

**HIGH RESOLUTION MULTI-PROTEIN NANOPATTERNS**S. R. Coyer,<sup>1,2</sup> A. J. García,<sup>2</sup> E. Delamarche<sup>1</sup><sup>1</sup>IBM Research GmbH, Zurich Research Laboratory, 8803 Rüschlikon, Switzerland. <sup>2</sup>Woodruff School of Mechanical Engineering, Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332-0363

**INTRODUCTION:** The organization of proteins on surfaces is critical to the design of bioactive and biocompatible surfaces for implantable biomedical devices and in vitro studies of cell biology. In many cases, extracellular matrix composition and organization determine functionality, for example in stem cell localization, differentiation, and proliferation. Much attention has been given to developing methods to produce surfaces with biologically relevant modifications [1]. We report here a powerful yet simple method in which multiple proteins can be patterned simultaneously into complex architectures with nanoscale resolution and high contrast [2].

**METHODS:** Nanotemplates were produced using standard electron beam lithography techniques. Poly(dimethylsiloxane) planar elastomers were made from Sylgard® 184. Atomic force microscopy (AFM) images were obtained using tapping mode. TRITC- and AlexaFluor 647-labeled goat anti-rabbit IgG were printed onto glass and visualized by fluorescence microscopy.

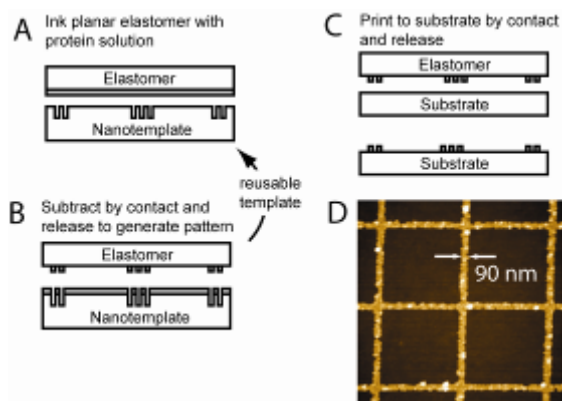


Fig. 1: (A-C) Experimental design to transfer patterns of proteins from a silicon nanotemplate to substrates using a planar elastomer. (D) Resulting pattern of proteins visualized by AFM.

**RESULTS:** Figure 1 presents the three steps in the “ISP” strategy: 1) a planar elastomer is inked (I) with a monolayer of fluorophore-labeled IgG and then brought into contact with the nanotemplate, 2) subtraction (S) occurs in regions of contact between the hydrophobic elastomer and hydrophilic nanotemplate, 3) the remaining protein pattern is printed (P) onto a final substrate. AFM visualization of the final substrate shows a pattern

of proteins with high resolution (90 nm) and high contrast. As shown in Table 1, the individual steps of the ISP strategy can be combined to produce a wide variety of protein patterns. Several geometries, sizes, and spacing between features can be produced. Spacing of up to 64  $\mu\text{m}$  between nanoscale features is achieved. Multiple proteins can be printed simultaneously in either overlapping patterns or patterns that are intrinsically self-aligned.

Table 1. Combinations of the ISP Strategy produce unique protein patterns.

| Protein  | Patterning Steps          | Results |
|----------|---------------------------|---------|
| B        | $I_B S_B P_B$             |         |
| A then B | $I_A S_A P_A I_B S_B P_B$ |         |
| A and B  | $I_A S_A I_B S_B P_{AB}$  |         |

**DISCUSSION & CONCLUSIONS:** The flexibility of the ISP strategy allows production of a wide variety of patterns with high resolution and high contrast. Single and multiple proteins can be printed in self-aligned patterns with precise control over feature geometry, size, and spacing. This technique is applicable to biological systems in which functionality is achieved through the combination of individual proteins into complex architectures. The ISP strategy provides a robust platform in which the role of individual components in the larger complex can be deduced through systematic variation of their organization.

**REFERENCES:** <sup>1</sup>S.J. Xiao, M. Textor, N.D. Spencer, H. Sigrist (1998) *Langmuir* 14 (19): 5507-5516. <sup>2</sup>S.R. Coyer, A.J. García, E. Delamarche (in press) *Angew. Chem. Int. Ed.*

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