BioNanoAdhesion: atomic force microscopy study of the electrostatic properties of pyridine-and imidazole-based polycationic surfaces

Conference or Workshop Item

BioNanoAdhesion: Atomic Force Microscopy Study of the Electrostatic Properties of Pyridine- and Imidazole-based Polycationic Surfaces

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Introduction

Atomic force microscopy (AFM)[1] is widely recognised as a highly versatile scientific tool, which can be used for numerous types of investigations. One of the most fundamental AFM measurements is the investigation of the force which exists between two interacting surfaces.[2] Such measurements can be performed by attaching a probe to the apex of a suitable AFM cantilever,[3] as shown in **figure 1**. A spherical microparticle will provide a tip with defined geometry, enabling the interaction force to be calculated accurately.

The objective of this investigation is to assess the effect of environmental pH on the degree of protonation of a self-assembled monolayer (SAM) formed from an amino-containing moiety in which the pK_a is tuneable. An AFM cantilever modified with a silica microparticle (6.62 μm mean diameter) will be used to investigate the protonation of the SAM over a range of pH. The surface of the particle will be polyanionic when the environmental pH is greater than the isoelectric point of silica, pH 2.[4] Therefore, an electrostatic force will exist between the silica and SAM surfaces, which can be measured quantitatively using the AFM.

The results obtained from this investigation could be used in the development of non-viral nanoparticulate vectors for gene delivery. Gene delivery has the potential to treat cancer, cystic fibrosis and cardiovascular disorders.[5] Non-viral gene delivery involves complexing DNA with an amino-containing polycationic vector, which is then delivered to the nucleus of target cells.[6] The electrostatic interaction between the DNA and the vector plays a crucial role in the efficiency of the delivery process. The interaction between the SAM and the silica microparticle can be treated as a model system for the DNA-vector interaction. A cartoon representation of the transfection process is shown in **figure 2**.

Synthesis

A series of pyridine and imidazole derivatives are currently being prepared. They will be used to form SAMs, whose electrostatic properties can be assessed using AFM. Their syntheses are illustrated in **schemes 1 & 2**. Compounds (**4**), (**5**) and (**9**) are the molecules to be used for SAM formation and AFM analysis in the first instance. Commercially available mercaptoundecanoic acid (**1**) is converted into the disulfide form (**2**) via iodine-induced oxidation. This prevents the thiol group from competing with the hydroxyl group on the pyridine and imidazole molecules during the subsequent esterification reaction.[7] The oxidation of the thiol group in (**1**) produces HI in the formation of (**2**). The HI catalyses the formation of the bis-ethyl ester (**2**). Thus, the next step is to hydrolyse the ethyl ester, affording the bis-acid (**3**). (**4**) is prepared via a DCC coupling reaction between (**3**) and 3-pyridinepropanol. N-methylation of (**4**) affords (**5**). The nitrogen at position 1 in (**6**) is protected with the *tert*-butoxycarbonyl group (Boc), affording (**7**). This allows selective coupling of (**7**) to (**3**) in an esterification reaction. The Boc protecting group in (**8**) is removed using trifluoroacetic acid (TFA), affording (**9**).

SAM formation

When performing AFM force measurements, the presence of asperities on either of the surfaces will reduce the measured interaction force.[8] Surfaces with low roughness are produced by depositing gold onto freshly cleaved mica using an Agar automatic sputter coater. **Figure 3** shows a typical region of the sputter deposited gold surface over a 1 μm^2 area. AFM characterisation of the gold surface revealed a mean RMS roughness of 0.670 nm and a mean peak-to-valley distance of 4.213 nm.

The SAMs are formed by immersing the substrates in a 1 mM ethanolic solution of SAM molecules (**4**), (**5**) or (**9**) for 24 hours. The molecules will coordinate strongly to the gold surface through Au-S bonds.[9] The van der Waals forces between alkyl chains will cause them to pack closely together, yielding a two-dimensional quasi-crystalline molecular assembly.[10] The formation process is followed by contact angle measurements and verified by X-ray photoelectron spectroscopy (XPS). **Figure 4** shows an idealised arrangement of the SAM molecules on the gold surface.

Conclusion and outlook

SAMs of pyridine- and imidazole-based disulfides are currently being produced on low roughness gold surfaces. The electrostatic interaction between these surfaces and an AFM cantilever, modified with a silica microparticle, will subsequently be investigated as a function of environmental pH. The results can be used towards the development of improved nanoparticulate non-viral gene delivery vectors.

