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## Summary

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This thesis describes the selective attachment of proteins to  $\beta$ -cyclodextrin ( $\beta$ CD) self-assembled monolayers (SAMs), termed molecular printboards through multivalent orthogonal interactions. It is shown that the molecular printboards allow different assembly pathways for the build-up of (complex) bionanostructures. In the assembly of these bionanostructures, control over stability, stoichiometry of binding, and orientation is achieved. A monovalent supramolecular blocking agent can be applied to prevent nonspecific immobilization of proteins to the molecular printboard, while the specific attachment of proteins via multivalent interactions is still possible.

A review of the development of the  $\beta$ CD molecular printboard is given in Chapter 2. It is shown that the stable (reversible) attachment of molecules to the molecular printboard is based on the valency of the interaction. In multivalent binding to  $\beta$ CD molecular printboards, the high effective concentration of  $\beta$ CD at the surface is responsible for the high stability. This allows the patterning and the build-up of 3-dimensional (3D) structures on these molecular printboards. Furthermore, protein and cell attachment to surfaces in general is reviewed. Prerequisites are selectivity, function, stability, and control over orientation. Surfaces consisting of poly(ethylene glycol), as developed by amongst others Whitesides, are discussed as a manner to avoid nonspecific protein immobilization. Control over orientation is possible by making use of bio-engineered His<sub>6</sub>-tags in proteins. These proteins can be attached to surfaces which contain Ni-*N*-nitrilotriacetic acid. This is also a method which yields a high coverage of functional protein, in contrast to immobilization methods in which lithography is used.

The stepwise reversible attachment of a noncovalent capsule on the molecular printboard is discussed in Chapter 3. The capsule is based on two calix[4]arenes. The bottom half of the capsule being a calix[4]arene modified with four adamantyl functionalities at the lower rim to ensure stable positioning at the molecular

printboard, and four guanidinium groups at the upper rim, the top part calix[4]arene modified with four sulfonate groups at the upper rim to ensure binding to the guanidinium groups at the lower half of the capsule. The association constants ( $K_a$ ) for capsule formation in solution and at the surface are comparable. The possibility of stepwise adsorption and desorption of the capsule on the molecular printboard is shown using surface plasmon resonance spectroscopy (SPR) experiments.

The attachment of streptavidin (SAv) to the molecular printboard via orthogonal linkers and the hetero-functionalization of surface-confined SAv is shown in Chapter 4. SAv is assembled via orthogonal linkers bearing a biotin moiety on one side and either a single (monovalent) or two (divalent) adamantyl groups on the other. The tetravalent linker-protein complex is much more stable at the surface than the divalent linker-protein complex, as demonstrated in competition experiments. The divalent linker allowed the stepwise adsorption of SAv to the molecular printboard, which is confirmed by SPR and atomic force microscopy (AFM) measurements. The availability of the free biotin-binding pockets in the stepwise immobilized SAv at the printboard is shown by patterning studies in which the divalent ligand is patterned to which SAv is attached. The subsequent attachment of biotin-4-fluorescein showed the availability of the free binding pockets. Cytochrome *c* (cyt *c*) is the first functional protein that is attached to stepwise immobilized SAv. It is proven that the cyt *c* coverage at the molecular printboard is similar to the theoretical coverage.

In Chapter 5, the possibility of antibody (AB) attachment to the molecular printboard via multiple orthogonal binding motifs is described. Patterning studies in which a bionanostructure of SAv, biotinylated protein A (bt-PA), and a fluorescently labeled Fc fragment of a human immunoglobulin (IgG-Fc) are built up, show that the assembly process is selective. AB bionanostructures are built on top of the molecular printboard via two different assembly schemes: via a biotinylated immunoglobulin and via an Fc receptor protein. Both SPR and AFM measurements confirm the build-up of these bionanostructures. AB-coated surfaces are used as a platform for specific cell attachment. From fluorescence studies it appears that CD3<sup>+</sup> lymphocytes can be attached to the molecular printboard in a specific manner. Cell attachment appeared to be approximately linear with concentration. For the attachment of proteins inside microchannels, a chip with a large channel splitting up into four smaller, parallel channels is designed. In this chip, a  $\beta$ CD SAM is immobilized in a stepwise fashion.

The four smaller channels can be addressed individually by the stepwise and selective immobilization of the bionanostructures.

The development of a supramolecular method for the inhibition of nonspecific protein interactions to surfaces is shown in Chapter 6. To this purpose an adamantyl-modified hexa(ethylene glycol) guest molecule (AdHEG) has been synthesized. The hexa(ethylene glycol) chain prevents nonspecific protein adsorption, while the adamantyl part ensures specific interaction to the molecular printboard. It is shown that AdHEG is efficient in preventing the nonspecific interactions of SAV, the histidine-tagged maltose binding protein (His<sub>6</sub>-MBP), and bovine serum albumin (BSA) to the molecular printboard. The concentration of AdHEG functional for the inhibition of nonspecific protein immobilization is about an order of magnitude lower than for the standard protein-repellent poly(ethylene glycol) PEG-surfaces, as developed by, amongst others, Whitesides. Furthermore, AdHEG still allows the specific immobilization of SAV and His<sub>6</sub>-MBP through the divalent adamantyl-biotin linker and an adamantyl-modified Ni-*N*-nitrilotriacetic acid (Ni-NTA) linker, respectively.

The attachment of His<sub>6</sub>-tagged proteins to the molecular printboard in a specific fashion is described in Chapter 7. Titration experiments with His<sub>6</sub>-MBP to the molecular printboard were modeled, and from these modeling studies it could be concluded that the binding of Ad-NiNTA to His<sub>6</sub>-MBP in solution is to a large extent monovalent. On the surface, however, the majority of the His<sub>6</sub>-MBP is bound in a trivalent fashion, via three Ad-NTA-Ni linker complexes. This difference is caused by the high effective concentration of  $\beta$ CD at the surface, which induces a high stability of binding in a multivalent fashion, to molecular printboards. The surface enhancement factor for the formation of the trivalent species at the surface is a factor 30 higher than for the divalent species. Patterning of His<sub>6</sub>-tagged proteins is shown with (His<sub>6</sub>)<sub>4</sub>-DsRed-FT. This auto fluorescent protein was patterned on the molecular printboard with a high level of specificity. When (His<sub>6</sub>)<sub>4</sub>-DsRed-FT is patterned on the molecular printboard in the absence of Ad-NiNTA, the patterns were not stable upon rinsing. In contrast, the formed patterns were stable when (His<sub>6</sub>)<sub>4</sub>-DsRed-FT is premixed with Ad-NiNTA. SPR experiments showed that the nonspecific interactions for the  $\alpha$ -(His<sub>6</sub>)<sub>14</sub>-20S proteasome could be suppressed by about 60%.

This thesis shows that the molecular printboard can be used as a general platform for the attachment of proteins and cells. It is possible to build up complex bionanostructures at the molecular printboard, consisting of multiple orthogonal binding motifs, which resulted in control over thermodynamics, orientation, and functionality. It is shown that, by means of supramolecular chemistry and multivalency, a very effective scheme for the blocking of nonspecific protein immobilization is possible, while maintaining the specific binding via multivalent interactions. These results constitute a starting point for the development of applications for the immobilization of proteins, such as ABs, cells, and the selective immobilization of proteins inside microchannels. The results described in this thesis can be applied in the development of optical and electrochemical biosensors, which may be useful for medical and environmental applications. Another possibility is the development of more complex protein or DNA assays based on the immobilization schemes that have been developed in this thesis.