

## Engineered mannose-presenting platform for the study of *E. coli* adhesion under static and dynamic conditions

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**INTRODUCTION:** Bacterial adhesion to carbohydrates of eukaryotic cell surfaces is involved in a plethora of infections. Often, mannoside-containing *N*-linked glycoproteins are targeted by the pathogen lectins,<sup>1</sup> though molecular details are not entirely understood. Therefore, the study of bacteria adhesion at the molecular level and their carbohydrate binding fingerprint are believed to contribute to a better understanding of adhesion mechanisms, important for bacterial infections and biofilm formation on medical devices. In the present study, we investigated the specific interactions between surface immobilized mannosides and the bacterium *Escherichia coli* (*E. coli*). Synthetic oligomannoside determinants, derived from high-mannose glycoproteins, were conjugated to the polycationic graft copolymer poly-(L-lysine)-*graft*-poly(ethylene glycol) (PLL-*g*-PEG) and adsorbed on Nb<sub>2</sub>O<sub>5</sub>-coated surfaces. *E. coli* adhesion was studied upon the sugar epitope, PEG-carbohydrate density and linker both under static and dynamic conditions.

**METHODS:** Surfaces were incubated with a genetically modified K-12 *E. coli* strain presenting fully functional type I fimbriae with mannose binding lectin FimH<sup>2</sup> at 38 °C during 30 min both under stagnant (10<sup>9</sup> cells/ml) and flow conditions (10<sup>8</sup> cells/ml). Stable adhered bacteria were observed by phase contrast microscopy and quantified by image analysis. A photolithography based patterning method (MAPL) was used to create bacteria adhesive patterns in a non-interactive background.<sup>3</sup>

**RESULTS:** *E. coli* adhered to surfaces via specific interactions with the adhesin FimH to the immobilized mannosides. No adhesion was observed with FimH deficient *E. coli* strains, on pure PLL-*g*-PEG surface, nor in the presence of high concentration of the inhibitor  $\alpha$ -Me-mannose. Although, strong multivalent interactions occurred at all the studied PEG-mannose densities (6.7x10<sup>4</sup>-2.2x10<sup>5</sup> mannosides/ $\mu$ m<sup>2</sup>), the number of adherent bacteria varied with the saccharide density. Furthermore, the *E. coli* binding affinity varied with the mannoside epitopes. The highest binding was shown to the branched trimannoside Man

(1 $\rightarrow$ 3)Man(1 $\rightarrow$ 6)Man (M3) compared to the monosaccharide (M1) while low affinity was observed to hexa- (M6) and nona-structures (M9) exposing (1 $\rightarrow$ 2)-linked mannoses (Figure 1a). Interestingly, it was also shown that a propyl linker stabilized the binding compared to an ethylene glycol chain. As bacterial adhesion in the body often occurs in the presence of fluid flow, *E. coli* adhesion was also studied under dynamic conditions. It showed that the binding of *E. coli* to the monosaccharide was enhanced by the shear stress while about no effect was seen for the binding to M3 or reduced as a result of kinetic effect on the initial attachment.<sup>4</sup> Finally, bacteria micropatterns in a bioinert non-functionalized PLL-*g*-PEG background were produced (Fig. 1b).

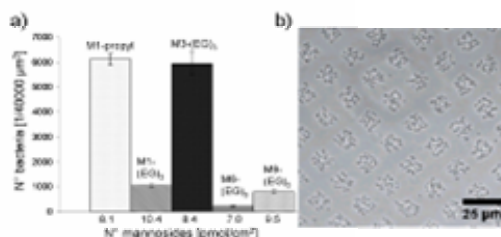


Fig. 1: *E. coli* adhering to PLL-*g*-PEG/PEG-mannoside: a) homogeneous surface containing various mannose epitope at ca. 10 pmol/cm<sup>2</sup>; b) 10 x 10  $\mu$ m<sup>2</sup> patterns of trimannose.

**DISCUSSION & CONCLUSIONS:** Mannoside functionalized PLL-*g*-PEGs are useful tools mimicking glycoproteins to study *E. coli* adhesion using well defined carbohydrate structures and controlled density. The presented platform can be easily extended for studies of other bacteria strains. Bacteria micropatterns are of interest to study mechanistic aspects of bacteria communication such as quorum sensing and biofilm formation in small and well-defined bacterial colonies.

**REFERENCES:** <sup>1</sup>N. Sharon (2006) *Biochem Biophys Acta* **1760**, 527-537. <sup>2</sup>W. Thomas et al. (2002) *Cell* **109** (7), 913-923. <sup>3</sup>D. Falconnet et al. (2004) *Adv Funct Mater* **14**, 749-756. <sup>4</sup>L. Nilsson et al. (2006) *J. Biol. Chem.* **281**, 16656-16663.

**ACKNOWLEDGEMENTS:** ETH Research Grant TH-38/04-2: "GlycoSurf" for funding.