Assembly of Oriented Virus Arrays by Chemo-Selective Ligation Methods and Nanolithography Techniques

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ABSTRACT

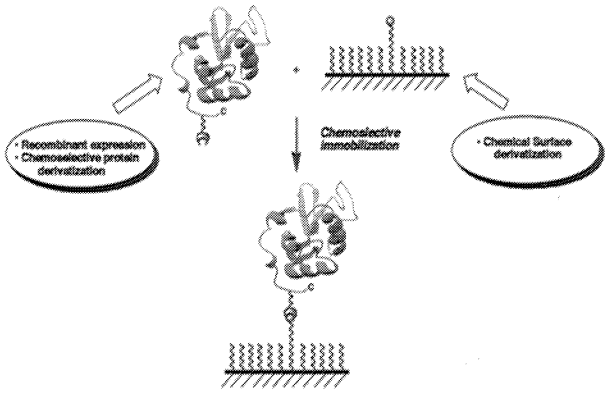
The present work describes our ongoing efforts towards the creation of nano-scaled ordered arrays of protein/virus covalently attached to site-specific chemical linkers patterned by different nanolithograpy techniques. We will present a new and efficient solid-phase approach for the synthesis of chemically modified long alkyl-thiols. These compounds can be used to introduce chemoselective reacting groups onto gold and silicon-based surfaces. Furthermore, these modified thiols have been used to create nanometric patterns by using different nanolithography techniques. We will show that this patterns can react chemoselectively with proteins and/or virus which have been chemically or recombinantly modified to contain complementary chemical groups at specific positions thus resulting in the oriented attachment of the protein or virus to the surface.

Keywords: Chemoselective Attachment, Protein Arrays, Dip Pen Nanolithography, Cow Pea Mosaic Virus (CPMV)

1 INTRODUCTION

Many experimental techniques in biology and biophysics, and applications in diagnosis and drug discovery, require proteins immobilized on solid substrates. In fact, the concept of arrays of proteins attached to a solid support has attracted increasing attention over the last three years due to the sequencing of several genomes, including the human genome. Protein arrays can be used easily for the parallel analysis of whole proteomes. Another powerful application employs ordered nanometric arrays of proteins as nucleation templates for protein crystallization. Recent advances in nanoprinting techniques have allowed the creation of sub-micrometer arrays of proteins [1,2]. All these applications demonstrate the use of protein arrays and also highlight the need for methods able to attach proteins in a well defined and ordered way onto a solid supports.

Various methods are available for attaching proteins to solid surfaces. Most rely on non-specific adsorption, or on the random cross-linking of proteins to chemically reactive surfaces. In both cases the protein is attached to the surface in random orientations. The use of recombinant affinity tags addresses the orientation issue. However, in most cases the interactions of the tags are reversible and therefore not stable over the course of subsequent assays or require large mediator proteins. For the Covalent attachment and orientation of a protein to a solid support requires two unique and mutually reactive groups on the protein and the support surface are required. The reaction between these two groups should be highly chemoselective, thus behaving like a molecular '*velcro*' (Scheme 1).



Scheme 1: General concept of chemoselective reaction between a functionalized protein and an appropriately modified surface.

2 RESULTS

The principle of chemoselectivity is fundamental to modern protein chemistry, and simply refers to the ability to chemically modify a functional group even in the presence of other chemical groups. In our case this modification involves the reaction between a unique chemical group present in the protein or virus with a complementary one contained on the surface where the protein has to be attached (Scheme 1). This reaction has not to be affected by the presence of the other reactive groups normally present in a protein.

Most of the methods suitable for the chemoselective attachment of proteins to surfaces are based on ligation methods originally developed for the synthesis, semisynthesis and selective derivatization of proteins by chemical means (see reference [3] for a complete review). These methods involve the derivatization of the protein with a unique chemical group at a defined position, which will later reacted chemoselectively with a complementary group previously introduced into the surface.

2.1 Chemical Modification of Surfaces

When trying to attach proteins to surfaces the most common surfaces employed are silicon based (e.g. glass slides or Si/SiO_2 wafers) or metals (mainly Au [111] surfaces). Modified silanes and thiols are usually employed for the chemical derivatization of these surfaces, respectively.

