

## Protein Self-Assembly on Micro-Contact Printed Surface Patterns

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**INTRODUCTION:** Functional protein arrays have a major potential in basic research, drug discovery, drug target identification and the development of novel pharmaceutical therapies. They contain proteins in their native state and are submitted to the analysis of a wide range of biochemical activities.

Micro-contact printing ( $\mu$ CP) has been used to pattern proteins both by direct placement, using the protein solution as the ink<sup>1</sup>, or indirectly by selective protein adsorption to a pre-patterned surface. Such self-assembly of proteins to surfaces aims at positioning the protein of interest on a pre-stamped template pattern via affinity tags allowing a both gentle and specific immobilization of proteins, as well as a controlled and uniform protein orientation at the surface. The recently developed SNAP-tag technology<sup>2</sup> has been shown to be suitable for surface immobilization of proteins<sup>3</sup>. The SNAP-tag protein, a mutant of the human DNA repair protein O<sup>6</sup>-alkylguanine-DNA-alkyltransferase covalently transfers its substrate (Benzyl Guanine (BG)) onto itself. In this study, we have combined the SNAP-tag technology with  $\mu$ CP in order to achieve high-resolution parallel patterning of protein, combined with a gentle, specific, oriented and versatile immobilization method, applicable to any soluble protein fused to a SNAP-tag.

**METHODS:** As a model protein we use a non-fluorescent SNAP-FLAG-His10 fusion construct, which enables us to purify the protein via the decahistidine tag, test the array compatibility with standard immunofluorescent staining, using a Cy3-labeled anti-FLAG antibody, and immobilize the protein to surfaces via the SNAP tag. Protein patterns were done by  $\mu$ CP, according to Stamou *et al.*<sup>4</sup>. Samples were characterized by confocal fluorescence microscopy.

**RESULTS & DISCUSSION:** The model protein SNAP-FLAG was not directly stamped to the surface but meant to self-assemble via the SNAP tag to streptavidin surfaces, via a biotinylated BG substrate. Two options of self-assembly to the surface are compared: (a) the "standard" biotinylation strategy, where the SNAP-tag moiety is biotinylated by pre-incubation with BG-biotin.

The biotin tag directs the immobilization to pre-patterned fluorescently labeled streptavidin on the surface; (b) the "in situ" strategy where the surface is pre-patterned with fluorescently labeled streptavidin preincubated with BG-biotin. The SNAP-tag moiety directs in this case the immobilization by recognizing the BG group from solution. We show that both approaches performed on different kinds of streptavidin surfaces were successful for specific positioning of the protein to the streptavidin layer and its correct orientation, as revealed by subsequent binding of a fluorescent Anti FLAG antibody.

The versatility of the SNAP-tag technology appears to be suitable for successive immobilization of distinct proteins on a single surface, without need of engineering the distinct proteins with various tags.

**REFERENCES:** <sup>1</sup> A. Bernard, J.P. Renault, B. Michel, H.R. Bosshard, E. Delamarche, E. (2000) *Advanced Materials* **12**: 1067-1070; <sup>2</sup> A. Keppler, S. Gendreizig, T. Gronemeyer, H. Pick, H. Vogel, K. Johnsson, (2003) *Nature Biotechnology* **21**: 86-89; <sup>3</sup> M. Kindermann, N. George, N. Johnsson, K. Johnsson (2003) *J. Am. Chem. Soc.* **125**: 7810-7811. <sup>4</sup> D. Stamou, C. Duschl, E. Delamarche, H. Vogel, (2003) *Angew. Chem. Int. Ed Engl.* **42**: 5580-5583.

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