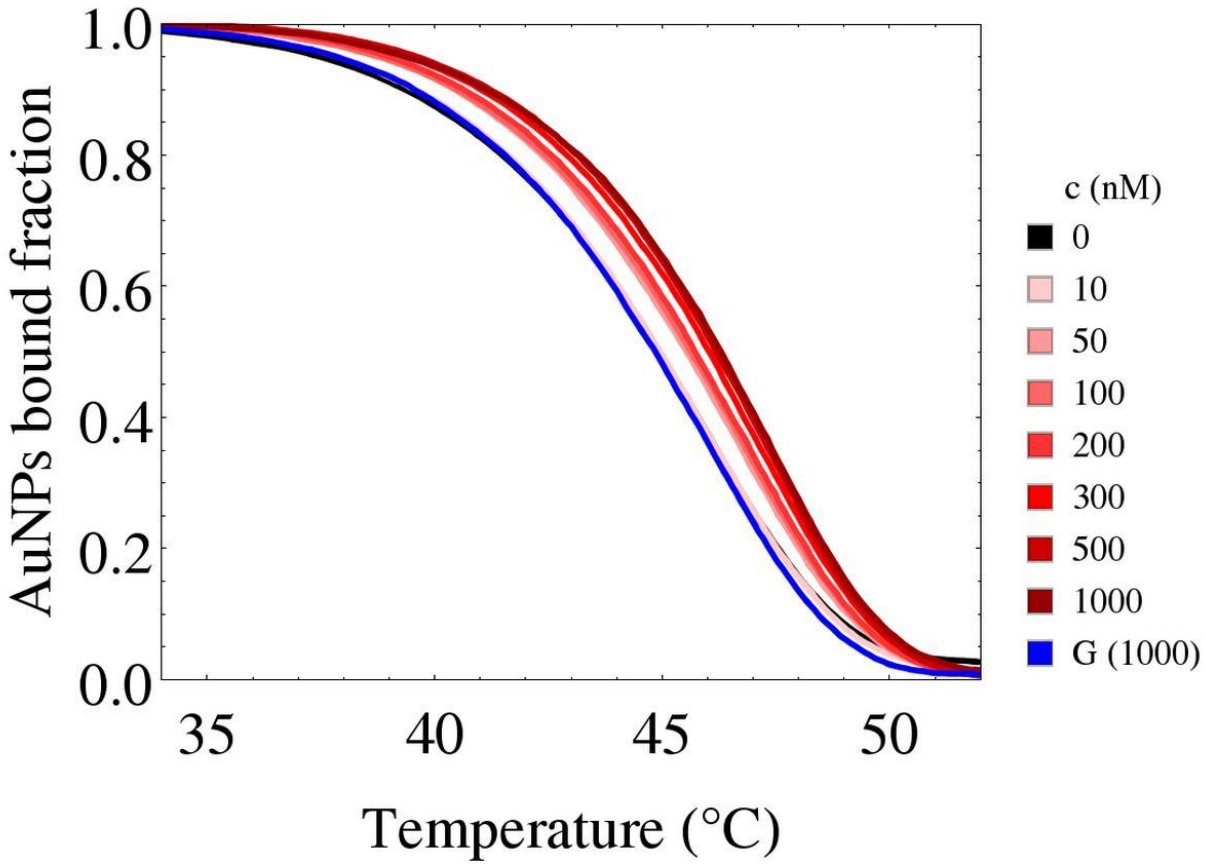


Figure S5. Melting curves for the complex formed by AuNPs functionalized with **AuSplitAPT** and the arrayed **SplitApt8** spots for various concentration of Adenosine (from 10 nM light pink to 1,000 nM dark red) or with 1,000 nM Guanosine (blue) or without Adenosine nor Guanosine (black).

