FABRICATION OF METAL-ION PATTERNS BY μCP AND DPN FOR PROTEIN IMMOBILIZATION

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We have demonstrated that calcium and copper ions can be transferred onto fluorescent SAMs using dip-pen nanolithography (DPN) at μm and sub-μm scale[1]. Subsequent modulations of the fluorescent signal were visualized in situ by using a hybrid atomic force fluorescence microscope (AFFM). This approach enables several applications, including the selective and controlled immobilization of proteins via a specific metal–protein interaction.

Nitrilotriacetic acid (NTA)-terminated SAMs are particularly suitable for protein immobilizaton experiments, because they allow the oriented binding of His-tagged proteins via the complexation with metal ions (Ni^{2+}, Cu^{2+}, or Co^{2+})[2]. We present data using two lithography techniques, microcontact printing (μCP) and DPN, to fabricate nickel ion patterns on NTA-functionalized glass slides as templates for the immobilization of His-tagged enhanced green fluorescent protein (EGFP) (Figure 1).

We demonstrate that His-tagged EGFP patterns at μm scale can be achieved by microcontact printing of Ni^{2+} ions (Figure 2-a). The protein and complexed Ni^{2+} patterns can be removed easily by using EDTA solution (Figure 2-b). To demonstrate that the functionalized surfaces can be re-used, a PDMS stamp with a different feature size was used for microcontact printing of Ni^{2+} ions for immobilization of His_6-EGFP (Figure 2-c). The difference of fluorescence intensities in Figure 2-a and c may result from the varied amount of Ni^{2+} ions adsorbed onto the surface of PDMS stamp.

In order to obtain protein patterns at smaller scale, a cleaned Si_3N_4 tip dipped in $NiCl_2$ solution was used to write metal ion patterns. Figure 3-a and c depict fluorescence images of His-tagged EGFP line and dot patterns, respectively. Protein patterns with line width about 1.2 μm are presented. The smallest diameter of the dots in Figure 3-c is ~ 0.6 μm which is close to the resolution limit of the confocal fluorescence microscope. AFM topography experiments yield further information about the exact sizes of the nanofabricated protein patterns.

References:

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[2] M. J. W. Ludden, A. Mulder; R. Tampé, D. N. Reinhoudt and J. Huskens, Angew. Chem. Int. Ed., **46** (2007), 4104.

Acknowledgement:

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Figures:

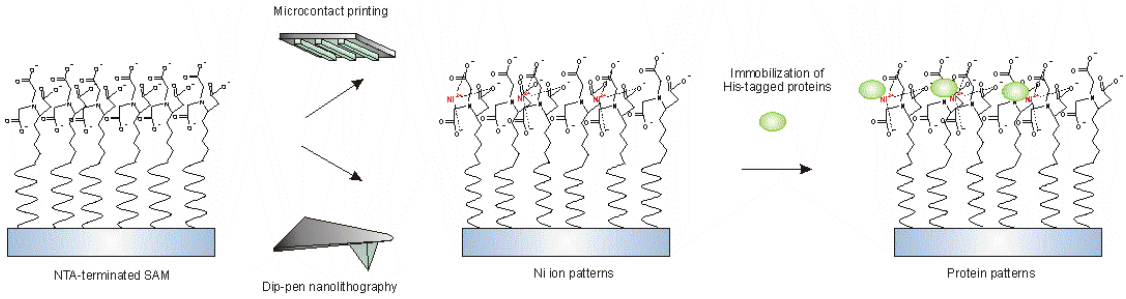

Figure 1 *Illustration of fabricating nickel ion patterns on a NTA-functionalized surface with microcontact printing and dip-pen nanolithography techniques for immobilization of His-tagged proteins.*

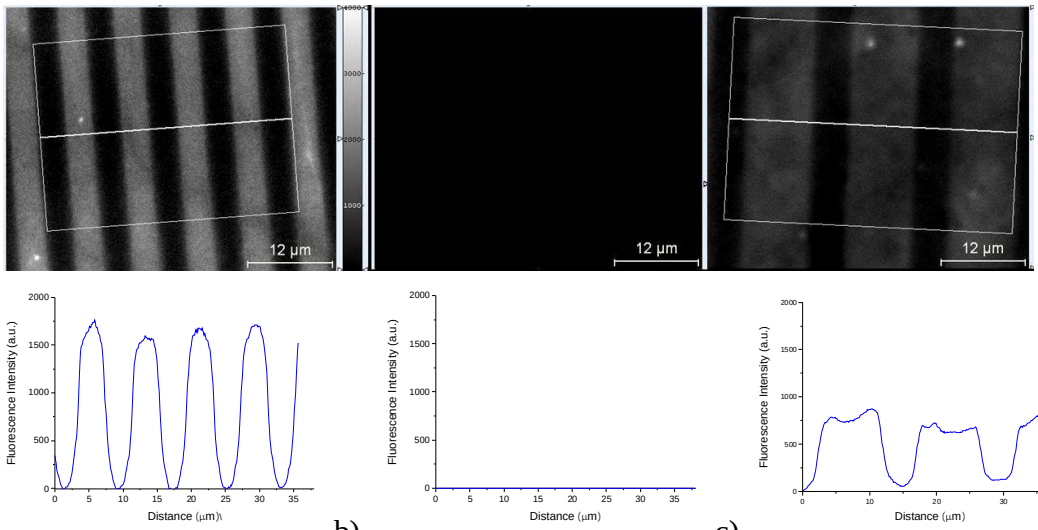

Figure 2 *Fluorescence images in gray scale and average fluorescence intensity profiles of the area inside the rectangle of His_6- tagged EGFP immobilized on Ni^{2+} patterns created after a) 1st µCP, b) after rinsing with 0.1M EDTA solution and c) after 2nd µCP). The integration time of (a), (b) and (c) is 200 ms, 200ms and 800ms, respectively. The printing time of 1st and 2nd µCP is 2 minutes without applying extra load.*

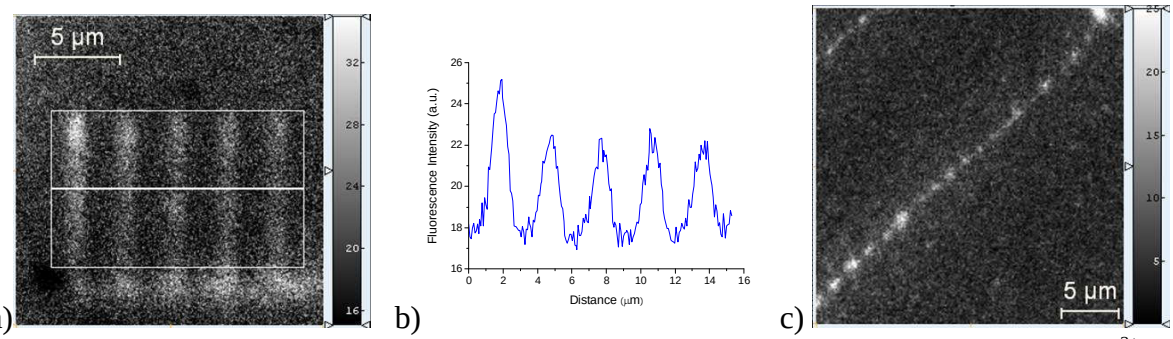

Figure 3 *a) and c) Fluorescence images in gray scale of His_6- tagged EGFP immobilized on Ni^{2+} line and dot patterns created by DPN. b) Average fluorescence intensity profile of the area inside the rectangle indicated in (a).*