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## Small molecule SPR imaging detection from split aptamer microarrays

Feriel Melaine<sup>a,b,c</sup>, Yoann Roupioz<sup>a,b,c</sup> and Arnaud Buhot<sup>a,b,c\*</sup>

<sup>a</sup> Univ. Grenoble Alpes, INAC-SPRAM, F-38000 Grenoble, France

<sup>b</sup> CEA, INAC-SPRAM, F-38000 Grenoble, France

<sup>c</sup> CNRS, INAC-SPRAM, F-38000 Grenoble, France

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### Abstract

The small molecules detection remains a challenge for diagnostics. Due to their size, difficulties arise in finding selective and sensitive probes as well as developing appropriate detection format based on sandwich and/or signal amplification assays. In this study, we combine multiple strategies: a) the use of aptamer probes, short oligonucleotides strands selected in order to present strong selectivity for the small molecule target, b) the sequence engineering of split versions of the aptamer for a sandwich assay and c) the amplification of Surface Plasmon Resonance imaging (SPRi) signal by the use of gold nanoparticles (AuNPs). Combining those three strategies lead to state-of-the-art limit of detection (LOD = 50 nM) for the adenosine target.

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*Keywords:* Gold nanoparticles; Surface Plasmon Resonance imaging; small molecule detection; split aptamers; sequence engineering.

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Small molecules detection is one of the major challenge facing biosensors with potential applications in many areas: pharmacology, environment or security. The main difficulty in such diagnostic relates to the small size and low concentrations of the targets. The first challenge resides in finding a probe with high selectivity and affinity towards the target. Secondly, direct detection is generally impossible as the effect of the probe-target binding is usually close to noise signal. Indirect strategies like sandwich assays or signal amplification are to be developed.

In this study, we combine multiple strategies to overcome those limitations. The choice of aptamers as probes is particularly adapted for small molecules [1]. Those are short oligonucleotides selected specifically from a large library of RNA or DNA sequences for their affinity towards a predefined target. Recently, aptamer sequences have been engineered and cut in two parts to enable sandwich-like assays. This splitting was realized without significant loss of affinity towards the target in comparison with the original single strand sequence. This is the case of the

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\* Corresponding author. Tel.: +33-438 78 38 68; fax: +33-438 78 56 91.

E-mail address: [arnaud.buhot@cea.fr](mailto:arnaud.buhot@cea.fr)

adenosine aptamer whose hairpin sequence may be cut in the loop as this region is not directly involved in the adenosine binding pocket [2]. We performed a sequence screening of multiple split-aptamer probes thanks to a microarray format [3]. Surface Plasmon Resonance imaging (SPRi) allows for the multi-parametric and real-time detection. SPRi has been known so far to be limited in term of resolution for the direct detection of small molecules. The use of gold nanoparticles functionalized by the second half of the split-aptamer AuSplitAPT allows for an amplification of the SPRi signal. We developed dedicated functionalization chemistries based on self-assembling monolayers of thiols on both gold surfaces, the SPRi prisms and the nanoparticles, in order to limit the non-specific adsorption [3,4,5]. We measured the limit of detection (LOD) for two particular adenosine split aptamer sequences SplitAPT4 and SplitAPT8 (see Table 1 for sequences). Three orders of magnitude difference in the LOD were observed depending on the existence or not of SPRi signal in absence of adenosine. For the split-aptamer sequence SplitAPT4 presenting a signal OFF-signal ON behaviour, state-of-the-art performances have been obtained with a LOD as low as 50 nM. Injection of guanosine, instead of adenosine, confirm the good selectivity of the aptasensor.

**Table 1:** Oligonucleotide sequences: AuSplitAPT grafted on AuNPs. SplitAPT4 and SplitAPT8 grafted to the microarrays along with the negative Control sequence. In blue, hybridizing part. In red, adenosine binding pocket.

Name	Sequence (5' -> 3')
SplitAPT8	HS- T <sub>10</sub> -TGC GGAGGAAGGTTCTC
SplitAPT4	HS- T <sub>10</sub> -TGC GGAGGAAGGTAGAG
AuSplitAPT	HS-T <sub>10</sub> -AGAGAACCTGGGGGAGTAT
Control	HS-T <sub>5</sub> -GACCATCGTGCGGGTAGGTAGACC

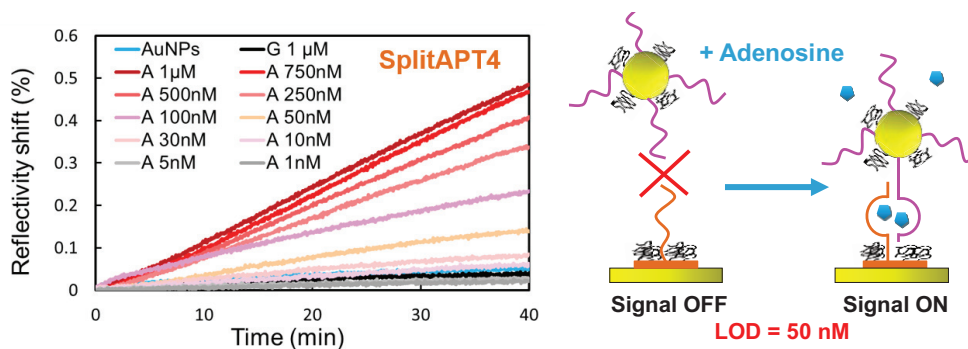


Fig. 1. SPR imaging detection of adenosine (A) with split-aptamers at LOD = 50 nM. Guanosine (G) injection serves as selectivity control.

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