In the present work we have developed an efficient solid-phase approach for the rapid synthesis of chemically modified long alkane thiols (Figure 1).

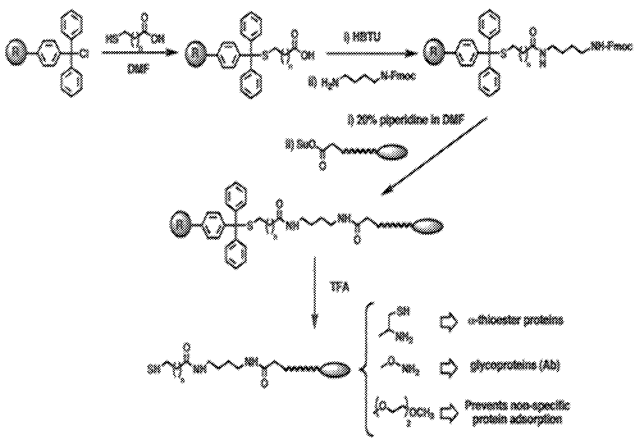


Figure 1. Synthetic scheme for the rapid and efficient preparation of chemically modified thioalkanes. The solid elipsoid represents the different reactive groups that can be introduced in the surface.

The first step involves the immobilization of the corresponding ω-mercaptoalkanoic acid on a trityl chloride resin. The thiol moiety of the carboxylic acid reacts selectively with the trityl chloride resin forming a thioether which results in the immobilization of the ω-mercaptoalkanoic acid on the solid-support. The free carboxylic function is then activated with HBTU (2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate) (HBTU) in DMF (dimethylformamide) after which is reacted with a mono-protected diamine. This step transforms the previous carboxylic function in a more versatile amino group. After the deprotection step, the amino group is acylated with the appropriate complementary reactive moiety (Figure 1) to yield the corresponding modified alkane thiol. Among others, the introduction of a Cys moiety allows the selective reaction with C-terminal α-thioester proteins [3,6], the amino-oxy group allows the reaction with carbonyl-containing proteins (which can be easily generated by mild oxidation of glycoproteins) and the tri-(ethyleneglycol) (TEG) moiety prevents non-specific adsorption of proteins to surfaces. The final of the synthesis step involves the cleavage and deprotection of the product from the resin, which is accomplished by treatment with trifluoroacetic acid. The whole synthetic process is extremely efficient and the final product can be obtained with an overall yield bigger than 95% thus not requiring further purification.

We have shown that these chemically modified thiols can be used to generate nanometric chemical patterns on Au and Si-based surfaces using micro-contact printing [5] and dip pen nanolithography (DPN) [1,2] techniques (Figure 2).

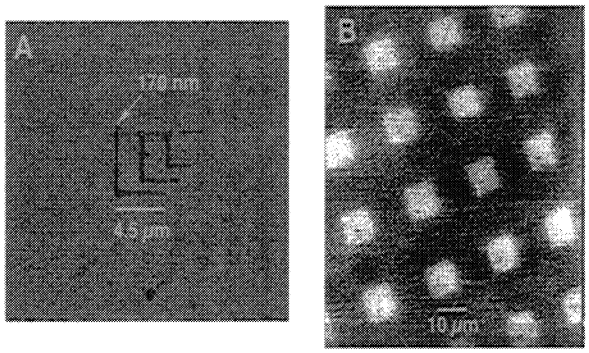


Figure 2. Producing chemical templates with modified alkylthiols on gold (A) and Si-based (B) surfaces. A. Friction-mode AFM image of $HS-(CH_2)_{15}-CONH-(CH_2)_4-NH_2$ patterned on gold using DPN. B Friction-mode AFM image of $HS-(CH_2)_{15}-CONH-(CH_2)_4-NH_2$ patterned on a Si/SiO_2 wafer previously reacted with $Br-(CH_2)_{11}-Si(OCH_3)$ using the micro-printing contact technique.

