Supporting information for

A sandwich-like strategy for the label-free detection of oligonucleotides by surface plasmon fluorescence spectroscopy

by

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Optimization of the buffer solution

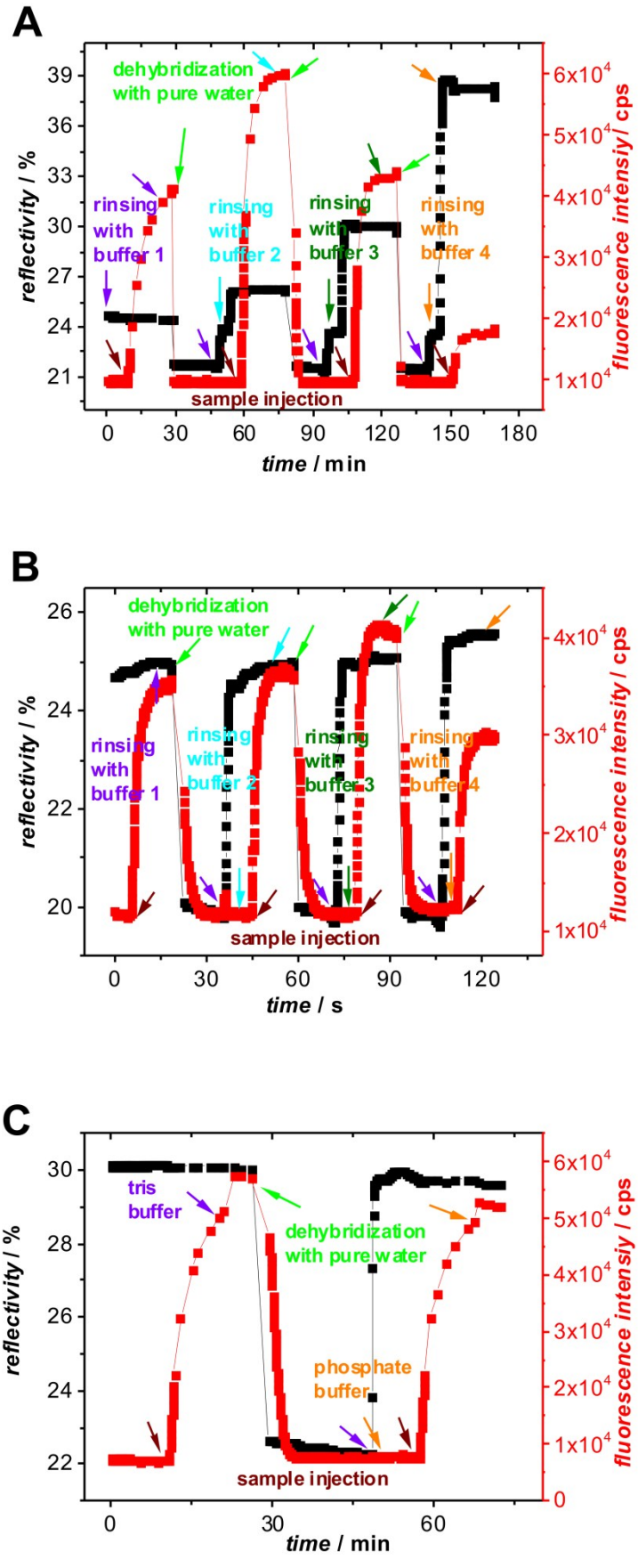

Figure S1. SPR (black) and SPFS (red) kinetic scan curves collected during the optimization of the buffer composition. The sensor chip has been prepared by adsorption of the capture probe at a concentration 5 µM. For signal readout 100 µL of a 10 nM solution of the target comprising 40 bases ($\mathbf{T_{40}}$) has been mixed (hybridized) with 100 µL of a 100 nM solution of the reporter probe. Before injection of the resulting mixture (wine arrow), the flow cell was rinsed by the same buffer in which the target and the reporter probe were dissolved. The mixture comprising a target concentration of 5 nM was incubated at the sensor chip for 10 min. Thereafter the cell was rinsed by the buffer in which the target and reporter probe dissolved to remove unbound target and the excess of reporter probe. After each detection step the sensor chip was regenerated, i. e. the captured target $\mathbf{T_{40}}$ and the reporter probe were dehybridized by rinsing with pure water (green arrow). In (**A**) the NaCl concentration was varied (concentrations of 100 mM, violet, 200 mM, cyan, 400 mM, olive, and 800 mM, orange, were evaluated), as buffer 2 mM tris-buffer at pH 7.5 with 6 mM $MgCl_2$ was used. In (**B**) the $MgCl_2$ concentration was varied (concentrations of 2 mM, violet, 4 mM, cyan, 6 mM, olive, and 8 mM, orange, were evaluated), as buffer 