

## Site-Specific Sorting of Proteoliposomes for High Density Parallel Screening of Membrane Receptor Function

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**INTRODUCTION:** Membrane proteins have particularly high impact in drug discovery with at least 50% of all targets being membrane proteins [1]. As such, it would be ideal integrate membrane proteins on a sensor substrate to investigate their properties. Membrane protein function is strongly dependent on assuming the correct conformation within a cell membrane, resulting in that such a sensor should incorporate the membrane proteins within a lipidic structure, e.g. liposomes or planar lipid membranes. We propose to build a platform, which in addition to incorporating membrane proteins in a near native environment on a sensor substrate, will also be able to sort the membrane proteins according to their functionality. We here present the first steps towards this platform in terms of fabricating of fabricating micron to sub-micron wells and surface functionalization for selective adsorption of proteins and liposomes only inside the wells.

**METHODS:** First, a 350 nm layer of silicon nitride is deposited onto a Si wafer. High aspect ratio straight wells are etched through the silicon nitride using RIE with a chrome mask patterned by colloidal lithography. The well size can be tuned to between 50 nm and 1  $\mu\text{m}$  depending on the requirement [2]. A PAAM-stamp “inked” with PLL-g-PEG will then be employed to passivate the top part of the sensor substrate [3]. Fibrinogen will then be used as a testbed subject to ensure that the substrate surface is non-fouling.

**RESULTS:** Wells were successfully etched into silicon nitride. Figure 1 shows the SEM top view of a silicon nitride surface where wells of 1  $\mu\text{m}$  diameter have been etched into the silicon nitride. Subsequently, the top part of the silicon nitride layer was successfully passivated against fibrinogen adhesion. Fibrinogen only adhered to the insides of the wells (Figure 2).

**DISCUSSION & CONCLUSIONS:** A porous substrate was successfully fabricated and initial tests with proteins indicated that this platform could also be used for the capture and subsequent investigation of proteoliposomes. Subsequent steps include backfilling the insides of the wells with PLL-g-PEG/Biotin or BSA/Biotin, and specific

adhesion of DNA strands to capture tagged proteoliposomes [4]. The proteoliposome adhesion will be studied using fluorescence microscopy and AFM.

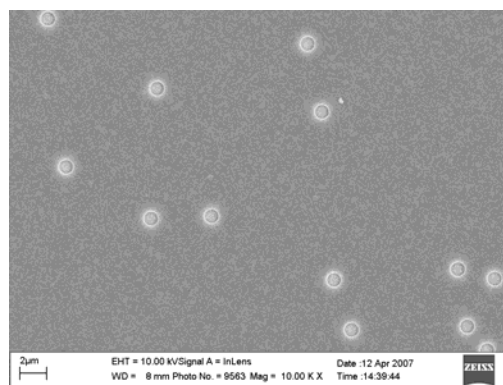


Figure 1: Wells of 1  $\mu\text{m}$  diameter were successfully etched into a silicon nitride layer.

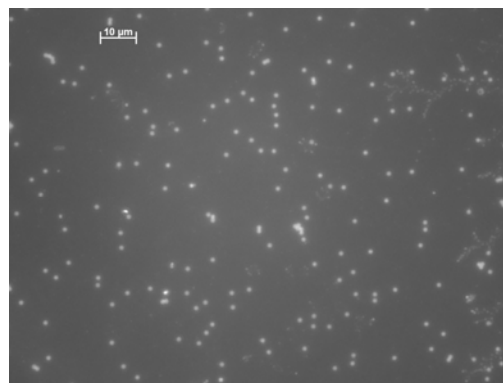


Figure 2: The top part of the sensor was passivated using PLL-g-PEG and the wells were backfilled with fibrinogen.

**REFERENCES:** <sup>1</sup> A.D. Howard, et al., (2001) *Trends in Pharmacological Sciences* **22**(3): p. 132-140. <sup>2</sup> E. Reimhult, K. Kumar, and W. Knoll, (2007) *Nanotechnology* **18**(27). <sup>3</sup> M. Ochsner, et al., (2007) *Lab on a chip* (Accepted). <sup>4</sup> B. Stadler, et al., (2004) *Langmuir*, **20**(26): p. 11348-11354.

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