

Supplementary information

Specific and reversible immobilization of histidine tagged proteins on functionalized silicon nanowires

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Characterization of the functionalization process on the glass support

The modification of the glass/silicon surface with Ni²⁺:NTA motifs via silanization of the surfaces with GPTMS, followed by the coupling of AB-NTA to the epoxy terminated groups via the amine-epoxid reaction, and finally the Ni²⁺ loading, is well reported in the literature [1-3].

In order to simplify the verification of the different functionalization steps selected for modification of the Si NWs, the entire process was carried out on glass solid supports, which is regarded as an analogue of the SiO₂ on the Si NW surface. The modified glass surfaces were characterized by both contact angle measurements (to check the GPTMS silanization and the AB-NTA coupling) and fluorescence techniques (to demonstrate the Ni²⁺ chelation and the specific protein immobilization via the His-tag) beforehand.

1. Contact angles at different stages of the functionalization process on the glass solid support

Surface/Layer	Contact angle deg.
SiO ₂ (clean glass)	< 5°
Epoxy SAM (GPTMS SAM)	51° ± 2°
AB-NTA	< 10°

Table S1: The contact angle demonstrates the different functional groups exposed on the surface in the different functionalization steps. The clean SiO₂ layer with hydroxyl groups on the surface gives a very low contact angle, which increases significantly after the formation of the GPTMS SAM with epoxy terminated groups [3]. Finally, the coupling of AB-NTA gives a low contact angle, characteristic of carboxylic acid terminated surfaces [4]

The reported contact angles, θ , are the average value of at least 12 drops of ultra pure water for each surface treatment on the glass coverslip. The shape of the water-drop was recorded by an equipped video camera and the contact angle between a given water-drop and the functionalized coverslip was given by an image analysis tool, *PixelStick* (pixilated software, freeware).

2. Demonstration of the presence of Ni²⁺ and the specific interaction between His-tagged protein and the Ni²⁺:NTA modified surface by EDTA elution

EDTA is a strong chelating agent. Upon addition to Ni²⁺-NTA modified surfaces, it chelates the Ni²⁺ ions, thereby simultaneously removing the bound protein since it is attached through its His-tag to the Ni²⁺. Hence a consequence of this mechanism of action is that if the protein

were not immobilized through the specific His-tag/ Ni^{2+} interaction, addition of EDTA would not result in protein release from the surface. This experiment is thus an indirect, but relevant proof of principle of Ni^{2+} on the surface and its important role in protein immobilization [5-7,9).