2 mM tris-buffer at pH 7.5 with 200 mM NaCl was used. In **C** the detection of $\mathbf{T_{40}}$ in 2 mM tris buffer (violet arrow) and in 2 mM phosphate buffer (orange arrows), both containing 200 mM NaCl and 6 mM $MgCl_2$ at pH 7.5, were compared.

Comparison of different thiol spacers

As shown in **Figure S2** different thiol spacers were compared with the mixture of 4-mercapto-1-butanol/3-mercaptopropionic acid (MCB/MPA). The thiols 6-mercaptohexanol (MCH), 11-mercapto-1-undecyl dihydrogen phosphate (MDPA), and 3-mercapto-1-propanesulfonic acid sodium salt (MPS) were evaluated. The highest sensitivity was obtained with the mixture of the short thiols MCB and MPA, possibly because the short thiols do not hamper the hybridization of the target at the surface.

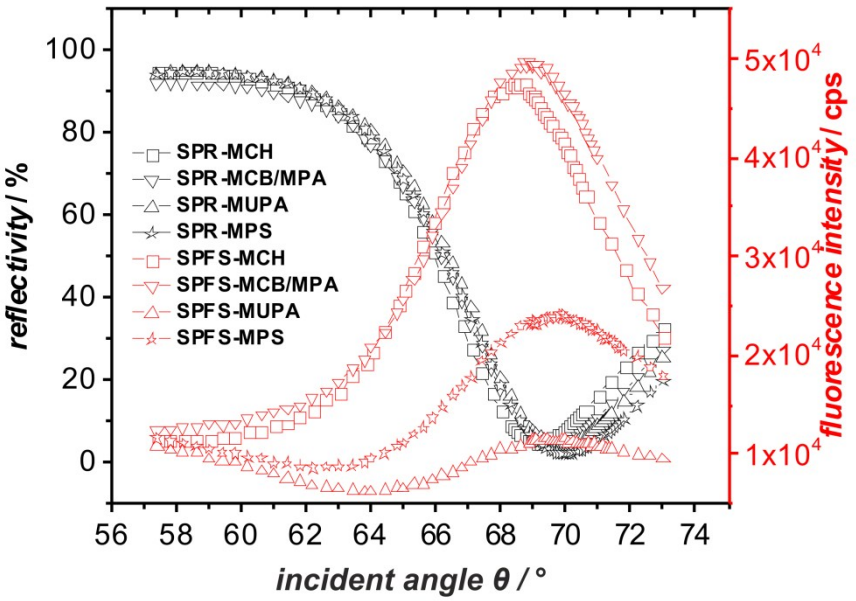

Figure S2. Comparison of different thiol spacers. SPR (black) and SPFS (red) angular scan curves collected during the target detection procedure with sencor chips prepared with different thiol spacers. The sensor chips were prepared by adsorption of 5 µM capture probe in the absence of additional thiols for 1 h followed by the adsorption of a mixture of 1 mM MCH (squares), 1 mM MCB/MPA (down triangles), 1 mM MUPA (up triangles) and 1 mM MPS (stars), respectively. Thereafter the surface was rinsed with buffer. Subsequently 5 nM T_{40} mixed with 50 nM reporter probe was incubated for 10 min, followed by rinsing with buffer for 5 min before the angular scan curves were collected.

Determination of the detection limit for the target T_{40} following a stepwise hybridization procedure as outlined in Scheme 3A.