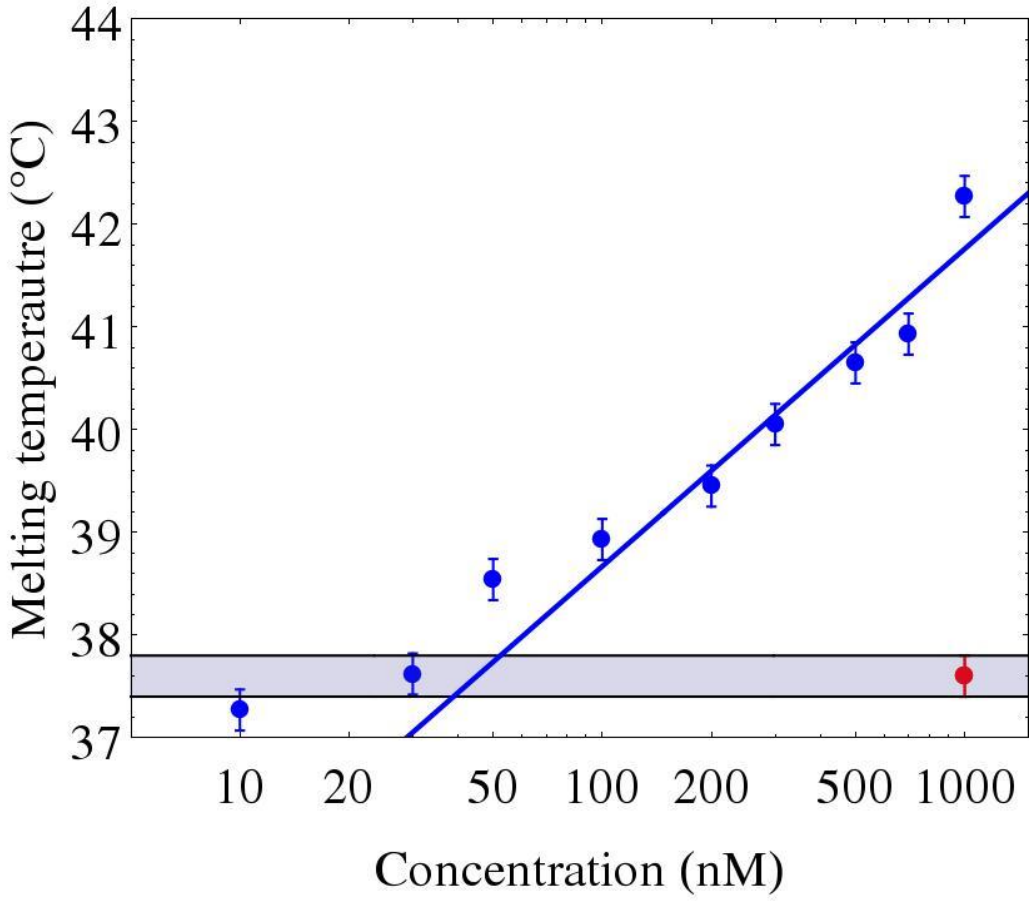


Figure S6. Melting temperatures for the **SplitApt6** functionalized surface as function of Adenosine concentration (in log scale). The grey bar corresponds to the melting temperature range expected without Adenosine and the red dot to the melting temperature for 1,000 nM of Guanosine. Error bars represent the standard deviation of signal measurements from spots triplicate of the same probe.