2.2 Chemoselective Attachment of Cow-Pea Mosaic Virus on Chemically Modified Surfaces

As a model for testing the chemoselective attachment of proteins to chemically patterned surfaces we used the Cowpea Mosaic Virus (CPMV). The CPMV particles are ≈30nm-diameter icosahedra and they are formed by 60 copies of two different types of viral proteins. The virus is remarkably stable and it can be genetically modified to display the thiol function (i.e. by introducing Cys mutations) on specific locations of the exterior surface of the viral capsomer (Figure 3) [4].

It is well known that the thiol group reacts with high chemoselectivity with some chemical groups like α-haloacetyl and maleimido compounds [3]. Moreover, these mutated virus have been shown to react chemoselectively

with derivatized gold-nanoparticles and fluorescent dyes [4].

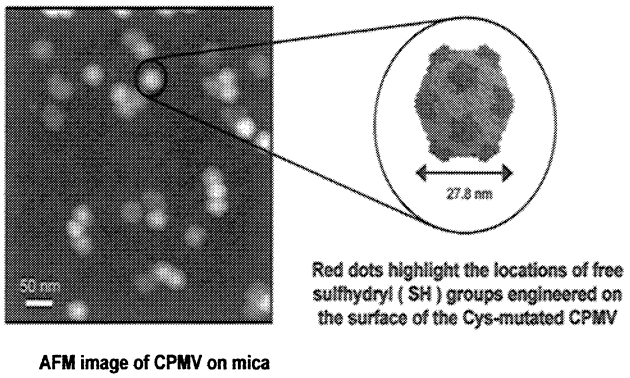


Figure 3. Structure of the Cowpea Mosaic Virus (CPMV).

Using the highly selective reaction between the thiol and the maleimido groups, we have shown that the Cys-mutated CPMV particles can be chemoselectively attached on a chemical template containing the maleimido functionality (Figure 4).

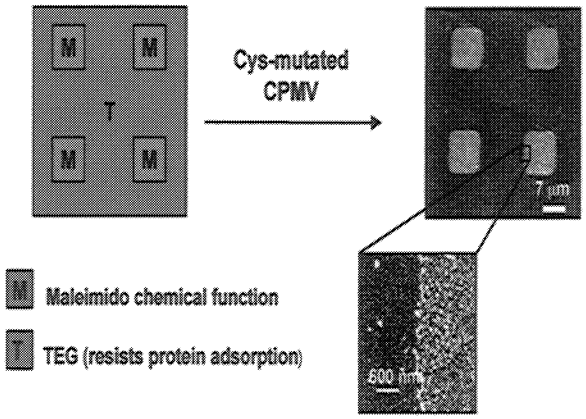


Figure 4. Preferential covalent attachment of Cys-mutated Cow Pea Mosaic Virus (CPMV) on a maleimido template fabricated by micro-contact printing.

The chemical template was produced by micro-contact printing on a gold surface using $HS-(CH_2)_{15}-CONH-(CH_2)_4-NH_2$ as ink. The gold surface outside of the patterned area was then capped with an alkylthiol containing a TEG moiety to prevent any non-specific adsorption of the virus. The maleimido function was finally introduced by reacting the amino group with 3-maleimidopropionate N-hydroxysuccinimide ester (MPS). The freshly reduced Cys-mutated virus was then left to react with the chemically patterned surface on degassed PBS overnight at 4° C. Once the ligation reaction was finished the surface was extensively washed with PBS to remove any virus not covalently attached to the surface. As shown in Figure 4 the Cys-mutated virus was found preferentially (with a selectivity of 1 to 10000) on the squares containing the maleimido function.

3 SUMMARY OF RESULTS

In summary, we have developed a general approach which combines nanolithographic techniques with chemoselective linkers to fabricate chemical templates for creating oriented protein and virus arrays. We have also developed efficient and rapid chemical routes for the synthesis of modified alkane thiols which can be used for the chemoselective attachment of proteins as well as surface passivation. Finally, we have demonstrated that a maleimido-containing template in combination with TEG can be used for making templates of CPMV viruses in micron-scale.

Future work will involve the fabrication of nano-scaled template arrays of chemoselective linkers on flat gold surfaces by using DPN. This templates will be designed with different geometric designs in order to investigate the inter-virus interactions.

4 AKNOWLEDGMENTS

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