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FNSNF

Comparison Between SiNWs and Optical Sensors Functionalized with Ligand Recognizing the Bacterial Lectin FimH

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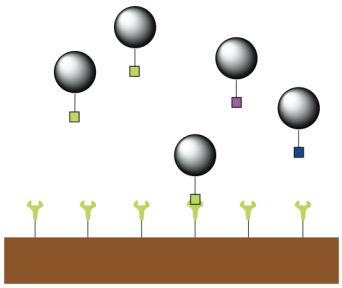
Abstract

The conversion of a silicon nanowire (Si-NW) field effect transistor into a chemical sensor is done by applying a receptor layer, which selectively interacts with the specific targeted analyte molecule. Si-NWs have shown great potential as biosensors for ultrasensitive and label-free detection of biomolecular interactions. Their sensitivity depends not only on the device properties, but also on the type of recognition event at their surface. We want to apply this technology to the screening of clinical samples infected by FimH bearing bacteria. One of our FimH ligand showed positive results, but the system needs to be optimized. This process could be helped by a comparison with a stable, well established method. We decided to use gold optical biosensors, based on Surface Plasmon Resonance, which can give important insights into the binding events, under dynamic conditions.



Methods

Biacore setup:

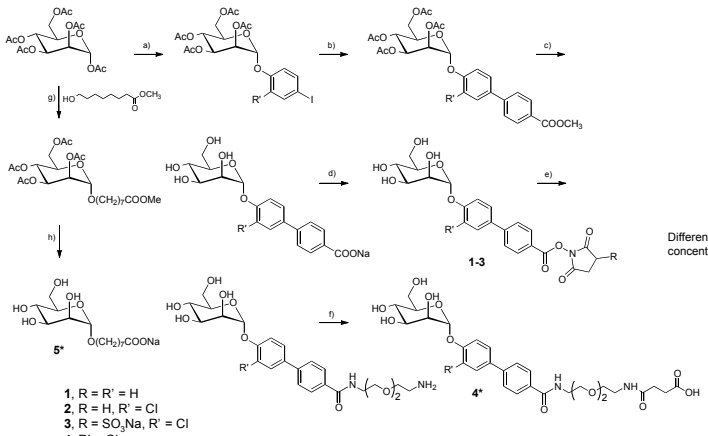
The surface of a CM4 dextran matrix covered gold chip (Biacore) was reacted with 1,2-ethylenediamine linker (i. activation of the carboxylic groups on the surface, NHS, EDC, 150μL, 5μL/min, then ii. 0.1 M solution of 1,2-ethylenediamine in borate buffer, pH 8.5, 100μL, 5μL/min), and then with carboxylic acid *N*-hydroxysuccinimidyl esters (NHS-esters) of the test compounds (2mM solution, 10% DMSO, 90% borate buffer, pH 8.5, 200μL, 5μL/min). This strategy was applied to mimic the surface chemistry of the nanowires. A dilution series of the lectin protein FimH in HBS-P buffer (10mM HEPES, 150mM NaCl, 0.005% P20, pH = 7.4) was then assayed in an affinity test.

Nanowire setup:

The surface of a Nanowire-chip was functionalized with 3-aminopropyl-dimethylsilane (gas phase deposition, 80°C, sealed vessel, overnight), and then reacted with *N*-hydroxy-3-sulfo-succinimidyl esters of the test compounds (1.5μL drop on each active area, 100mM in PB 50mM, pH 7.5, culture dish, overnight). A flow-cell was then fixed to the chip, and a dilution series of the protein FimH was passed through it by a peristaltic pump.

Synthesis:

The ligands were chosen from a series of FimH antagonists with known affinities for the target FimH protein. The NHS and Sulfo-NHS esters were synthesized in water, following literature procedures.



1, R = R' = H
2, R = H, R' = Cl
3, R = SO_3Na, R' = Cl
4, R' = Cl

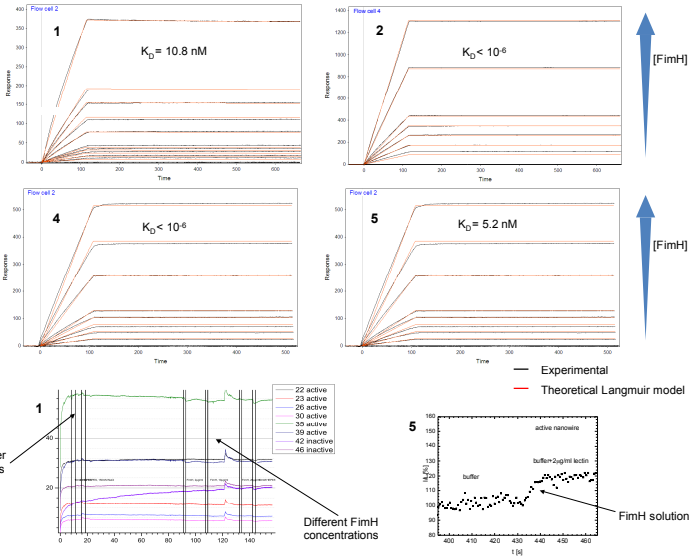
* Compounds number **4** and **5** were converted to their NHS esters without isolating them, and they were used directly for the functionalization of a CM4 chip.

a) 2-chloro-4-iodophenol, $BF_3{\cdot}Et_2O$, DCM, MS 4A, 70%.
b) Pd(dppf)DCM, p-carbomethoxyphenylboronic acid pinacol ester, K_3PO_4, 70%
c) i. NaOMe, MeOH, rt; ii. NaOH 0.5M, MeOH, rt 65%
d) EDC, NHS, H_2O, rt quant
e) PB 50mMpH 7.7/THF 2:9, EBE, rt 50-60%
f) Succinic anhydride, THF, rt, 45%
g) $SnCl_4$, DCM, rt, 30%
h) i. NaOMe, MeOH, rt; ii. NaOH 0.3M, MeOH, rt 81%

Results

The Biacore experiments showed that the NHS/EDC-diamine-NHS ester two-steps functionalization approach is effective on CM4 surfaces, and that the ligand used are all very strong binders of the FimH lectin protein. Calculated K_Ds are in the nM range for compound **1** and **5,** and out of range of Biacore experiment determination for compound **2** and **4** that means, the value is expected to be in the pM range. The sensograms show that once immobilized on the surface, the protein is very strongly retained, and that the signal response increases with increasing concentrations of protein.

The Nanowire-chip experiments showed that the surface, similarly functionalized, does not respond to the FimH protein, when functionalized with either **1** or **2** (data shown only for compound **1**). This could be due to limited flexibility of the aromatic ligands on the silicon surface, which would obstacle the protein-ligand interaction; this is in good agreement with the observation that the surface respond to the FimH protein when functionalized with compound **5.**



Outlook

The Biacore experiment will be repeated on a CM1 gold chip, which present a flat gold surface, without a dextran matrix; this should mimic more closely the Si-NW surface, giving a better insight in the binding event under these conditions. The Si-NW will be functionalized with ligands through different linkers, like in case of compound **4,** to understand whether a higher flexibility can restore the protein-ligand affinity.