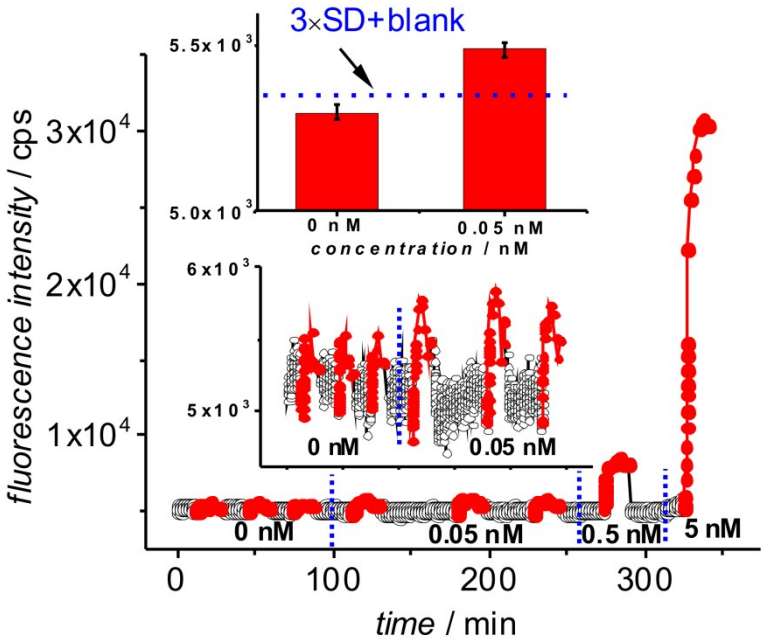

Figure S3. Determination of the dection limit for the 40 bases target (**T_{40}**) for stepwise hybridization at the sensor surface. The SPFS kinetic scan curve was collected for three times blank followed by three times detection of the target at concentration of 0.05 nM, as well as one time at 0.5 nM and 5 nM. Injection of 200 µL target solution was followed by injection of 200 µL of the reporter probe at a concentration of 50 nM (periods for incubation with the reporter probe (10 min) and subsequent rinsing with buffer (5 min) is shown in red). After each detection step the sensor surface was regenerated by dehybridization with pure water followed by equilibration with buffer (black). In the inset the average fluorescence signal of three measurements of the blank and the target a concentration of 0.05 nM on the same sensor chip is displayed. The fluorescence signal was detected after 10 min incubation with the reporter probe and 5 min subsequent rinsing with buffer. The threshold (dashed line) is equal the sum of the blank and three times the standard deviation (SD). The blank is the average value for the negative control (0 nM target). The error bars show the confidence interval for P = 0.95.

Determination of the detection limit for the target T_{60} target by incubating mixtures of target and reporter probe followed by incubation with the patch.