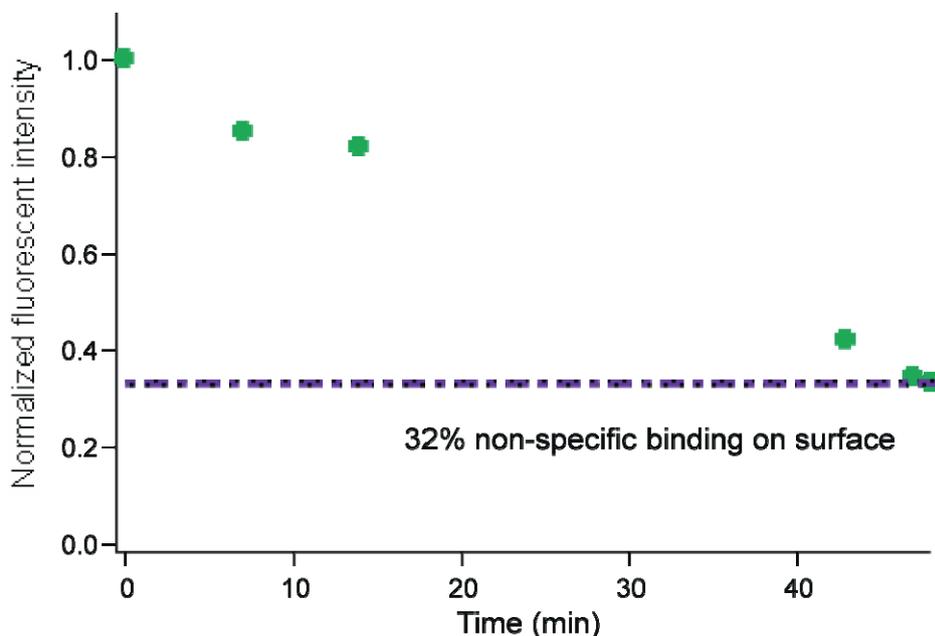


Figure S1: The His-tagged SFH-600 was immobilized on the Ni^{2+} :NTA modified coverslip and the fluorescence intensity with time was recorded in the presence of 500 mM EDTA. The drop of the intensity reveals the presence of Ni^{2+} on the surface at the beginning of the experiment. The figure also demonstrates the non-specific binding of the protein on the modified surface.

3. Demonstration of the specific interaction between His-tagged protein and the Ni^{2+} :NTA modified surface by elution with different concentrations of imidazole.

The ring structure of imidazole mimics the ring structure in the histidine amino acid and will thus compete with histidine for interaction with the Ni^{2+} . This experiment is a direct proof of the specific His-tag mediated interaction between the protein and the surface [5, 8].

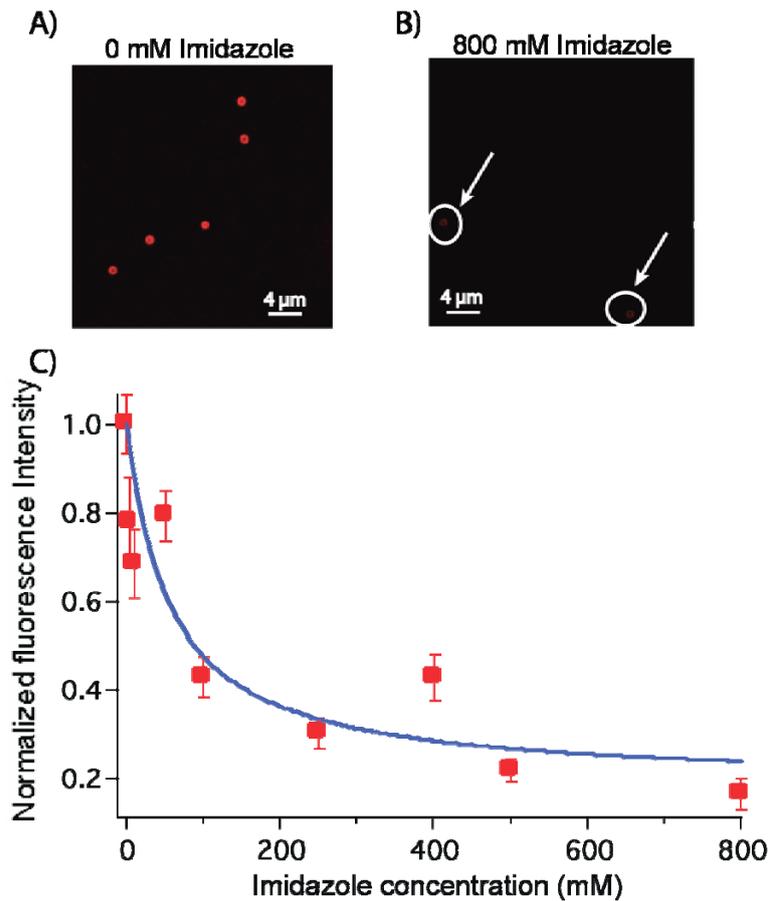


Figure S2: The His-tagged SFH-600 was immobilized on Ni²⁺:NTA modified glass beads. The confocal micrographs display the glass beads A) upon protein immobilization and B) after elution with 800 mM imidazole for 50 min. The white circles in B) point out the position of two glass beads. The fluorescence intensity of the glass beads *versus* different imidazole concentrations used to elute protein away from the beads is shown in C). Each data point represents the averaged intensity of at least 50 beads. The data points were fitted to the half maximum inhibitor concentration (IC50) formula ($f(\text{Conc}) = \text{max} - (\text{max} - \text{min}) / (1 + \text{Conc} / \text{IC50})$). The first point and last point on the curve correspond to A) and B) respectively.

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