Acknowledgements

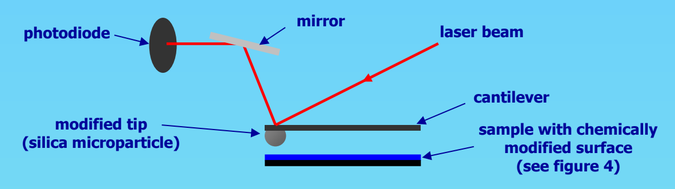


Figure 1. Measuring surface interactions using AFM

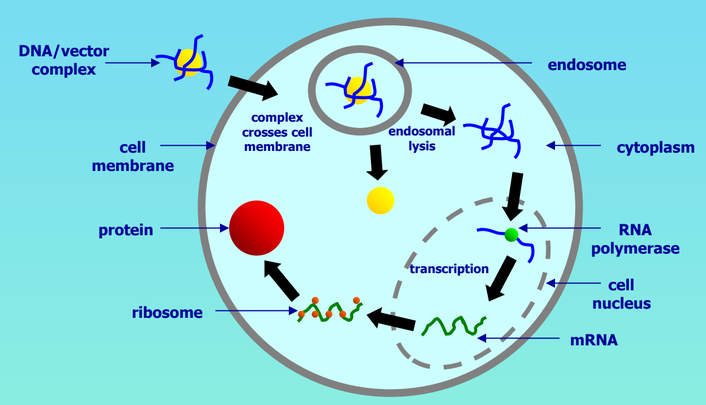
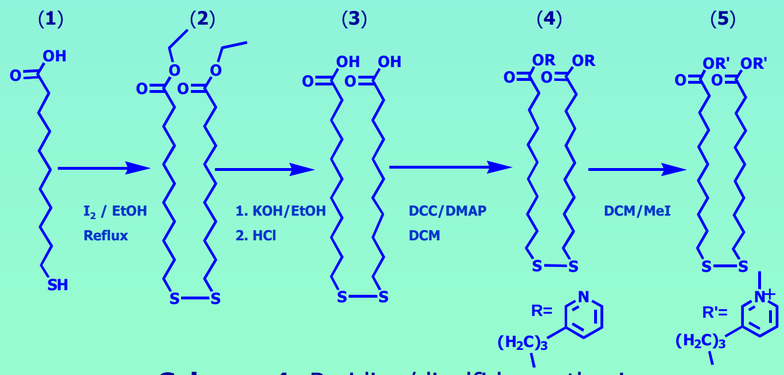
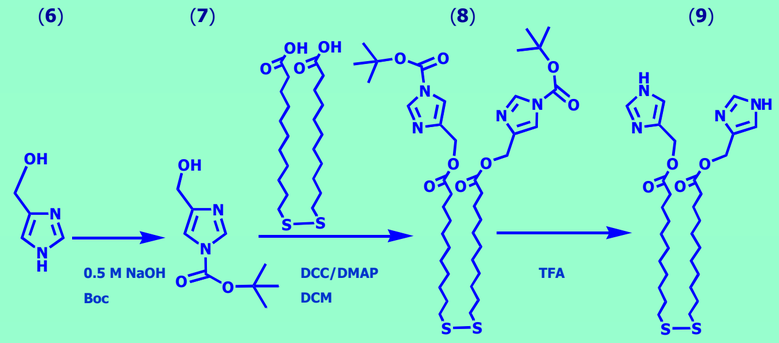


Figure 2. Transfection of a cell by a non-viral gene delivery vector



Scheme 1. Pyridine/disulfide synthesis



Scheme 2. Imidazole/disulfide synthesis

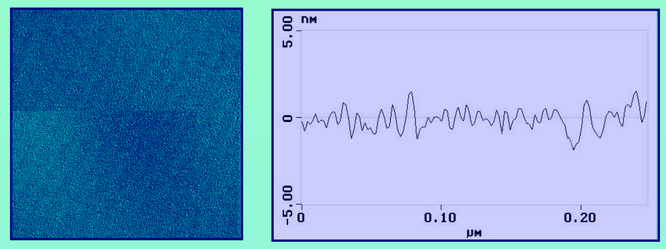


Figure 3. 1 μm^2 region and section analysis of a sputter deposited gold-on-mica substrate obtained using contact mode AFM (10 nm height scale)

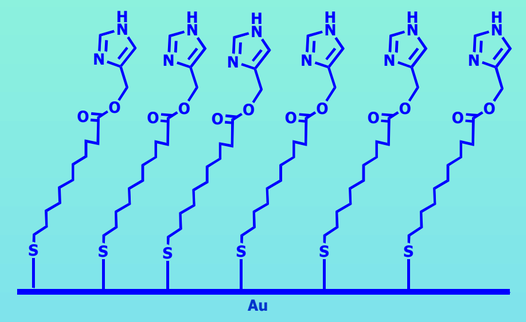


Figure 4. Imidazole/disulfide SAM on gold surface

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