Electronic Supplementary Information

**Surface plasmon field-enhanced fluorescence reversible split aptamer biosensor**

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**Characterization of the biointerface architecture**

The surface mass density of neutravidin and S1-Biotin segment at the sensor surface was measured as 1.49 ng/mm$^2$ and 0.47 ng/mm$^2$, respectively. These values were determined from shifts of SPR dip in the angular reflectivity spectra presented in Figure S1. The surface mass density of neutravidin (molecular weight of 60kDa) is comparable to full packed monolayer [1]. The three – fold smaller surface mass density of the aptamer segment S1-Biotin (molecular weight of 9.37kDa) corresponds to about 2 strands immobilized per attached neutravidin.

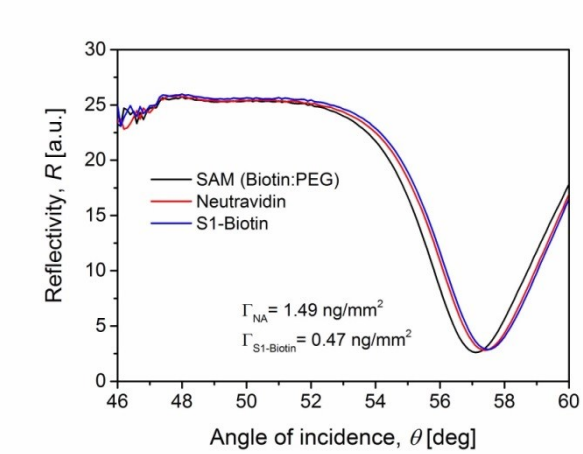


Figure S1. Angular reflectivity spectra $R(\theta)$ measured at sensor chip functionalization steps.

Thicknesses of immobilized NA and S1-Biotin were calculated by fitting the angular reflectivity spectra using transfer matrix-based model with the software Winspall (Max Planck Institute for Polymer Research, Germany). In the fitting, refractive index of buffer was assumed as $n_b$= 1.333, $n_{SAM}$ and refractive index n of polymer layer with a thickness $d_p$ on the top of the gold surface was $n_p$=1.45. The surface mass density $\Gamma$ is calculated with the obtained thickness values using the formula $\Gamma=(n_p-n_b) \cdot d_p \cdot \partial c/\partial n$ , where $\partial n/\partial c$= 0.2 $mm^{-3}$ $mg^{-1}$.

**Optimization of S2-AF647 concentration**

In the fluorescence assay, the concentration of S2-AF647 aptamer segment was chosen based on data presented in Figure.S2. Fluorescence signal intensity was recorded for ATP-assisted formation of the complex between S1-Biotin and S2-AF647 segments. S2-AF647 concentration of 10 nM and 100 nM and varying ATP concentrations between 1 µM and 1 mM were used and the limit of detection (LOD) determined as a concentration for which the calibration curve intersects with the baseline noise. As the LOD for 100 nM concentration out-performed that for 10 nM S2-AF647 concentration, the higher one was used in the assay. The even higher concentration was not considered as the further increased background signal $F_0$ would limit the dynamic range of the fluorescence detection.

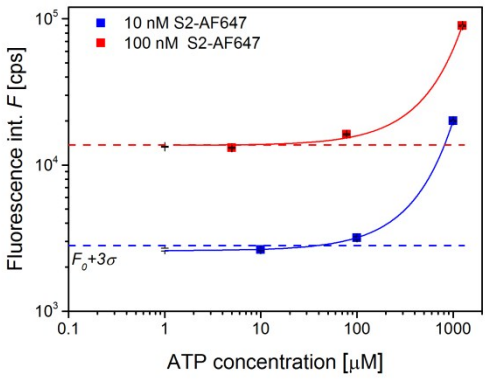


Figure S2. Titration measurements performed with S2-AF647 concentration of 10 nM and 100 nM and varying ATP concentrations between 1 µM and 1 mM.