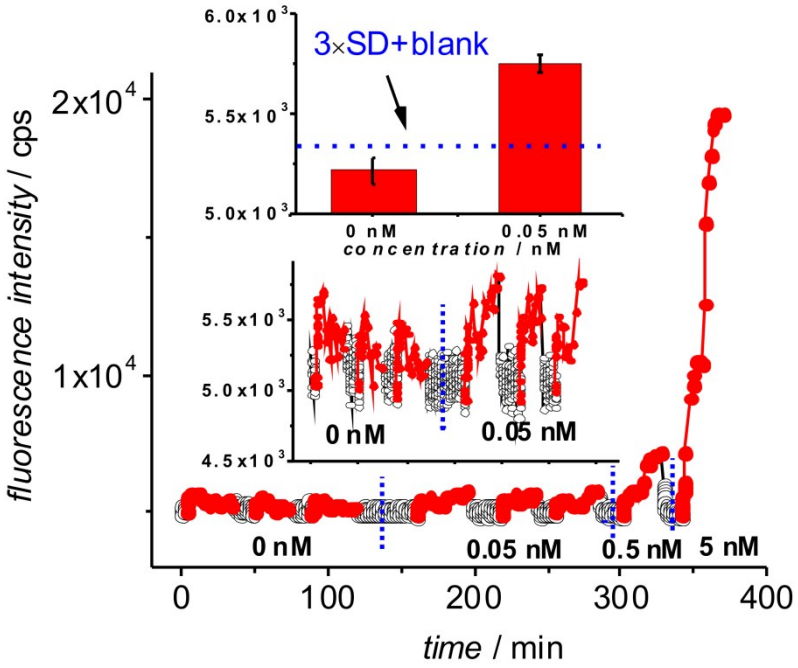

Figure S4. Determination of the detection limit for the 60 bases target ($\mathbf{T_{60}}$) by incubation after hybridization (mixing) 100 µL of target solution with 100 µL of a 100 nM solution of the reporter probe. The SPFS kinetic scan curve was collected for three times blank followed by three times detection of the target at concentration of 0.05 nM, as well as one time at 0.5 nM and 5 nM. After binding target and reporter probe the patch was incubated at a concentration of 10 nM (periods for incubation with the target plus reporter probe as well as the patch is shown in red). Especially from the data collected for the target concentrations 0.5 nM and 5 nM it can be seen that by incubation with the patch the fluorescence significantly increases. After each detection step the sensor surface was regenerated by dehybridization with pure water followed by equilibration with buffer. In the inset the average fluorescence signal of three measurements of the blank and the target a concentration of 0.05 nM on the same sensor chip is displayed. The fluorescence signal was detected after 10 min incubation with the patch and subsequent rinsing with buffer. The threshold (dashed line) is equal the sum of the blank and three times the standard deviation (SD). The blank is the average value for the negative control (0 nM target). The error bars show the confidence interval for P = 0.95.

Determination of the detection limit for the target T_{60} following a stepwise hybridization procedure as outlined in Scheme 3B.

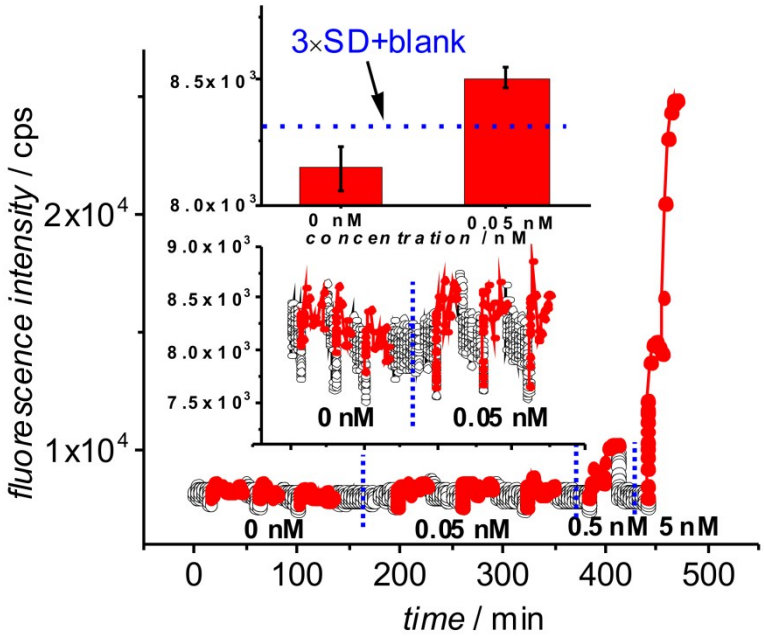

Figure S5. Determination of the detection limit for the 60 bases target ($\mathbf{T_{60}}$) by stepwise incubation starting with 200 µL of target solution followed by 200 µL of a 50 nM solution of the reporter probe and then 200 µL of the patch containing buffer solution at a patch concentration of 10 nM. The SPFS kinetic scan curve was collected for three times blank followed by three times detection of the target at concentration of 0.05 nM, as well as one time at 0.5 nM and 5 nM. Periods for incubation with the reporter probe as well as the patch are shown in red. Especially from the data collected for the target concentrations 0.5 nM and 5 nM it can be seen that by incubation with the patch the fluorescence significantly increases. After each detection step the sensor surface was regenerated by dehybridization with pure water followed by equilibration with buffer. In the inset the average fluorescence signal of three measurements of the blank and the target a concentration of 0.05 nM on the same sensor chip is displayed. The fluorescence signal was detected after 10 min incubation with the patch and subsequent rinsing with buffer. The threshold (dashed line) is equal the sum of the blank and three times the standard deviation (SD). The blank is the average value for the negative control (0 nM target). The error bars show the confidence interval for P = 0.95.