Application Note # 117

# Quantification of single stranded oligonucleotides with SPR Navi 200

## Introduction

dNA assays can be applied for detecting genetic material in order to detect genetic disorders, mutations, gene transfection or species from a large variety of samples. By assaying specific single stranded oligonucleotides of over 20 nucleotides in length, it is possible to detect and quantify unique gene sequences from large amounts of genetic material [1]. BioNavis SPR Navi 200 can be utilized easily into a dNA assay that can detect and quantify single-stranded oligonucleotides with high precision over a large concentration range from micro to nanomolar concentrations.

## Materials and methods

27-unit oligonucleotide modified at the 5' end to have a disulfide group was self adsorbed onto a purified gold surface for 15 minutes. In order to prevent non-specific binding interactions of analyte with the gold surface, *N,N*-bis (2-hydroxyethyl)-α-lipoamide molecule was self adsorbed to the surface for 15 minutes.

Samples of PCR-amplified single stranded dNA of 123 having a complementary sequence to the probe dNA (dNA-123) were used as a model sample for gene partially digested dNA. The assay specifity was tested with non-complementary dNA of the same length, and bovine serum albumin (BSA). The experiments were performed in pH 7.5 20 mM PBS with 300 mM NaCl and 1 mM EDTA. 0.1% SDS in 10 mM NaOH was used to regenerate the surface between samples. The dNA binding was measured in fixed angle mode of the BioNavis SPR Navi 200 equipped with the SPR Navi Autosampler.

## Results and discussion

An example of the sensogram resulting from the baseline-analyte-regeneration cycle for two consecutive injections is presented in Figure1. A compilation of several injections in Figure2 and the exponential fit (Figure3) created from this compilation show wide detection range (1000 nM and 0.1 nM) and sensitivity of the assay. The excellent compliance with the one-to-one Langmuir exponential fit of the data shows that the interactions were single site, as expected. The injections with non-complementary dNA and BSA with 1000 nM concentration did not result to any signal changes after buffer rinsing ("non-comp" in Figure2), showing that the assay was highly selective. The assay sensitivity and detection limits are identical to a similar assay described in the literature [1].

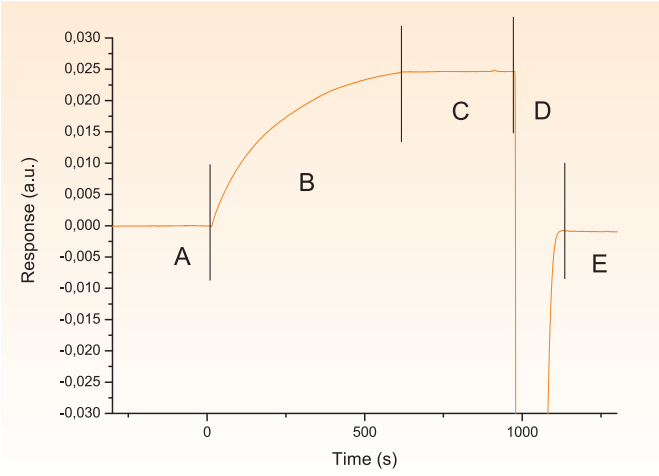


**Figure 1.** A sensogram from 100 nM injection of the dNA showing the analysis cycle. A) Baseline, B) dNA injection, C) Buffer rinsing, D) Regeneration and E) Background for next injection.


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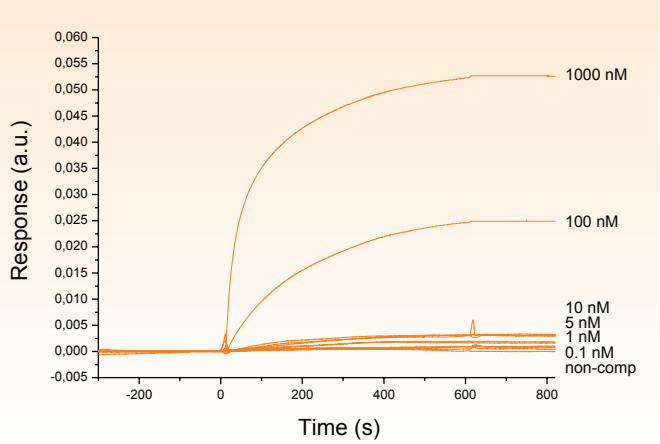


**Figure 2.** A compilation of the all the injections used in the experiment, concentrations ranging from 1000 nM to 0.1 nM.

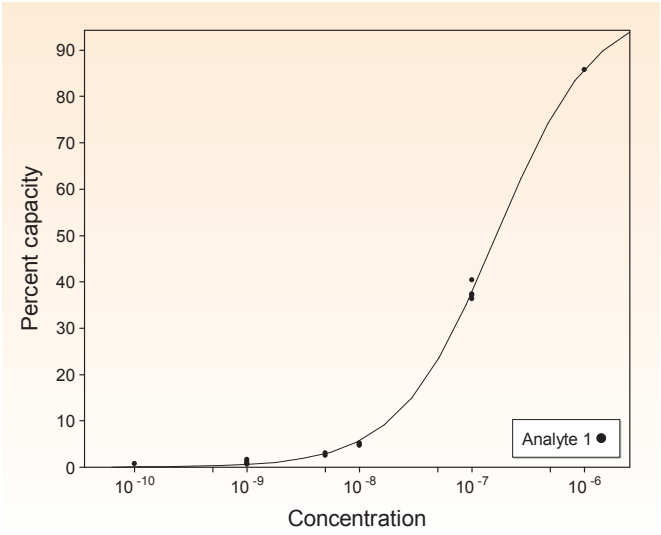


**Figure 3.** A semi-logarithmic plot of the SPR signal after 20s of flushing the sample with buffer. The solid line is One-to-One exponential fit to the data.

## Conclusions

SPR Navi 200 is an effective tool for detecting and quantifying oligonucleotides with high sensitivity and precision. The simple self assembly dNA assay creation is higly effective in the oligonucleotide quantification, and the assay performance is identical to the same assay performed with a different instrument in the literature [1].

[1] Vikholm-Lundin et al. Surface Science 603 (2009) 620–624



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