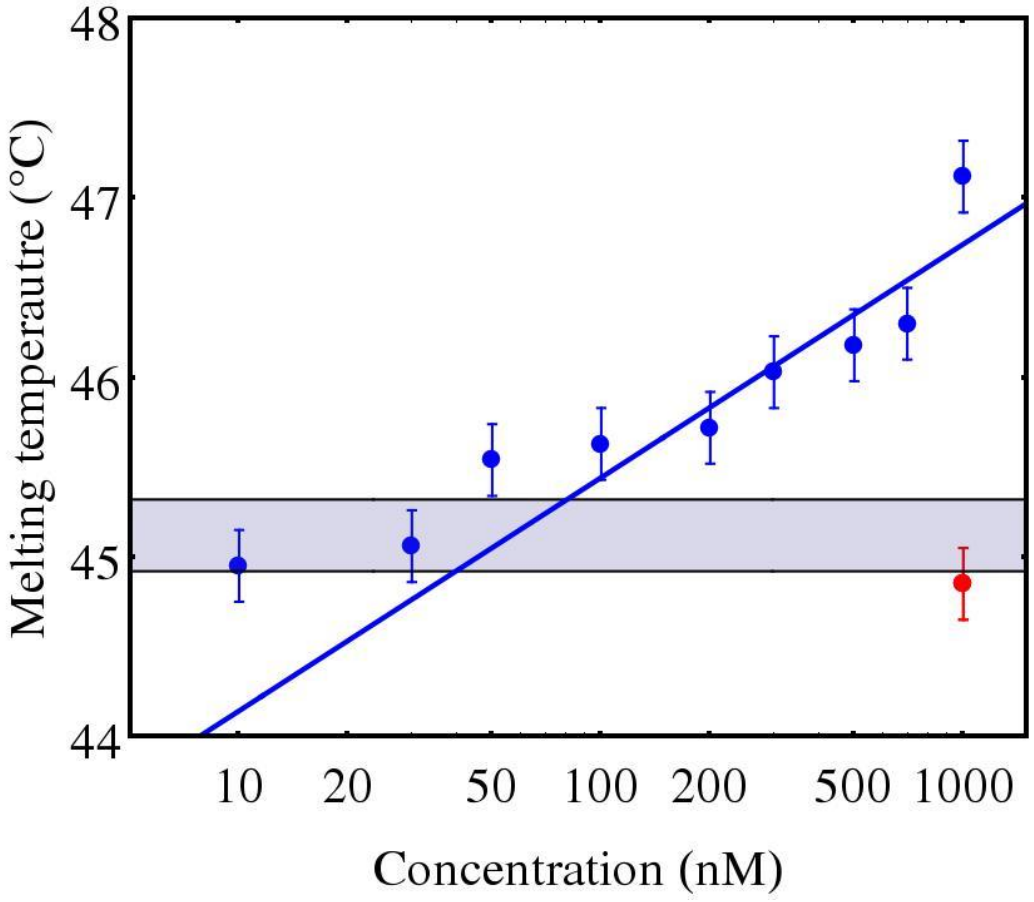


Figure S7. Melting temperatures for the **SplitApt8** functionalized surface as function of Adenosine concentration (in log scale). The grey bar corresponds to the melting temperature range expected without Adenosine and the red dot to the melting temperature for 1,000 nM of Guanosine. Error bars represent the standard deviation of signal measurements from spots triplicate of the same probe.

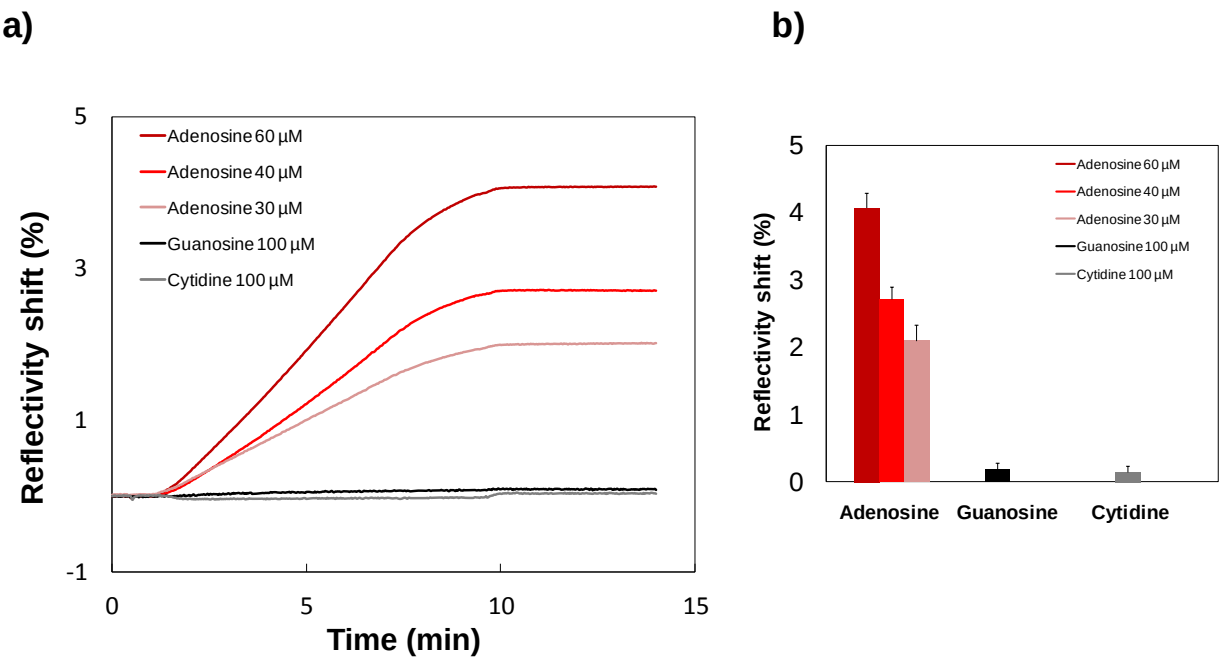


Figure S8. Selectivity test for the designed split aptamer sequences towards Adenosine. (**a**) Three different concentrations of Adenosine (60 µM, 40 µM and 30 µM) and its structural analogues: Guanosine (100 µM) and Cytidine (100 µM) were incubated in presence of AuSplitAPT functionalized-AuNPs. The specific SPRi signal on SplitAPT8 spots is observed in the presence of Adenosine and increases in function of the concentration. The presence of Guanosine or Cytidine does not induce a significant change in the SPRi signal, implying a high selectivity of the biosensor toward Adenosine. (All the SPRi signals were subtracted from the reference: injection of AuSplitAPT functionalized-AuNPs alone, C = 50 pM). (**b**) Differential reflectivity shift for Adenosine (60 µM, 40 µM and 30 µM), Guanosine (100 µM) and Cytidine (100 µM). All data (from three different spots) are expressed as mean ± standard deviation. (NB. After these preliminary selectivity tests, only Guanosine was chosen as a negative control for the Melting Curve experiments).