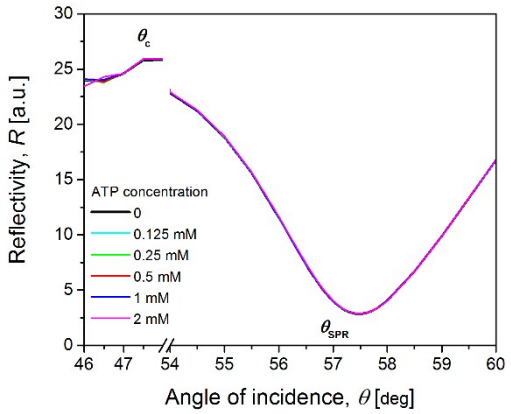


Figure S3. Angular reflectivity spectra $R(\theta)$ measured upon the affinity binding of ATP to the sensor surface from samples with ATP concentration from 0.125 to 2 mM (Figure 2 of manuscript).

**Determination of equilibrium affinity binding constants**

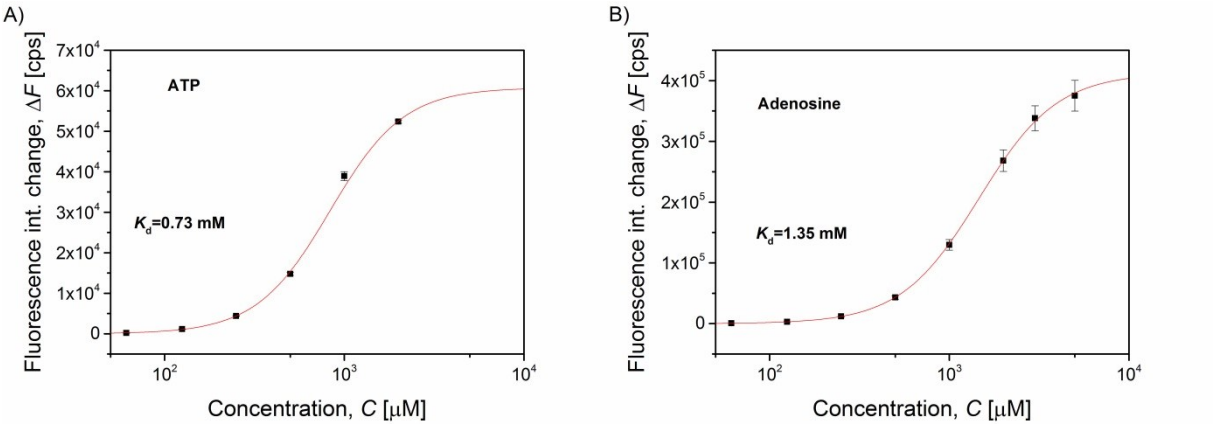


Figure S4. Calibration curves of A) ATP and B) Adenosine detection fitted with the Langmuir isotherm model. Dissociation constant $K_d$ values determined as the half saturation concentration of the analyte.

**Reversibility of the assay performed in 10% serum**

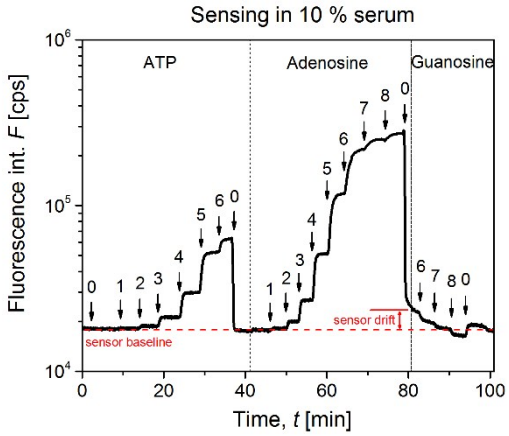


Figure S5. The titration measurements illustrating the reversibility of the split aptamer based assay in 10 % serum. Analyte concentrations indicated in sequential numbers are as follows: 0- 0; 1- 0.062 mM; 2- 0.125 mM; 3- 0.25 mM; 4- 0.5 mM; 5- 1 mM; 6- 2 mM; 7- 3 mM; 8- 5 mM, respectively.

**References**

[1] T. Liebermann and W. Knoll, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2000, **171**, 115-130.